World Journal of Food Science and Technology
2020; 4(1): 23-30
http://www.sciencepublishinggroup.com/j/wjfst
doi: 10.11648/j.wjfst.20200401.14
ISSN: 2637-6016 (Print); ISSN: 2637-6024 (Online)

Effect of Ethanol on Physical Chemistry Characterization, Microorganism, and Toxicity of Carrageenan Extracted with the Assistant of Enzyme Viscozyme L

Bui Huy Chich[1, *], Do Van Ninh[2], Vu Ngoc Boi[3], Dang Xuan Cuong[4, *]

[1]Department of Science and Technology, Ba Ria-Vung Tau Province, Vung Tau, Vietnam

[2]Pacific Ocean University, MOET, Nha Trang, Vietnam

[3]Faculty of Food Technology, Nha Trang University, Nha Trang, Vietnam

[4]Organic Matterial from Marine Resource, Nhatrang Institute of Technology Application and Research, Vietnam Academy of Science and Technology, Nha Trang, Vietnam

Email address:

chichcntp@gmail.com (B. H. Chich), vaninhcb@yahoo.com (Do V. Ninh), minhboint@yahoo.com (Vu N. Boi), cuong_mails@yahoo.com.vn (D. X. Cuong)

[*]Corresponding author

To cite this article:

Bui Huy Chich, Do Van Ninh, Vu Ngoc Boi, Dang Xuan Cuong. Effect of Ethanol on Physical Chemistry Characterization, Microorganism, and Toxicity of Carrageenan Extracted with the Assistant of Enzyme Viscozyme L. *World Journal of Food Science and Technology.* Special Issue: *Marine Bio-Polymer: Bio-Activity, Extraction and Application*. Vol. 4, No. 1, 2020, pp. 23-30.
doi: 10.11648/j.wjfst.20200401.14

Received: March 17, 2020; **Accepted**: April 3, 2020; **Published**: April 29, 2020

Abstract: Carrageenan is a biopolymer found in red algae with high potential in food, functional food, pharmaceutics, and cosmetics. The study focused on the effect of ethanol on physical chemistry characterization and microorganisms of carrageenan that extracted by the enzyme – assisted method and the purification by using ethanol. The results showed the moisture of carrageenan varied from 10.9 to 9.5% DW. After the impact of ethanol, the purification and physical (dispersal in water and rheological) characterization of carrageenan was higher than before the impact of ethanol. For example, dispersal in water, the viscosity of the solution, the solution strength (1.5% of carrageenan and 0.2% of potassium chloride), and carbohydrate content at 20°C corresponded to 1.06, 1.18, 1.07, and 1.11 times, compared to before the impact of ethanol. The content of ethanol-insolubility impurities, total ash, acid-solubility ash, acid-insolubility ash, total protein, sunphat content (SO_4^{2-}), and lipid content was 43%, 94.6%, 42.9%, 44.44%, 3.9%, 97.2%, and none-detected in comparison to before the impact of ethanol. The content of lead, arsenic, cadmium, and mercury was 0.01, < 0.01, 0.05, and < 0.01 ppm, respectively. Total aerobic bacterial of carrageenan got the highest value of 2.1 x 102 cells/g. *E. coli, coliforms, staphylococcus aureus, salmonella*, and *bacillus cereus* did not occur in carrageenan.

Keywords: Carrageenan, NMR, Rheology, Mineral, *Kappaphycus alvarezii*, Cam Ranh

1. Introduction

Carrageenans are linear polysaccharides, possess repeating sequences of α-D-galactopyranose and β-D-galactopyranose residues with the 1,3 and 1,4 linkage, named the A residue and B residues, respectively. The difference in the extraction method and the algae species, types of carrageenans can be obtained different, for example, kappa (κ), iota (ι), and lambda (λ) [1-3]. Carrageenans possess good rheological characterizations (forming thermoreversible gels, viscosity) in the salt solutions of small concentration with widely applicating into food [4, 5], functional food [5], pharmaceutics [6, 7], and cosmetics [8] in the role of texturing, thickening, suspending, or stabilizing agents [9, 10]. Carrageenan is non-toxic, induces thrombosis, anti-cancer, and anti-inflammatory [11, 12].

Carrageenan content in red algae is up to 40% DW and extracted by using acidic, alkaline, or enzyme depending on the algae species [13]. Almost studies on the carrageenan

extraction used the chemistry method leading to environmental pollution. The enzyme-assisted extraction method was environmental pollution less than the chemistry method. The carrageenan separation out of the cell membrane is effectively better than the chemistry method. Carrageenan is usually purified by the column causing the difference in the application into the food [13-17].

Thus, the study focused on the effect of ethanol on physical chemistry characterization and microorganisms of carrageenan for finding the solution of carrageenan purification easier.

2. Material and Methods

2.1. Material

Kappaphycus alverazii (Doty) cultivated commonly in Nha Trang Bay was harvested, and after cleaning by seawater, they were transferred to the laboratory at the condition under 10°C for further study.

All chemicals using in the analysis were from Sigma – Aldrich. The distilled water and 96% ethanol was of Vietnam.

2.2. Sample Preparation

2.2.1. Enzyme-assisted Extraction of Carrageenan

K. alvarezii was macerated in the buffer (pH 5.1) at 42°C for 60 minutes with 1.45% of enzyme according to the solution and algae ratio of 20/1 (v/w). After filtration, the residue was soaked in aqueous at 90°C for 80 minutes with the aqueous to residue ratio of 50/1 (v/w) and collecting the supernatant through the membrane. Carrageenan was continuously precipitated in 80% ethanol and dried by using the method of freeze-drying for the further studies.

2.2.2. Purification of Carrageenan by Using Ethanol

The solution composed of 5% of carrageenan and 25% ethanol was kept at 70°C for 15 minutes for precipitating dissolved protein and impurities. The supernatant was continuously collected by the centrifugation at 10.000 rpm for 15 minutes, and precipitating in 60% ethanol for 40 minutes. After precipitation, the residues were filtered, cleaned twice in 96% ethanol, and dried at 45±2°C by using the freeze drying with the velocity ratio of 2 m/s.

2.3. Quantification Methods

Quantification of moisture was according to the AOAC method [18].

Quantification of solubility in water

The determination of rheology characterization (viscosity and gell strength) was by the machine (Brookfield (American) and CR 500DXS - SunScientific (Japan)), respectively [19].

Quantification of total ash, acid-insoluble ash, and ash soluble in acid was in acordance to the AOACA method (AOAC. 975.12) and Nancy et al. [20].

Quantification of protein content was according to the AOAC method (920.103) based on the nitrogen content with the factor 6.25 [21].

Quantification of sulfate content (SO_4^{2-}): One gram of carrageenan was soaked in 50 mL of 0.2 N HCl and boiled for 01 hours. 25 mL of H_2O_2 was then added to the mixture and heated for 05 h. After 05 hours, this solution added to 10 mL of 10% $BaCl_2$ and boiled for 02 hours. The residues were filtered through an ashless filter (Whatman No. 42) and removed the residual chloride by using the hot distilled water. The filter paper and precipitate were finally burned at 650°C in a furnace and calculating based on equation 5 (JECFA 2007).

Quantification of carbohydrate content was according to the method of Roman (1946) with the standard of glucose, and the absorbance measurement at the wavelength of 490nm [22].

The quantification of lipid content was to base using n-hexane [23].

Quantification of the content of Pb, As, Cd, and Hg was by using inductively coupled plasma mass spectrometry [24].

Quantification of total aerobic bacterial

Quantification of *Escherichia coli* and *Coliforms* was according to Method 1604 (2002) [25].

Quantification of Staphylococcus aureus was based on the method of AOAC 975.55 [26].

Quantification of *Salmonella* was according to Denise et al. [27].

Quantification of *Bacillus cereus* was according to Irena et al. [28].

2.4. Evaluation of Toxicity

The toxicity assay of single-dose (safety) was on Swiss white mice consisting of four groups and twelve mice per group (ten male and ten female). Group A, B, C, and D drunk the carrageenan solution of 1.5% (w/v), 1.0% (w/v), 0.5% (w/v), and physiological saline, respectively. Clinical manifestations and weight of each rat were observed daily for seven consecutive days. All mice were operated on to see the whole organ in the abdominal and thoracic cavity (Table 1). The tissue samples will be taken and sent to histopathology at the Department of Pathology and Forensic Medicine, Hue University of Medicine and Pharmacy as finding any abnormalities. Mice numbers were from 101 to 120, 201 to 220, 301 to 320, and 701 to 720, corresponding to group A, B, C, and control (salt solution), respectively (Table 5). Numbered mice were to the first male and late females.

Table 1. *Criteria for evaluating clinical manifestations in safe laboratory rats.*

Symptom	Evaluation (%, appearance date)
Struggling / stimulating / tiptoeing	No / Yes
Sluggishness, poor reflexes with the outside	No / Yes
Ruffled feathers	No / Yes
Shortness of breath	No / Yes
Exudation (watery eyes, runny nose, saliva)	No / Yes
Shivering/sweating	No / Yes
Distention	No / Yes
Vomiting	No / Yes
Diarrhea	No / Yes
Paralysis or increase/decrease in muscle tone	No / Yes

2.5. Determination of Carrageenan Purification

The determination of carrageenan purification was by using the NMR spectrum, and carrageenan content before and after purification in 96% ethanol. 1H-NMR (500 MHz, $CDCl_3$) and 13C-NMR (125 MHz, $CDCl_3$) spectrum were determined on the machine Bruker Avance-500 MHz with internal standards of TMS.

2.6. Data Analysis

All experiments were in triplication (n=3) and removing unnormal value by the method of Duncan. Statistic analysis was by using the software of MS. Excel 2010.

3. Results and Discussion

3.1. Physical Characterization of Carrageenan

The results showed ethanol affected the physical chemistry of carrageenan that extracted with the assistance of enzyme Viscozyme L and the purification by using ethanol. For example, before the impact of ethanol, viscosity of solution (1.5% of carrageenan) at 75°C and the solution strength (1.5% of carrageenan and 0.2% of postassium chlorua) at 20°C the content of the moisture corresponded to 80.5±2.01 (cPs) and 615±22.76 (g/cm^2), respectively. After the impact of ethanol, the viscosity and the strength of the carrageenan solution were 95.3±2.76 (cPs) and 657±15.11 (g/cm^2) (Table 2), respectively. Therefore, ethanol caused the improvement of the physical characterization (viscosity and strength of the solution) of carrageenan.

Table 2. *Effect of ethanol on physical chemistry characterization of carrageenan.*

Order	Analysis target	Unit	The results	
			Before purification	After purification
1	Moisture	% DW	10.9±0.23	9.5±0.27
2	Dispersal in water	% DW	92.5±1.76	98.2±2.46
3	Ethanol-insolubility impurities	% DW	1.74±0.04	0.74±0.02
4	Viscosity of solution (1.5% of carrageenan) at 75°C	cPs	80.5±2.01	95.3±2.76
5	The solution strength (1.5% of carrageenan and 0.2% of potassium chloride) at 20°C	g/cm^2	615±22.76	657±15.11
7	Total ash content	% DW	20.3±0.37	19.2±0.52
8	Acid-solubility ash	% DW	0.7±0.02	0.3±0.01
9	Acid-insolubility ash	%	0.9±0.02	0.4±0.01
10	Total protein content	%	5.1±0.17	0.2±0
11	Sunphat content (SO_4^{2-})	%	17.8±0.52	17.3±0.4
12	Carbohydrat content	%	45.5±1.64	50.6±1.42
13	Lipid content	%	0.4±0.02	-
14	Lead content (Pb)	mg/kg	0.023	0.01
15	Arsenic content (As)	mg/kg	0.038	< 0.01
16	Cadmium content (Cd)	mg/kg	0.105	0.05
17	Mercury content (Hg)	mg/kg	0.026	< 0.01

3.2. Microorganisms on Carrageenan

The microorganisms causing the human diseases did not occur in carrageenan, except for total aerobic bacterial. For example, before and after the impact of ethanol, total aerobic bacterial of carrageenan corresponded to 2.1×10^3 and $10^{\wedge2}$ Cells/g, respectively. According to the standard of FAO on carrageenan [29], total aerobic bacterial was not excess 5000 CFU/g (Table 3). Therefore, carrageenan in the current study got the standard of FAO.

Table 3. *Effect of ethanol on the mircoorganisms of carrageenan.*

Order	Microorganism	Unit	The results	
			Before purification	Before purification
1	Total aerobic bacterial	Cells/g	$2.1.10^3$	10^2
2	*Escherichia coli*	Cells/g	None detected	None detected
3	*Coliforms*	Cells/g	None detected	None detected
4	*Staphylococcus aureus*	Cells/g	None detected	None detected
5	*Salmonella*	Cells/25g	None detected	None detected
6	*Bacillus cereus*	Cells/g	None detected	None detected

3.3. Chemical Composition and Characterization

The results showed ethanol affected the chemical composition and characterization of carrageenan that extracted with the assistance of enzyme Viscozyme L and the purification by using ethanol. For example, before the impact of ethanol, the content of the moisture, the ethanol-insolubility impurities, total ash, acid-solubility ash, acid-insolubility ash, total protein, sulfate (SO_4^{2-}), carbohydrate, and lipid of carrageenan corresponded to 10.9±0.23, 1.74±0.04, 20.3±0.37, 0.7±0.02, 0.9±0.02, 5.1±0.17, 17.8±0.52, 45.5±1.64, 0.4±0.02% DW, respectively, and was 1.14, 2.35, 1.05, 2.33, 2.25, 25.5, 1.03, and 0.9 times, compared to after the impact of ethanol, respectively (Table 2). Lipid did not exist in carrageenan after

the impact of ethanol. the viscosity of solution (1.5% of carrageenan) at 75°C, and the solution strength (1.5% of carrageenan and 0.2% of potassium chloride) at 20°C of carrageenan after the impact of ethanol were higher than before the impact of ethanol. Heavy metal content (lead, arsenic, cadmium, and mercury) of carrageenan after the impact of ethanol was lower than before the impact of ethanol. Cadmium content got the highest value, compared to other metal content for both carrageenan kinds. The maximum value of the content of lead, arsenic, and mercury was ≤ 0.01 ppm.

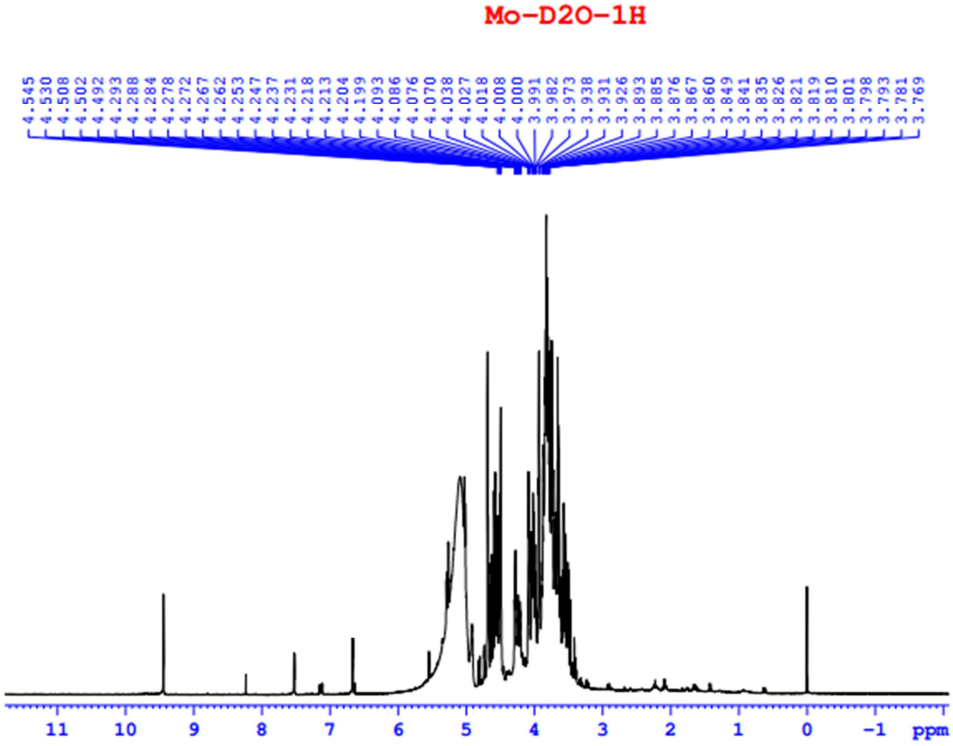

Figure 1. *The 1H-NMR spectrum of carrageenan before the impact of ethanol.*

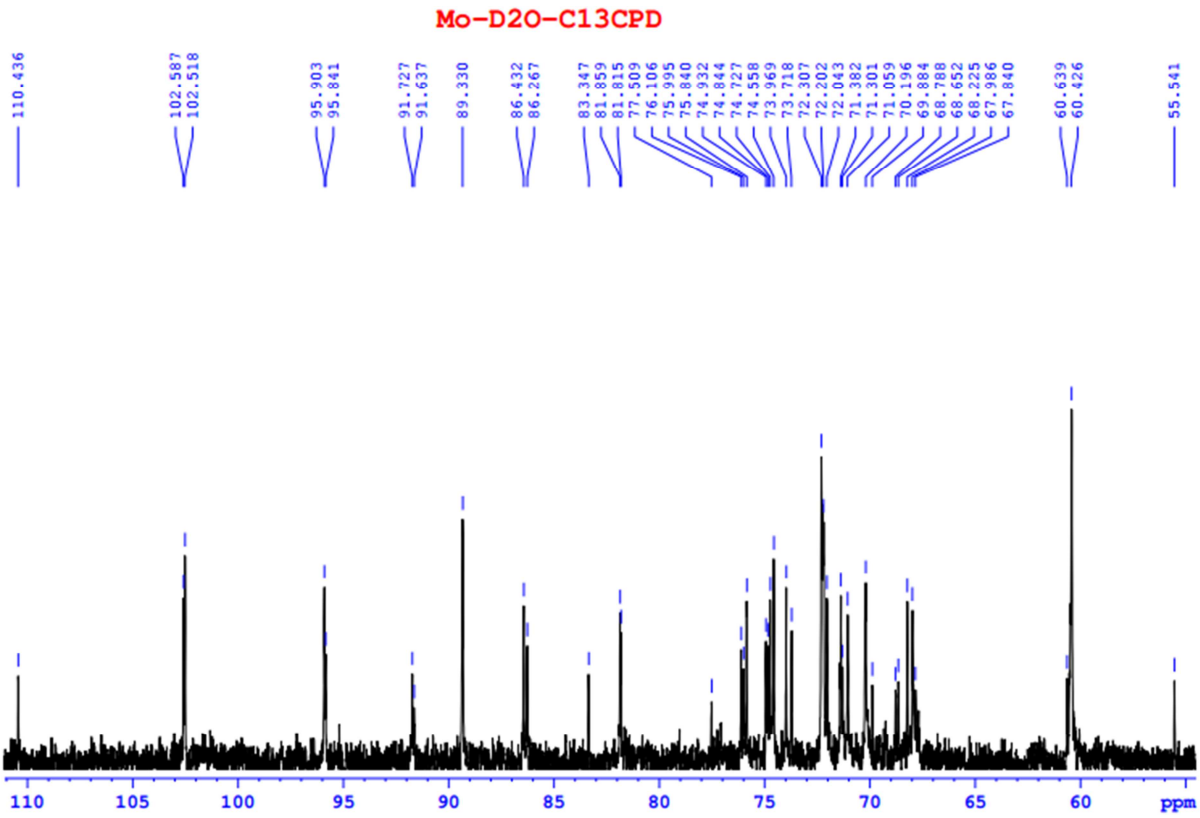

Figure 2. *The 13C-NMR spectrum of carrageenan before the impact of ethanol.*

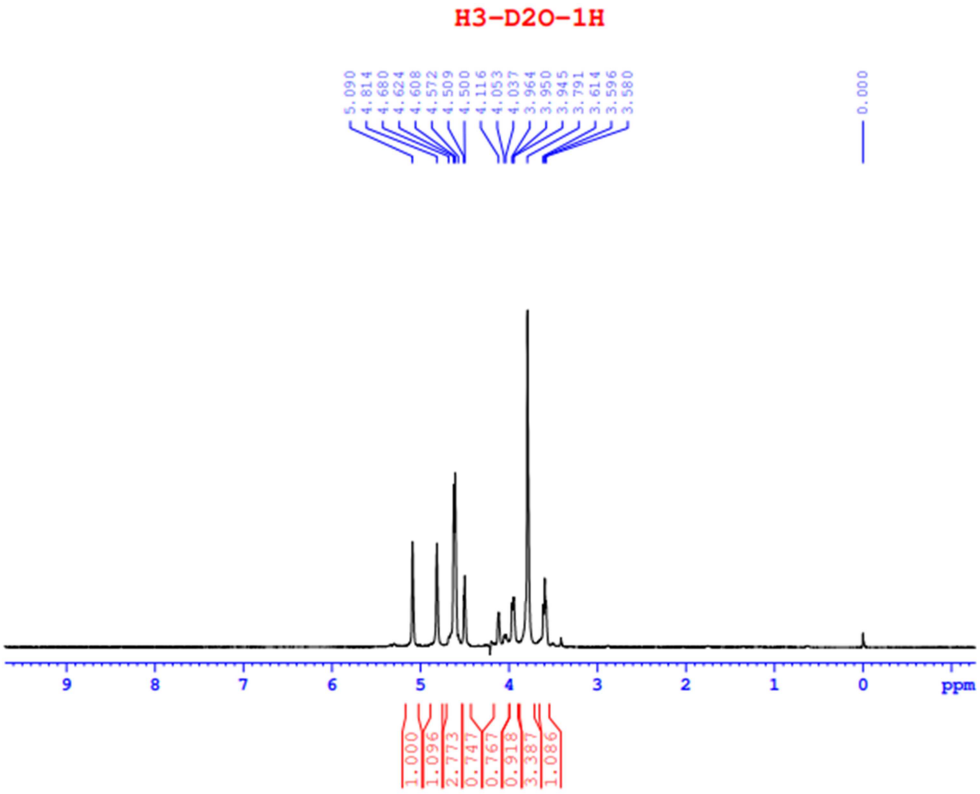

Figure 3. *The 1H-NMR spectrum of carrageenan after the impact of ethanol.*

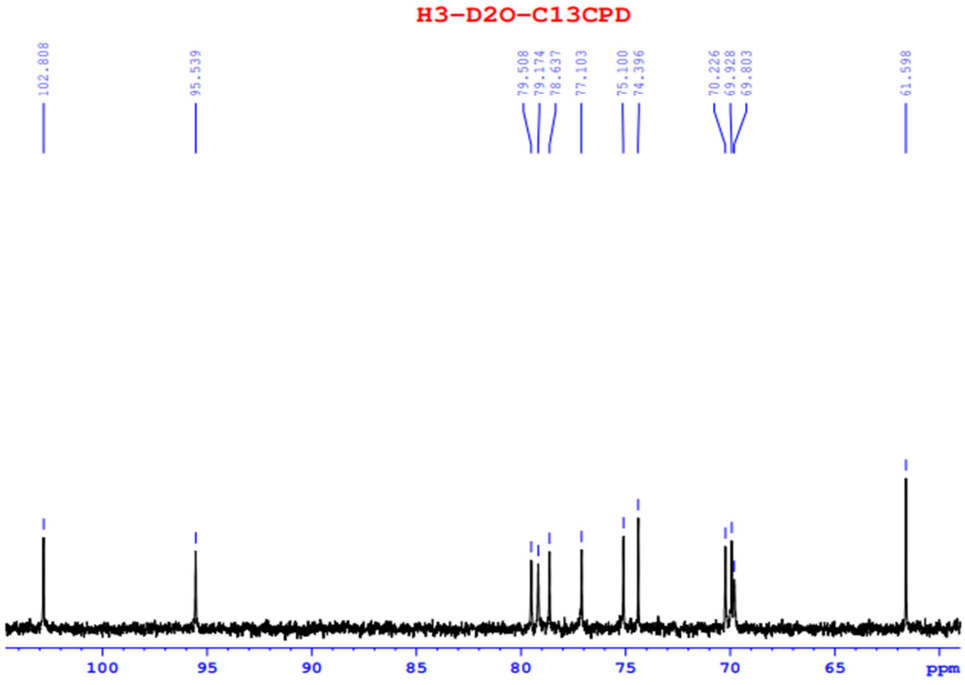

Figure 4. *The 13C-NMR spectrum of carrageenan after the impact of ethanol.*

The anomeric proton signals (1H in the β-D-Gal residue of carrageenans) exhibited in the range of 4.49 to 4.54 ppm (Figure 1) and 4.5 to 4.57 ppm (Figure 3) in the 1H NMR spectrum. The signals of 1H in α-D-AnGal residue, αD-AnGal residue, the methyl proton in 6-O-methyl Gal, and methyl hydrogen of carrageenan did not occur in both of 1H spectrum. Methylene and methine hydrogens of the carrageenan exhibited in the range of 3.76 to 4.5 (Figure 1) and 3.5 to 4.81 ppm (Figure 3). The signal range at 102.5 & 91.72, 91.7, and 95.8 & 95.9 ppm exhibited anomeric carbon resonance pairs attributed to the pyruvated α-, methylated α- and ι carrageenans, respectively (Figure 2). The anomeric carbon

resonance pairs belonging to pyruvate and ι carrageenans occurred in the signal range at 102, 95.5 (Figure 4). 60.4 & 60.6 (Figure 2), and 61.3 (Figure 4) of the carbon resonance were belonging to the methylated C-6 of 3-linked galactose. 13C NMR resonances at 101.0 ppm indicated the acetal group of the pyruvate unit. C-4 and C-5 of the 3-linked pyruvate galactose unit exhibited in the signals at 67.8 and 67.9 ppm. The signal at a range of 6 to 7 ppm was the characterization for protein impurities that existed in carrageenan. This signal was consistent with the results of the analysis of physical and chemical indicators of pre-purified carrageenan samples and showing that the protein existed in the initial carrageenan sample. The peak at 6 ÷ 7ppm did not occur in figure 4. Some peaks at the range of 4 ppm in figure 2 were more than figure 4, was the characterization of protein and lipid. The information was suitable for the analysis results of physical chemistry of carrageenan before and after the purification by ethanol. Thus, ethanol was useful to the purification of carrageenan.

3.4. Toxicity of Carrageenan After Purification

3.4.1. Clinical Manifestations in Mice

After seven days of testing, mice were given carrageenan at different concentrations without any clinical symptoms compared to the control samples (Table 4).

Table 4. *Clinical manifestations of mice drinking Carrageenan and control.*

Symptoms	Group A	Group B	Group C	Control group
Struggling / stimulating / tiptoeing	None	None	None	None
Sluggishness, poor reflexes with the outside	None	None	None	None
Ruffled feathers	None	None	None	None
Shortness of breath	None	None	None	None
Exudates (watery eyes, runny nose, saliva)	None	None	None	None
Shivering/sweating	None	None	None	None
Distention	None	None	None	None
Vomiting	None	None	None	None
Diarrhea	None	None	None	None
Paralysis or increase/decrease in muscle tone	None	None	None	None

Note: None: none-detection.

3.4.2. Mice Weight

Table 5. *Mice weight drinking Carrageenan and control after seven days.*

Mice code	Weight (g) per day							
	D0	D1	D2	D3	D4	D5	D6	D7
101	20.5	21.6	22.8	24.3	25.4	26.7	28.3	30.2
102	20.2	21.5	22.7	24.2	25.8	26.8	28.3	30.2
103	19.5	20.5	21.7	23.2	24.2	25.5	27.0	28.9
104	20.0	21.5	22.6	24.1	25.5	26.7	28.2	29.8
105	20.7	21.9	23.3	24.7	26.1	27.3	28.4	30.1
106	20.3	21.3	22.4	23.8	25.2	26.4	27.7	29.8
107	18.4	19.5	20.6	22.0	23.4	24.4	25.7	27.3
108	18.1	19.5	20.7	22.1	23.3	24.6	25.9	27.5
109	18.6	19.9	21.4	22.9	24.1	25.4	26.4	29.5
110	20.0	21.1	22.6	23.9	25.1	26.1	27.8	30.2
111	18.3	19.4	20.9	22.2	23.6	25.0	26.7	29.1
112	18.7	19.9	21.1	22.4	23.8	25.2	26.9	29.0
113	18.6	20.0	21.2	22.5	24.2	25.6	27.4	28.3
114	19.0	20.1	21.7	23.4	25.1	26.5	27.9	30.0
115	19.6	20.9	22.5	24.0	25.5	27.1	28.5	31.0
116	19.9	21.3	23.1	24.6	26.0	27.4	28.8	30.5
117	18.8	19.8	21.4	23.0	24.4	25.7	27.1	28.7
118	18.4	19.5	20.7	22.3	23.8	25.1	26.7	29.2
119	20.0	21.3	22.5	24.1	25.6	27.2	28.8	30.2
120	19.3	20.7	22.0	23.6	25.1	26.7	28.3	30.2
201	18.3	19.6	21.3	22.9	24.4	26.0	27.6	28.3
202	20.5	21.9	23.2	24.8	26.2	27.5	29.0	32.2
203	18.0	19.1	20.4	21.9	23.3	24.6	26.1	28.9
204	18.3	20.1	21.1	22.6	24.0	25.2	26.7	29.0
205	18.2	19.7	20.9	22.4	24.0	25.2	26.7	28.8
206	18.0	19.6	21.0	22.5	24.1	25.3	26.9	27.9
207	19.4	20.8	21.8	23.1	24.7	26.0	27.2	29.1
208	19.5	21.0	22.4	23.7	25.4	26.7	27.9	29.7
209	19.7	21.0	22.2	23.5	24.9	26.3	27.9	29.2
210	18.1	19.7	20.9	22.2	23.6	25.0	26.6	28.3
211	20.5	21.9	23.1	24.6	26.2	27.5	29.1	31.0
212	20.2	21.5	22.7	24.2	25.8	27.1	28.6	30.5
213	19.5	20.9	22.1	23.6	25.2	26.5	28.0	29.9
214	20.0	21.5	22.6	24.1	25.5	26.7	28.2	29.8
215	20.7	21.9	23.0	24.4	25.8	27.0	28.6	30.1
216	20.3	21.7	22.8	24.2	25.6	26.8	28.1	29.8
217	18.4	20.0	21.1	22.5	23.9	25.1	26.4	27.3
218	18.1	19.5	20.7	22.1	23.3	24.6	25.9	27.5
219	18.6	19.9	21.4	22.9	24.1	25.4	27.0	29.5
220	20.0	21.1	22.6	23.9	25.1	26.5	28.2	30.2
301	18.3	19.4	21.2	22.2	23.6	24.6	26.3	29.1
302	18.7	19.9	21.5	22.5	23.9	25.3	27.0	29.0
303	18.6	20.0	21.9	22.9	24.6	26.0	27.6	28.3
304	19.0	20.3	21.9	22.1	23.8	25.2	26.6	30.0
305	19.6	20.6	22.2	23.2	24.7	25.8	27.2	31.0
306	19.9	21.3	22.9	23.9	25.3	26.7	28.1	30.5
307	18.8	20.5	21.9	22.3	23.7	24.9	26.3	28.7
308	18.4	19.5	20.7	21.7	23.2	24.5	26.1	29.2
309	20.0	21.3	22.5	23.5	25.0	26.6	28.2	30.2
310	19.3	20.7	22.0	23.0	24.5	25.7	27.3	30.2
311	18.3	19.6	20.9	22.5	24.0	25.6	27.2	28.3
312	20.5	21.9	23.2	24.8	26.2	27.5	29.0	32.2
313	18.0	19.5	20.8	22.3	23.7	25.0	26.5	28.9
314	18.3	19.9	21.1	22.6	24.0	25.2	26.7	29.0
315	18.2	19.4	20.6	22.1	23.7	24.9	26.4	28.8
316	18.0	19.3	20.7	22.2	23.8	25.0	26.6	27.9
317	19.4	20.8	22.2	23.5	25.1	26.4	27.6	29.1
318	19.5	21.0	22.4	23.7	25.1	26.4	27.6	29.7
319	19.7	21.5	22.7	24.0	25.4	26.8	28.4	29.2
320	18.1	19.7	20.9	22.2	23.6	25.0	26.6	28.3
701	19.0	20.3	21.6	22.8	24.0	25.6	27.6	30.1
702	19.0	20.2	21.5	22.7	23.9	25.5	27.5	28.7
703	18.0	19.1	20.4	21.6	22.8	24.4	26.4	28.3
704	18.1	19.4	20.7	21.9	23.1	24.7	26.7	27.8
705	18.6	20.0	21.3	22.5	23.7	25.3	27.3	29.8

Mice code	Weight (g) per day							
	D0	D1	D2	D3	D4	D5	D6	D7
706	18.9	20.4	21.7	22.9	24.1	25.7	27.7	29.5
707	18.6	20.0	21.3	22.5	23.7	25.3	27.3	29.0
708	18.4	20.0	21.3	22.5	23.7	25.3	27.3	28.8
709	19.4	20.7	22.0	23.2	24.4	26.0	28.0	29.3
710	18.7	20.2	21.5	22.7	23.9	25.5	27.5	28.7
711	18.9	20.3	21.6	23.0	24.4	25.5	26.6	28.1
712	18.7	19.9	21.2	22.3	23.7	24.8	25.9	27.9
713	18.7	20.2	21.5	22.7	24.1	25.2	26.3	28.0
714	19.6	20.9	22.2	23.4	24.6	26.2	28.2	31.0
715	19.7	21.1	22.4	23.6	24.8	26.4	28.4	30.0
716	20.5	21.8	23.1	24.3	25.5	27.1	29.1	33.0
717	19.0	20.2	21.5	22.7	23.9	25.5	27.5	28.9
718	19.2	20.7	22.0	23.2	24.4	26.0	28.0	29.4
719	19.4	20.7	22.0	23.2	24.4	26.0	28.0	29.3
720	18.4	20.0	21.3	22.5	23.7	25.3	27.3	28.6

Note: D_i: day i. i was from 0 to 7.

Carrageenan oral mice: mice weight increased from 9.7 to 10.5 g/rat (increased by 50 - 55%, respectively), compared to the initial weight. The increase in mice weight was not different insignificance ($p>0.05$) between other groups. Therefore, purified carrageenan by ethanol was non-toxicity.

3.4.3. Pathology

Abnormalities in the organs belong to the abdominal and thoracic of the rat were not found after surgery (Figure 5). The lymph nodes, tumors, bleeding signs, abnormal fluid retention in the abdominal and chest cavities did not appear.

(a) (b)

Figure 5. *Mice drunk physiological saline (a) and carrageenan (b).*

With the results of clinical observation, weight monitoring, and anatomy, it said that carrageenan was non-toxic and safe.

4. Conclusion

After the impact of ethanol, the purification and physical (dispersal in water and rheological) characterization of carrageenan was higher than before the impact of ethanol. For example, dispersal in water, the viscosity of the solution, the solution strength (1.5% of carrageenan and 0.2% of potassium chloride), and carbohydrate content at 20°C corresponded to 1.06, 1.18, 1.07, and 1.11 times, compared to before the impact of ethanol. The content of ethanol-insolubility impurities, total ash, acid-solubility ash, acid-insolubility ash, total protein, sunphat content (SO_4^{2-}), and lipid content was 43%, 94.6%, 42.9%, 44.44%, 3.9%, 97.2%, and none-detected in comparison to before the impact of ethanol. The content of lead, arsenic, cadmium, and mercury was 0.01, < 0.01, 0.05, and < 0.01 ppm, respectively. Total aerobic bacterial of carrageenan got the highest value of 2.1 x 10^2 cells/g. *E. coli, coliforms, staphylococcus aureus, salmonella,* and *bacillus cereus* did not occur in carrageenan. Purified carrageenan by using ethanol was non-toxic.

Acknowledgements

The authors thank Vietnam Ministry of Agriculture and Rural Development, Department of Science and Technology (Ba Ria-Vung Tau province), Faculty of Food Technology (Nha Trang University), Pacific Oceanography University, and Nha Trang Insitute of Technology Application and Research for helping.

References

[1] Álvarez R., Araya, H., Navarro-Lisboa, R. and Lopez, d. D. C. (2016). Evaluation of polyphenol content and antioxidant capacity of fruits and vegetables using a modifi ed enzymatic extraction. Food Technol Biotechnol, 54 (4): 462-467.

[2] Necas J. and Bartosikova, L. (2013). Carrageenan: a review. Vet Med-Czech, 58 (4): 187-205.

[3] Sankalia M., Mashru, R., Sankalia, J. and Sutariya, V. (2006). Stability improvement of alpha-amylase entrapped in kappa-carrageenan beads: physicochemical characterization and optimization using composite index. Int J Pharm, 312 (1-2): 1-14.

[4] Sarah H., Mariel, B., Ross, C., Kevin, P. and Angie, T. (2016). Chapter 10 - The use of carrageenan in food. Carrageenans. Leonel, P, Nova Science Publishers, Inc.

[5] Błażej B., Grażyna, G. and Alexander, S. (2018). Carrageenan as a functional additive in the production of cheese and cheese-like products. Acta Sci Pol Technol Aliment, 17 (2): 107–116.

[6] Li L., Ni, R., Shao, Y. and Mao, S. (2014). Carrageenan and its applications in drug delivery. Carbohydr Polym, 103 1-11.

[7] Abida K. K., et al. (2017). Carrageenan based bionanocomposites as drug delivery tool with special emphasis on the influence of ferromagnetic nanoparticles. Oxid Med Cell Longev 1-13.

[8] Abdul B. A. A., Mohaddeseh Adel, Pegah, K. and Mahvash, P. (2014). Chapter Ten - Pharmaceutical, Cosmeceutical, and Traditional Applications of Marine Carbohydrates. Adv Food Nutr Res, 73 197-220.

[9] Roos G., Cheshire, A., Nayar, S., Clarke, S. M. and Zhang, W., Harnessing marine macroalgae for industrial purposes in an australian context: emerging research and opportunities: Emerging research and opportunities, Hershey PA, USA: IGI Global, 2018, pp. 34.

[10] Nussinovitch A., Hydrocolloid Applications, Doyle Graphics, Tullamore, Ireland: Springer Science+Business Media Dordrecht, 1997, pp. 40-58.

[11] Zhiwei L., et al. (2019). Anti-Cancer Activity of Porphyran and Carrageenan from Red Seaweeds. Molecules, 24 (23): 4286-4299.

[12] Ning M., et al. (2015). Preventive effect of aspirin eugenol ester on thrombosis in κ-carrageenan-induced rat tail thrombosis model. PLoS One, 10 (7): e0133125.

[13] Vanessa W., Sabrina, M. D. C., Paulo, J. O., Leila, H. and Pedro, L. M. B. (2012). Optimization of the extraction of carrageenan from Kappaphycus alvarezii using response surface methodology. Ciênc Tecnol Aliment Campinas, 32 (4): 812-818.

[14] Sitti N., Syarief, R. S., Peranginangin, R., Nurtama, B. and Jaswir, I. (2017). Production of refined carrageenan from Kappaphycus alvarezii on pilot plant scale: optimization of water extraction using response surface methodology. Int Food Res J, 24 (Suppl): S522-S528.

[15] Nanna R.-K., Marcel, T. A. and Anne, S. M. (2015). Seaweed hydrocolloid production: An update on enzyme assisted extraction and modification technologies. Mar Drugs, 13 (6): 3340–3359.

[16] Blanco-Pascual N., Aleman, A., G ´ omez-Guill´ en, M. and Montero ´, M. (2014). Enzyme-assisted extraction of k/i-hybrid carrageenan from Mastocarpus stellatus for obtaining bioactive ingredients and their application for edible active film development. Food Funct, 5 319-329.

[17] Soma C. (2017). Carrageenan for encapsulation and immobilization of flavor, fragrance, probiotics, and enzymes: A review. J Carbohydr Chem, 36 (1): 1-19.

[18] William H. and George, W. L., Official methods of analysis of AOAC International, 18th, Arlington, VA, USA: Gaithersburg, Md.: AOAC International, 2005.

[19] Awang B., Anisuzzaman, S. and Wan, D. O. (2014). Effect of process conditions on the gel viscosity and gel strength of semi-refined carrageenan (SRC) produced from seaweed (Kappaphycus alvarezii). JKSUES, 26 (1): 3-9.

[20] Nancy J. T., Lawrence, N. and Andy, C. (2012). Quantification of ash soluble in acid (Determination of Ash in Animal Feed: AOAC Official Method 942.05). Journal of AOAC International, 95 (5): 1392-1397.

[21] Latimer G., Official Methods of Analysis of AOAC International, Gaithersburg, MD, USA: AOAC International, 2016.

[22] Roman D. (1946). Qualitative test for carbohydrate material. Ind Eng Chem Anal Ed, 18 (8): 499-499.

[23] AOAC, Fat in foods. Chloroform-methanol extraction method: Method 983.23, 19th, Gaithersburg, MD, USA: AOAC International, 2012.

[24] Julshamn K., et al. (2013). Determination of arsenic, cadmium, mercury, and lead in foods by pressure digestion and inductively coupled plasma/ mass spectrometry: first action 2013.06. J AOAC Int, 96 (5): 1101-1102.

[25] Method 1604 (2002). Total Coliforms and Escherichia coli in water by membrane filtration using a simultaneous detection technique (mi medium). EPA-821-R-02-024. (4303T) 1200 Pennsylvania Avenue, NW Washington, DC 20460., U.S. Environmental Protection Agency Office of Water.

[26] Silbernagel K., Jechorek, R., Carver, C., Horter, B. and Lindberg, K. (2003). 3M Petrifilm staph express count plate method for the enumeration of Staphylococcus aureus in selected types of processed and prepared foods: collaborative study. J AOAC Int, 86 (5): 954-962.

[27] Denise H., Angela, E. D. and Louise, H. (2003). Salmonella in Foods: new enrichment procedure for tecra salmonella visual immunoassay using a single RV (R10) only, TT only, or Dual RV (R10) and TT selective enrichment broths (AOAC Official method 998.09): Collaborative study. J AOAC Int, 86 (4): 775-790.

[28] Irena N., Kateřina, S., Petr, R., Barbora, U. and Eva, Š. (2011). Methods for detection of Bacillus sp., B. cereus, and B. licheniformis in raw milk. Czech J Food Sci, 29 (S55-S60).

[29] FAO/WHO, Compendium of food additive specifications, Rome, Italy: FAO/WHO, 2014, pp. 7-16.