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Production of semi-refined carrageenan from locally available red seaweed, *Eucheuma cottonii* on a laboratory scale

(Penghasilan karaginan separa tulen daripada rumpair laut merah tempatan, *Eucheuma cottonii* pada peringkat makmal)

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Key words: semi-refined carrageenan, process optimisation, red seaweed *(Eucheuma cottonii)*

Abstrak

Kaedah penghasilan karaginan separa tulen (SRC) daripada rumpair laut merah tempatan, *Eucheuma cottonii* melibatkan proses praperlakuan, pengekstrakan alkali, peneutralan, pengeringan dan pengisaran. Keadaan optimum bagi praperlakuan adalah membasuh rumpair laut kering di dalam air yang mengalir selama 2 minit. Untuk pengekstrakan alkali, keadaan optimum adalah dengan menggunakan kalium hidroksida pada pH 13 selama 1 jam pada suhu 80 °C, diikuti dengan proses peneutralan iaitu SRC direndam di dalam air (1:3) selama 4 jam kemudian direndam semalaman. Bagi proses pengeringan, terdapat pilihan tiga proses iaitu pada 70 °C selama 5 jam, 60 °C selama 8 jam atau 50 °C selama 24 jam. SRC yang dihasilkan mempunyai sifat fizikal dan kimia setanding dengan spesifikasi ketulenan EEC Council Directive (78/663/EEC)(1978) bagi karaginan tulen.

Abstract

Procedures for the production of semi-refined carrageenan (SRC) from local red seaweed, *Eucheuma cottonii* involved pre-treatment, alkali extraction, neutralisation, drying and grinding. The optimal conditions for pre-treatment involved washing the dried seaweed under running water for 2 minutes. For alkaline extraction, the optimum conditions were extraction using potassium hydroxide at pH 13 for 1 hour at temperature of 80 °C followed by neutralisation whereby the SRC was soaked in water (1:3) for 4 hours followed by second soaking overnight. Drying can be carried out using a choice of three optimal conditions namely drying at 70 °C for 5 hours, 60 °C for 8 hours or 50 °C for 24 hours. The SRC produced had physical and chemical properties comparable to the purity specifications of the EEC Council Directive (78/663/EEC)(1978) that is specifications for pure carrageenan.

Introduction

Seaweeds belong to the simplest group of plants, the algae. With a few exceptions, these plants are so simple that they have no distinguishable roots, stems or leaves, and all parts of the plant looking much alike (Rabanal and Trono 1983). The algae vary in size, from microscopic single celled forms to gigantic macrophytes. There are four main sub-groups of algae, each

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distinguished by the predominant colouring pigment in their cells. They are Cyanophyta (blue-green), Chlorophyta (green), Rhodophyta (red) and Phaeaophyta (brown). The economic marine seaweeds belong to the red, brown and green algae; most blue-greens as well as some greens, are fresh water forms.

For centuries, seaweed of various kinds had been put to use in the countries of South and Southeast Asia. Development of the uses of the seaweeds was favoured by their ready availability and proximity to centres of human population (Rabanal and Trono 1983). In Malaysia, seaweeds have been used as food, fertilisers, animal feed and in traditional medicine (Burkill 1966). Industrial use of seaweed on a commercial basis is a recent development (Infofish 1983). This is especially so in developing countries where only a few have set up sizeable seaweed processing industries. The most dynamic sector of the industry is the manufacturing of a wide range of seaweed colloids and its remarkable variety of commercial applications, ranging from air fresheners and textiles to pharmaceuticals and processed foods.

One of these commercially important hydrocolloids is carrageenan that occur as matrix materials in numerous species of red seaweed (Rhodophyta) wherein they serve a structural function analogous to that of cellulose in land plants (Stanley 1987). Seaweeds which have been used for carrageenan production are *Chondrus cripus* (France, North Atlantic), *C. ocellanus, Gigartina stellata* (France), *G. acicularis* and *G. pistillata* (Morocco), *G. canaliculata* (Mexico), *G. chamissoi* (Peru, Chile), *G. radula* (Chile), *G. skottsbergii* (Argentina, Chile), *E. cottonii* and *E. spinosum* (Philippines, Indonesia), *Hypnea musciformis* (Brazil), *H. spiciferea, Gymnogongrus, E. gelatinae* and *Furcellaria fastigiata*. *Eucheuma cottonii* and *E. spinosum* are heavily utilized by carrageenan producers and widely cultivated in the Philippines and Indonesia (Infofish 1991).

In Malaysia, the Fisheries Department of Sabah has an on-going project to promote seaweed mariculture of the *Eucheuma* species amongst local fishermen around the Semporna area and this project has been very successful whereby the production of fresh seaweed has drastically increased from 200 t in 1990 to more than 2 000 t in 1997 (Anon. 1998). Export of dried seaweed has increased from 3 t/month in 1989 to more than 100 t/month presently. The areas of mariculture have also expanded to Kunak, Lahad Datu and Kota Belud. The dried seaweeds produced are exported to Denmark, France and other western countries to be processed into refined carrageenan.

Refined carrageenans are categorised based on their three major fractions, known commercially as ‘kappa’, ‘iota’ and ‘lambda’, which differ essentially in the degree of sulphation (Morris 1986). Carrageenans are widely used as thickening agents and stabilizers in the food industry (Glicksman 1969), especially in emulsion stabilisation, for syneresis control and for bodying, binding and dispersion. Carrageenan is unique in its ability to suspend cocoa in chocolate milk at very low concentration. Major uses of carrageenan in foods are in frozen desserts, pasteurised milk products, cream mixture for cottage cheese, sterilised milk products, whipped products, acidified milk, jellies, syrups, fruit drink powders, frozen concentrates, imitation milk and others (Stanley 1987). Carrageenan has also found an important use in many dietetic and dietary products, cosmetics and photographic films (Huffman and Shah 1995). Carrageenan is used in combination with locust bean and guar galactomannans as a gelling agent in pet foods. However, extracted carrageenans have now almost been entirely replaced by semi-refined carrageenan from *E. cottonii*.

Carrageenans are produced by the extractive and non-extractive methods (Llana 1991). The extractive method, used in refined carrageenan processing, involves

alkaline cooking of dried seaweed materials and more sophisticated and technically advanced potassium chloride or alcohol extraction system. On the other hand, non-extractive method, used for semi-refined carrageenan involves cooking of dried seaweed materials in alkali solution to a desired modification level, washing, drying, grinding and blending to customers' satisfaction.

Carrageenans may occur under the names 'carraghenate', 'Danish agar', 'eucheuman', 'fercellaran', 'gelose', 'hynean', 'Irish moss', 'carragheen', 'refined carrageenan', 'semi-refined carrageenan', 'Philippine natural grade (PNG) carrageenan', 'processed eucheuma seaweed' (PES) and 'red algae extract' (Bixler 1994). The efforts being made by the Philippines and other countries to combine all the different types of carrageenan above under one EC classification E407 are being opposed by certain manufacturers and trade organisations who have proposed new names for the semi-refined grades and change the specifications in favour of refined grade carrageenan in order to deny food grade status to less expensive semi-refined grades. However, in 1995, the Codex Alimentarious Commission (CAC) approved an International Numbering System (INS) – E407a – for semi-refined (PNG) carrageenan to be listed as additive for food (Anon. 1997).

The production of semi-refined carrageenan in Malaysia is relatively new. The process is distinguished by its low energy inputs and the utilisation of locally available *Eucheuma cottonii* as a raw material. The specific details for the extraction process are closely guarded as trade secrets by several manufacturers of carrageenan. Hence, this study was undertaken to determine the optimum parameters in the extraction process for the production of semi-refined carrageenan from locally available seaweed, *E. cottonii*.

Materials and methods

Acquisition of samples

Dried seaweeds, *E. cottonii* were obtained from the district of Semporna, Sabah. Three lots were obtained over the year in consideration for seasonal variations, if any. The analyses were carried out in triplicates.

Pre-treatment of dried seaweed

Pre-treatment was carried out on 100 g dried seaweed to remove foreign matter such as sand, stones, dried sea animals as well as nylon strings used in the cultivation process. Pre-treatment of the dried seaweed was carried out with the main aim of cleaning and reducing the sea salt content in the raw materials used. The dried seaweed contained impurities and foreign matter that have to be sorted out carefully before the extraction process. Salt content in the dried seaweed needs to be reduced as this will affect the final gelling property of the SRC. The carrageenan from *E. cottonii* is mainly made up of *kappa* fractions and this *kappa*-carrageenan was reported to form a strong and brittle gel in the presence of K^+ (Rees 1969). Manual sorting was done to remove any physical matter visible to the naked eye. In order to reduce the sodium chloride content, six treatments were carried out such as washing under running water for 1 min and 2 min; soaking in water for 5 min and 10 min at ratio of 1:1 seaweed to water; and soaking in water for 5 min and 10 min at ratio 1:2 seaweed to water. The final sodium chloride content was determined as chloride content (Egan et al. 1981). The conditions that resulted in minimal sodium chloride content were selected.

Extraction process (alkali cooking)

The extraction process involved the use of dilute potassium hydroxide solution at alkaline pH (10; 11; 12; 13) at the temperature ranges of 80, 90 and 100 °C for time duration of 1, 2 and 3 hours. Optimal condition chosen as those giving the least breakage (evaluated visually).

Neutralisation
Seaweed was neutralised by soaking in water for 4 h or soaking for 4 h, followed by a second soaking overnight. The condition that resulted in efficient pH reduction to neutral was considered as optimum.

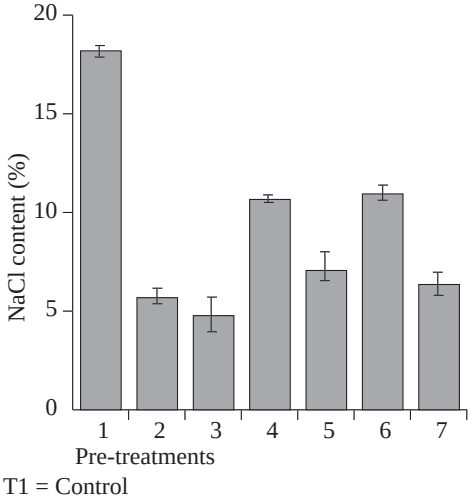
Drying
The neutralised product was dried in a convection oven at temperatures of 50, 60 and 70 °C for the duration of 5 h, 8 h and overnight. The process parameters that effectively reduced the moisture content to less than 10% were chosen as criterion for product stability.

Analysis of semi-refined carrageenan (SRC)
For the analysis of the SRC, the dried product was chipped and ground. The analysis included physical, chemical and microbiological tests. The physical tests conducted were gel strength, viscosity, freeze-thaw property and syneresis of gel (Lawrence 1973). Chemical analysis included total moisture, total ash, acid insoluble ash and sulphate content (AOAC 1990). Microbiological test conducted was total plate count (APHA 1976). All analyses were done in triplicate and the results presented in this paper are the mean values of three batches.

Results and discussion
The production of semi-refined carrageenan (SRC) from local seaweed was carried out using the seaweed species of *E. cottonii* obtained from Semporna district in Sabah. The samples had been sun-dried and baled before processing. In the production of semi-refined carrageenan (SRC), the main processes involved are pre-treatment, alkali cooking, neutralisation and drying of the final product (Infofish 1991).

Six pre-treatment processes were evaluated and the results on the sodium chloride content after each treatment are shown in *Figure 1*. T3 resulted in the highest reduction in salt content, followed by T2, T7, T5, T4 and T6. All the treatments except for T4 and T6 showed more than 50% reduction in the sodium chloride content in the raw materials. Consequently, it could be inferred that optimal conditions for pre-treatment involved washing the dried seaweed under running water for 2 min viz. T3.

The SRC extraction involves a hot water, high pH process. High pH must be maintained as carrageenan rapidly degrades under hot acidic conditions. A range of temperatures (80–100 °C), pH (10–13) and time of extraction (1–3 hours) were evaluated to determine the optimal conditions for the alkaline cooking. Doty (1986) reported the alkali-treatment process for dried *E. cottonii* being treated with hot alkali solution containing about 8.5% potassium hydroxide and maintaining pH at 13. During the extraction process, breakage of the seaweed was observed and it can be seen that lower pH resulted in higher breakage *(Table 1)*. High breakage would



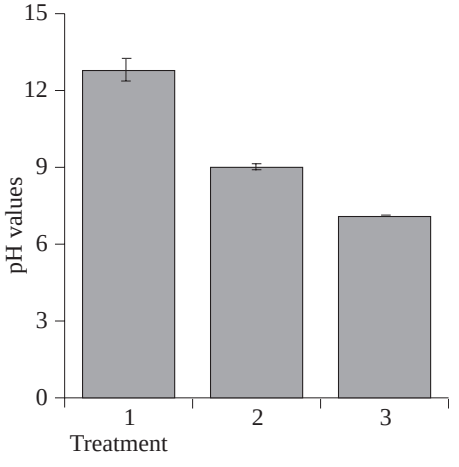
T1 = Control
T2 = Washing under running water for 1 min
T3 = Washing under running water for 2 min
T4 = Soaking in water (1:1) for 5 min
T5 = Soaking in water (1:2) for 5 min
T6 = Soaking in water (1:1) for 10 min
T7 = Soaking in water (1:2) for 10 min

Figure 1. Effect of pre-treatments on the NaCl content

Table 1. Effect of extraction process (alkali cooking) on the breakage of seaweed

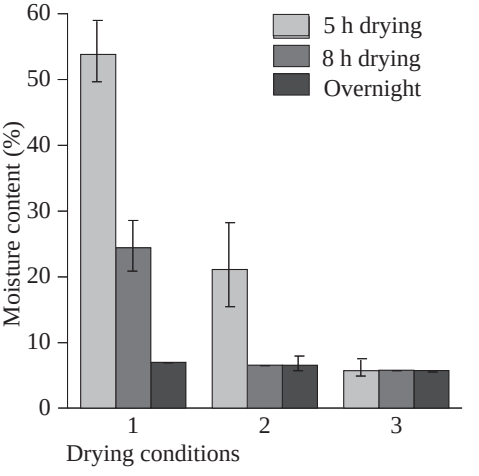
	80 °C			90 °C			100 °C		
	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
pH 11	+++	+++	+++	+++	+++	+++	+++	+++	+++
pH 12	+++	+++	+++	+++	+++	+++	+++	+++	+++
pH 13	+	++	+++	++	+++	+++	+++	+++	+++

+++ = high breakage (more than 50%)
++ = less breakage
+ = little breakage (less than 5%)



T1 = Control
T2 = Soaking in water (1:3) for 4 h
T3 = Soaking in water (1:3) for 4 h followed by second soaking overnight

Figure 2. Effect of neutralisation treatments on the final pH of SRC



T1 = Drying at 50 °C for 5 h, 8 h and overnight
T2 = Drying at 60 °C for 5 h, 8 h and overnight
T3 = Drying at 70 °C for 5 h, 8 h and overnight

Figure 3. Effect of drying conditions on final moisture content of SRC

result in loss of materials when the extracted SRC is rinsed and soaked for the process of neutralisation. From these results, it could be deduced that optimal alkaline extraction was performed at pH 13 for 1 hour at 80 °C.

After the hot alkaline extraction, the pH of the extracted carrageenan remained high (more than pH 12) and it needs to be adjusted to neutral before drying. Two processes for neutralisation were evaluated. From the results obtained in *Figure 2*, it can be seen that T3 (soaking in water (1:3) for 4 h followed by second soaking overnight) was able to bring the initial pH to neutral (about pH 7).

Drying of the SRC was carried out to reduce the moisture content for product stability. Drying was conducted in a forced air convection oven and three drying conditions were evaluated. It was observed that the rate of moisture loss was highest at 70 °C for time duration of 5 hours as shown from the results in *Figure 3*. However, after overnight drying (more than 15 hours), the moisture content of each drying treatment was more or less similar to each other. From the results obtained, there exist a choice of three drying conditions i.e. drying at 70 °C for 5 h, 60 °C for 8 h or 50 °C for an overnight drying period, depending on the timing at the end of earlier process step. The

Table 2. Physical, chemical and microbiological properties of semi-refined carrageenan

	Value	EEC 78/663
Gel strength (g/cm^2)	69.50 ± 2.12	Not available
Viscosity, 1.5% sol (cP)	12.33 ± 1.15	Min. 5
Freeze-thaw property (g/cm^2)	61.67 ± 10.02	Not available
Syneresis (%)	0.94 ± 0.06	Not available
Total moisture (%)	6.51 ± 0.08	Not available
Total ash (%)	23.50 ± 1.50	15–40
Acid insoluble ash (%)	0.56 ± 0.02	Max. 2
Sulphate (%)	5.03 ± 0.95	15–40
Total plate count (cfu/g)	34 x 10^2	Not available

dried SRC was then ground to the required mesh size before evaluation of its physical and chemical properties as well as microbiological status were carried out.

Table 2 shows the physical and chemical properties as well as microbiological status of SRC produced. Formation of gel is one of the characteristics of *kappa*-carrageenan whereby *kappa*-carrageenan binds with water to form a strong rigid gel with some syneresis. The gel formed is slightly opaque since the production of SRC did not leach out the cellular materials especially cellulose. The viscosity of the SRC was much higher than that specified in the EEC Council Directive (78/663/EEC)(1978) with a minimum value of 5 cP. Freeze-thaw property is the measure of the gel strength as affected by freezing and thawing of the gel. This influences the performance of SRC in frozen products. A slight drop in the gel strength from 69.50 ± 2.12 g/cm^2 to 61.67 ± 10.02 g/cm^2 was observed when the frozen gel was thawed. Only a slight syneresis of the gel also occurred indicating good water binding capability as measured by the exudate. The chemical properties of the SRC produced were all within the purity specifications of the EEC Council Directive (78/663/EEC)(1978), i.e. total ash of 15–40%, acid insoluble ash of 2% maximum and sulphate content of 15–40%. The total plate count (TPC) is commonly used to indicate the overall microbiological status of a food, taking into account the determination of viable microorganisms. It was observed that the microbiological counts for SRC were 3.4 x 10^3 cfu/g. However since SRC is to be used as an ingredient in other products whereby in most cases thermal processing is involved, the final product microbiological load would be within acceptable limits with proper handling and processing procedures.

Conclusion

Procedures for the production of semi-refined carrageenan (SRC) from local red seaweed, *E. cottonii* involved pre-treatment, hot alkali extraction, neutralisation, drying and grinding. SRC produced from this process had physical and chemical properties comparable to the purity specifications of the EEC Council Directive (78/663/EEC)(1978) that is the specifications for pure carrageenan.

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