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Yield prospection of carrageenan extracted from *hypnea musciformis* seaweed collected on the Coqueiro Beach, Piaui state, submitted to four previous treatments

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Abstract

Carrageenan is a polysaccharide extracted from the red seaweed *Hypnea musciformis*, and it has been widely studied due to its abundance on the coast of several countries, including Brazil. It is used in the production of aqueous, meat and dairy products, such as dessert gels, gels products made from fish, fruit juices and powder concentrates, animal feed (canned), spices and sauces, puddings, ice cream, chocolate, among others. Due to the wide applicability of carrageenan and the fact that industries are becoming increasingly dependent on it, there is debate nowadays on the large-scale production of carrageenan, both through cultivation and with methods for obtaining higher yield of this polysaccharide. In this sense, this study aimed to make a comparison between four different methods of carrageenan extraction, seeking to prove the best route to achieve the highest level of performance. In the methods discussed, seaweeds were bleached or remained unbleached, and were subsequently sun-dried or oven-dried, in the following treatments: (T1), sun-dried and bleached; (T2) sun-dried and unbleached; (T3) oven-dried and bleached, and (T4) oven-dried and unbleached. There was a difference in the yield of carrageenan obtained by these pre-extraction treatments; T2 was the most satisfactory, with a yield of 21.63%.

Key words: Extraction. Polysaccharides. Seaweed. Yield.

Introduction

Polysaccharides extracted from seaweeds have characteristics that differ from those found in other higher plants, especially because of the presence of 3,6-anhydrogalactose units of sulfate groups.[1] One of these natural polysaccharides is particularly relevant: carrageenan, which is currently the object of a great deal of research, given its biocompatibility and non-toxicity.

Carrageenans can be found in some seaweeds of the class *Rhodophyceae*, and they are well known for their gelling properties. They are very often used in the pharmaceutical and food industries as gelling, thickening or stabilization agents. Chemically, carrageenans are polygalactanes, that is, sulfated polymers of alternating molecules of D-galactose and 3-6 anhydro-D-galactose (3,6-AG) linked by glycosidic bonds α (1-3) and β (1-4).[2]

Research shows that, depending on the species, different types of carrageenan can be extracted: lambda (represented by the Greek letter λ), which is soluble in cold and produces high viscosity; Kappa (κ), characterized by the formation of high-strength and brittle, hard gel; and the Iota form (ι), i.e., more elastic gel with thixotropic properties. Some seaweed species produce mixed forms, for example, kappa/iota, kappa/lambda or iota/lambda. Other less common forms that may still be found are carrageenans Mu and Nu.[3]

Among the various species of carrageenan-producing seaweeds, *Hypnea musciformis* is worth of notice, and kappa carrageenan ca be extracted from it. This red seaweed inhabits shallow marine environments of tropical and subtropical seas; it can be found in Senegal, Vietnam, USA, the Philippines, India, Brazil, Myanmar, Bangladesh and the Bahamas.[4] *H. musciformis* is an important resource for Brazil because it is widely used in the production of phycocolloids.[5] The species is considered a resistant seaweed, mainly due to its tolerance to wide ranges of water temperature, salinity and light intensity.[4]

The extraction of polysaccharides from seaweed often uses seaweeds taken directly from natural beds, which can lead to environmental impacts if there is over-exploitation. Aiming at sustainable development, there has been research on the use of drifting seaweeds (seaweeds scattered across the beachfront, often thought of as organic waste) in the production of new products.

In this sense, the objective of this research was to evaluate the yield of carrageenan extracted from drifiting *Hypnea musciformis* seaweeds, collected on the Coqueiro beach, on the coast of Piauí state, against four previously applied treatments, with the purpose of assessing the use of this phycocolloid in the production of future food products.

Material and Methods

The seaweeds were collected in October (2013) on the beachfront of Coqueiro Beach (Piauí) (Figure 1) and transported in seawater to the Fish Technology Laboratory of the Federal University of Piauí/LATEP-UFPI. They were then stored under refrigeration (± 5 ° C) in saline to 3% by the time of completion of the treatments. The latter were prepared by combining two variables: blanching and drying. Four treatments were performed to prepare the samples.

Figure 1. Satellite image of Coqueiro Beach, Piauí (collection site), 2013.
Source: http://maps.google.com.br/

In treatment 1 (T1), the seaweeds were subjected to sodium hypochlorite solution at a ratio of 1:1000 (v/v) for about 5 minutes to bleach. Then, they were sun-dried according to solar peak hours (12h00min to 13h30min.) for two consecutive days. In treatment 2 (T2), the seaweeds were also sun-dried, as in the previous treatment; however, there was no bleaching stage. Treatments 3 and 4 differ from the ones described above as regards the drying of samples. In treatment 3 (T3), the seaweed species of interest was dried in an oven at 45 ° C for 20 hours, after previous bleaching, whereas in treatment 4 (T4), the seaweeds were also oven-dried as in treatment 3, but without the bleaching stage (Figure 2).

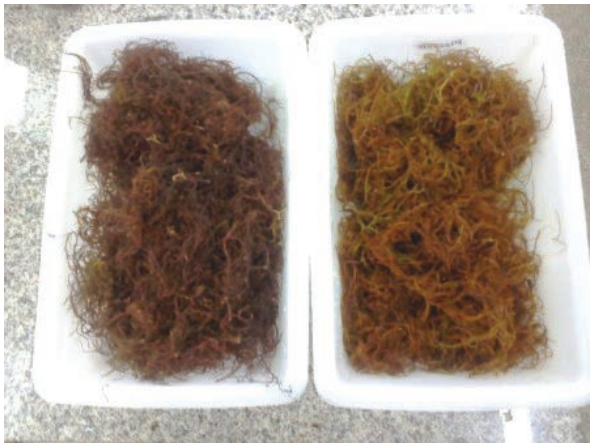

Figure 2. Bleached (right-hand side) and unbleached (left-hand side) seaweeds. UFPI, Parnaíba Campus-PI, 2013.

The extraction was based on a method described in the literature,[1] but with some modifications: First, 1 g of dried seaweed was weighed for each study, after previous treatment. Then, the samples were treated with 0.1M NaOH alkaline solution for an hour and a half. After that, the seaweeds were washed in (distilled) running water to pH 7.0 After neutralization of pH, they were subjected to extraction in 50ml distilled water for one hour under 85 °C. After extraction, the material was filtered, and the solid part was disregarded. The collected filtrate was precipitated in 30 ml ethyl alcohol solution (P.A. 99%) at the ratio of 2:1 (v/v) and dried in an oven at 70 °C to constant weight.

All analyses were performed in triplicate, with a replicate for increased reliability of the results.

Carrageenan yield was calculated with the mathematical methodology of the rule of three. The results of observation of treatments were analyzed statistically using analysis of variance (ANOVA) at 5% probability by Tukey's test to detect a possible similarity in the comparison of means, with the help of statistical software Excel.

Results and Discussion

Table 1 shows the results for carrageenan yield in this research. It can be seen that the sun-drying treatments (T1 and T2) provided higher gel content acquired during the extraction process for unbleached seaweeds, with a 21.33% yield. The bleaching stage performed in treatment 2 (T2) reduced the percentage of extracted carrageenan, with a 15.99% yield. The treatments in which the seaweeds were oven-dried at 45 °C (T3 and T4) also showed lower yield values when compared with those of T1 and T2. Oven-dried, bleached seaweeds (T3) had only 13.88% yield, while those which did not undergo bleaching (T4) showed higher carrageenan yield (17.70%). It can be seen that for this type of extraction, seaweed bleaching is not advisable, because gel is probably lost during this process (Table 1).

Table 1. Mean results of carrageenan extraction yield in *Hypnea musciformis* depending on the treatment applied. Parnaíba-PI, 2013.

Treatments (T)	Dried seaweed weight (g)	Carrageenan weight (g)	Yield (%)
T1	1.01 ± 0.004	0.16 ± 0.021	15.99 ± 2.108
T2*	1.01 ± 0.004	0.22 ± 0.033	21.63 ± 3.292
T3	1.01 ± 0.004	0.14 ± 0.024	13.89 ± 2.425
T4	1.02 ± 0.004	0.18 ± 0.029	17.71 ± 2.850

Notation (*): Significant values at $p < 0.05\%$.

There were significant differences ($p < 0.05\%$) in yield for the treatments applied, and treatment 2 (T2) was identified as the most effective for carrageenan extraction.

As far as yield values are concerned, it should be noted that the yield of the phycocolloid extracted from certain seaweed species may vary due to seasonality, time of year, and abiotic factors such as light, temperature, salinity, etc.[6] Thus, future studies should calculate the yield of carrageenan extracted from *H. musciformis* and its relationship to the physical and chemical parameters of the environment, as well as time of year.

Conclusions

Samples of drifting *Hypnea musciformis* seaweeds used for carrageenan extraction showed yield values that varied depending on drying method and presence or absence of bleaching. However, bleached seaweeds had lower yield than unbleached ones.

Under the conditions of this study, sun-dried, unbleached seaweeds had higher carrageenan content.

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