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RESEARCH ON SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND BIOLOGICAL ACTIVITY OF CARRAGEENAN–LECTIN MICROPARTICLES FROM RED ALGAE

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PREFACE

The necessary of the thesis

In Vietnam, red algae have been widely cultivated or harvested wild, they are a source of income for coastal households and a source of raw materials for lectin and carrageenan. However, to improve the usability and economic value of red algae, it is necessary to research new products from red algae, especially carboxymethyl derivatives from red alga polysaccharides. This has led the researcher to conduct the thesis: "***Research on synthesis, structural characterization, and biological activity of carrageenan–lectin microparticles from red algae***", with the aim of developing red algae resources as well as biological materials for use in food and medicine**.**

The objectives of the thesis

The dissertation focuses on the extraction and evaluation of the hemagglutination activity of lectins from the two red algae species *Kappaphycus striatus* and *Betaphycus gelatinus*, as well as the characterization of the carrageenan obtained from these species. Subsequently, the study involves the synthesis of carboxymethyl-carrageenan derivatives and the analysis of their structural features. Based on these results, carboxymethyl-carrageenan microcapsules encapsulating lectin were fabricated, structurally characterized, and their lectin release behavior was also investigated.Evaluate the hemagglutination activity of lectin extracted from two red algae *Kappaphycus striatus* and *Betaphycus gelatinus*.

The thesis contents

- After collecting the seaweed samples from March to June, the materials were processed, and lectins were extracted and purified from the red algae *K.striatus* and *B.gelatinus*. The hemagglutination activity of the purified lectins was subsequently determined.

- The algal residues remaining after lectin extraction were used for carrageenan isolation. The extracted carrageenan was purified, and its structural characteristics were analyzed for both *K.striatus* and *B. gelatinus*.

- Carboxymethyl-kappa-carrageenan derivatives were synthesized from kappa-carrageenan originating from *K. striatus*, and their structural features were characterized. The optimal chemical ratios required to obtain carboxymethyl-kappa-carrageenan with a high degree of substitution were also determined.

- Microcapsules composed of carboxymethyl-kappa-carrageenan encapsulating lectin from *K. striatus* were fabricated, followed by structural characterization and evaluation of their biological activity.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. This is the first scientific study in Vietnam to successfully extract and integrate lectin and carrageenan from two red algae species: *K. striatus*, cultivated in Van Phong Bay, Khanh Hoa Province, and *B. gelatinus*, naturally harvested from the coastal waters of Ninh Thuan (currently part of Khanh Hoa). The dissertation investigates the structural characteristics and biological activities of lectins and carrageenans extracted from these red algae: The carrageenan extracted from *K. striatus* consists predominantly of kappa-carrageenan, whereas the carrageenan from *B. gelatinus* is a hybrid carrageenan, composed of beta-carrageenan and kappa-carrageenan. Both carrageenan and its carboxymethyl-kappa-carrageenan derivative from red algae exhibited hemagglutination activity and antioxidant properties.

2. For the first time, carboxymethyl-kappa-carrageenan has been synthesized from kappa-carrageenan derived from *K. striatus* cultivated in Van Phong Bay, Khanh Hoa Province, Vietnam. This derivative exhibits pH-responsive swelling behavior, suggesting its potential application as a biocompatible carrier for bioactive compounds in biomedical fields.

CHAPTER 1. INTRODUCTION

1.1. Overview of red algae

1.1.1. Red algae

Red algae such as *Kappaphycus alvarezii*, *Kappaphycus striatus*, *Eucheuma denticulatum*, and *Betaphycus gelatinus* are economically important species that are widely cultivated or harvested from the wild in Vietnam. These species are not only valuable sources of lectins but also serve as significant sources of carrageenan for use in food, cosmetics, and as carrier materials in pharmaceuticals.

1.1.2. *Kappaphycus striatus* and *Betaphycus gelatinus*

Table 1.2. Classification system of *K. striatus* and *B. gelatinus*

Alga	***Kappaphycus striatus***	***Betaphycus gelatinus***
Regnum	*Plantae*	*Plantae*
Division	*Rhodophyta, Wettstein*	*Rhodophyta, Wettstein*
Class	*Florideophyceae, Cronquist*	*Florideophyceae, Cronquist*
Order	*Gigartinales, F. Schmitz*	*Gigartinales, F. Schmitz*
Family	*Solieriaceae, J. Agardh*	*Solieriaceae, J. Agardh*
Genus	*Kappaphycus, Doty*	*Betaphycus*
Species	*Kappaphycus. striatus* (F. Schmitz, 1895) Doty ex P.C. Silva, 1996	*Betaphycus gelatinus* (Esper) Doty ex P. C. Silva 1996

1.2. Carrageenans from red algae

1.2.1. Structure of carrageenan

The structure of carrageenan consists mainly of D-galactose residues, which form repeated disaccharides through 3-β-D-galactopyranose bonds and 4-α-D-galactopyranose bonds.

1.2.2. Physicochemical properties, biological activities, and applications of carrageenan

1.2.2.1. Physicochemical properties and biological activities of carrageenan: Carrageenans possess a range of biological activities,

including anti-influenza A virus (H1N1), anticancer, cholesterol-lowering, and antioxidant effects.

1.2.2.2. Potential applications of carrageenan

Carrageenan has been widely applied in various pharmaceutical formulations, including tablets, coatings, fast-dissolving inserts (FDIs), microparticles, inhalation systems, injectable preparations, and hydrogels.

Chemical modification of carrageenan such as the introduction of carboxyl (-COOH) groups onto its molecular chains can yield effective drug delivery carriers. These modified systems enhance bioavailability, prevent premature release or degradation of active compounds during gastrointestinal transit, and enable site-specific drug release.

1.3. Lectin from red algae

1.3.1. Structure of lectin

Red algae including *Kappaphycus alvarezii, K. striatus, Eucheuma denticulatum,* and *Betaphycus gelatinus*, are not only valuable sources of carrageenan for the food industry but also promising sources of bioactive lectins for biomedical applications.

1.3.2. Biological activities and potential applications

1.3.2.1. Hemagglutination activity

Lectins derived from red algae demonstrate strong hemagglutination activity, particularly against rabbit and sheep erythrocytes, with relatively lower reactivity toward human ABO blood groups.

1.3.2.2. Applications

Due to their carbohydrate-binding specificity and structural diversity, red algal lectins exhibit considerable potential in biomedical applications, including anti-inflammatory, antibacterial, antiviral, and anticancer effects, as well as in disease diagnosis and targeted therapy.

1.4. Carrageenan microcapsules from red algae

1.4.1. Biological activities and applications

Marine polysaccharides such as carrageenan, agar, and alginate extracted from algae - demonstrate significant potential as drug delivery matrices, especially for anti-inflammatory and anticancer therapeutics.

1.4.2. Microcapsule fabrication methods

The ionic gelation technique has been employed to fabricate polysaccharide-based microcapsules from algae - derived carrageenan for the encapsulation of bioactive agents such as insulin or lectins.

1.5. Research status worldwide and in Vietnam relevant to the dissertation

1.5.1. International research Status

Carrageenan obtained from red algae has been explored as a pharmaceutical excipient and as a component of various drug delivery systems, including nanoparticles, microcapsules, microspheres, and hydrogel formulations.

1.5.2. Research status in Vietnam

Numerous studies on the isolation and characterization of lectins from red algae have been conducted by Dr. Le Dinh Hung and collaborators (2009–2021). These lectins exhibit high-mannose type N-glycan binding activity and share structural features with known antiviral and anticancer lectins (active against HIV, SARS-CoV, and influenza viruses), as reported in international publications.

In Vietnam, various studies have focused on the extraction and purification of carrageenan from red algae for food industry applications. However, there remains a lack of published research on carrageenan derivatives such as carboxymethyl-kappa-carrageenan and their potential applications in food or medical fields. Research on the development of microcapsules using carboxymethyl-carrageenan derivatives for the encapsulation of red algal lectins is, therefore, both novel and of high practical relevance.

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1. Research Objects

Red algae *Kappaphycus striatus* "sacol" brown strain (F. Schmitz Doty ex P. C. Silva) was collected from Van Phong Bay (12°29′N, 109°10′E), Khanh Hoa Province, Vietnam, during the period from August 2020 to March 2021.

Figure 2.2. *K. striatus*: a) fresh sample, b) dried sample, c) herbarium specimen

The red alga *Betaphycus gelatinus* strain (Esper Doty ex P. C. Silva) was collected from Ninh Thuan Province (11°35′N, 109°02′E) (currently part of Khanh Hoa Province), Vietnam during the period from April to June 2022.

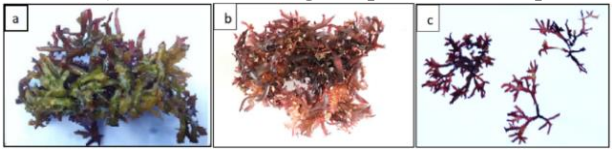

Figure 2.3. *B. gelatinus*: a) fresh sample, b) dried sample, c) herbarium specimen

The research was conducted from January 2021 to December 2024 at the Institute of Research and Technology Application of Nha Trang (now the Institute of Oceanography).

2.2. Extraction and purification of lectin and carrageenan from red algae

2.2.1. Extraction, purification, and protein content determination of lectin: Lectin was first extracted from red algae, followed by carrageenan extraction from the remaining algal biomass. Lectin was extracted and purified using the method described by Le Dinh Hung et al., 2011, 2015a.

The residual algal biomass was dried and powdered, stored at −20°C, and subsequently used for carrageenan extraction.

2.2.2. Extraction and purification of carrageenan: Carrageenan was extracted from the post-lectin-extraction algal biomass using both the natural extraction method of Craigie and Leigh (1978) and the alkaline treatment method of Ohno (1994).

2.3. Preparation of carrageenan-lectin microparticles and evaluation of lectin release

2.3.1. Synthesis of carboxymethyl-carrageenan derivatives: The carboxymethylation of carrageenan was conducted following the Williamson ether synthesis procedure.

2.3.2. Preparation of carrageenan-lectin microparticles: Microparticles encapsulating lectin were prepared using the ionic gelation method described by O. Sipahigil et al. (2001) and K.H. Leong et al. (2011b).

2.3.3. Determination of lectin release from carrageenan–lectin microparticles: The release of lectin from the polysaccharide–lectin microparticles was evaluated by UV–Vis spectrophotometry through measuring protein absorbance at 280 nm, based on the method of Harold Edelhoch (1967).

2.4. Chemical composition and structural characterization of carrageenan, carboxymethyl-carrageenan, and carrageenan–lectin microparticles

2.4.1. Chemical composition analysis

The molecular weight (MW) of carrageenan and its carboxymethylated derivatives was determined by gel permeation chromatography (Shimadzu LC-20AD, Japan). The content of 3,6-anhydro-D-galactose in the polysaccharides was quantified using the resorcinol method by Yaphe and Arsenault (1965) with D-fructose as the standard. The sulfate content was measured by the method of Terho and Hartiala (1972) using Na_2SO_4 as the

reference standard. Gelation and melting temperatures were determined following Craigie and Leigh (1978) using 1.5% carrageenan solution in 0.2% KCl; Gel strength was measured according to the method of Ohno et al. (1994).

2.4.2. Structural characterization

FT-IR spectroscopy and nuclear magnetic resonance (NMR) were employed to analyze the structure of carrageenan, carboxymethyl-carrageenan, and the microparticles.

The degree of substitution (DS) was determined by: a) Potentiometric titration, and b) Integration of ^{1}H NMR spectra.

The morphology and surface characteristics of the carrageenan–lectin microparticles were examined using scanning electron microscopy (FE-SEM) (HITACHI S-4800).

2.5. Evaluation of biological activity and swelling behavior of carrageenan, carboxymethyl-carrageenan, and carrageenan–lectin microparticles

2.5.1. Antioxidant activity determination: The antioxidant activity of carrageenan and its carboxymethyl derivatives was evaluated using the method described by Shimada et al. (1992).

2.5.2. Hemagglutination activity of lectin, carrageenan, and microparticles: Hemagglutination activity was assessed for lectin, carrageenan, the carrageenan samples (KC-0/1), carboxymethylated samples (CMKC-1/1 to CMKC-5/1), and lectin released from polysaccharide–lectin microparticles.

2.5.3. Swelling behavior of carrageenan and carboxymethyl-kappa-carrageenan microparticles: The swelling behavior of carrageenan and carboxymethyl-kappa-carrageenan microparticles was determined according to the method of K.H. Leong et al. (2011).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Results of chemical composition, structural characterization, and properties of lectin and carrageenan from the red alga *K. striatus*

3.1.1. Composition, properties, and structure of lectin from *K. striatus*

Table 3.2. Protein content and hemagglutination activity of lectin extracted from the red alga *Kappaphycus striatus*

Lectin from *K.striatus*	**Crude**	**After precipitation**	**After GFC**
Volume (mL)	800	19.4	600
Protein (mg/mL)	1.77	1.73	0.053
Protein tổng (mg)	1416.00	33.56	31.80
HA (HU/mL)	128	256	8
HTTS (HU)	102400	4996	4800
HTR (HU/mL)	72.31	148.87	150.95
LHC(µg/mL)	16.39	6.76	6.63

HA: Activity per 1 mL of lectin extract, HTTS (*Total Specific Activity*): *Total hemagglutination activity, HTS* (*Specific Activity*): Specific *hemagglutination activity,* LHC (*Lowest Hemagglutination Concentration*): *The minimum concentration required to induce hemagglutination*, *GFC*: *Gel Filtration Chromatography.*

Results of the investigation on the effects of metal ions, temperature, and pH range on the hemagglutination activity of lectin

Table 3.3. Experimental results on the effect of divalent cations on the hemagglutination activity of lectin extracted from *Kappaphycus striatus*

Sample	**HA (HU/mL)**	**HA (%)**
Metal-free sample	128	100
EDTA	128	100
Ca^{2+}	128	100
Mg^{2+}	128	100

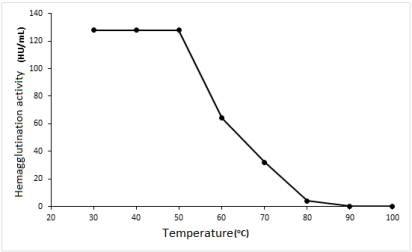

Figure 3.1. Hemagglutination activity of lectin from *K. striatus* at different temperatures..

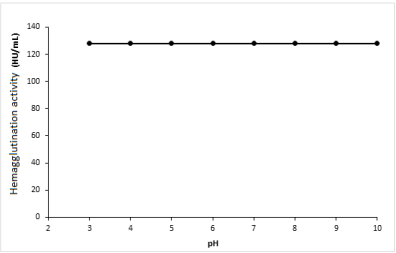

Figure 3.2. Hemagglutination activity of lectin from *K. striatus* at different pH values.

Carbohydrate-binding specificity of lectin from the red alga *Kappaphycus striatus*

Table 3.4. Minimum concentrations of monosaccharides and glycoproteins capable of inhibiting the hemagglutination activity of lectin from *K.striatus*

No.	**Monosaccharide and Glycoprotein**	**Concentration**
	Monosaccharide	C_{min} *(mM)*
1	Glucose	-
2	N-acetyl-D-glucosamine	-
3	D-galactose	-
4	N-acetyl-D- galactosamine	-
5	D-mannose	-
6	N-acetyl-D-manosamine	-
7	Xylose	-
8	N-acetyl neuraminic axit	-
	Glycoprotein	C_{min} *(µg/mL)*
9	Transferrin	-
10	Asialo-Transferrin	-
11	Fetuin	-
12	Asialo-Fetuin	31.2
13	Yeast mannan	7.8
14	Porcine thyroglobulin	7.8
15	Porcine stomach mucin	62.5
16	Asialo-porcine stomach mucin	62.5

Lectin extracted from the red alga *K. striatus* exhibited specific binding affinity to high-mannose type N-glycans. This suggests that the lectin may

possess antiviral properties, highlighting its potential applications in medicine.

3.1.2. Chemical composition, Properties, and Structural Characteristics of Carrageenan from *Kappaphycus striatus*

Carrageenan extracted using two different methods showed variations in composition and properties, as presented in Table 3.5 and Figure 3.5.

Table 3.5. Chemical composition of carrageenan extracted from *K. striatus.*

Thành phần	Carrageenan từ *K. striatus*
Native carrageenan	
Yield (% of dry weight w/w)	45.8 ± 2.8
Alkali modified carrageenan	
Yield (% of dry weight w/w)	31.3 ± 3.5
DA (% carrageenan w/w)	27.7 ± 0.7
Sulfate (% carrageenan w/w)	27.7 ± 0.9
Gelting temp (°C)	44.5 ± 0.5
Melting temp (°C)	61.5 ± 0.5
Gel strength (g/cm^2)	595 ±10
MW (kDa)	791 ± 9.3

Figure 3.5. Images of carrageenan extracted from *K. striatus* , native carrageenan, alkali modified carrageenan

The structural characteristics of carrageenan extracted from the red alga *Kappaphycus striatus* were evaluated based on Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectral analyses. The FT-IR spectra (Figure 3.6) of polysaccharides extracted from *K. striatus* using both the natural extraction method and the alkaline treatment (KOH) method revealed characteristic absorption bands corresponding to sulfated polysaccharides of the kappa-carrageenan type.

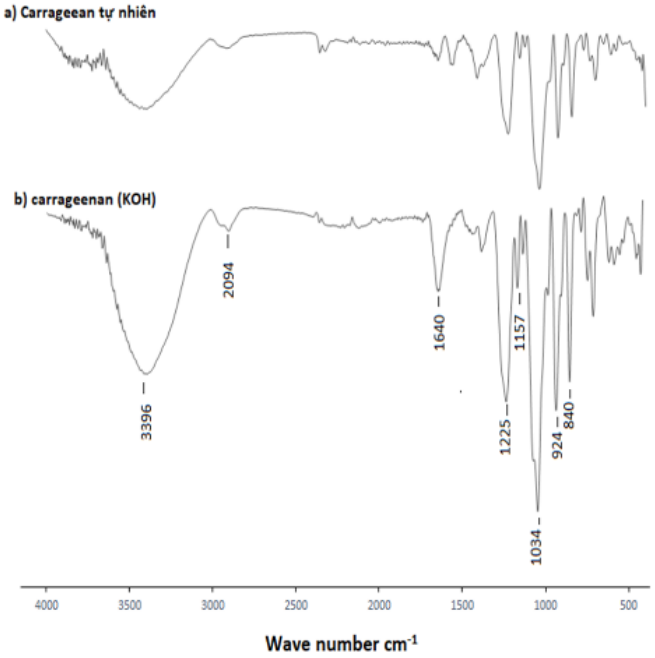

Figure 3.6. FT-IR spectra of carrageenan from *K. striatus*: a) native carrageenan, b) alkali modified carrageenan (KOH).

NMR spectral results of carrageenan from *Kappaphycus striatus*

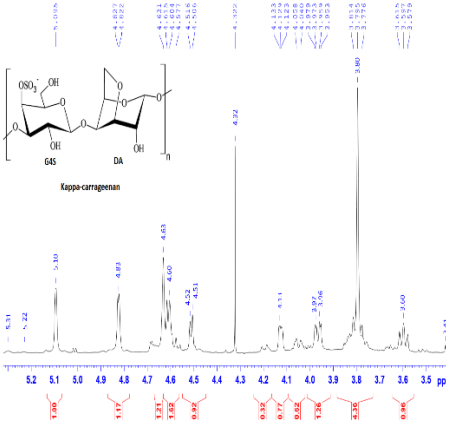

Figure 3.7. ^{1}H NMR spectra of carrageenan from *K. striatus*.

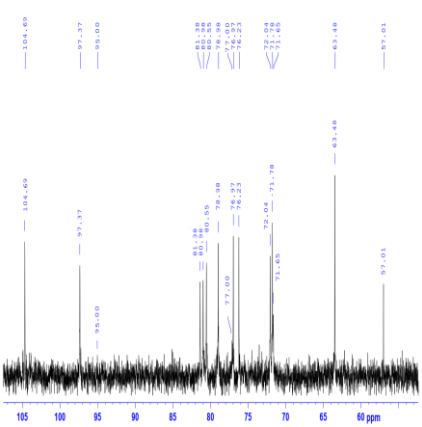

Figure 3.8. **^{13}C NMR** spectra of carrageenan from *K. striatus*.

Based on the results of FT-IR and NMR spectral analyses, the carrageenan extracted from *Kappaphycus striatus* cultivated in Van Phong Bay was identified to consist primarily of kappa-carrageenan**,** with a small amount of iota-carrageenan**.**

3.2. Results of composition, properties, and structural characterization of lectin and carrageenan from the red alga *Betaphycus gelatinus*

3.2.1. Composition and properties of lectin from *Betaphycus gelatinus*

The protein content and hemagglutination activity of lectin extracted from the red alga *Betaphycus gelatinus* are presented in table 3.8.

Table 3.8. Protein content and hemagglutination activity of lectin extracted from *Betaphycus gelatinus*

Lectin from *B. gelatinus*	**Crude**	**After precipitation**	**After GFC**
Volume (mL)	1650	307.5	1320
Protein (mg/mL)	1.31	1.26	0.034
Protein tổng (mg)	2161.50	386.86	44.88
HA (HU/mL)	64	256	8
HTTS (HU)	105600	78720	10560
HTR (HU/mL)	48.85	203.57	235.29
LHC(µg/mL)	8.44	4.91	4.10

HA: Activity per 1 mL of lectin extract, HTTS (*Total Specific Activity*): *Total hemagglutination activity, HTS* (*Specific Activity*): Specific *hemagglutination activity,* LHC (*Lowest Hemagglutination Concentration*): *The minimum concentration required to induce hemagglutination, GFC*: *Gel Filtration Chromatography*

Carbohydrate-binding specificity of lectin from the red alga *Betaphycus gelatinus:* The hemagglutination activity of lectin from *B. gelatinus* was not inhibited by monosaccharides or glycoproteins such as transferrin, asialo-transferrin, and fetuin. Similarly, the activity was not affected by glycoproteins like transferrin and fetuin. Lectin from *B. gelatinus* demonstrated specific binding to high-mannose type N-glycans, a property

that is highly relevant for potential medical applications, such as antiviral and anticancer activities.

3.2.2. Chemical composition, properties, and structural characteristics of carrageenan from *Betaphycus gelatinus*

The compositional analysis revealed data on carrageenan content, 3,6-anhydro-D-galactose content, and sulfate content in carrageenan extracted from *B. gelatinus* at different harvest times. A slight variation was observed across the months in terms of carrageenan content, 3,6-anhydro-D-galactose levels, and sulfate concentration.

Table 3.10. Chemical composition of the monthly native and alkali modified carrageenans from the red alga *B. gelatinus*.

Month	Native carrageenans			Alkali modified carregeenans		
	Yield [a]	DA [b]	Sufate [b]	Yield [a]	DA [b]	Sufate [b]
April	68.2±2.1	23.1±0.5	17.9±1.7	24.2±1.7	42.3±0.6	8.7±0.4
May	73.1±2.3	24.8±0.6	20.3±0.6	31.5±1.8	43.9±0.7	8.6±0.5
June	68.4±2.2	23.4±0.4	20.0±0.7	23.7±1.6	41.6±0.6	8.8±0.5

[a] Calculated on % of dry weight of alga, [b] Calculated on % of the carrageenan, DA, 4-linked 3,6-anhydro-α-D-galactose.

FT-IR and NMR analyses indicated that the polysaccharide extracted from *Betaphycus gelatinus* is a hybrid carrageenan, consisting of beta-carrageenan and kappa-carrageenan, with a relatively low kappa-carrageenan content, making it difficult to separate the two components. Due to the heterogeneous composition of carrageenan from *B. gelatinus*, the carboxymethyl-carrageenan derivative in this study was synthesized from kappa-carrageenan extracted from the red alga *Kappaphycus striatus*.

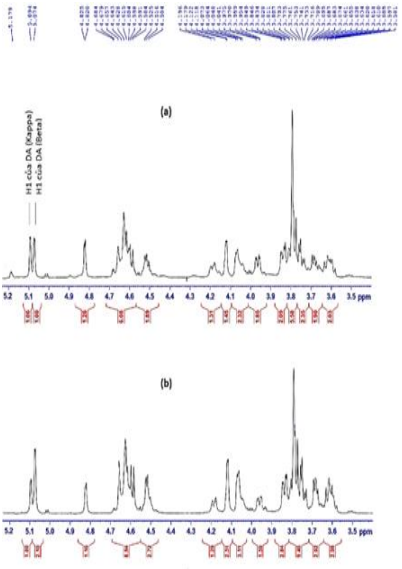

Figure 3.10. ^{1}H NMR spectra of (a) Native and (b) alkali-modified carrageenan extracted from *B. gelatinus*.

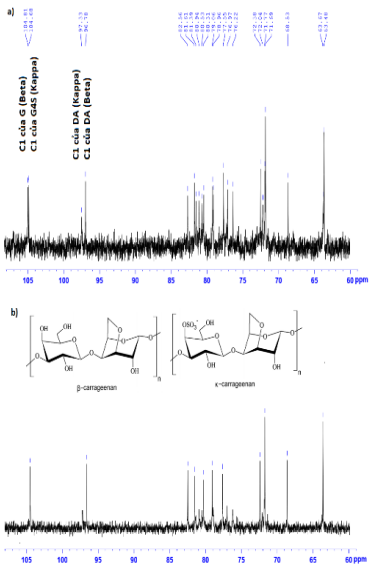

Figure 3.11. ^{13}**C** NMR spectra of (a)Native and (b) alkali-modified carrageenan extracted from *B. gelatinus*.

3.3. Results of characterization and structural analysis of CMKC and carrageenan–lectin microcapsules

3.3.1. Chemical composition and Properties of CMKC

Table 3.12. Chemical composition of CMKC

Sample	DA (% w/w)	Sulfate (%w/w)	MW (kDa)
KC-0/1	28.5 ± 0.8	23.9 ± 0.7	747.4 ± 8.3
CMKC-1/1	27.5 ± 0.6	23.8 ± 0.7	756.1 ± 7.5
CMKC-2/1	26.2 ± 0.6	23.7 ± 0.6	772.8 ± 7.3
CMKC-3/1	23.6 ± 0.5	23.4 ± 0.5	779.6 ± 9.9
CMKC-4/1	19.5 ± 0.4	22.5 ± 0.5	775.0 ± 6.2
CMKC-5/1	18.4 ± 0.4	22.3 ± 0,.5	774.3 ± 6.3

DA: 3,6-anhydro-D-galactose, MW: Average Molecular Weight GPC

3.3.2. Structural characteristics of carboxymethyl-kappa-carrageenan from *k. striatus*

The spectral analysis results of carboxymethyl-kappa-carrageenan samples, in comparison with the original kappa-carrageenan, are presented in Figure 3.12. The appearance of three new absorption bands confirms that the carboxymethylation process of kappa-carrageenan was successful, resulting in the formation of carboxymethyl-kappa-carrageenan.

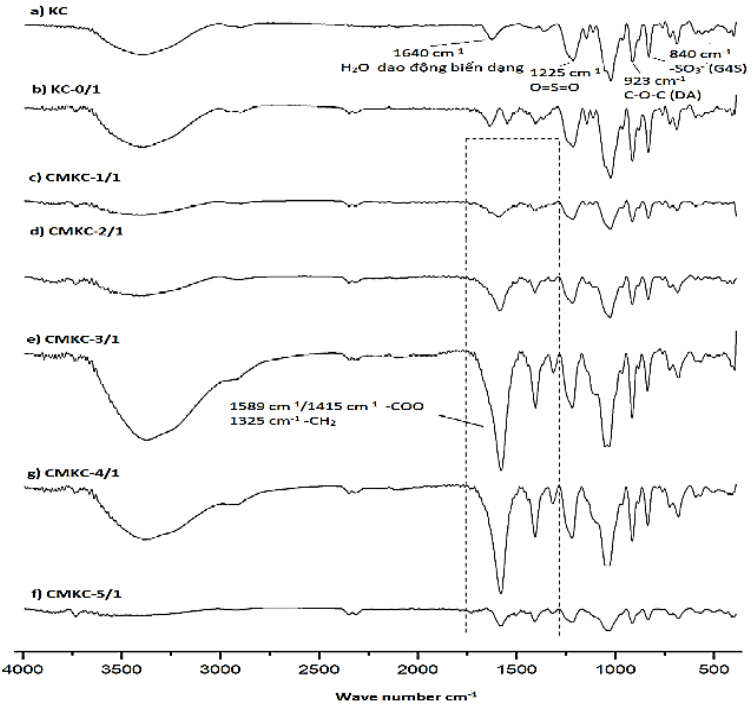

Figure 3.12. FT-IR spectra of carboxymethyl-kappa-carrageenans

The degree of substitution (DS) values were analyzed on a relative basis, with slight discrepancies observed between the two measurement methods. The DS values, calculated from the ^{1}H NMR spectra, ranged from 0.24 to 1.12. The highest substitution level was obtained at a molar ratio of MCA/KC = 3:1. At higher molar ratios, the DS decreased. This reduction is

attributed to side reactions between sodium monochloroacetate (MCA) and NaOH, leading to the formation of sodium glycolate. As the MCA/KC molar ratio increases, the MCA concentration increases, which in turn decreases the amount of NaOH available to react with KC to form alkoxide (due to a fixed volume of NaOH), resulting in a lower DS.

Among the ^1H NMR spectra of the CMKC samples, the spectrum of CMKC-3/1 was selected to represent the appearance of new proton signals (Figure 3.13e), owing to its highest degree of substitution. A comparison of the chemical shift values from KC in Table 3.6, KC-0/1 in Table 3.13, and CMKC-3/1 in Table 3.13 revealed the emergence of new proton peaks that were absent in the KC-0/1 spectrum, indicating successful carboxymethylation of the hydroxyl groups in KC-0/1.

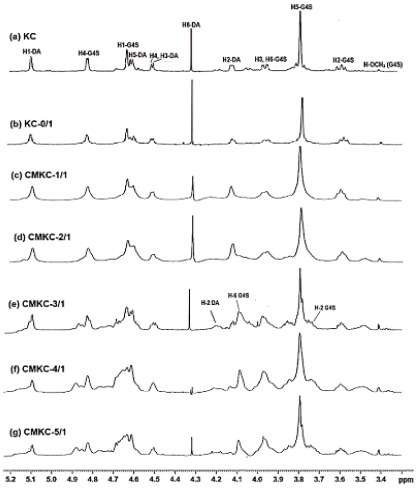

Figure 3.13. [1]H NMR spectra of kappa-carrageenan and CMKCs.

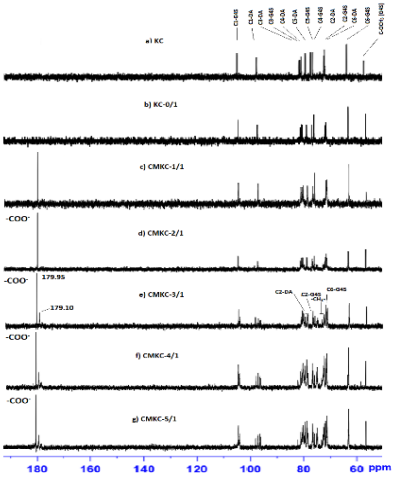

Figure 3.13. [13]C NMR spectra of kappa-carrageenan and CMKCs.

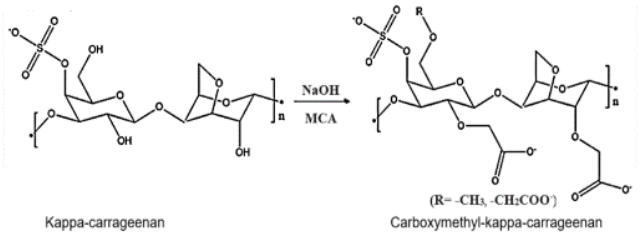

Figure 3.17. Synthesis of carboxymethyl-kappa-carrageenan.

The analysis of the ^{13}C NMR spectra in Figure 3.15 reveals a comparison of the chemical shifts among the KC, KC-0/1 (Figure 3.15a, b), and CMKC samples (Figure 3.15c - g). Prominent signals observed at δ = 179.95 and 179.10 ppm correspond to the carbon atoms of carboxylate groups (COO^-), indicating different substitution positions of the carboxymethyl groups on KC-0/1. Signals within the range of 177.7 - 182.6 ppm have also been reported for carboxymethyl-substituted sites in kappa-carrageenan. Based on the peak intensity ratios in the ^{13}C NMR spectra and DS values obtained from ^1H NMR, it is evident that the hydroxyl groups in KC-0/1 were successfully carboxymethylated. The reactivity order of hydroxyl groups was determined as O-C2G4S > O-C6G4S > O-C2DA.

3.3.3. Determination of the swelling behavior of carrageenan and carboxymethyl-kappa-carrageenan microspheres

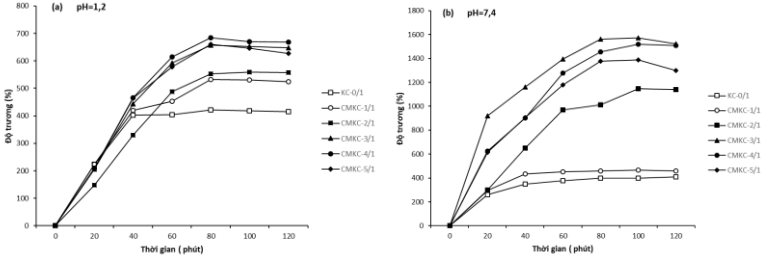

Figure 3.18. Swelling behavior of KC-0/1 and carboxymethyl-kappa-carrageenan (CMKC-1/1 to CMKC-5/1) microspheres in different media: (a) pH = 1.2, (b) pH = 7.4.

The carboxymethyl-kappa-carrageenan (CMKC) microspheres (CMKC-1/1 to CMKC-5/1) exhibited lower swelling degrees in an acidic medium at pH = 1.2 (ranging from 1.47 to 6.84) and significantly higher swelling degrees in a neutral/basic medium at pH = 7.4 (ranging from 2.97 to 15.74). The variation in the swelling behavior of CMKC microspheres under different pH conditions is attributed to the presence of carboxymethyl groups in the kappa-carrageenan structure.

3.3.4. Structural characteristics of carageenan–lectin microspheres

Microspheres formed from kappa-carrageenan encapsulating lectin (denoted as KC-L) and from carboxymethyl-kappa-carrageenan at a molar ratio of 3:1 encapsulating lectin (CMKC-3/1-L) were spherical in shape, transparent, and exhibited a pink color due to the lectin solution. After drying, the microspheres showed a porous structure, and their color intensity varied depending on the ratio between polysaccharide and lectin. The supernatant obtained after microsphere formation was colorless and transparent, indicating high encapsulation efficiency. The encapsulation efficiency of CMKC-3/1 for lectin was approximately 90%. After drying, the microspheres became less spherical and exhibited a more porous morphology.

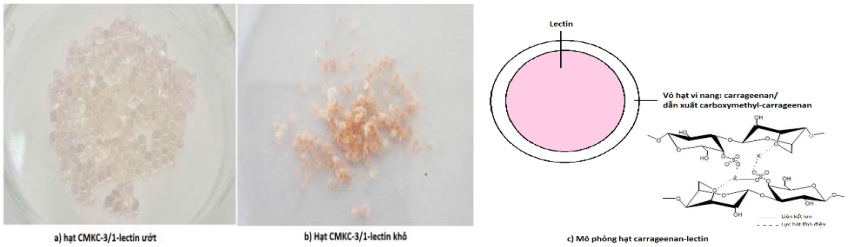

Figure 3.19. CMKC-3/1-L microspheres in different forms: a) hydrated (wet) microspheres, b) dried microspheres, c) schematic representation of carrageenan–lectin microspheres. The concentration of CMKC-3/1 was 3.0%.

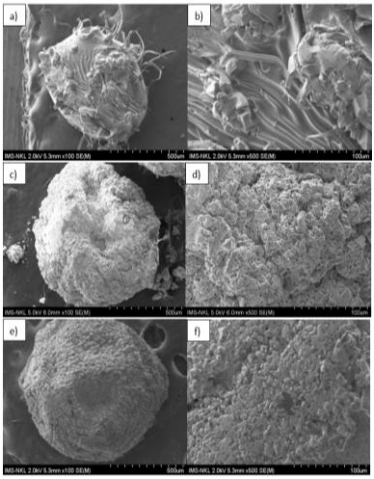

Figure 3.20. SEM images of microspheres: (a) KC-L microspheres, (b) surface morphology of KC-L microspheres, (c) CMKC-3/1 microspheres without lectin, (d) surface morphology of CMKC-3/1 microspheres without lectin, (e) CMKC-3/1-L microspheres, (f) surface morphology of CMKC-3/1-L microspheres.

3.4. Results of biological activity evaluation of carrageenan, CMKC, and carrageenan-lectin microcapsules

3.4.1. Antioxidant activity of carrageenan and CMKC

Carrageenans have been demonstrated to be potential antioxidants capable of preventing oxidative damage in living organisms. Most carboxymethylated polysaccharides exhibit improved antioxidant activity due to their ability to directly scavenge free radicals.

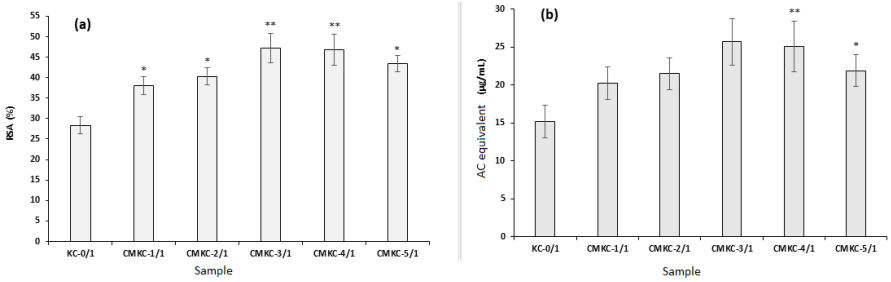

Figure 3.21. Antioxidant activity of KC-0/1 and carboxymethyl-kappa-carrageenans (CMKC-1/1 to CMKC-5/1) at a concentration of 500 µg/mL. (a) Free radical scavenging activity – RSA (%) of DPPH, and (b) Ascorbic acid equivalent activity (AC). Values are expressed as mean ± SD (n = 3). Differences are indicated by asterisks. *P < 0.05 and P < 0.01 for CMKC compared with KC-0/1

3.4.2. Hemagglutination activity

Hemagglutination activity of carboxymethyl-kappa-carrageenan on human blood groups A, B, AB, and O: The hemagglutination activity of CMKC is dependent on the degree of substitution (DS) of carboxymethyl groups on the kappa-carrageenan backbone.

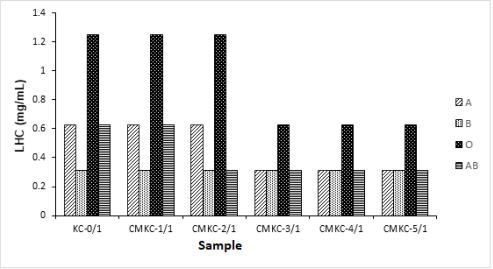

Figure 3.23. Lowest hemagglutination concentration (LHC, mg/mL) of KC-0/1 and CMKC (CMKC-1/1 to CMKC-5/1) on human blood groups. The initial concentration of each KC-0/1 or CMKC sample was 10 mg/mL, using a twofold serial dilution method.

Hemagglutination activity of CMKC on trypsin-treated rabbit erythrocytes: Analysis shows that CMKC's hemagglutination activity on trypsinized rabbit erythrocytes also depends on the degree of carboxymethyl substitution. Derivatives with higher carboxymethyl group content tend to exhibit increased erythrocyte adhesion and aggregation.

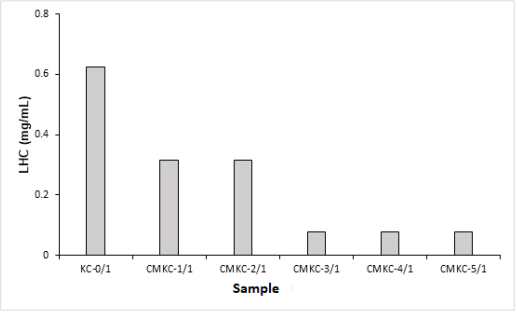

Figure 3.24. Lowest hemagglutination concentration (LHC, mg/mL) of KC-0/1 and carboxymethyl-kappa-carrageenan (CMKC-1/1 to CMKC-5/1) on rabbit erythrocytes.

Hemagglutination activity of CMKC and lectin solutions:

Interaction between KC and CMKC with Lectin: An experiment was conducted to analyze the carbohydrate-binding specificity of lectin with various sugars and glycoproteins, following a similar protocol. The results indicated that there were no specific interactions between KC, CMKC and the lectin.

Hemagglutination activity of CMKC-Lectin mixtures: The hemagglutination activity of CMKC-3/1 and lectin mixtures increased as the CMKC concentration rose from 2.0% to 3.0%. Further increase to 4.0% resulted in no additional enhancement of activity.

3.5. Evaluation of lectin release from polysaccharide–lectin microcapsules

Lectin release was monitored over time from the microcapsules in simulated gastric fluid (pH = 1.2) and simulated intestinal fluid (pH = 7.4,). Initially, the soaking solution was colorless and gradually turned pink, indicating lectin diffusion. KC-based microspheres released lectin more rapidly in both environments compared to CMKC-based microspheres.

This slower release helps protect lectin from degradation in acidic conditions and prolongs its retention in alkaline environments. Increasing CMKC-3/1 concentration resulted in a denser microsphere surface, requiring more time for CMKC-3/1 to diffuse in acidic medium and swell in alkaline conditions, thus slowing lectin release.

CONLUSION

The dissertation successfully achieved the proposed objectives and yielded the following main findings:

1. This is the first scientific study in Vietnam to extract and integrate lectin and carrageenan from two red algae species: *Kappaphycus striatus*, cultivated in Van Phong Bay (Khanh Hoa Province), and *Betaphycus gelatinus*, naturally harvested from the coastal waters of Ninh Thuan Province (currently part of Khanh Hoa). The structural properties and biological activities of lectins and carrageenans extracted from these red algae were thoroughly investigated:

- The carrageenan composition of *Kappaphycus striatus* consists mainly of kappa-carrageenan, with a minor fraction of iota-carrageenan; while the carrageenan from *Betaphycus gelatinus* is a hybrid carrageenan, comprising 68.6% beta-carrageenan and 31.4% kappa-carrageenan.
- Lectins extracted from *Kappaphycus striatus* and *Betaphycus gelatinus* were structurally characterized and exhibited hemagglutination activity on rabbit erythrocytes.

2. For the first time, carboxymethyl-kappa-carrageenan (CMKC) was successfully synthesized from kappa-carrageenan derived from *Kappaphycus striatus* cultivated in Van Phong Bay, Khanh Hoa Province, Vietnam. The structural characterization revealed that both kappa-carrageenan and its CMKC derivative possess hemagglutination and antioxidant activities. The CMKC derivatives exhibited pH-responsive swelling behavior. Among the samples, CMKC-3/1 showed the highest degree of substitution (DS = 1.12) and superior biological activity compared to other derivatives. CMKC-3/1-based microparticles demonstrated low swelling at pH 1.2 and significantly higher swelling at pH 7.4, indicating potential for targeted drug delivery.

3. Microparticles were successfully formulated from kappa-carrageenan and CMKC-3/1, encapsulating lectin extracted from *Kappaphycus striatus.* A 3% concentration of CMKC-3/1 was found optimal for achieving high encapsulation efficiency. The encapsulated lectin retained its hemagglutination activity during the microparticle formation process. Preliminary in vitro release studies in different pH environments (1.2 and 7.4) indicated that lectin encapsulated in CMKC-derived microparticles exhibited a slower release profile over time compared to those made from unmodified kappa-carrageenan.

RECOMMEDATIONS

1. Continue to evaluate the lectin release kinetics of microcapsules in varying pH environments.
2. Determine the the hemagglutination activities of lectin released from microcapsules in varying pH environments.
3. Study on creating microcapsules from carboxymethyl-kappa-carrageenan encapsulating other bioactive substances such as curcumin from turmeric, beta-carotene from Gac fruit, with the aim of increasing solubility when used through the digestive tract.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. Le Dinh Hung, **Hoang Thi Trang Nguyen**, and Vo Thi Dieu Trang, 2021, Kappa carrageenan from the red alga *Kappaphycus striatus* cultivated at Vanphong Bay, Vietnam: physicochemical properties and structure, *Journal of applied phycology*, 33, 1819–1824.

2. Le Dinh Hung**, Hoang Thi Trang Nguyen**, Vo Thi Dieu Trang, Le Trong Nghia, Dinh Thanh Trung, Thanh Thi Thu Thuy, 2024, Hybrid beta/kappa/gamma-carrageenan from the red alga *Betaphycus gelatinus* in Vietnam, *Journal of applied phycology*, 36, 3689–3695.

3. **Hoang Thi Trang Nguyen**, Dinh Thanh Trung, Vo Thi Dieu Trang, Pham Duc Thinh, Thanh Thi Thu Thuy, Le Dinh Hung, 2025, Carboxymethyl-kappa-carrageenan derivatives synthesized from kappa-carrageenan of the red alga *Kappaphycus striatus* cultivated in Vietnam: Characterization, structure and biological activity, *J*ournal of Applied Phycolog**,** 37, 527–537.

4. **Hoàng Thị Trang Nguyên**, Lê Đình Hùng, Thành Thị Thu Thủy, 09/2024, Nghiên cứu carboxymethyl-kappa-carrageenan bao gói lectin từ rong đỏ *Kappaphycus striatus*, *Tạp chí hóa học và ứng dụng*, 3B (71), 281-287

5. **Hoàng Thị Trang Nguyên**, Lê Đình Hùng, 2022, Kappa carrageenan từ rong đỏ *Kappaphycus striatus*, *Hội nghị quốc tế "Biển Đông 2022",* 508-518. (ISBN: 978-604-357-067-0)

6. Le Dinh Hung, **Hoang Thi Trang Nguyen**, 2022, The red algae, carrageenophytes: source of potential lectins for application, *Hội nghị quốc tế "Biển Đông 2022"*, 444-455. (ISBN: 978-604-357-067-0)