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Optimization of the extraction of carrageenan from *Kappaphycus alvarezii* using response surface methodology

Otimização da extração de carragenana de Kappaphycus alvarezii utilizando metodologia de superfície de resposta

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Abstract

This study aims to optimize an alternative method of extraction of carrageenan without previous alkaline treatment and ethanol precipitation using Response Surface Methodology (RSM). In order to introduce an innovation in the isolation step, atomization drying was used reducing the time for obtaining dry carrageenan powder. The effects of extraction time and temperature on yield, gel strength, and viscosity were evaluated. Furthermore, the extracted material was submitted to structural analysis, by infrared spectroscopy and nuclear magnetic resonance spectroscopy (^{1}H-NMR), and chemical composition analysis. Results showed that the generated regression models adequately explained the data variation. Carrageenan yield and gel viscosity were influenced only by the extraction temperature. However, gel strength was influenced by both, extraction time and extraction temperature. Optimal extraction conditions were 74 °C and 4 hours. In these conditions, the carrageenan extract properties determined by the polynomial model were 31.17%, 158.27 g.cm^{-2}, and 29.5 cP for yield, gel strength, and viscosity, respectively, while under the experimental conditions they were 35.8 ± 4.68%, 112.50 ± 4.96 g.cm^{-2}, and 16.01 ± 1.03 cP, respectively. The chemical composition, nuclear magnetic resonance spectroscopy, and infrared spectroscopy analyses showed that the crude carrageenan extracted is composed mainly of κ-carrageenan.

Keywords: *red algae; biopolymer; gelificant agent.*

Resumo

Este estudo tem como objetivo otimizar um método alternativo para extração de carragenana sem tratamento alcalino prévio e sem precipitação com etanol através da Metodologia de Superfície de Resposta (RSM). A fim de inovar a etapa de isolamento, a secagem por atomização foi adaptada, o que reduziu o tempo para a obtenção do pó seco. Os efeitos da temperatura e do tempo de extração sobre o rendimento, força do gel e de viscosidade foram avaliados. Além disso, o material extraído foi submetido a análises estruturais por espectroscopia de infravermelho e de ressonância magnética nuclear, e composição química. Os resultados mostraram que os modelos de regressão gerados explicam adequadamente a variação de dados. Apenas a temperatura de extração afetou o rendimento e a viscosidade do gel. Entretanto, a força do gel foi influenciada tanto pelo tempo como pela temperatura de extração. As condições ótimas de extração foram 74 °C durante 4 horas. Nessas condições, as propriedades da carragenana bruta determinadas pelo modelo polinomial foram 31,17%, 158,27 g.cm^{-2} e 29,5 cP, para rendimento, força do gel e viscosidade, respectivamente, enquanto que em condições experimentais foram 35,8 ± 4,68%, 112,50 ± 4,96 g.cm^{-2} e 16,01 ± 1,03 cP. A composição química e as análises estruturais mostraram que a carragenana extraída é principalmente κ-carragenana.

Palavras-chave: *alga marinha; biopolímero; agente gelificante.*

1 Introduction

Carrageenans are sulfated galactans, extracted from red algae (Rhodophyta), composed of D-galactose residues linked alternately in a α-1,3 and β-1,4 bonds. They are classified as *kappa* (κ), *iota* (ι), and *lambda* (λ) according to their sulfate substitution pattern and 3,6-anhydrogalactose content. These natural polymers have the ability to form thermoreversible gels or high viscous solutions, and they are commonly used as gelificant, stabilizing, and emulsifying agents in several foods and pharmaceutical and cosmetic products. Food industry is particularly responsible for using 70 to 80% of carrageenans world production, mainly in meat and dairy products (HILLIOU et al., 2006; PRADO-FERNÁNDEZ et al., 2003; VAN DE VELDE et al., 2002).

Studies on carrageenan extracted from *Kappaphycus alvarezii* in water showed that they are mostly composed of strong κ-carrageenans gelling agents, significant amounts of low-molecular-weight galactans with κ-structure, and small quantities of non-gelling carrageenans and agaroids (ESTEVEZ; CIANCIA; CEREZO, 2000, 2004). The production of κ-carrageenan in Brazil is low and restricted to the evaluation of native cultivations of *Hypnea musciformis* (Wulfen) Lamouroux (SAITO; OLIVEIRA, 1990), and some experimental cultivations

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(BULBOA; PAULA; CHOW, 2007; PAULA; PEREIRA; OHNO, 1999) and a commercial cultivation of *K. alvarezii* at the Southeastern coast (CASTELAR; REIS; BASTOS, 2009). Nowadays, the cultivation of *K. alvarezii* has been studied in experimental farms in the Southern Region, in Florianópolis, Santa Catarina State, Brazil (27° 29' 19" S and 48° 32' 28" W) (HAYASHI et al., 2011).

K. alvarezii was first introduced in 2008 into a cultivated area in Florianópolis, Santa Catarina State under the auspices of the Brazilian Environmental Institute (IBAMA) (HAYASHI et al., 2011). Hayashi et al. (2011) showed that the cultivation of *K. alvarezii* can be technically feasible in subtropical water and can be associated with local mussel farms, mitigating eutrophication and, eventually, increasing the profitability of farmers.

Carrageenan's quality is usually evaluated according to its technical performance as gelling (gel strength) and thickening (viscosity) agent. The chemical composition of *K. alvarezii* carrageenan should be taken into consideration by industries since it can be significantly affected by different extraction processes, *i.e.*, extraction temperature and time affect the rheological properties of this polymer (MONTOLALU et al., 2008). However, these parameters vary in several studies. Besides, if carrageenan used for industrial purposes is obtained without alkaline treatment and alcoholic precipitation (HAYASHI; PAULA; CHOW, 2007; REIS; YONESHIGUE-VALENTIN; DOS SANTOS, 2008), extraction process will be more attractive from an ecological and economic point of view.

Therefore, the aims of this study were to determine the optimal conditions for carrageenan crude extraction from *K. alvarezii* cultivated in Florianópolis (SC, Brazil) using Response Surface Methodology (RSM) and to study the chemical structure of carrageenan crude extract.

2 Materials and methods

2.1 Experimental design optimization

A Central Composite Design (CCD) with two independent variables was applied. The independent variables were temperature (°C, X_1) and extraction time (h, X_2). The optimization was performed using the following five levels: one central point (level 0 = T: 60 °C and t: 4 hours); level 1 (T: 80 °C and t: 5 hours); level –1 (T: 40 °C and t: 3 hours); levels α (T: 88.28 °C and t: 5.41 hours); and level –α (T: 31.72 °C and t: 2.59 hours); with $\alpha = +/-\sqrt{2}$, for k = 2 (two independent variables).

The complete design consisted of 13 experiments including four factorial (levels –1 and +1) and four axial experiments (levels ± α), and five replicates at central point. These five replicates were carried out in order to estimate error. Carrageenan yield (%), gel strength ($g.cm^{-2}$), and viscosity (mPa.s) were considered as dependent variables. All experiments were performed randomly in order to minimize the effect of unintentional variability in responses due to systematic experimental errors.

2.2 Sample

Kappaphycus alvarezii samples of brown tetrasporophytic strain were obtained from the Biological Sciences Center (CCB) of the Federal University of Santa Catarina (UFSC), grown in an experimental cultivation in Sambaqui Beach, in Florianópolis, in Santa Catarina State, Brazil (27° 29' 19" S and 48° 32' 28" W). Some algae were collected in March 2009; they were air-dried for four days and, then, dried in oven (60 °C) for 48 hours before extraction. Commercial carrageenan pattern (type I, containing predominantly *kappa* (κ) with the least amount of *lambda*, C1013) and κ-carrageenan pattern from *Kappaphycus alvarezii* (C1263, type III, κ-carrageenan) were purchased from Sigma-Aldrich (São Paulo, Brazil).

2.3 Carrageenan aqueous extraction

Some previously dried seaweed was washed in water to remove salt and dirtiness. Samples of 10 g were soaked in distilled water (800 mL) for 1 hour, and then they were grounded with a mixer. This solution was placed in a water-bath (with different times and temperatures, according to the statistical design). Diatomaceous earth (25 g) was added in the solution 15 minutes before the process ended to help the filtration process. Separation of crude extract of carrageenan (filtrate) from residue (cellulose) was carried out in a filtration vacuum system with qualitative paper. Next, the filtrate was atomized using the Mini Spray Dryer B-290 (BÜCHI, Switzerland) to obtain the crude carrageenan extract powder using the following parameters: inlet temperature 200 °C and air flow 30 $mL.min^{-1}$ with an aspiration capacity of 100% and pump capacity of 25%.

The yield of crude carrageenan extract was determined using Equation 1:

$$y_{carrageenan} = (P/AS) \cdot 100\% \quad (1)$$

where $y_{carrageenan}$ is the yield of crude carrageenan extract (%), P is the amount of extracted carrageenan in grams, and AS is the seaweed amount (~10 g - dry weight) used for extraction.

2.4 Carrageenan extract analysis

The analyses of gel strength and viscosity, which are the response function variables in the statistical method, was performed in an aqueous solution of 1.5% (w/w) carrageenan crude extract prepared at 80 °C. For the gel strength analysis, this solution was placed in plastic tubes (30 mL in volume, 50 mm height, and 50 mm diameter) which were kept under refrigeration (8 °C ± 2 °C) for ~24 hours before analysis. The analyses were performed using a Stevens LFRA 1000 texturometer coupled to a cylindrical probe with 5 mm diameter using the following parameters: velocity of 2 $mm.s^{-1}$ and sample penetration of 20 mm. Maximal penetration strength was considered as the gel strength, which was registered by the Texture Expert software (Stable Micro Systems Ltd., England). All analyses were carried out in triplicate.

Viscosity of carrageenan crude extract solutions (1.5% w/w) was measured using a Brookfield RVDV-III rheometer (Brookfield Engineering Laboratories, model DVIII Ultra,

Stoughton, MA, EUA) of concentric cylinder geometry coupled to a thermo-stabilized water bath (TECNAL model TE-184) at 75 °C using 16 mL of the carrageenan solution. The data were analyzed using the Rheocalc® 32 *v* 2.5 software (*Brookfield Engineering Laboratories,* Inc., Middleboro MA 02346 EUA). Viscosity analyses were carried out in duplicate. Solutions of carrageenan crude extract (1.5% w/w at 75 °C) presented Newtonian behavior, in which viscosity does not depend on the applied deformation rate and shear stress. An interval of deformation rate ranging from 12 s^{-1} to 95 s^{-1} was used.

Carrageenan crude extract extracted under optimum conditions and commercial carrageenan were analyzed for total ash, calcium, iron, sodium, potassium (ASSOCIATION..., 2005), protein (LOWRY et al., 1951), and phosphate content (INSTITUTO..., 2005). Sulfate content was measured turbidimetrically after hydrolyzing 40 mg of carrageenan crude extract in sealed tubes for 2 hours in 0.5N HCl at 105 °C (JACKSON; McCANDLESS, 1978). Total carbohydrate was determined by the phenol sulfuric acid method (DUBOIS et al., 1956).

Fourier transformed infrared spectroscopy (FTIR) of carrageenan crude extract (extracted under optimum conditions) was performed using a Perkin Elmer spectrometer (16PC) with a resolution of 4 cm^{-1} in the range of 4000-400 cm^{-1}. The spectra were obtained in KBr chips (spectrometric degree). Commercial and κ-carrageenan patterns were used as reference materials for infrared spectroscopy analysis. The ^1H NMR spectra were recorded at 80 °C on a Varian Mercury Plus spectrometer (400 MHz) using D_2O as solvent. The phycocolloid concentration was 3% (w/w).

2.5 Statistical analyses

The regression coefficients for linear and quadratic terms and interaction among terms were determined by multiple linear regression. The statistical significance of each regression coefficient was evaluated by the *t*-value from pure error obtained from replicates at the central point. Analysis of variance (ANOVA) was applied for model validation.

The regression coefficients were then used for determining the 2nd order model of each response. This model can be expressed with codified variables (temperature = X_1, time = X_2) according to the following Equation 2:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_1X_2 + \varepsilon \quad (2)$$

where Y is the observed response; B_0 is the constant for equation parameters; B_i represents linear terms; B_{ii} represents the quadratic terms for one variable; B_{ij} represents the interaction terms (I = 1, 2 e j = 1, 2); and ε is the random error. After adjusting the 2nd order model, optimal extraction conditions were obtained. A response surface plot was obtained using the software Statistica 6.0 (StatSotft Inc., Tulsa, OK, EUA) (5% of significance) as a function of the two independent variables.

3 Results

3.1 Optimization of carrageenan extraction – development of a surface response model

The values obtained for yield, gel strength, and viscosity are shown in Table 1.

The significance of each regression coefficient was statistically evaluated by entering *t*- value from pure error obtained from replicates at the central point. In order to develop the model, the multiple regression coefficients related to independent variables which were significant in the *t*-test ($p \leq 0.05$) were used. Extraction time did not affect ($p > 0.05$) the crude carrageenan extraction (Y_1) and the solution viscosity (Y_3). Thus, multiple regression coefficients related to this independent variable were not used for obtaining equations representing the models of these two responses. As for the yield of crude carrageenan, only the linear effect of temperature was observed. However, with regard to the solution viscosity, a quadratic effect of temperature was observed. Both time and temperature affected ($p < 0.05$) the gel strength (Y_2). The interaction between independent variables was not significant for the observed responses.

Table 1. Results of yield, gel strength and viscosity of carrageenan extracted from *Kappaphycus alvarezii* based on extraction time and temperature.

Experiment	Real variables		Assigned variables		Responses		
	Temperature (°C)	Time (h)	X_1	X_2	Yield (%)	Gel strength (g cm^{-2})	Viscosity (cP)
1	40	3	−1	−1	22	36.80	10.28
2	80	3	1	−1	35	128.05	21.99
3	40	5	−1	1	18	47.26	9.11
4	80	5	1	1	34	115.11	22.23
5	32	4	−1.41	0	19	18.33	3.02
6	88	4	1.41	0	32	130.39	17.73
7	60	2.59	0	−1.41	26	126.29	21.46
8	60	5.41	0	1.41	27	131.36	37.78
9	60	4	0	0	27	158.31	31.96
10	60	4	0	0	25	135.96	26.10
11	60	4	0	0	34	168.86	45.91
12	60	4	0	0	23	134.66	40.83
13	60	4	0	0	27	147.14	26.26

Equations for RSM were obtained based on the significant coefficients of *t*-test ($p \leq 0.05$). The coefficients of determination (R^2) for Y_1, Y_2, and Y_3 were 0.74, 0.94 and 0.71, respectively (Table 2). The models presented here are well adjusted and randomness of residue was reached, as supposed.

Variance analysis was performed in order to evaluate the significance of the quadratic polynomial equation. Regression was significant for all responses, and the lack of adjustment was not significant ($p > 0.05$). Therefore, the three models adequately explain the variation of responses.

The yield of carrageenan crude extract increased linearly with the increase of temperature. Extraction temperature and time affected gel strength, which was higher when carrageenan crude extract was extracted at 74 °C for 4 hours (Table 2). At higher temperatures, biopolymer degradation may occur. Carrageenan crude extract solution viscosity increased when the extraction temperature reached 60 °C, and at higher temperatures this parameter decreased. Extraction time did not influence solution viscosity.

3.2 Chemical composition

The chemical composition of commercial and native carrageenan (crude carrageenan extracted from *K. alvarezii* under optimized conditions) is described in Table 3.

The total carbohydrate determined for commercial (64.82 g.100 g^{-1}) and native carrageenan (56.44 g.100 g^{-1}) showed no statistic significance difference. However, native carrageenan contains significantly higher ($p < 0.05$) amount of ash compared to commercial carrageenan. This can be explained by the fact that the native carrageenan also has higher amounts of protein, iron, phosphorus, and sodium (Table 3).

3.3 Infrared spectroscopy

Several authors have used infrared spectroscopy (IR) to characterize and differentiate carrageenans κ, ι, and λ (HILLIOU et al., 2006; MENDOZA et al., 2006; PEREIRA et al., 2009; PRADO-FERNÁNDEZ et al., 2003; ROCHAS; LAHAYE; YAPHE, 1986). Figure 1a-c shows the FTIR spectra of native carrageenan and the carrageenan references for commercial and κ-carrageenan, respectively. The study of carrageenans by FTIR spectroscopy shows the presence of very strong absorption bands in the 1210-1260 cm^{-1} region (due the S = O of sulfate esters) and 1010-1080 cm^{-1} region (ascribed to the glycosidic linkage) for all types of carrageenan. A particularly intense signal was recorded in all samples at 803-805 cm^{-1}, which is specific to 3,6-anhydrogalactose-2-sulfate. Another signal was observed at 840-850 cm^{-1} (attributed to D-galactose-4-sulfate). Peaks were also observed at 925-935 cm^{-1} in all samples (3,6-anhydro-D-galactose) (PEREIRA et al., 2009; PRADO-FERNÁNDEZ et al., 2003).

3.4 Nuclear magnetic resonance spectroscopy

Figure 2 shows the ^{1}H NMR spectrum of native carrageenan extracted from *K. alvarezii*. The peaks at 5.32 and 5.11 ppm show the presence of ι- and κ-monomers, respectively, and the peak at 5.59 ppm shows the presence of α-D-galactose 2,6-disulfate, found in λ- and ν-carrageenans. (VAN DE VELDE et al., 2002; HILLIOU et al., 2006). The ^{1}H NMR spectra of the carrageenan references (commercial and κ-carrageenan standard) showed the same peaks although with less intensity than that of native carrageenan at 5.32 and 5.59 ppm (data not showed). No peak could be observed at 5.26 ppm, which refers to the presence of μ- monomers (precursor of κ-carrageenan) (VAN DE VELDE et al., 2002).

4 Discussion

The extraction performed at 74 °C for 4 hours can be considered a satisfactory condition which results in a high carrageenan extract yield, high gel strength, and high viscosity. At this condition, the carrageenan yield determined by the polynomial model (Table 2) was 31.17%, and the carrageenan yield determined experimentally was 35.8 ± 4.68%. Additionally, gel strength determined by the model was 158.27 g.cm^{-2}, and gel strength determined experimentally was 112.50 ± 4.959 g.cm^{-2}. Viscosity was determined by the model as 29.5 cP and calculated experimentally as 16.01 ± 1.025 cP.

Comparing these results with those of other reports, native carrageenan yield and gel strength data (which ranged from 18 to 35% and from 18.33 to 168.86 g.cm^{-2}, respectively Table 1), are similar to the data obtained by Hayashi, Paula and Chow (2007) (21-35% and 18.33 to 168.86 g.cm^{-2}). These authors have also used native carrageenan from *K. alvarezii*. However, they did not use atomization drying but alcohol precipitation and oven drying for the extraction process. Furthermore, they used gels prepared in saline solutions, which induced the formation of carrageenan gel (DE RUITER; RUDOLPH, 1997; VIEBKE; PICULELL; NILSSON, 1994). However, the values for native carrageenan gel strength were higher than those found by Freile-Pelegrín, Robledo and Azamar (2006) and by Freile-Pelegrín and Robledo (2008), who used alkaline extraction but no salt in sample preparation, who obtained weak gels (<50 g.cm^{-2}) of carrageenan extracted from *Eucheuma isiforme* from different regions.

Table 2. Surface response model for carrageenan extracted from *K. alvarezii*.

Response	Quadratic polynomial model		
	Real variables	Assigned variables	R^2
Carrageenan yield	$Y_1 = 8.97 + 0.3\ X_1$	$Y_1 = 27.19 + 5.96\ X_1$	0.74
Gel strength	$Y_2 = -598.75 + 14.89\ X_1 - 0{,}107\ X_1^2 + 120\ X_2 - 14.93\ X_2^2$	$Y_2 = 149.09 + 39.9\ X_1 - 42.99 X_1^2 + 0.58\ X_2 - 14.93\ X_2^2$	0.94
Viscosity	$Y_3 = -99.96 + 4.11\ X_1 - 0.0319\ X_1^2$	$Y_3 = 34.23 + 5.73\ X_1 - 13.16\ X_1^2$	0.71

Y_1 (carrageenan yield, %); Y_2 (gel strength, g cm^{-2}); Y_3 (viscosity, cP); X_1 (temperature, °C); X_2 (time, h).

Viscosity had a high variation (3.02 to 45.91cp, reported on Table 1) related to the temperature used for extraction. The values obtained for central point were similar to those reported by Freile-Pelegrín, Robledo and Azamar (2006) (39 to 57 cP), who used native extraction of carrageenan from *E. isiforme* cultivated in Mexico, and the values were lower than those found by Freile-Pelegrín and Robledo (2008) (114.6 ± 3.3 cP) for carrageenans from *E. isiforme* cultivated in Nicaragua and extracted without alkaline treatment.

Carrageenan properties can vary extensively depending on the harvest time (AZANZA-CORRALES; SA-A, 1990; TRONO JUNIOR; LLUISMA, 1992), region, growth conditions (salinity, deepness, nutrients), time of growth (HAYASH; PAULA; CHOW, 2007), extraction process, and parameters (HILLIOU et al., 2006; MONTOLALU et al., 2008). This explains the differences previously reported in several studies.

The total carbohydrate determined for commercial and native carrageenan was 64.82 g.100 g^{-1} and 56.44 g.100 g^{-1}, respectively (Table 3). Estevez, Ciancia and Cerezo (2004) found a value of 54% for the total carbohydrate content of κ-carrageenan for an analytical standard (Sigma-c1263) purified by reprecipitation in 0.125 M KCl and by dialysis.These authors found a value of 45% for total carbohydrates for carrageenan extracted from *K. alvarezii* by alkali treatment. The higher value obtained in the present study (Table 3) is certainly due to the fact that native carrageenan used in this experiment does not gone through any prior purification process.

The content of sulfate groups in commercial and native carrageenan (21.65% and 20.02% respectively, Table 3) is in agreement with the expected value for κ-carrageenan, which is roughly 20% (DE RUITER; RUDOLPH, 1997). Hayashi et al. (2007) found values of sulfate content ranging from 23.08 to 33.48% for carrageenan extracted from four strains of *K. alvarezii* (brown, green, and red and one strain derived from tetraspores progeny, called G11) submitted to alkali treatment, harvested in May, August, and October and cultivated along the coast of São Paulo state, Brazil.

Other authors (MENDOZA et al., 2006, VILLANUEVA; HILLIOU; SOUSA-PINTO, 2009) also obtained values close to those found for native carrageenan (Table 3) when they analyzed the extract of other seaweed which produce carrageenan (*Kappaphycus striatum* - predominantly κ-carrageenan and *Chondrus crispus* - producer of carrageenan hybrid κ/ι, respectively).

The ratio sulphate/carbohydrate showed similar patterns for the commercial (0.33) and native carrageenan (0.35). Therefore, it can be suggested that the two samples of carrageenan have the same number of sulfate groups per carbohydrate unit. Thus, it can be expected that both native and commercial carrageenans contain large amount of a less sulfated carrageenan, i.e., κ-carrageenan.

The commercial and native carrageenans showed large amounts of potassium (about 11 g.100 g^{-1} sample). Furthermore, these carrageenan samples differed ($p < 0.05$) in the amount of iron, phosphorus, calcium, and sodium. The higher sodium content in native carrageenan is noteworthy, showing that

Table 3. Chemical composition of commercial and native carrageenan.

Composition (g.100 g^{-1})	Carrageenan	
	Commercial	Native
Total carbohydrates	64.82[a] ± 4.0376	56.44[a] ± 3.1396
Sulfate ester	21.65[a] ± 1.1971	20.02[a] ± 0.5400
Moisture	2.50[a] ± 0.0575	2.89[a] ± 0.4119
Total ash	31.38[a] ± 0.0907	32.46[b] ± 0.2994
Protein	1.35[a] ± 0.13	6.12[b] ± 0.51
Iron	0.0085[a] ± 0.0004	0.0051[b] ± 0.0003
Phosphorus	0.0164[a] ± 0.0011	0.0973[b] ± 0.0030
Calcium	1.7784[b] ± 0.0266	0.1507[a] ± 0.0166
Potassium	11.1985[a] ± 0.1730	11.2165[a] ± 0.2277
Sodium	0.4719[a] ± 0.0083	3.0167[b] ± 0.1483

[a,b]Lowercase superscripts in the same column indicate significative differences ($p < 0.05$) among the carrageenans studied.

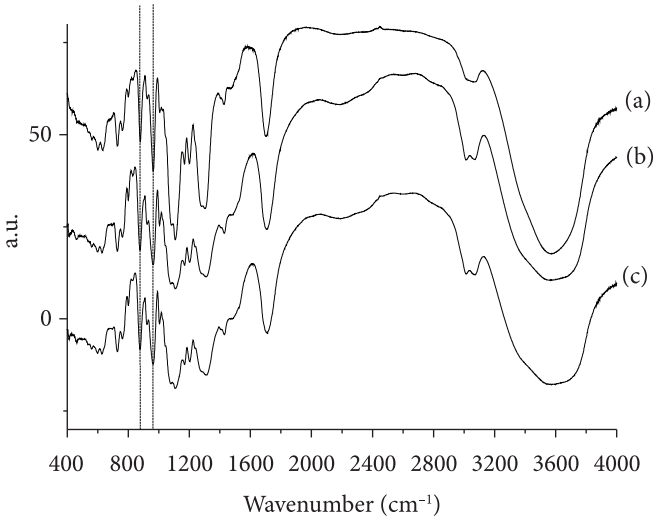

Figure 1. FTIR Spectra of (a) κ-carrageenan (b) commercial carrageenan and (c) native carrageenan.

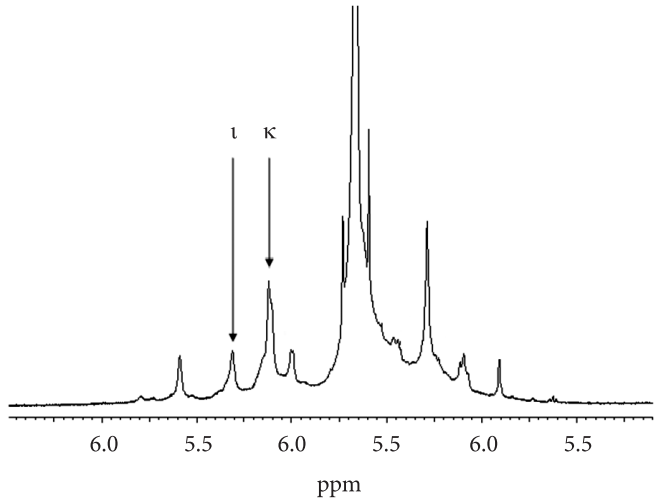

Figure 2. H NMR spectrum of native carrageenan from *K. alvarezii*.

the extraction methodology employed does not remove residual sodium chloride derived from the marine cultivation. The protein content of native carrageenan (6.12 ± 0.51%) is higher ($p < 0.05$) than that of the commercial carrageenan (1.35 ± 0.13%), probably due to the precipitation process (alcohol or potassium chloride) applied in industrial processes (commercial carrageenan).

The FTIR spectra are almost identical (Figure 1), which confirms the hypothesis that the carrageenan extracted under the optimized conditions is manly of κ-carrageenan type (bands at 933 and 847 cm^{-1}). Sulfates in the C4 position (the ring of galactose) are shown in the band 845-850 cm^{-1}, as seen in the two spectra presented indicating the simultaneous presence of κ-carrageenan. The ι-carrageenan has an additional feature band near 805 cm^{-1} associated with the structure 3,6-anhydro-D-galactose, which is not observed in the spectra in Figure 1. Commercial and native carrageenans do not present any of the bands corresponding to λ-carrageenan (1026 cm^{-1}, 867 cm^{-1}, 830 cm^{-1}, and 820 cm^{-1}). Furthermore, it was observed a higher intensity of absorbance at 1250 cm^{-1} in native carrageenan in comparison to commercial carrageenan in the FTIR spectrum, which is in agreement with the data of the sulfate content analysis by turbidimetric method (Table 3).

The ^1H NMR spectrum demonstrates that the biopolymer extracted from *K. alvarezii* is essentially composed of κ-monomers, and to a lesser extent, it contains minor quantities of ι-monomers. Therefore, the raw native carrageenan can be seen as hybrid carrageenans (blocks of κ-, ι-monomers distributed along the same macromolecule) or equally as mixtures of κ- and ι-carrageenan biopolymers, as reported by previous studies (ESTEVEZ; CIANCIA; CEREZO, 2000, 2004; VAN DE VELDE et al., 2002). Furthermore, a peak at 5.59 ppm (Figure 2) indicates the presence of sulfated galactans (VAN DE VELDE et al., 2002), and it confirms the greatest amount of sulphate groups in native carrageenan reported by the FTIR analysis (band 1250 cm^{-1}, Figure 1) and turbidimetric analysis (Table 3). These results prove that the crude carrageenan obtained from *K. alvarezii* is a complex system mainly composed of κ-carrageenan, but it also contains ι-carrageenan, non-gelling carrageenans, and agaroids, as reported by Estevez, Ciancia and Cerezo (2000, 2004).

5 Conclusion

Extraction time did not affect yield and viscosity, and extraction temperature was considered the most important parameter for all the responses. The models developed and statistically analyzed are adequate for optimization of extraction of crude carrageenan from *K. alvarezii* using the method described in the present study. The optimal conditions determined for native carrageenan extraction were 74 °C and 4 hours resulting in a satisfactory yield, higher gel strength, and high viscosity. The FTIR spectra and the ^1H NMR spectrum showed the predominance of κ-carrageenan, and to a lesser extent, minor quantities of ι-monomers and sulfated galactans. These results showed the success of carrageenan crude extraction from *K. alvarezii* providing potential benefits for industrial extraction from an ecological and economic point of view.

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