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Characteristics of Carrageenan Extracted from Commercially Important Seaweeds from the MIMAROPA Region, Philippines

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Abstract

This study analyzed the physicochemical attributes of raw dried seaweed samples *(Eucheuma striatum, Kappaphycus alvarezii* and *Eucheuma denticulatum)* obtained from different seaweed culture sites in the MIMAROPA Region. The results showed that the moisture content ranged from 35.70% to 38.67%, ash content from 16.22% to 18.55%, impurities from 1.42% to 1.59%, and CAW (clean anhydrous seaweed) from 40.40% to 50.03%. All parameters tested met the local and international standards for dried seaweeds. The samples underwent an alkali extraction process to obtain Philippine natural grade (PNG) carrageenan. The physicochemical attributes analyzed included yield (35.36% to 47.96%), moisture (15.38% to 16.99%), insoluble ash (0.96%), and sulfate content (27.45% to 37.33%). The factors such as yield, insoluble ash, and sulfate content were consistent with previous studies. However, the moisture content did not meet the required level, which is an important parameter for dry additives like carrageenan. The rheological properties of the extracted carrageenan from the three samples were assessed, including gel strength (205 g/cm^2 to 320.22 g/cm^2), gel viscosity (12.54 Cps to 13.59 Cps), gelling point (18.59ºC to 23.57ºC), and melting point (47.15ºC to 60.25ºC). Except for the gel strength, all other parameters met the standards for carrageenan. Overall, the physicochemical attributes and rheological properties of the carrageenan extracted from the analyzed seaweed samples were mostly compliant with the established standards, highlighting their potential for various applications in the food and pharmaceutical industries.

Keywords: Carrageenan, physicochemical properties, raw dried seaweeds, rheological properties

Introduction

The seaweed industry in the Philippines holds a prominent position as one of the primary contributors to fisheries production in the country. In terms of volume, it stands at the forefront among aquaculture commodities, accounting for approximately 60-70% of the total aquaculture production (BFAR, 2022). Key production areas for these seaweeds are concentrated in BARMM, MIMAROPA, Zamboanga Peninsula, and Central Visayas regions (PSA, 2017). Due to the growing global demand for high-quality carrageenan as a food ingredient, as well as its applications in cosmetics and pharmaceuticals, Eucheumatoids (specifically *Kappaphycus* and *Eucheuma)* have emerged as the primary red seaweeds cultivated in Southeast Asia, especially in the Philippines (Valderrama et al., 2015). *Kappaphycus* and *Eucheuma* varieties are widespread in the Philippines, with cultivation taking place in 15 different regions across the country. This expansion in cultivation is driven by the increasing market demand for these seaweeds and their carrageenan products (Buschmann et al., 2017).

Carrageenan is a naturally occurring polysaccharide derived from various species of red seaweeds (Campo et al., 2009). It is highly valued for its versatile applications in the food, pharmaceutical,

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and cosmetic industries. It functions as a gelling agent, thickener, stabilizer, and emulsifier, adding texture, viscosity, and stability to various products. It is widely used in dairy products, processed meats, desserts, personal care products, and pharmaceutical formulations (Liu et al., 2015; Zia et al., 2017). Furthermore, carrageenan is known for its potential health benefits. Some studies suggest that carrageenan may have antimicrobial, anti-inflammatory, and antioxidant properties (Chen et al., 2019; Yew et al., 2020).

The qualities of carrageenan can vary depending on the place of origin and the species of seaweed from which it is extracted (Lomartire & Gonçalves, 2022). Different species of seaweed, such as *Kappaphycus* and *Eucheuma*, can yield carrageenan with varying properties (Bui et al., 2018; Jiang et al., 2022). The chemical composition and structure of carrageenan molecules can differ among seaweed species, resulting in variations in gelling properties, viscosity, and functionality (Aguilan et al., 2003). Furthermore, environmental factors, including temperature, salinity, water quality, and nutrient availability, can vary across different geographical regions. These variations can influence the growth, composition, and physiological characteristics of seaweeds (Liang et al., 2022). As a result, carrageenan extracted from seaweeds in different regions may exhibit differences in gel strength, rheological behavior, and other physical properties (Rhein-Knudsen et al., 2017).

Hence, this study aims to understand the specific characteristics of carrageenan from different seaweed sources for quality control and standardization in the production process. By evaluating parameters such as gel strength, viscosity, and other rheological behavior, producers can ensure consistent product quality and performance. Also, by studying the characteristics of carrageenan from various seaweed species, researchers can identify the most suitable sources for specific applications.

Materials and Methods

Dried samples of three commercially important seaweeds namely *Eucheuma striatum*, *Kappaphycus alvarezii,* and *Eucheuma denticulatum* were obtained from different seaweed culture sites in the MIMAROPA Region from March to April 2023. Identification of the samples was based on Hurtado & Agbayani (2000) and Trono (1992). Samples were delivered to the Center for Lake and Sustainable Development (CLSD) laboratory of the College of Fisheries, Laguna State Polytechnic University, Los Baños Campus, Los Baños, Laguna.

The Philippine natural grade (PNG) from the three seaweed samples was extracted following the method of Bono et al. (2014) and Moses et al. (2015), with some modifications. Dried seaweeds were washed several times to remove sand and debris. Then, the samples were alkali-treated with 1% w/v potassium hydroxide (KOH) for 3 h at 80^{o}C to produce semi-refined carrageenan (SRC). Samples were washed with distilled water 3-4 times until neutral pH was achieved. Seaweeds were chopped and soaked in 5% sodium hypochlorite (NaCIO) for 15 min. Afterward, samples were washed with distilled water to remove excess NaCIO. Seaweed samples were oven-dried for 15 h at 60^{o}C, micronized into powder, and sieved through #80 mesh. The yield of carrageenan as a result of extraction was calculated based on the percentage between the carrageenan weight produced and the weight of dried seaweed (AOAC, 1990). The yield was calculated using the following equation:

$$Yield = \frac{weight\ carageenan}{weight\ seaweed}\ x\ 100$$

Raw dried seaweeds were subjected to moisture and ash analyses. The extracted PNG carrageenan powder was analyzed for moisture, ash, acid-insoluble ash, and sulfate contents (AOAC, 1990; PNS, 2012; Diharmi et al., 2020).

Fifty grams of dried seaweed was weighed in a glass cup (Wo). Then the unwanted objects (other types of seaweed, plastic, shells, coral, etc.) were separated from the seaweed and weighed (Wd). Clean Anhydrous Weed (CAW) was analyzed and the impurities were calculated using the following equation (Neish, 2008):

$$Crude\ impurities = \frac{Wd}{Wo}\ x\ 100$$

Fifty grams of dried seaweed (Wo) were soaked for 30 minutes and stirred every 5 minutes. Furthermore, the water was removed and added repeatedly while stirring again. Then, the seaweed samples were filtered and drained for 2 minutes. Meanwhile, samples were placed in prepared aluminum foil, dried in an oven at 60°C for 60 minutes, and weighed (Wa). When constant weight was achieved, the dried seaweed was weighed (Wd). The CAW content was calculated using the following equation:

$$CAW = \frac{Wd - Wa}{Wo} \times 100$$

Meanwhile, the gelling and melting point of the gel was determined using the method of Luhan (1992), with some modifications. About 2g of powdered sample was mixed with 100ml distilled water (2% solution) and boiled until observation of carrageenan sol. Three tubes were filled with 10ml of the solution and gelling and melting temperatures of the three samples were determined by heating the carrageenan in a water bath until the samples melt. The melting point temperature was recorded when the colored glass beads (6mm diameter) dropped at the test tube's bottom. On the other hand, gelling point temperature was obtained when the last colored glass bead remained at the surface. A modification was made by adding ice to the water bath when gelling point was observed to be lower than the ambient temperature. The water bath temperature was lowered to 10-15^{o}C to facilitate gelling. All analyses were done in triplicates.

The gel strength and viscosity were determined using Bono et al. (2014) and Wardhana et al. (2022), with some modifications. Gel strength was analyzed by dissolving 3g of samples in 197mL of distilled water. The solution was heated until it reached 80^{o}C, cooled, and stored at 10^{o}C until it hardens. The gel strength was measured using a gel tester. For viscosity, about 2.7g of carrageenan sample was dissolved and heated in 170mL of distilled water. After dissolving the samples, the weight was set to 180g, so the concentration becomes 1.5% (w/w). The solution was heated in a water bath while stirring until it reached a temperature of 80^{o}C. Viscosity was measured using a gel viscometer.

All experiments were done in triplicates. Values were expressed as mean and standard deviation. Data were analyzed using a one-way analysis of variance ($p<0.05$) followed by Tukey's posthoc in PAST v 4.0.

Results and Discussions

The physicochemical properties of the three raw dried seaweed samples are shown in Table 1. Significant differences were observed among the percent CAW of the samples, while there are no significant differences in terms of moisture, ash, and impurities. The moisture contents of *E. striatum, K. alvarezii,* and *E. denticulatum* were 35.30%, 35.70%, and 38.67%, respectively. The values in this study comply with the PNS BAFPS 85:2012 specifications, which require *Kappaphycus* and *Eucheuma* to have 40% and 30% moisture content, respectively. Moisture is a crucial parameter that the industry closely monitors as it significantly impacts the quality of the final product. Manufacturers aim to reduce moisture levels to meet standard limits, ensuring that the resulting product, in this case, raw dried samples attained an acceptable quality standard (Zambrano et al., 2019). In terms of ash content, the percentage values ranged from 16.22 to 18.55. The results were in accordance with international standards and previously published literature.

The ash content of the three samples was acceptable since it is within the international standard range of 15-40% (FAO, 2007). Similarly, Hussin et al. (2014) reported an ash content of 23-26% for *Kappaphycus* and 28-30% for *Eucheuma*. The ash content is a crucial parameter that determines the nutritional quality of seaweed. High levels of ash content in algae make them unsuitable for human consumption and also restrict their inclusion in animal diets. This is because a high ash content hampers the usability of seaweed as a food source, both for humans and animals (Alghazeer et al., 2022).

Table 1. Physicochemical properties of raw dried seaweeds

	E. striatum	*K. alvarezii*	*E. denticulatum*
Moisture (%)	35.30 ± 1.89^{a}	35.70 ± 0.45^{a}	38.67 ± 1.57^{a}
Ash (%)	16.44 ± 2.22^{a}	18.55 ± 1.04^{a}	16.22 ± 0.70^{a}
Impurities (%)	1.44 ± 0.21^{a}	1.59 ± 0.18^{a}	1.32 ± 0.20^{a}
CAW (%)	48.74 ± 1.24^{a}	34.14 ± 3.23^{a}	50.03 ± 0.84^{b}

Values were expressed in mean ± standard deviation. Values with different superscripts are statistically significant ($p<0.05$)

The percent impurities values observed in all the samples are significantly lower than the maximum limit of 3% set by the PNS BAFPS 85:2012 for raw dried seaweeds, applicable to both the *Kappaphycus* and *Eucheuma* species. Similar results were reported by Shanmugam et al. (2017) wherein the impurities derived from *K. alvarezii* were 0.56% only, while Diharmi et al. (2020) revealed that *E. cottonii* from Indonesia has 1.04-3.79% impurities. However, Katili et al. (2019) reported high impurities in *K. alvarezii* (7.23%) because of the drying methods wherein drying was done on bamboo racks placed on the ground. In the present study, the acceptable results can be attributed to the post-harvest practices adopted by farmers, wherein they manually remove foreign matter that may be attached to the seaweeds before the drying process. This intervention helps to ensure that the dried seaweeds meet quality standards by minimizing the presence of extraneous materials. Also, to facilitate the drying process, the farmers construct drying facilities with roofs made of plastic material. These facilities are designed to provide a controlled environment for drying. The air-drying method is employed, wherein the seaweed is suspended or hung within the facility. This method involves the circulation of air to remove moisture from the seaweed, effectively facilitating the drying process (Zhao et al., 2022).

CAW is the parameter used to determine the salt-free dry matter content in seaweed. The results of this study are in agreement with the PNS BAFPS 85:2012 for raw-dried seaweeds which sets the minimum limit for *Kappaphycus* and *Eucheuma* seaweed as 30% and 40%, respectively. Furthermore, the samples also satisfy the international standard which sets the minimum CAW at 40% (Periyasamy et al., 2015). The results of this study are also in agreement with other published studies wherein CAW from dried *Kappaphycus* and *Eucheuma* varied from 39-45% (Periyasamy et al., 2015; Subaryono & Kusumawati, 2020).

The term Philippine Natural Grade (PNG) is used to refer to *Eucheuma* seaweeds that are harvested in the Philippines and Indonesia. These seaweeds are then subjected to a direct treatment process using alkali to alter the properties of carrageenan found within the seaweed (FDA, 1994). In the present study, carrageenan from the three seaweed samples was extracted using alkali, resulting in PNG carrageenan. The physicochemical qualities of the extracted carrageenan are shown in Table 2. Significant differences were observed in the yield of *E. denticulatum* with the lowest yield. The low yield in this species can be due to several reasons such as the extraction method and culture conditions. Lomartire & Gonçalves (2022) reported that the characteristics of carrageenan can differ based on its geographical origin and the specific seaweed species used for extraction. Different species of seaweed, such as *Kappaphycus* and *Eucheuma*, can yield carrageenan with varying properties (Bui et al., 2018; Jiang et al., 2022). Nevertheless, the yield from the three samples using the alkali extraction method described in this study has similar results to Ohno et al. (1996) (54.5%), Hasizah et al. (2021) (29.6-62.4%), and Darmawan et al. (2013) (30-41%).

Meanwhile, the moisture content of the carrageenan extracted from the three samples has higher values (15.38% to 16.99%) than other reported studies. Wullandari et al. (2021) recommended a 7-9% moisture content, while Basmal & Ikasari (2014) and Sari et al. (2021) reported values of 11.73% and 8%, respectively. Meanwhile, the EU requires an 8% moisture content for food additive E407 or carrageenan (EFSA, 2018). The high moisture content of the three samples can be due to insufficient drying time.

Table 2. Physicochemical properties of PNG carrageenan

	E. striatum	*K. alvarezii*	*E. denticulatum*
Yield (%)	45.81±1.56[a]	47.96±2.03[a]	35.36±2.16[b]
Moisture (%)	15.38±0.96[a]	16.65±0.70[a]	16.99±1.16[a]
Insoluble Ash (%)	0.90±0.10[a]	0.96±0.06[a]	0.96±0.07[a]
Sulfate Content (%)	27.45±1.55[b]	36.88±2.57[a]	27.33±1.63[b]

Values were expressed in mean ± standard deviation. Values with different superscripts are statistically significant ($p<0.05$).

For the insoluble ash, no significant differences were observed among the samples. The values ranging from 0.90 to 0.96 comply with the international standards for insoluble ash content of <1 (FAO, 2007; National Research Council, 1981). The acid-insoluble ash content in seaweed is an indicator of the presence of mineral or metal contaminants that cannot be dissolved in an acid solution, such as silica (Si) commonly found in quartz, stone, and sand (Darmawan et al., 2013). The sorting process, whether during the handling of raw materials or the processing of the PNG carageenan, plays a crucial role in minimizing the acid-insoluble ash content. A high acid insoluble ash content indicates the presence of mineral residue or insoluble metals that cannot be effectively reduced during processing (Syamsuar, 2006). On the other hand, low values of acid-insoluble ash indicate that the PNG carrageenan produced was free from contamination during the processing steps. The sorting process is essential to ensure the removal of any impurities or foreign materials that could contribute to elevated levels of acid-insoluble ash in the final product.

The sulfate content between *Kappaphycus* and *Eucheuma* is significantly different with the former having the highest value of 36.88%. In the study of Basmal & Ikasari (2014), 6.74% sulfate was from *K. alvarezii*, while Sari et al. (2021) reported that *E. cottonii* has 11% sulfate content. Also, the results were in accordance with CFR (2023) and FAO (2007) wherein acceptable sulfate content is 20-40% and 15-40%, respectively. Commercially available k-carrageenan typically consists of approximately 22% (w/w) sulfate, while iota-carrageenan contains around 32% (w/w) sulfate, and lambda-carrageenan contains about 38% (w/w) sulfate (De Ruiter & Rudolph, 1997). However, it is important to note that significant variations in sulfate content can occur due to variations between seaweed species or different batches (Jiang et al., 2022; Bui et al., 2018).

The rheological properties of PNG carrageenan are represented in Table 3. Results showed significant differences in gel strength between *Kappaphycus* and *Eucheuma* species, wherein the former has a gel strength of 320 g/cm^2. The gel strength of the three samples was below the recommended values of FAO (2007) of >400 g/cm^2. In the study of Basmal & Ikasari (2014) and Sari et al. (2021), the gel strength of *Kappaphycus* and *Eucheuma* is 746 g/cm^2 and 715 g/cm^2, respectively. One essential property of carrageenan is its ability to transform from a liquid state to a solid state or convert a solution into a gel-like substance (Necas & Bartokisova, 2013). Hence, gel strength is a significant physical characteristic that plays a crucial role in determining the rheological properties of seaweeds. The gel strength of carrageenan is primarily influenced by two factors: sulfate content and 3,6-anhydrogalactose (3,6-AG) content. The resulting colloid exhibits a higher gel strength when the extracted carrageenan has a low sulfate content or a high 3,6-AG content. In other words, a decrease in sulfate content or an increase in 3,6-AG content contributes to the formation of stronger gels in carrageenan-based products (Azevedo et al., 2013). The results of the sulfate content of the three samples were relatively high, hence, the low gel strength results. However, this study's results agree with Ohno et al. (1996) with extracted carrageenan having a gel strength ranging from 247-557 g/cm^2.

The gel viscosity of the three samples is not significantly different and was compliant with the FAO (2014) standard of not >5 Cps at 75^{o}C. The gel viscosity range from 12.54 to 13.59 Cps which is also in agreement with 16-97 Cps (Ohno et al., 1996) and 7 Cps (Sari et al., 2021). This means that the extracted carrageenan from the samples was of good quality and indicate good handling and proper temperature application during the drying and extraction process of the raw seaweeds. In the study

Table 3. Rheological properties of PNG carrageenan

	E. striatum	*K. alvarezii*	*E. denticulatum*
Gel Strength (g/cm^2)	205±4.34^a	320.22±9.90^b	297.25±5.91^a
Gel Viscosity (Cps)	12.55±1.52^a	13.59±1.31^a	12.54±1.64^a
Gelling Point (^{o}C)	23.57±2.80^{ab}	18.59±0.54^a	20.47±0.47^b
Melting Point (^{o}C)	50.48±0.73^a	47.15±0.84^a	60.25±0.92^b

Values were expressed in mean ± standard deviation. Values with different superscripts are statistically significant ($p<0.05$).

of Jiang et al. (2022), calcium-induced carrageenan (Ca-IC) has a low viscosity which can be attributed to the degradation of carrageenan molecules, due to prolonged exposure to high temperatures. This degradation process can lead to changes in the structure and properties of carrageenan, including its viscosity and gel-forming ability. Additionally, the low molecular weight of carrageenan could also contribute to the observed low viscosity of calcium-induced carrageenan (Ca-IC). The smaller molecular size of carrageenan molecules in Ca-IC may result in reduced viscosity compared to other forms of carrageenan.

The gelling point of carrageenan was found to vary significantly between *K. alvarezii* and *E. denticulatum,* indicating distinct gelation properties between these two species. However, no significant differences in gelling points were observed between *E. striatum* and E. denticulatum. This suggests that *E. striatum* and *E. denticulatum* share similar gelation characteristics. Despite the differences, the values of all samples satisfy the >35ºC gelling point stipulated in FAO (2014). Meanwhile, the melting points vary significantly between *K. alvarezii* and *E. denticulatum*, with the latter having the highest value of 60.25ºC. The results for the three samples were in accordance with the FAO (2014) which sets the melting point of carrageenan at above 60ºC. The gelling point is a crucial factor for the food industry when selecting materials for their products. It represents the temperature at which the food substance, such as jelly, forms a gel-like consistency. Knowing the gelling point allows food producers to determine the minimum temperature required to maintain the desired quality of the jelly. On the other hand, the melting point is useful for determining the maximum temperature at which a food product can be stored before it is consumed. By understanding the melting point, food producers can establish the temperature limits necessary to preserve the food's quality (Jiang et al., 2022; Darwaman et al., 2013; Necas & Bartosikova, 2013).

Both *Kappaphycus* and *Eucheuma* species are commercially important seaweeds in the Philippines. The seaweeds harvested from the different culture sites in the MIMAROPA region exhibited physicochemical and rheological characteristics that were compliant with the Philippine and international standards for raw dried seaweeds and carrageenan. The variations of the present results to the existing studies can be attributed to numerous intervening factors originating from both internal and external aspects of seaweed biology and post-harvest processes.

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