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Research article

Physicochemical Properties of Carrageenan Extracted from Raw Dried Seaweed of Caluya, Antique, Philippines

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Abstract: Marine hydrocolloids, such as carrageenan extracted from seaweeds, are widely used in food, industrial, and commercial applications. The Philippines has great potential to export carrageenan, but the industry requires strict quality regulations for the product. The specifications for raw dried seaweeds (RDS), including chemical and gel rheological properties, of *Eucheuma denticulatum "Milyon milyon"* and *Kappaphycus striatum "Sacol"*, collected from Caluya, Antique, Philippines, were investigated and compared to the national standards used by the carrageenan production industry. The RDS (moisture, ash, clean anhydrous weed, impurities) of *E. denticulatum* and *K. striatum* were within the limits of the Philippine National Standards. The chemical properties (moisture, ash, acid-insoluble ash, and sulfate content) of carrageenan extracted from *E. denticulatum* were within the standards, except for the acid-insoluble ash and sulfate content of *K. striatum*, which exceeded the limit. On the other hand, the rheological properties of carrageenan, including gel viscosity, gelling and melting temperatures, and hysteresis, were also within the standard limits. The study suggests using RDS from *E. denticulatum* to produce carrageenan, as it complies with the Philippine National Standards utilized by the seaweed industry.

Keywords: Hydrocolloids; carrageenan; physicochemical properties; rheological properties

1. Introduction

Marine macroalgae, commonly known as seaweed, are primarily found in coastal regions and adhere to rocks and other rigid substrates. Many types of these plant-like organisms thrive in rivers, lakes, seas, and oceans [1]. In Asian nations, including China, Japan, and South Korea, seaweed has long been a staple food. This practice spread to other parts of the world, where eating seaweed is uncommon due to migration [2], prompting a global appreciation for seaweed as a vital component of a healthy diet. The widespread use of seaweed-derived hydrocolloids across dietary, industrial, and commercial applications has driven their growing popularity [3]. The three main hydrocolloids that provide different products with stabilizing, emulsifying, preserving, and textural improvements are agar, alginates, and carrageenan [4]. As a cheap source of these compounds, seaweeds are in worldwide demand. Among the three hydrocolloids, carrageenan is highly favored due to its flexibility and cheapness. This compound accounts for 18% of the global ingredient market, the highest among all [5]. In the local scene, the Philippines is the leading carrageenan exporter in the world, exporting 94% of its seaweed production as Philippine Natural Grade (PNG) carrageenan, with significant markets in the USA, China, Spain, Russia, and Belgium [6].

A fair proportion of the country's seaweed production has been contributed by Western Visayas, or Region 6 [7]. The Province of Antique is the leading producer and is dubbed the "seaweed basket" of the region, with vast areas of seaweed farms located in the island municipality of Caluya, primarily culturing *Eucheuma* and *Kappaphycus* species [8, 9]. The RDS from the area are then transported to Cebu and Laguna for carrageenan processing. But to our knowledge, no work has been conducted to analyze the physical and chemical attributes of carrageenan extracted from the RDS in Caluya, Antique. Thus, the objective of this study was to determine whether the quality attributes of carrageenan extracted from the commonly cultured and dried seaweeds of Caluya, Antique complied with the Philippine Natural Grade (PNG) standard limit for carrageenan by conducting a physicochemical quality assessment.

2. Materials and Methods

2.1 Raw material

The RDS varieties of *Eucheuma denticulatum "Milyon milyon"* and *Kappaphycus striatum "Sacol"* were purchased from the island of Caluya, Antique, Philippines, where these species are abundantly cultivated. The seaweeds were grown in natural coastal seawater with an average salinity of 30–34 ppt, temperature of 28–31 °C, and moderate tidal water movement, conditions favorable for carrageenophyte production. Culture was conducted using the monoline hanging method for 1.5 months. Harvesting and sample collection were conducted during the dry season, specifically in March, to minimize the impact of rainfall and excessive moisture on the quality of the raw, dried seaweeds. After harvest, the seaweeds were thoroughly washed with seawater to remove foreign materials and epiphytes, and then dried using the conventional sun-drying method. The secondary information on culture and drying practices was obtained from the seaweed producer. One kilogram of dried samples per variety was packed in clean straw sacks and transported to the Fish Processing Laboratory at the University of the Philippines Visayas, Miagao, Iloilo, for laboratory analyses.

The study focused on the analysis of samples collected from Caluya, Antique, and its limitation was not comparing them to commercially available seaweed powder on the market.

2.2 Characterization of raw dried seaweeds (RDS)

Clean anhydrous seaweed and impurity/debris tests were conducted to determine the specifications of the RDS. The clean anhydrous weed method was used to determine the weight of seaweeds free of impurities and moisture. Impurities determine the total percentage of sand, straw, and other foreign matter attached to the dried seaweed samples. It is essential to consider these factors because they often influence the weight of the dried seaweed products delivered to the seaweed processor. The analyses were based on the Philippine National Standards [10] procedures for RDS. The moisture and ash content of the RDS were determined using methods by the Association of Official Analytical Chemists [11].

2.3 Alkali pretreatment and carrageenan extraction

The RDS were washed thoroughly with distilled water to remove contaminants. These were boiled in a water bath with 10% (w/w) potassium hydroxide (KOH) solution at 60˚C for 5 hours. As recommended by Peter McHugh [12] in *A Guide to the Seaweed Industry*, a bleaching step was performed after alkali pretreatment to improve color and for microbial control during the production of processed natural grade (PNG) seaweed. Accordingly, the pretreated seaweeds were soaked in a sodium hypochlorite solution prepared by diluting 0.06 mL of sodium hypochlorite into 500 mL of distilled water, yielding a 0.012% (v/v) solution. Samples were rinsed five times and drained to remove excess water. Then, these were dried in an oven at 60˚C for *Eucheuma spp.* and 80˚C for *Kappaphycus spp.* for 12-16 hours. The final dried seaweeds were chopped and ground using the laboratory milling machine and stored in a resealable pouch at ambient temperature until further carrageenan analysis was conducted. After bleaching, the samples were rinsed 5 times with distilled water and thoroughly drained to remove excess water. The seaweeds were then oven-dried at 60 °C for *Eucheuma* spp. and 80 °C for *Kappaphycus* spp. for 12–16 h, or until a constant weight was achieved. The dried samples were finally chopped and milled using a laboratory milling machine, and then stored in resealable polyethylene pouches at ambient temperature until further carrageenan analysis was conducted.

2.4 Chemical properties of carrageenan

Yield, moisture content, and insoluble ash of the gel were determined using the AOAC methods [11]. Sulfate content is a vital factor to monitor in the gel, as it is directly correlated with gel strength, and was determined through a precipitation method using barium chloride [13].

2.5 Gel Rheological properties

Carrageenan extracted from the samples was subjected to gel viscosity analysis, gelling, and melting-point determination. For the gel viscosity, 1.5 g of powdered seaweed was cooked to a viscous solution in 100 ml of distilled water, and its viscosity was analyzed using the gel viscometer (Ametek Brookfield, USA). On the other hand, the gelling and melting points were determined using the method of Rhein-Knudsen et al. through a basic laboratory setup (water bath, thermometer, glass tubes) [14].

2.6 Statistical analysis

The significant differences between variables were determined using a *t*-test. The statistical analysis was conducted using SPSS version 20.

3. Results and Discussion

3.1 Physicochemical properties of RDS

The RDS specifications of *Eucheuma denticulatum* and *Kappaphycus striatum* were presented in Figure 1. The study utilized the parameters based on the Philippine National Standard [10] for the seaweed industry, particularly for RDS production. As can be observed, *E. denticulatum* has a moisture content of 35.98%. These values are within the standard moisture content limit for *Eucheuma* species [10]. The moisture content of *K. striatum* was 40.51%, which is 0.51% higher than the limits for *Kappaphycus* species set by the Philippine National Standards [10] for RDS. The values were not statistically significant among the samples ($p > 0.05$). The ash content of *E. denticulatum* was 34.2%. This falls within the 15-40% standard limit set by FAO JECFA [2] for *Eucheuma* species. The ash content of *K. striatum* was 33.8%, slightly higher than that of *Kappaphycus alvarezii,* which is 28.9%, as studied by Abirami and Kowsalya [15]. There was no significant difference in ash noted among samples ($p>0.05$). For the clean anhydrous weed, which pertains to seaweeds free of moisture, salt, sand, and impurities, the *E. denticulatum* had a content of 35.26%, which is below the 40-45% limit set by the Philippine National Standard [10] for *Eucheuma* species. The *K. striatum* had a content of 23.6%, also lower than the 30-35% limits for *Kappaphycus* species of PNS [10]. There was a significant difference in the *E. denticulatum* samples ($p<0.05$). The *Kappaphycus* and *Eucheuma* species had impurity values lower than the allowable impurity level of 3%, as specified in PNS [10]. *E. denticulatum* had 0.58%, while *K. striatum* had 1.34%. A significant difference was observed between variables, with *K. striatum* showing greater deviation ($p < 0.05$).

Moisture is one of the vital parameters monitored in the industry because it affects manufacturers' final products. For this study, the moisture content of *E. denticulatum* and *K. striatum* is 35.98% and 40.51%, respectively. The moisture content is similar to that reported by Abel and Tolentino [16] for *Eucheuma* and *Kappaphycus*, which range from 35.30% to 38.67%. The standard moisture limit set by PNS [10] specifications for *Eucheuma* and *Kappaphycus* is 30% and 40%, respectively. The *K. striatum* from this study had a moisture content 0.51% above the desired limit, but it is still within the acceptable range and is therefore considered acceptable. The varying drying temperatures and long drying times directly affect the final moisture content of dried seaweeds [17]. Thus, it is essential to maintain the moisture content at the level established by government agencies that govern the industry to prevent the high perishability of seaweeds [18]. Furthermore, it must also be lowered to the standard limit to ensure that the RDS is of good quality [10].

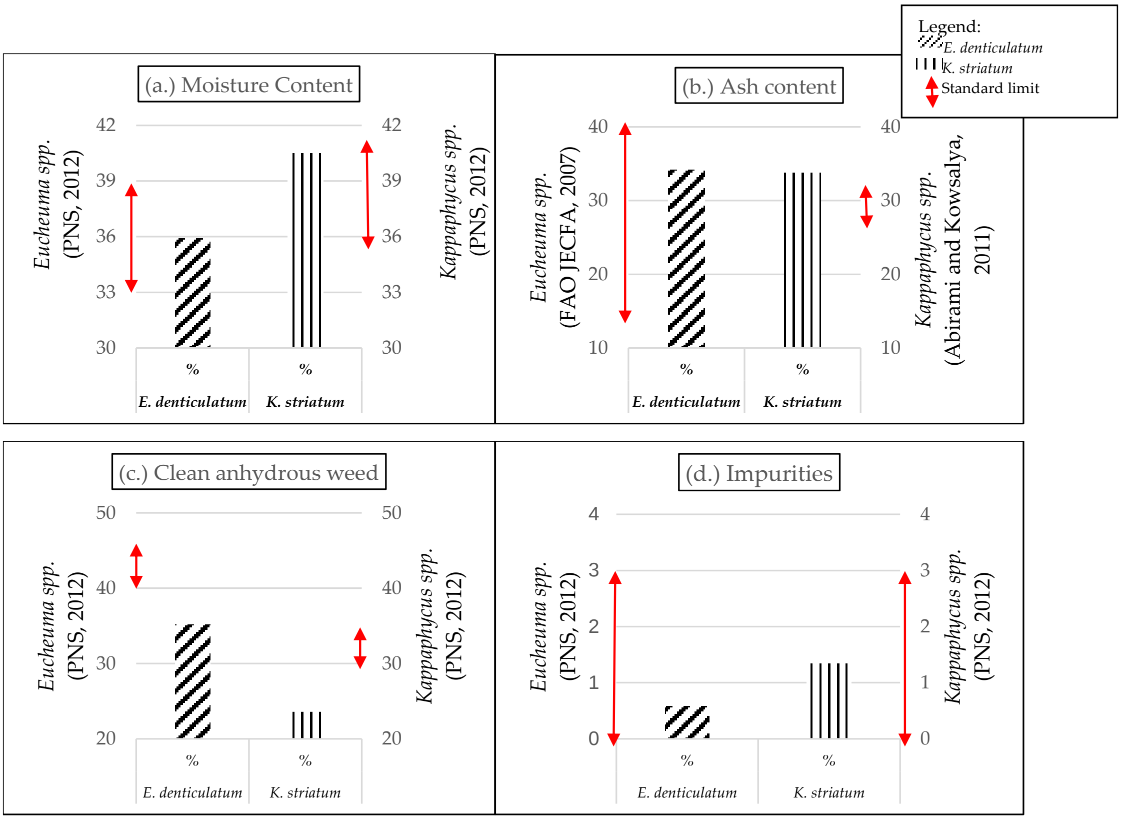

Figure 1. The RDS specifications (a. moisture content; b. ash content; c. clean anhydrous seaweed; d. impurities) of *E. denticulatum* and *K. striatum*.

The ash content represents the total mineral component of the seaweeds [19]. In this study, the ash content of the samples is 34.2% for *E. denticulatum* and 33.8% for *K. striatum*, which are considered acceptable within the FAO JECFA standard range of 15-40% [2]. In comparison, the ash content of *Kappaphycus alvarezii,* studied by Abirami and Kowsalya [15], was 28.9%, which is slightly lower than the result obtained in this study. The ash and mineral content of seaweed are influenced by factors such as drying methods and environmental conditions. Sun-drying is known to cause water leaching and exposure time, which are directly related to lower ash content in dried seaweeds [18]. The high ash content of algae makes it unsuitable for human consumption and limits its application in animal feeds. This is necessary because a high percentage renders seaweed less beneficial for both humans and animals as a food source [20]. The clean anhydrous weed is determined for dried seaweed products because it is used to analyze their salt-free dry matter content [16]. For this study, the seaweeds free of moisture, salt, sand, and impurities, in *E. denticulatum* and *K. striatum*, are 35.26% and 23.6%, respectively. The results are acceptable and fall below the standard limits set by the Philippine National Standard [10] for *Eucheuma* species (40-45%) and for *Kappaphycus* species (30-35%). The lower values obtained from this study indicate that the samples have a more accurate weight when purchased, as dirt and impurities in seaweeds can add extra weight. The impurities collected from the samples included other types of seaweed, plastic straws, dirt, sand, stones, and other foreign materials. The variation in the value of clean, anhydrous seaweed is influenced by the drying method used and the environment in which the seaweed is placed during drying. Drying trays may also affect this value, as well as the position or overlapping, which can cause crystals to form due to low evaporation and allow dirt to adhere easily to seaweed [21].

3.2 Chemical properties of carrageenan

The chemical properties of carrageenan derived from the two samples are presented in Table 1.

Table 1. Chemical properties of extracted carrageenan from *E. denticulatum* and *K. striatum*

Hydrocolloid source	Parameters			
	Yield (%)	**Moisture (%)**	**Insoluble ash (%)**	**Sulfate content (%)**
E. denticulatum	24.84 ± 0.40[a]	22.31 ± 0.04[a]	1.66 ± 0.23[a]	18.22 ± 0.78[a]
K. striatum	11.00 ± 0.47[b]	13.53 ± 0.10[a]	2.12 ± 0.92[a]	38.96 ± 0.08[b]

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

The yields of the samples were 24.84% (initial weight: 250 gms; final weight: 62.10 gms) and 11.00% (initial weight: 250 gms; final weight: 27.50 gms) for *E. denticulatum* and *K. striatum*, respectively. The yield value of *K. striatum* is statistically different among samples (p<0.05). While the moisture content for *E. denticulatum* was 22.31%, and for *K. striatum* was 13.53%. There was no significant difference among the moisture samples (p> 0.05). The acid-insoluble ash content of *E.denticulatum* was 1.66% and 2.12% for *K.striatum*. According to the FAO JECFA [2], the standard limit for acid-insoluble ash of *Eucheuma* species was 1%, similar to the value of acid-insoluble ash of semi-refined carrageenan set by PNS [10]. There was no significant difference noted among samples (p>0.05). The sulfate content of *E. denticulatum* was 18.22%, while that of the *K. striatum* was 38.96%. There was a significant difference in sulfate content between *E. denticulatum* and *K. striatum* samples (p<0.05). Yield is the ratio of dried carrageenan weight to dried seaweed weight [13]. The yield reported by Freile-Pelegrin and Robledo [22] for *Eucheuma isiforme* was 33.8-43.5%, similar to that of *E. denticulatum*. Mendoza et al. [23] studied *Kappaphycus striatum* and achieved a yield of 56.4%, which is significantly higher than the yield in this study. The yield differences between the same species across studies can be attributed to the methods used for seaweed extraction. The cited research used Aspergillus oryzae for degradation, whereas this study used an alkali pretreatment for extraction. Freile-Pelegrin and Robledo [22] emphasized that carrageenan yield decreases during hot alkaline operations because polysaccharide degradation occurs due to the rigors of processing. Diharmi et al. [24] also noted that carrageenan sourced from various regions exhibits a range of chemical and physical characteristics, particularly those associated with hydrocolloid properties, such as yield.

The moisture content of the carrageenan extracted from *E. denticulatum* was 22.31%, and for *K. striatum* was 13.53%. The values were lower than the PNS standard limit because they were further subjected to various heat applications and had a larger surface area exposed to heat. Thus, the moisture content was lower than the 33-38% limit for *Eucheuma* species and the 35-40% limit for *Kappaphycus* species. Furthermore, the EU requires a moisture content of 8% for food additives, such as carrageenan [25]. Abel and Tolentino [16] reported a moisture content of 16.99% for *E. denticulatum* and 16.65% for *K. alvarezii* in PNG carrageenan. Variation in the moisture content of dried samples can be attributed to factors such as drying temperature, time, method, and speed [26]. The acid-insoluble ash content of carrageenan extracted from *E.denticulatum* was 1.66% and 2.12% for *K. striatum*. Similar to the value of acid-insoluble ash of semi-refined carrageenan set by PNS [10], the standard limit for acid-insoluble ash of *Eucheuma* species was set by FAO JECFA [2] at 1%. The presence of a high acid-insoluble ash content indicates the presence of mineral residue or insoluble metals that remain unaltered during processing [27]. Thus, the drying and sorting processes are important to eliminate impurities in dried seaweeds, which may lead to increased levels of acid-insoluble ash in the final product.

Sulfate groups are important structural substituents of the galactose backbone of carrageenan. The method for its determination is based on the selective hydrolysis of sulfate ester by acid and subsequent selective precipitation of the sulfate ions of barium sulfate [28]. Its value is directly proportional to the gel strength of the hydrocolloid; as potassium hydroxide concentration increases, the value of sulfate decreases, and gel strength increases [13]. According to FAO JECFA [2], the sulfate content of *Eucheuma* species should be 15-40%, which was satisfied by *E. denticulatum* with 18.22%. Meanwhile, Mendoza et al. [23] reported a sulfate content of 23.5% in *K. striatum,* which was lower compared to the result from this study, which was 38.96%. Another survey of the characteristics of carrageenan had reported a sulfate content of 27.33% for *E. denticulatum* and 36.88% for *K. alvarezii* [16]. Commercially available k-carrageenan typically contains 22% (w/w) sulfate, while iota-carrageenan has 32% (w/w) sulfate, and lambda-carrageenan includes 38% (w/w) sulfate [29]. Parameters such as seasonal variation and environmental conditions are known to affect the chemical composition of carrageenan extracted from dried seaweed products [30].

3.3 Rheological properties of carrageenan

Table 2 presents the rheological properties of the samples. The gel viscosity of *E. denticulatum* was 11.05 cPs, while the *K. striatum* had a gel viscosity of 29.25 cPs. No significant difference was observed among samples ($p>0.05$). The gelling point of *E. denticulatum* was 9.83˚C and 15.67˚C for *K. striatum*. The gelling points were statistically different among the samples ($p < 0.05$). The melting point of *E. denticulatum* was 47.67˚C and 37.67˚C for *K. striatum*. The *K. striatum* was significantly different among the samples ($p < 0.05$). Hysteresis is the difference between the gelling point and the melting point. The hysteresis value for *E. denticulatum* was 37.83˚C, and 22˚C for *K. striatum*, and a significant difference was observed among samples ($p<0.05$).

Table 2. Gel rheological properties of *E. denticulatum* and *K. striatum*

Hydrocolloid source	Parameters			
	Gel viscosity (cPs)	Gelling Temperature (ºC)	Melting Temperature (ºC)	Hysteresis (ºC)
E. denticulatum	11.05 ± 0.78^a	9.83 ± 0.29^a	47.67 ± 0.58^a	37.83 ± 0.29^a
K. striatum	29.25 ± 0.72^a	15.67 ± 0.58^b	37.67 ± 0.58^b	22.00 ± 0.10^b

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

Gel formation is the most important feature of hydrocolloids; only *kappa* and *iota* carrageenan can form a gel, as other carrageenans lack the essential conformation to gel [31]. Carrageenan forms a highly viscous solution above its coil helix transition temperature. Viscosity increases exponentially with concentration, and adding salts to carrageenan reduces viscosity by shielding the charges on the polymer. It is known to increase with the molecular mass of carrageenan [32]. The gel viscosity of *E. denticulatum* and *K. striatum* is within the limit set by FAO JECFA [2], which is greater than 5 cPs. The results indicate that the correct temperature was used during the drying and extraction process of the RDS, and the extracted carrageenan from the samples was of good quality. The gelling point of *E. denticulatum* was 9.83˚C and 15.67˚C for *K. striatum*. According to Imeson [33], the gelling point of PNG carrageenan ranges from 5-20˚C, and the result of the *E. denticulatum* falls within this range. In comparison, Mendoza et al. [23] reported that the gelling point of *Kappaphycus striatum* was 34.5˚C. At the same time, the melting point of *E. denticulatum* and *K. striatum* was 47.67˚C and 37.67˚C, respectively. The values for *E. denticulatum* fall within the range cited by Imeson [33], which was 40-60˚C, while Mendoza et al. [23] reported a melting point of 56.2˚C for *K. striatum*. Finally, the hysteresis values for *K. striatum* and *E. denticulatum* were 22 °C and 37.83 °C, respectively.

Carrageenan is commonly used in the food sector; therefore, understanding the gelling point is an important consideration when making any food products. It refers to the temperature at which food materials, such as jelly, take on a gel-like consistency. Knowing the gelling point, food manufacturers can calculate the lowest temperature required to maintain the jelly's desired quality. The melting point, on the other hand, can be used to determine the highest temperature at which food items can be stored before consumption. Knowing the melting point enables food manufacturers to establish the temperature restrictions necessary to maintain the food's quality [34-36]. The gelling point of carrageenan from *E. denticulatum* is lower than that of *K. striatum* because it predominantly contains ι-carrageenan, which is more highly sulfated and therefore undergoes coil–helix transition and gelation at lower temperatures [12]. However, the melting point of *E. denticulatum* is higher because ι-carrageenan forms stronger, calcium-mediated ionic cross-links, resulting in a more thermally stable gel network that requires more energy to disrupt. In contrast, *K. striatum*, which is richer in κ-carrageenan, forms a firmer gel at higher temperatures due to its higher 3,6-anhydro-D-galactose content; however, this network is mainly stabilized by potassium ions and weaker intermolecular forces, resulting in a lower melting temperature [37]. Hysteresis, as the temperature difference between gel melting and gel setting, is a well-recognized phenomenon in carrageenan systems and reflects the thermal stability and functional performance of carrageenan gels [12, 33]. A larger hysteresis value indicates a more thermally stable, strongly cross-linked gel network that requires greater thermal energy to melt once formed. In contrast, a smaller hysteresis suggests a less stable gel structure with weaker intermolecular interactions. In carrageenan, this temperature gap is governed by the degree of sulfation, the 3,6-anhydro-D-galactose content, and the dominant cations (K+ or Ca2+) that stabilize the polymer matrix [37]. Environmental factors and the different physiological and environmental tolerances influence variation in carrageenan content [38]. This phenomenon has been observed in individuals of the same species reared under various environmental conditions [39]. Its properties vary widely depending on harvest time, region, growth conditions (salinity, depth, and nutrient levels), growth stage, extraction method, and other parameters [40]. Thus, it was expected that the results of this study would show both similarities and differences compared with the same or related species. These variations are likely due to differences in internal and external factors that affect seaweeds.

4. Conclusions

Based on the extraction process using the two seaweed species from Caluya, Antique, it can be concluded that *Eucheuma denticulatum* "*Milyon milyon*" exhibited better quality properties than *Kappaphycus striatum* "*Sacol*". Most of its parameters met the industry standards. It was also further observed that the standard drying method for *Kappaphycus* spp. is unsuitable for *Kappaphycus striatum* "*Sacol*" as it negatively affects the final dried product and results in a lower yield than the other variable. Therefore, further studies are needed to optimize the drying temperature for *Kappaphycus striatum* "*Sacol*" and explore its other potential industrial applications.

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