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USP Grade Lambda-Like Carrageenan From Halymenia Durvillaei Bory De Sainte Vincent (Short Communication)

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A *lambda*-like carrageenan was produced from *Halymenia durvillaei*, a red seaweed that grows widely in almost all parts of the Philippines. The extraction procedure was optimized at a seaweed to hot water ratio of 1:40 (W/V). An average yield of 29.10% was obtained using two (2) extractions followed by precipitation of the carrageenan with isopropyl alcohol. The physicochemical properties of the product conform with the standard *lambda* carrageenan obtained from Sigma Chemical Company and the USP specifications for carrageenan.

Key words: lambda carrageenan, *Halymenia durvillaei*, isopropyl alcohol

Carrageenan are extracts of red seaweeds found in various parts of the world. The most commercially important raw materials and their locales are *Chondrus crispus* from Canada, New England, France and Korea; *Eucheuma cottonii* and *Eucheuma spinosum* from Philippines and Indonesia; *Gigartina acicularis* from Morocco and *Gigartina radula* from Chile. Extracts from these seaweeds are primarily sulfated polysaccharides of varying ester content that give the three basic types of carrageenans (*kappa, iota, lambda*) their interesting and unique properties (Modliszewski 1983).

Lambda carrageenan is commercially produced from Gigartina acicularis and Gigartina pistillata, though *Chondrus crispus* is more commonly used in its manufacture. These red species only thrive in temperate countries, thus the Philippine carrageenan industry is only limited to the production of *kappa* (hard-type) and *iota* (soft type) carrageenan from *Eucheuma* species. This seaweed grows abundantly in the country making the Philippines a top producer of carrageenan in the world market.

Among the three types of carrageenan, *lambda* type is the most expensive. Due to its unique non-gelling property it is indispensable in the food, pharmaceutical and cosmetic industries. Structurally, it has a 6-sulfate similar to *mu* and *nu* carrageenan, however it lacks 4-sulfate. *Lambda* differs from *nu* - in that about 70% of the 1,3 linked units are sulfated at C_2 rather than C_4, the remainder being unsulfated.

Halymenia durvillaei (refer to Fig.1), a red alga is common in Philippine coastal waters. This species has been reported in Batanes; Cagayan; Ilocos Norte; Pangasinan; Bataan; Cavite (Corregidor I.); Batangas; Quezon; Sorsogon; Catanduanes; Oriental Mindoro; Western Samar; Eastern Samar; Aklan; Guimaras; Negros Oriental; Negros Occidental; Cebu; Siquijor; Zamboanga and Palawan including Sulu (Silva *et al.* 1987). The plant is red-orange or purple in color and may grow up to about 35 cm. This is somewhat soft and cartilaginous and slimy when fresh (Cordero 1977).

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A number of economic uses of *Halymenia durvillaei* have already been reported. Because of its striking color and flattened thallus, this species is ideal for use in Christmas cards and wall decors. It also contains the phycocolloid, carrageenan , which has various industrial uses. The utilization of *Halymenia* as glue was reported for the first time in the Philippines by Calmorin (1993). The development of this product stemmed from an earlier report by de Leon (1969) which mentioned that *Halymenia* gave a thick, viscous, but non-gelling solution. A red pigment, phycobiliprotein was extracted from *Halymenia durvillaei var. ceylanica* (Kuetzing) Weber-van Bosse by Gomez et al, 1997. The study showed the potentiality of said seaweed for natural colorants.

Halymenia species was also reported to contain *lambda*-like carrageenan (Montaño 1991). Preliminary investigation showed that this plant yielded about 30% of *lambda* type carrageenan. This study aims to determine if *lambda* type carrageenan can be extracted from *Halymenia* species, an indigenous raw material. Producing this hydro-colloid utilizing available local material, can possibly boost the Philippine carrageenan industry in the world market.

Materials and Methods

Fresh species of *Halymenia durvillaei* (Fig. 1) were collected from the coastal areas of Naic, Cavite (14^0 19' 15.6" Lat and 120^0 45' 46.09" Long) based on their Philippine distribution (Silva et al. 1987). Measurements were taken in a scale of 1:50,000 from the town center to the collection site. Collection was undertaken in the months of April to June 1996. The samples were then properly identified by the National Museum based on their herbarium specimen. All chemicals and reagents used were of analytical grade. IR spectra were obtained by using a film of 5 μm thick with Shimadzu 8101A FT-IR spectrophotometer.

The seaweeds were agitated in tap water for 10 minutes to remove adhering sands, shells and other impurities. The washed seaweeds were sun-dried or oven-dried to a moisture content of 20-25% then boiled with water using a ratio of 1:40 (seaweed: water) for 2 h. Diatomaceous earth was added to the mixture and filtered. The residue was collected and boiled again with water using the same parameters for second extraction. The filtrate or the liquid carrageenan was mixed with Isopropyl alcohol with a ratio of 1:3 (filtrate: alcohol) to precipitate out the carrageenan. The precipitate formed was collected and oven-dried.

Analysis of the physicochemical properties of carrageenan was based on the procedures found in USP XXII, 1990. Heavy metals such as Hg, Cd and Pb were determined according to the official methods of analysis for heavy metals, AOAC, 1990.

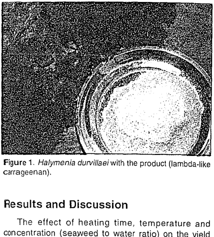

Figure 1. *Halymenia durvillaei* with the product (lambda-like carrageenan).

Results and Discussion

The effect of heating time, temperature and concentration (seaweed to water ratio) on the yield and recovery of *lambda*-type carrageenan was studied. The ratios 1:20 and 1:30 (dried seaweed: water) were not viable or sufficient enough to extract the carrageenan from the seaweed. The water added was absorbed mostly by the seaweed resulting to a thick and too viscous mixture. The ratio of water to seaweed was increased to 40. Table 1 showed the yield of *lambda* carrageenan using varying temperatures and heating time. The results showed that two (2) hours heating time at 100^{0}C gave the highest yield. Extraction of carrageenan was carried out thrice. An average yield of 26.10% was recovered from the first extraction. The second extraction yielded an average of 14.7% and 2.25% for the third extraction. Table 2 showed the average yield of *lambda*-carrageenan using the standardized parameters (laboratory scale and bench-scale studies). In the laboratory scale, one kilogram of dried *Halymenia* was used as starting material. Bench-scale studies make use of 5 kilograms of starting material. Process efficiency was determined by calculating the yield from the bench- scale over the

Table 1. Percentage yield of *Lambda*-type carrageenan using different parameters.

Temperature (°C)	Heating Time (minutes)		
	30	60	120
60	5.60	6.50	15.67
70	7.58	8.76	20.89
80	10.20	10.85	25.73
90	13.55	25.10	36.00
100	15.80	37.00	46.00

Table 2. Results on the yield of *lambda*-type carrageenan using the standardized parameters (laboratory scale and bench-scale studies).

Trial	Percent Yield (%)	
	Laboratory Scale	Bench-scale*
I	45.4	28.8
II	35.0	29.0
III	39.2	29.5
IV	36.2	
Average Yield	38.95	29.10
S.D	4.03	0.29

Processing Conditions: heating time, 2 hours; temperature: 100°C ratio: 1:40 (seaweeds:water)

yield of the laboratory scale. The results yielded 74.71% efficiency with only two extractions done.

The produced *lambda*-carrageenan was analyzed for its physicochemical properties. Identification test showed a solution that did not form a gel while allowed to cool at room temperature. A fibrous precipitate was formed upon addition with methylene blue. As shown in Table 3, the values obtained from the analysis are within the standard values. IR spectrum of the *lambda* - like carrageenan showed similar absorption curve with the standard. The results are as follows: *Lambda*-like carrageenan - 3400, 2945, 1642, 1374, 1251, 1044, 929, 830, 611 (cm^{-1}); Standard sample - 3291, 2958, 1643, 1357, 1240, 1026, 933, 830, 616 (cm^{-1}). Both samples have strong absorption bands at 830 cm^{-1} indicative of galactose-2-sulfate. The regions at 1240-1251 cm^{-1} strongly suggest the presence of ester sulfate.

Recovery process using alcohol precipitation method yielded an average of 29.10% with a process efficiency of 74.71%. The physicochemical properties

Table 3. Results of the physicochemical properties of *lambda-like* carrageenan.

	Batch			Standard Values
	I	II	III	USP XXII
Moisture (%)	10.90	12.00	11.20	<12.5
Sulfate (%)	38.80	38.40	39.06	15-40
Ash (%)	30.50	28.00	31.00	<35
Acid-insoluble ash (%)	0.70	0.40	0.15	<2.0
Viscosity, 1.5% solution (cp)	128	130	135	>5.0
Heavy metals (ppm)				
Hg	<1.10	<0.15	<0.10	-
Pb	3.9	3.5	3.80	max. 10
Cd	1.5	1.4	1.50	-

of the carrageenan produced from *Halymenia durvillaei* Bory De Sainte Vincent conforms with the USP specifications.

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