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Emulsifying potential of *Hypnea musciformis* carrageenan: A natural alternative for oil-in-water emulsions

Potencial emulsionante de carragenina de *Hypnea musciformis*: una alternativa natural para la formulación de emulsiones aceite en agua.

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ABSTRACT

Background: Polysaccharides such as carrageenans, extracted from red algae, are widely used in food and pharmaceutical industries due to their gelling, stabilizing, and emulsifying properties. However, the potential of carrageenan extracted from *Hypnea musciformis*, a red macroalga native to tropical coastal regions, remains underexplored, particularly in terms of its emulsifying properties. **Objective:** To evaluate the emulsifying potential of carrageenan extracted from *H. musciformis* by comparing the physicochemical stability of emulsions formulated with this biopolymer to those stabilized with sodium alginate (ALG), a widely used reference polysaccharide. **Methods:** Oil-in-water emulsions containing 20% sesame oil were elaborated using varying concentrations of carrageenan or ALG. Their physical stability was assessed through droplet size distribution, polydispersity index (PDI), zeta potential, surface tension, and visual inspection over 31 days of storage at room temperature. **Results:** Emulsions stabilized with carrageenan exhibited comparable droplet size, PDI, and surface tension values to those formulated with ALG, indicating similar emulsifying capacity. Notably, formulations containing 1% carrageenan demonstrated enhanced long-term physical stability. Zeta potential values remained consistently negative (−35 mV to −45 mV), suggesting electrostatic stabilization. **Conclusion:** Carrageenan extracted from *Hypnea musciformis* demonstrated effective emulsifying properties in 20% sesame oil emulsions, comparable to those of ALG. These findings support its potential as a natural emulsifier for food and cosmetic formulations, highlighting the relevance of exploring underutilized marine resources for biotechnological applications.

Keywords: *Hypnea musciformis* carrageenan, Marine-derived emulsifiers, Carrageenan-based emulsifier, polysaccharides.

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RESUMEN

Antecedentes: Los polisacáridos como la carragenina, extraídos de algas rojas, se utilizan ampliamente en las industrias alimentaria y farmacéutica por sus propiedades gelificantes, estabilizantes y emulsionantes. Sin embargo, el potencial de la carragenina extraída de *Hypnea musciformis*, una macroalga roja nativa de regiones costeras tropicales, permanece poco explorado, especialmente en lo que respecta a su comportamiento emulsionante. **Objetivo:** Evaluar el potencial emulsionante la carragenina extraída de *H. musciformis* mediante la comparación de la estabilidad fisicoquímica de emulsiones formuladas con este biopolímero frente a aquellas estabilizadas con alginato de sodio (ALG), un polisacárido de referencia ampliamente utilizado. **Métodos:** Se elaboraron emulsiones aceite-en-agua con 20% de aceite de sésamo utilizando diferentes concentraciones de carragenano o ALG. La estabilidad física se evaluó mediante el análisis del tamaño de gota, índice de polidispersidad (PDI), potencial zeta, tensión superficial e inspección visual durante 31 días de almacenamiento a temperatura ambiente. **Resultados:** Las emulsiones estabilizadas con carragenina mostraron valores comparables de tamaño de gota, PDI y tensión superficial respecto a las formuladas con ALG, lo que indica una capacidad emulsionante similar. En particular, las formulaciones con 1% de carragenina demostraron una mayor estabilidad física a largo plazo. Los valores de potencial zeta se mantuvieron consistentemente negativos (−35 mV a −45 mV), lo que sugiere una estabilización electrostática. **Conclusión**: La carragenina extraída de *Hypnea musciformis* mostró propiedades emulsionantes efectivas en emulsiones con 20% de aceite de sésamo, comparables a las del ALG. Estos hallazgos respaldan su potencial como emulsionante natural para formulaciones alimentarias y cosméticas, y resaltan la importancia de explorar recursos marinos poco aprovechados con aplicaciones biotecnológicas.

Palabras clave: Carragenina de *Hypnea musciformis*, Emulsificantes de origen marino, Emulsificantes a base de carragenina, Polisacáridos

1. INTRODUCTION

Hydrocolloids are biopolymers of natural origin that have gained increasing attention in recent years due to their multifunctionality and applicability across the food, pharmaceutical, and cosmetic industries. They are particularly valued for their biodegradability, renewable sourcing, and functional versatility, which includes their use as thickeners, stabilizers, gelling agents, and emulsifiers (1,2). Emulsification, the process of stabilizing immiscible liquids such as oil and water, is crucial in the development of various products, including creams, beverages, drug delivery systems, and functional foods. Traditional emulsifiers often rely on synthetic surfactants; however, the demand for natural, sustainable, and consumer-safe alternatives has led to a growing interest in hydrocolloids as structuring and stabilizing agents in emulsified systems (3).

Hydrocolloids such as carrageenan, alginate, pectin, and gum arabic function as emulsifiers through several mechanisms. These include increasing the viscosity of the continuous phase, forming a viscoelastic interfacial film around oil droplets, and imparting steric and electrostatic stabilization (3). Unlike small-molecule surfactants, hydrocolloids do not typically lower interfacial tension directly. Still, they provide physical barriers to coalescence and creaming by immobilizing droplets within a gel-like matrix or by adsorbing to interfaces in the case of amphiphilic structures. This stabilizing ability makes them particularly suited for formulations that require long-term stability and consistent rheological properties (4).

Marine algae are a major source of hydrocolloids, with polysaccharides comprising up to 70% of their dry biomass in some species (5). Red algae (Rhodophyta) in particular are recognized for producing galactans such as agar, agarose, and carrageenan (6). Carrageenans are linear sulfated polysaccharides composed of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose units, often containing a 3,6-anhydro bridge, and are classified as κ-, ι-, or λ-types depending on their sulfation pattern and gelling properties (7). Among these, κ-carrageenan is widely valued for its gel-forming capacity and has been extensively applied in food texturization, drug delivery, and biotechnological formulations (8).

Hypnea musciformis, a red alga of the order Gigartinales, is known for its high yield of κ-carrageenan, fast growth rate, and adaptability to environmental conditions. Several studies have reported the successful extraction of high-purity κ-carrageenan from *H. musciformis*, especially using hot-water extraction followed by potassium chloride precipitation (9). The extracted polysaccharides exhibit a predominantly κ-carrageenan structure with minimal contamination by other diads or galactan types. Moreover, optimized extraction conditions have been shown to yield sulfated galactan-rich fractions with bioactivities, including antioxidant, anticoagulant, immunostimulatory, and cytotoxic properties (10).

The physicochemical and functional properties of κ-carrageenan from *H. musciformis* make it a potential emulsifying agent in formulations requiring high stability and viscosity modulation (11). Although carrageenan has been extensively characterized for its gelling and thickening capacity, its emulsifying potential, particularly from specific algal sources such as *H. musciformis*, remains underexplored. Given the growing demand for natural, biodegradable emulsifiers, investigating the emulsifying behavior of κ-carrageenan from this red algae may offer novel insights and applications.

Therefore, the objective of this study is to evaluate the emulsifying properties of κ-carrageenan extracted from *H. musciformis*, to explore its potential applications in the pharmaceutical, cosmetic, and food industries. By characterizing its physicochemical behavior and assessing its performance in emulsion systems, this work aims to bridge existing knowledge gaps and contribute to the sustainable development of algae-derived functional ingredients.

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

Carrageenan was extracted from *H. musciformis* following the method described in Colombian Patent No. 08043691. Sodium alginate (ALG, medium viscosity, Cat. No. A2033-1KG, Lot SLBV4496) was purchased from Sigma-Aldrich® (St. Louis, MO, USA). Sesame oil used as the oil phase was of analytical grade and obtained from industrial suppliers. Ethanol (95%) and hydrogen peroxide (5%), used in the extraction and purification processes, were of analytical grade and purchased from Merck® (Darmstadt, Germany). Deionized water was used in all preparations. Instrumentation included the Ultra-Turrax homogenizer (IKA T-18 basic®, Staufen, Germany) for emulsion preparation, the Mastersizer 3000E (Malvern Panalytical®, Malvern, UK) for droplet size and polydispersity index measurements, and the Ramé-hart goniometer (Model 290-U4, Succasunna, NJ, USA) for surface tension analysis. Zeta potential was determined using the Zetasizer Nano ZS (Malvern Panalytical®, UK). Emulsifying capacity was assessed using a Hermle Z206A centrifuge (Wehingen, Germany). Rheological behavior was analyzed with a Discovery HR-2 rotational rheometer (TA Instruments®, New Castle, DE, USA). Droplet morphology was observed under an optical microscope (Motix®, China).

2.2 Extraction of the carrageenan (CAG)

The young growing apical fragments of *H. musciformis* were collected along the coastal area near Simón Bolívar Airport in Santa Marta, Colombia (11° 7′ 4″ N - 74° 14′ 57″ W), during the minor dry season. The sampling site corresponds to a rocky shore, characterized by generally moderate to high wave energy. The collection was carried out at low tide, ensuring a smooth and clean cut at the base of the thallus using a sharp stainless-steel knife. Epiphytic and associated organisms were carefully removed as thoroughly as possible.

To avoid direct sun exposure, the thalli were immediately placed in plastic containers with seawater and transported in a polypropylene cooler to the Marine Aquaculture Laboratory of the Universidad de Bogotá Jorge Tadeo Lozano, Santa Marta campus. During the sampling process, in situ physicochemical parameters were recorded, including salinity, surface water temperature, pH, nitrite, nitrate, and phosphate concentrations using colorimetric test kits, as well as light intensity using a lux meter.

The samples were subsequently acclimated for 10 days in transparent plastic aquaria filled with sterilized seawater under controlled conditions: a constant temperature of 24°C, salinity of approximately 37 ppm, pH of 8.0, continuous aeration, and a 12:12 h light-dark photoperiod.

The cultivation experiment was conducted at the Mundo Marino facilities of the Universidad Jorge Tadeo Lozano under the supervision of thesis student Dayan Vargas. Polypropylene ropes were used as support structures, and coral skeletons served as the growth substrate. The cultivation system was divided into two areas: a sterilization zone and a cultivation zone. In the sterilization area, natural seawater pumped from the facility was filtered and sterilized. In the cultivation area, skin-colored troughs with a capacity of 200 L each were used.

Each trough contained eight horizontally arranged polypropylene rope lines, on which plastic baskets were suspended 36 cm below the water surface. Coral skeletons were placed inside the baskets as substrate, and a total of 524.86 g of algal biomass was seeded, with an average of 5.467 ± 0.639 g per basket.

During the cultivation period, physicochemical parameters—salinity, temperature, pH, and light intensity—were maintained constant, replicating the acclimation conditions. The photoperiod consisted of 12 h of light and 12 h of dark, using T5 18W white LED lamps (120 cm, Mercury brand) combined with

blue light. The estimated photosynthetically active radiation (PAR) was 106 µmol·m^{-2}·s^{-1}. Sodium nitrate ($NaNO_3$) was added at a concentration of 15 mM. After 15 days, the algae were harvested, weighed, and a subsample was used for κ-carrageenan extraction.

CAG was extracted according to the Colombian patent 08043691(12). Briefly, *H. musciformis*, obtained from a semi-closed cultivation carried out in the city of Santa Marta in 2023, was used. Ten grams of the sample were cleaned, dried, and ground. Then, the sample was treated with 5% hydrogen peroxide to remove the pigments, washed with distilled water, and immersed in an alkaline buffer solution at pH 8.2. The mixture was heated to 80°C for a few minutes, and the resulting suspension was cooled to 40°C and neutralized. Once the sample was gelled, 95% ethanol was added to purify the polysaccharide, which was then filtered and dried at 30°C for 12 hours in a stream of hot air. The sample was brought to constant weight and stored in a desiccator for later use.

All the activities described are covered by the collection and research permit issued by the Ministry of Environment and Sustainable Development, under the framework of the Contract for Access to Genetic Resources No. 108, Addendum No. 7 to the framework contract for access to genetic resources and their derivatives No. 121, dated January 22, 2016.

2.3 Preparation of carrageenan and alginate solutions:

CAG and ALG solutions were prepared by dissolving 0.25%, 0.5%, 1% and 1.5 % w/v of the extracts in room temperature (20°C), in distilled water, using the *Ultra Turrax IKA T-18 basic* ® at speed 4 (15.500 rpm) for 5 minutes each dispersion, then they were stored at room temperature (20°C) for 24 hours for complete hydration.

2.4 Preparation of the emulsions

Oil-in-water (O/W) emulsions were prepared using 20% (v/v) sesame oil and 80% (v/v) of the previously prepared aqueous polymer solutions. Emulsification was carried out using an Ultra-Turrax IKA T-18 basic® homogenizer operating at 15,500 rpm for 5 minutes. Each emulsion had a final volume of 25 mL and was stored in 50 mL Falcon tubes, as illustrated in Figure 1. The concentrations of carrageenan (CAG) and alginate (ALG) in the final emulsions were 0.25%, 0.5%, 1%, and 1.5% (w/v), respectively.

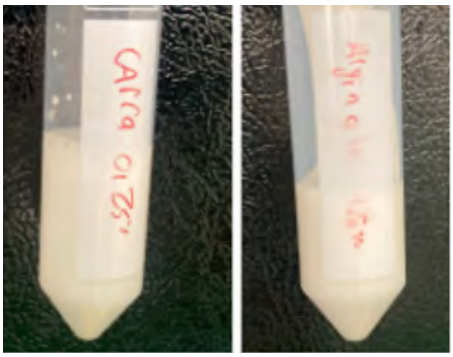

Figure 1. Emulsions O/W obtained Left: CAG emulsion at 0.25%. Right: ALG emulsion at 0.25%

2.5 Stability during storage and droplet size evaluation

The physical stability of the emulsions was assessed under accelerated storage conditions to determine potential changes over time. Stability testing was conducted at room temperature (20 °C) for a total period of 31 days. The emulsions were stored in sealed containers protected from light, and their physical integrity was monitored at predetermined intervals (days 1, 7, 14, and 31). The evaluation focused on visual inspection for signs of phase separation, creaming, or color change, which were used as indicators of physical instability (13).

Additionally, droplet size distribution and polydispersity index (PDI) were measured using a Mastersizer 3000E (Malvern Instruments, UK). For each time point, 3–5 mL of the emulsion was analyzed at 25 °C. The instrument automatically calculated the volume-weighted mean diameter (D[4,3] and PDI based on five consecutive readings per sample, using water as the dispersant and applying the Mie scattering model. These measurements allowed the evaluation of colloidal stability and droplet growth, which are essential parameters for determining the long-term performance and uniformity of emulsified systems (13).

2.6 Surface tension

The surface tension of CAG and ALG dispersions was measured using the automated goniometer ramé-hart instrument co. ® Model 290-U4 serial No. 200205, 0.5 mL of each emulsion was analyzed using the pendant drop method. A small droplet of the emulsion was suspended in air using a microsyringe, and software controlled the optical system to ensure accurate measurement (14). To validate the results obtained, the system was calibrated, measuring the tension of water/air, showing a tension of 72.8 mN at 20°C.

2.7 Zeta-potential

Zeta-potential was measured using the *Zetasizer Nano ZS Malvern®*. Each emulsion was diluted 1:100 in deionized water to avoid additional charges in the readings and was calculated automatically by the instrument, using 3 readings per sample, in triplicate (15). To validate the results, the zeta potential of deionized water was measured, resulting in a zeta potential of 0 mV.

2.8 Emulsifying capacity

10 mL of each sample, in duplicate, was centrifuged at 3500 rpm for 10 min in a Hermle® centrifuge Model Z206A, then the emulsifying capacity was calculated according to the equation 1 proposed by Ebrahimi, B. (13).

$$EC = \frac{fev}{It} * 100 \qquad \text{Equation 1}$$

Where f_{ev} was the final emulsion volume and It was the total volume.

2.9 Optical microscopy analysis

The structure of the emulsions was visualized and photographed using an optical microscope. Each emulsion was agitated in vortex IKA Vortex 3® at speed 4, then poured onto a microscope slide and covered by a cover slip.

2.10 Rheological behavior

The viscosity of each dispersion of ALG and CAG was measured by rotational rheometry Discovery HR-2® Serial Number 5332-2314 by TA Instruments at 25°C, the same method was used for the measurement of the viscosity of each emulsion of ALG and CAG using a flow-ramp system that was done at a shear rate of 0.1 - 300 s^{-1} at 25°C for 300 seconds (16).

2.11 Statistics

All measurements were performed in triplicate for each sample, and the data are presented as the mean ± SD. Data were examined by two-way analysis of variance, followed by the Bonferroni test with a significance level of $p < 0.05$, to confirm the difference in the mean value between samples using GraphPad Prism® software (version 6, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

3.1 Physical Characterization of Emulsions Stabilized with CAG and ALG

The physical characteristics of the emulsions formulated with carrageenan extracted from *H. musciformis* (CAG) and commercial alginate (ALG) are presented in Table 1. An inverse relationship was observed between polymer concentration and droplet size for both polysaccharides, indicating that increased concentration improves the stabilization of the emulsion. At 1.5% concentration, CAG-based emulsions exhibited the smallest initial droplet size (12.5 ± 0.2 µm on Day 1), which remained significantly smaller than those at lower concentrations throughout the storage period. A similar trend was observed for ALG emulsions, suggesting that both hydrocolloids effectively reduce droplet coalescence by enhancing the viscosity of the continuous phase and forming a steric barrier at the oil-water interface.

Table 1. Droplet Size and Polydispersity Index of CAG and ALG emulsions

		Droplet Size (µM)				PDI			
Concentration		0.25%	0.5%	1%	1.5%	0.25%	0.5%	1%	1.5%
CAG	Day 1	48.1 ± 1.2 ^{a,i}	20.0± 0.6 ^{b,i}	15.2 ± 0.1 ^{c,i}	12.5 ± 0.2 ^{c,i}	0.315± 0.02 ^{a,i}	0.332 ± 0.02^{a,i}	0.336 ± 0.02^{a,i}	0.282 ± 0.01^{b,i}
	Day 7	178± 2.4^{a,ii}	97.1± 0.6 ^{b,ii}	71.3 ± 0.2 ^{c,ii}	38.4 ± 0.2 ^{d,ii}	0.375 ± 0.02 ^{a,ii}	0.399 ± 0.02^{a,ii}	0.259 ± 0.01^{b,ii}	0.271 ± 0.01^{b,ii}
	Day 14	216± 2.5^{a,iii}	161± 1.0^{b,iii}	97.2 ± 0.5 ^{c,iii}	45.8 ± 0.2 ^{d,iii}	0.346 ± 0.02 ^{a,iii}	0.198 ± 0.01^{b,iii}	0.288 ± 0.01^{c,iii}	0.291 ± 0.01^{c,i}
	Day 31	239± 1.2^{a,iv}	172± 0.9^{b,iv}	173 ± 1.3^{b,iv}	82.2 ± 0.2^{c,iv}	0.368 ± 0.02 ^{a,ii}	0.322 ± 0.02^{b,iv}	0.218 ± 0.01^{c,iv}	0.219 ± 0.01^{c,iii}
ALG	Day 1	64.2± 0.8 ^{a,v}	33.5± 0.8 ^{b,v}	15.4± 0.1 ^{c,i}	12.3 ± 0.1 ^{c,i}	0.258 ± 0.02 ^{a,iv}	0.145± 0.01^{b,v}	0.207 ± 0.01^{c,v}	0.236 ± 0.01^{d,iv}
	Day 7	140± 1.2^{a,vi}	76.5 ± 0.1 ^{b,vi}	39.3± 0.2 ^{c,vi}	17.7 ± 0.2 ^{d,v}	0.250 ± 0.02^{a,iv}	0.123 ± 0.01^{b,vi}	0.265 ± 0.01^{c,vi}	0.245 ± 0.01^{a,v}
	Day 14	179± 0.9^{a,vii}	160 ± 1.0^{b,iii}	72.2 ± 0.2^{c,vii}	46.4 ± 0.3^{d,iii}	0.386 ± 0.02^{a,v}	0.193 ± 0.01^{b,ii}	0.347 ± 0.02^{c,vii}	0.264 ± 0.01^{d,vi}
	Day 31	242± 1.1^{a,i}	171 ± 1.2^{b,iv}	134.6 ± 0.4^{c,i}	85.2 ± 0.3^{d,iv}	0.381 ± 0.02^{a,v}	0.339 ± 0.02^{b,ii}	0.381 ± 0.02^{b,viii}	0.296 ± 0.01^{c,vii}

CAG: Carragenan from *H. musciformis*. ALG: Aginate from brown algae. Different letters (a-c) within one row show a significantly different ($p < 0.01$) between 4 time intervals. Different letters (i-viii) within one row show a significantly different ($p < 0.05$).

Over 31 days, all emulsions exhibited increases in droplet size, particularly at lower concentrations (0.25–0.5%), where size increments were more pronounced, indicating reduced stability. For instance, CAG emulsions at 0.25% exhibited an increase from 48.1 µm (Day 1) to 239 µm (Day 31). However, emulsions containing 1.0–1.5% ALG displayed slower rates of droplet growth and less variation in polydispersity index (PDI), reflecting superior physical stability compared to their CAG counterparts. This difference may be attributed to the higher molecular weight and more defined block structure of ALG, which favors the formation of stronger gel-like networks (17).

The PDI values (Table 1) further support these observations. Lower PDIs were associated with higher polysaccharide concentrations and later storage days, suggesting that emulsions became more homogeneous over time, particularly at concentrations ≥1%. Nonetheless, all systems remained within the limits indicative of polydisperse systems, likely due to sample handling before measurement. This variability may be minimized in future formulations by improving homogenization procedures and ensuring consistent sample loading (18).

The emulsification behavior observed aligns with previous studies that have highlighted the stabilizing potential of carrageenans in emulsions. Notably, previous works reported that emulsions stabilized with κ-, ι-, and λ-carrageenan exhibited progressive droplet aggregation during storage, with κ-carrageenan showing the highest resistance to destabilization (19). Similarly, in our study, CAG-based emulsions showed size increases over time, supporting the notion that carrageenans undergo time-dependent structural rearrangements that influence their emulsifying performance.

Interestingly, carrageenan extracted from *H. musciformis* has been previously characterized as predominantly κ-type, with some presence of hybrid structures containing ι-type disaccharide units. The major fraction precipitated with 0.125 M KCl was nearly pure κ-carrageenan, exhibiting strong gelling behavior and favorable rheological properties. However, minor fractions containing β-carrageenan diads or agarans may also be present, depending on extraction conditions and environmental factors affecting algal composition (9).

It is important to note the carrageenan used herein was extracted from *H. musciformis* following a patented method (20) , which employs hot aqueous extraction and ethanol precipitation to obtain a sulfated galactan-rich fraction predominantly composed of κ-type carrageenan. This process has been shown to selectively isolate the gelling polysaccharide fraction, minimizing the presence of non-gelling components such as agarans or carrageenan–agaran hybrids, which are unlikely to significantly influence the emulsion properties under the tested formulation conditions. No new compositional analysis was performed in this study, as its primary structural characteristics have already been studies in a prevoius work (21).

From a stability standpoint, the phase separation data further confirm the concentration-dependent stabilization effect. CAG emulsions at 0.25% and 0.5% separated within the first 7 days, while those at 1.5% remained stable for at least 30 days. ALG emulsions showed even greater resistance to phase separation at all tested concentrations, which may be related to the stronger gelling ability of alginate in aqueous media and its well-known affinity for forming ion-mediated networks in the presence of divalent cations (22).

In practical terms, the performance of CAG as a natural emulsifier supports its potential application in food and cosmetic products requiring moderate stability and biocompatibility. For example, in milk-based beverages, CAG could improve suspension and texture through interactions with casein micelles, even at low concentrations. Nevertheless, its emulsifying performance may benefit from co-formulation with more structurally robust polysaccharides like alginate or xanthan gum to enhance long-term stability (23,24).

3.2 Surface Tension and Emulsifying Capacity

Surface tension measurements were conducted using the pendant drop method and the results are summarized in Table 2. Figure 2 shows the shape of a pendant drop of a 0.25% carrageenan (CAG) aqueous dispersion captured using a goniometer (Ramé-hart, Model 290-U4, Serial No. 200205). This image was used to determine the surface tension via drop profile analysis. The symmetrical shape and well-defined contour of the drop allowed for accurate fitting of the Young–Laplace equation to calculate interfacial tension (25). Across all tested concentrations, both carrageenan (CAG) and alginate (ALG) solutions displayed surface tension values ranging between 49 and 52 mN/m, showing no significant concentration-dependent trend. This absence of clear variation suggests that the

tested concentration range may not have been broad enough to induce detectable surface activity changes. Furthermore, the surface tension values of CAG and ALG were closely aligned, indicating similar interfacial behaviors under the studied conditions.

Table 2. Surface Tension, Zeta Potential of emulsions stabilized with CAG and ALG Emulsifying Capacity of aqueous dispersions of CAG and ALG

	Concentration	Surface Tension (mN / m^{-1})	Zeta Potential mV	Emulsifying Capacity (%)
CAG	0.25%	50.33 ± 0.8 ^{a,b}	-85.2 ± 1.5^{a}	20.0 ± 0.2^{a}
	0.5%	51.50 ± 0.6 ^{b}	-93.7 ± 1.3^{b}	20.0 ± 0.1^{a}
	1%	50.31 ± 0.5 ^{a,b}	-92.3 ± 1.1^{b,c}	85.0 ± 0.2^{b}
	1.5%	49.53 ± 0.9 ^{a}	-88.3 ± 1.4^{b,d}	95.0 ± 0.2^{c}
ALG	0.25%	51.51 ± 0.9 ^{a,b}	-86.4 ± 1.8^{d}	15.0 ± 0.1^{d}
	0.5%	52.47 ± 1.0 ^{b,c}	-91.0 ± 1.2^{c,b,}	30.0 ± 0.2^{e}
	1%	50.35 ± 0.6 ^{a,b}	-89.5 ± 1.1^{c,d}	72.5 ± 0.1^{f}
	1.5%	49.24 ± 0.9 ^{a}	-92.3 ± 1.5^{b,c}	85.0 ± 0.1^{g}

Different letters (a-g) within one raw show a significantly different ($p < 0.01$)

Figure 2. A droplet of CAG 0.25%. Goniometer ramé-hart instrument co. Model 290-U4 serial No. 200205

Despite their limited reduction of surface tension, both CAG and ALG significantly improved the emulsifying capacity at higher concentrations, particularly at 1% and 1.5%. This effect is likely attributed not to classical surface activity, but rather to increased viscosity and steric stabilization, consistent with previous findings that hydrocolloids primarily stabilize emulsions by increasing the viscosity of the continuous phase and creating mechanical barriers at the oil–water interface (26).

This behavior is coherent with Garti and Leser (2001), who emphasized that hydrocolloids such as carrageenan are not true emulsifiers in the traditional sense since they do not actively adsorb at the interface, but instead act as stabilizers by increasing bulk viscosity and forming thick, gel-like interfacial layers. Similar mechanisms were also described for other anionic polysaccharides like xanthan and gum arabic (26).

The relatively stable surface tension values of κ-carrageenan measured in this study (around 50 mN/m) align with prior reports on carrageenan-based systems. Liszka et al. (2014) reported surface tension values of 64.1 mN/m at 25 °C for κ-carrageenan alone, which decreased in formulations containing xanthan gum, suggesting that the combination of polysaccharides may further impact interfacial properties and foam stability (27). Our findings are also in agreement with those of Chan et al. (2013), who highlighted the physicochemical stability and high water-holding capacity of κ-carrageenan gels, despite relatively modest surface activity (8).

Is important to note that CAG used in this study was not re-characterized, as its composition had already been validated in earlier work using methylation analysis and NMR spectroscopy. These previous studies confirmed that the extraction method employed (hot water extraction without alkali treatment) selectively yielded κ-carrageenan with a low content of agarans and minimal hybrid structures. While minor co-extraction of other polysaccharide types (e.g., agarans or κ/ι hybrids) cannot be entirely ruled out, they are not expected to significantly affect the observed emulsifying behavior given the formulation parameters and the predominance of κ-type structures (21).

Comparatively, ALG exhibited a similar surface tension profile but demonstrated slightly lower emulsifying capacity at low concentrations (e.g., 15.0 ± 0.1% at 0.25%), potentially due to differences in molecular conformation, charge density, and interaction with the oil–water interface. This is consistent with reports by Urbanová et al. (2019), who showed that alginate gel formation and interfacial activity are highly dependent on the type and concentration of divalent ions, chain flexibility, and structural organization (22).

Zeta potential is a key indicator of emulsion stability, reflecting the degree of electrostatic repulsion between dispersed particles. In this study, both carrageenan (CAG) and alginate (ALG) samples exhibited high negative zeta potential values,

ranging from –85.2 mV to –93.7 mV (Table 2). These values far exceed the conventional threshold for emulsion stability (±30 mV), suggesting strong electrostatic repulsion and, consequently, high kinetic stability of the emulsions.

This strong negative charge can be attributed to the molecular structure of the polysaccharides. Carrageenan contains abundant sulfate ester groups attached to galactose units, which confer a substantial negative charge to the polymer backbone (10). Similarly, alginate carries deprotonated carboxyl groups from its guluronic and mannuronic acid residues, contributing to its net negative charge under neutral to basic conditions (17).

The similar electrostatic profiles of CAG and ALG observed in this study are consistent with their structural classifications as anionic hydrocolloids. However, despite their comparable zeta potential values, differences in their emulsifying capacities were observed (Table 2). These variations may arise from differences in molecular weight distribution, chain conformation, and interaction with oil–water interfaces. Indeed, carrageenan's linear sulfated galactan backbone allows it to adopt a more rigid structure, which may enhance steric stabilization in addition to electrostatic repulsion (17).

As shown in Figure 3, emulsions stabilized with both CAG and ALG at 0.25% and 0.5% concentrations exhibited poor physical stability, characterized by irregular droplet sizes and non-uniform distributions. This behavior suggests the onset of coalescence processes, in which smaller oil droplets begin to merge, forming larger and unstable droplets. In contrast, systems formulated at 1% and 1.5% showed markedly improved droplet homogeneity and spatial distribution, indicating enhanced kinetic stability. These findings are consistent with the results obtained from droplet size and zeta potential measurements, which revealed that higher polysaccharide concentrations promote better electrostatic stabilization and steric hindrance.

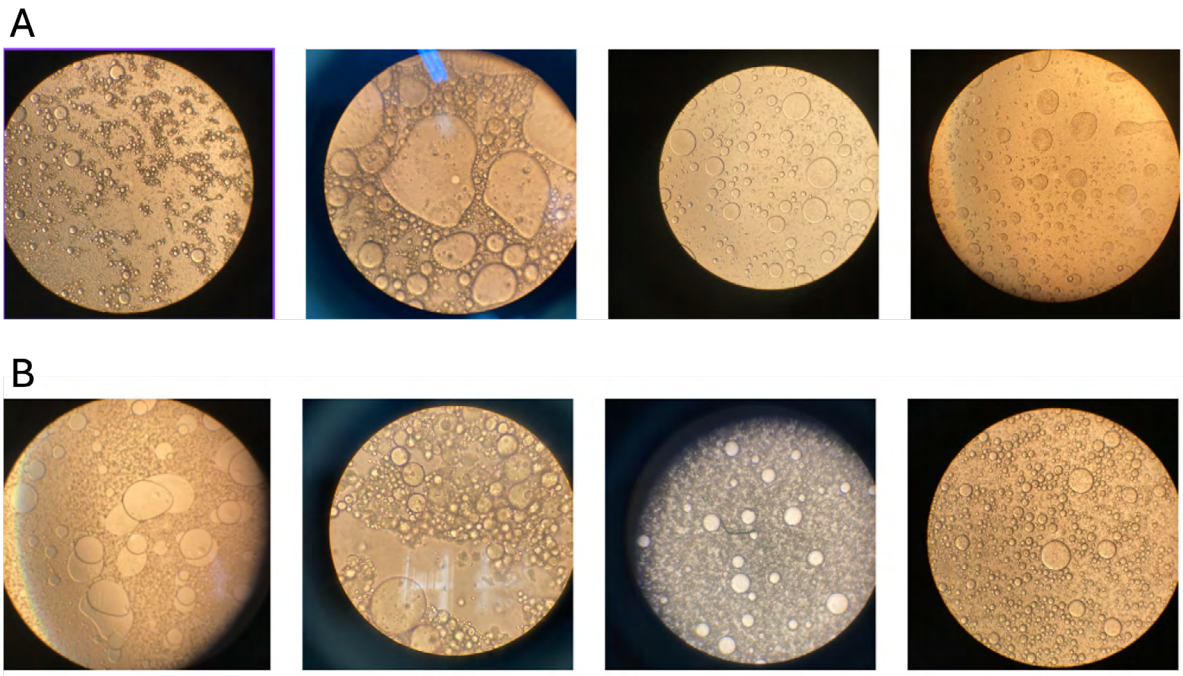

Figure 3. Droplet shape of A) CAG emulsions and B) ALG emulsions. 10x microscopy, from left to right: 0.25%, 0.5%, 1%, 1.5%.

This concentration-dependent stabilization capacity is a common feature of polysaccharide-based emulsifiers, including carrageenans and alginates, due to their amphiphilic nature and ability to form viscoelastic interfacial films (9). Specifically, κ-carrageenan, the major component of CAG extracted from *H. musciformis*, is known for its ability to form strong gels through the formation of helices in the presence of cations, which enhances its emulsifying potential at higher concentrations (21).

In comparison, ALG is a linear copolymer of β-D-mannuronic acid and α-L-guluronic acid, which contributes to a different rheological and interfacial behavior (17). According to Urbanova et

al. (2019), alginate forms less cohesive interfacial films and is more susceptible to ionic strength changes than carrageenan, which may explain the lower emulsifying capacity observed at similar concentrations (22).

These observations support the notion that polysaccharide concentration plays a pivotal role in determining the physical stability of emulsions. The improvement in stability at 1–1.5% w/v is likely due to the formation of a more cohesive and continuous interfacial layer, in line with prior studies on hydrocolloid-stabilized emulsions (18). Altogether, the structural attributes of CAG, including its high charge density and potential for gelation, contribute to its superior performance compared to ALG under the tested conditions.

3.3 Rheological Behavior

The rheological behavior of hydrocolloid-stabilized emulsions plays a critical role in their physical stability. Emulsions formulated with polysaccharides such as alginate and carrageenan exhibit increased viscosity, which reduces the mobility of dispersed oil droplets and delays creaming or sedimentation by hindering gravitational separation and droplet coalescence (18). Moreover, pseudoplastic (shear-thinning) behavior contributes to desirable flow properties during processing and use while maintaining structural integrity at rest. The formation of weak gel-like networks, particularly by κ-carrageenan, also provides structural rigidity that retards phase separation under storage conditions (28). Urbanová et al. (2019) further emphasized that the interplay between chain conformation, molecular interactions, and ionic environment influences the rheological contribution to emulsion stabilization, particularly in systems involving alginate or other charged polysaccharides (22). Therefore, the rheological properties of both dispersions and emulsions are central to predicting and optimizing long-term stability in food and cosmetic formulations.

According to this, viscosity and rheological behavior of the dispersions and emulsions formulated with carrageenan (CAG) and sodium alginate (ALG) were analyzed. As shown in Figures 4 and 5, the apparent viscosity of the ALG dispersions was consistently higher than that of the CAG dispersions across all concentrations. Both polysaccharides exhibited pseudoplastic (shear-thinning) behavior, particularly at higher concentrations (1.0% and 1.5%), which is characteristic of entangled polymeric networks in solution. At lower concentrations (0.25% and 0.5%), this behavior was less pronounced, possibly due to insufficient polymer chain interactions to induce shear thinning.

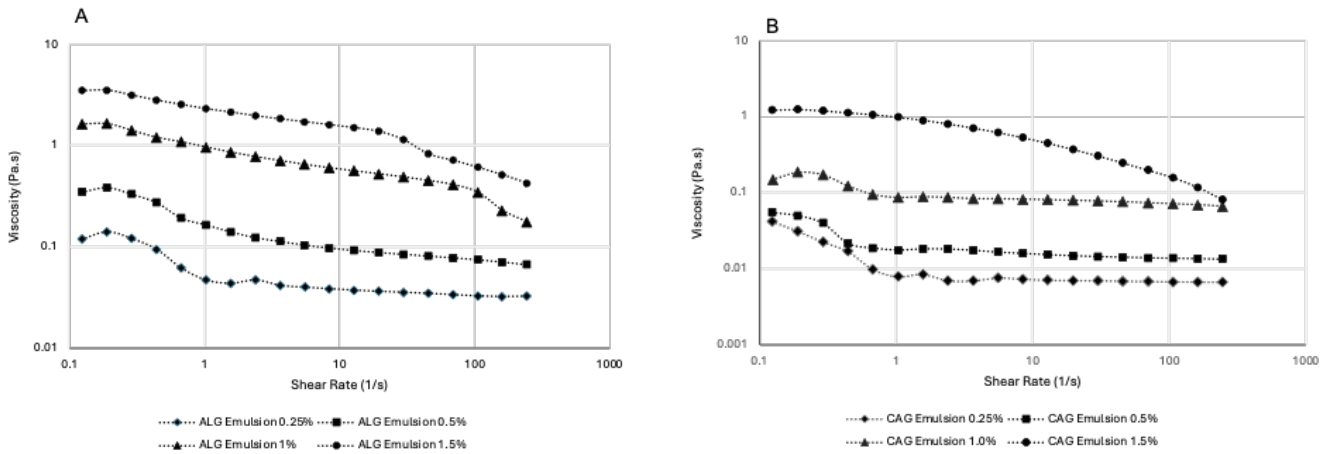

Figure 4. Apparent viscosity of the dispersions of A) Sodium Alginate and B) Carrageenan as a function of shear rate (0.01–300 s^{-1}).

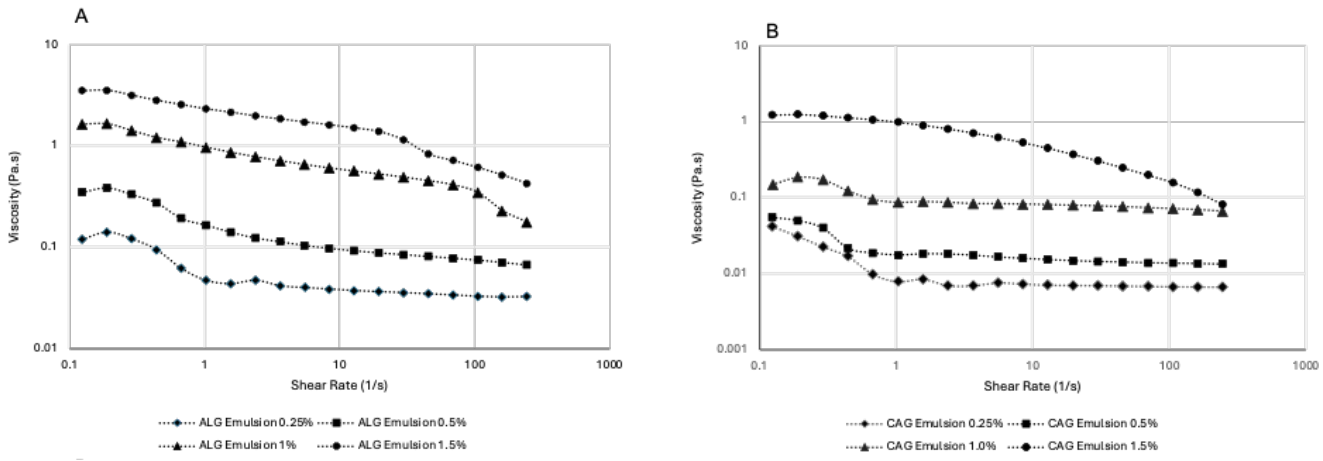

Figure 5. Apparent viscosity of the emulsions stabilized with A) Sodium Alginate and B) Carrageenan as a function of shear rate (0.01–300 s^{-1}).

A similar trend was observed in the emulsion systems: ALG-based emulsions exhibited higher viscosities than those stabilized with CAG, and viscosity increased with polysaccharide concentration (Figures 7 and 8). This behavior aligns with previous findings on polysaccharides such as xanthan gum and guar gum, which are known to stabilize emulsions primarily through viscosity enhancement of the continuous phase rather than interfacial adsorption (26). ALG's higher viscosity can be attributed to its linear structure composed of β-D-mannuronic and α-L-guluronic acid residues, which form stiff, extended chains and strong intermolecular associations, particularly at higher concentrations (29). In contrast, Carrageenan's pseudoplasticity arises from its coil-to-helix transitions and interchain interactions at higher concentrations, which contribute to the formation of weak gel-like networks under shear (28). However, its overall lower viscosity compared to ALG may also be due to differences in molecular weight distribution and chain rigidity (30,31).

4. PERSPECTIVES

The findings of this study demonstrate that carrageenan extracted from *H. musciformis* (CAG) possesses emulsifying properties comparable to those of commercial alginate (ALG), positioning this biopolymer as a promising alternative in multiple industrial contexts. This is especially relevant given the increasing demand for clean-label, sustainable, and marine-derived ingredients in food, pharmaceutical, and cosmetic formulations.

From a strategic standpoint, the cultivation of *H. musciformis* represents an opportunity to valorize underutilized native seaweed species, particularly in tropical regions with favorable conditions for macroalgal farming. The use of cultured biomass not only ensures supply chain consistency but also aligns with sustainable blue economy models by minimizing pressure on wild populations and fostering local biotechnological development.

Future research should focus on to develop innovative formulations, optimizing cultivation and extraction conditions to ensure compositional consistency, scaling up production under good manufacturing practices (GMP), and performing techno-economic and regulatory feasibility studies for targeted applications. The current results lay the groundwork for considering CAG from *H. musciformis* not merely as a functional ingredient but as a strategic bioproduct that bridges marine biotechnology and sustainable industrial innovation.

5. CONCLUSIONS

This study evaluated the emulsifying potential of carrageenan (CAG) extracted from *H. musciformis* and compared it with that of sodium alginate (ALG), a widely used polysaccharide in the food and pharmaceutical industries. The results showed that CAG exhibited comparable performance to ALG in terms of droplet size, polydispersity index (PDI), zeta potential, surface tension, and storage stability. These parameters consistently demonstrated that CAG is capable of stabilizing oil-in-water emulsions effectively, without significant phase separation over time.

The research addressed a critical gap in the literature concerning the functional application of

H. musciformis-derived carrageenan as a natural emulsifier. While carrageenan is well recognized for its gelling and thickening capabilities, its emulsifying role—particularly when extracted from non-commercial algae species—remains underexplored. This study contributes to bridging that gap by providing empirical data that supports the potential of *H. musciformis* as a sustainable and regionally available source of food-grade carrageenan.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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AUTHORS´ CONTRIBUTIONS

Conceptualization: D.M.A. and G.R.T ; Data curation: J.L.N. Formal analysis: J.L.N. and I.A.R ; Funding acquisition D.M.A. Investigation: J.L.N. and I.A.R; Methodology: J.L.N. ; Supervision: D.M.A.; Writing – original draft: J.L.N. and I.A.R; Writing – review and editing: D.M.A. and G.R.T

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