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Comparative study on the physicochemical properties of κ-carrageenan extracted from *Kappaphycus alvarezii* (doty) doty ex Silva in Tawau, Sabah, Malaysia and commercial κ-carrageenans

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ABSTRACT

κ-Carrageenan is a linear, sulphated polysaccharide that is widely used in the food industry as a gelling agent due to its lack of toxicity and biocompatibility. In this study, the physicochemical properties of κ-carrageenan (TA150) derived from *Kappaphycus alvarezii* (formerly *Eucheuma cottonii*) in Tawau, Sabah were investigated and compared to commercial κ-carrageenan (SeaKem CM611, Gelcarin GP812, Gelcarin GP911 NF, and Grindsted® carrageenan CL220). TA150 exhibited the lowest lightness but highest yellowness, with L^* and b^* values reported as 82.69 and 17.16, respectively. The rupture strength of κ-carrageenan increased significantly with increasing concentration ($p < 0.05$). The water losses from κ-carrageenan gel increased with increasing storage times. TA150 lost the most water within 10 days of storage time. The water-holding capacity (WHC) of κ-carrageenan gel was reported to be excellent (>90%) under all storage temperatures (25 °C, 4 °C and −18 °C). The moisture content, ash, acid-insoluble matter, and sulphate levels of κ-carrageenan samples were reported as 3.65–11.41%, 17.75–33.18%, 0.22–3.74%, and 12.00–19.71%, respectively. These samples were low in fat, protein, and crude fibre contents. The potassium content in κ-carrageenan was highest in Gelcarin GP812 (100.42 g/kg), followed by Grindsted® carrageenan CL220 (61.92 g/kg), TA150 (54.60 g/kg), Gelcarin GP911 NF (40.90 g/kg) and SeaKem CM611 (15.76 g/kg). No heavy metals were detected in TA150 and the other commercial κ-carrageenan samples except for lead. However, the concentration of lead detected in the κ-carrageenan samples fell within the acceptable ranges (<5 mg/kg) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

1. Introduction

κ-Carrageenan, a marine-based polysaccharide, is extracted from red seaweed (*Eucheuma cottonii)*, in which it is the major structural component of cell walls (Burey, Bhandari, Howes, & Gidley, 2008; Pereira & van de Velde, 2011). *E. cottonii*, typically known as *Kappaphycus alvarezii*, is a spiny, bushy plant consisting of numerous round branches (Imeson, 2000; Rudolph, 2000). It is abundant on the inner sides of coral reefs around the Philippines, Indonesia and the island coasts in East Africa (Imeson, 2000; Rudolph, 2000). The extract of *E. cottonii* contains almost pure κ-carrageenan, with less than 10% ι-carrageenan (Lee, Lo, & Chye, 2008). Its high level of κ-carrageenan makes it an economically important red seaweed (Lee et al., 2008).

In the food industry, κ-carrageenan is used as a gelling, thickening, stabilising, and water-binding agent in various food products, such as instant products, dessert, sauces, milk, yogurt, and meats (Dyrby et al., 2004; Mou, Jiang, Liu, & Guan, 2004). Moreover, κ-carrageenan is able to interact with other food polymers, such as proteins and starches, in meat and dairy products and is used in the confectionary and beer industries (Al-Alawi, Al-Marhubi, Al-Belushi, & Soussi, 2011). In addition to its typical function, κ-carrageenan was recently studied with the aim to produce an effective controlled release carrier in matrix, bead, microcapsule, and microgel forms, which is useful for the encapsulation of bioactive compounds (Ellis & Jacquier, 2009; Keppeler, Ellisa, & Jacquier, 2009).

Due to its wide range of applications, the carrageenan industry has grown rapidly in the last 15 years, with an annual growth rate

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of 8%, producing 28,000 metric tonnes of carrageenan with a value of US$ 270 million (Freile & Robledo, 2008). The carrageenan industry represents a multimillion-dollar growing worldwide market. Therefore, Malaysia has taken a great interest in *E. cottonii*, the κ-carrageenan-producing red seaweed. For the last three decades, *E. cottonii* has been largely cultivated on the east coast of Sabah, Malaysia (Lee et al., 2008). In the early 1970s, *E. cottonii* was manufactured as a component of canned pet food. By the early 1980s, the quality of alkali-treated flour had been improved with respect to colour, odour, and taste and was exported as food grade κ-carrageenan (Lee et al., 2008). The first food-grade natural κ-carrageenan in Malaysia was produced in Tawau, Sabah. Tawau is located near the world's largest seaweed-producing areas of Tawi-Tawi in the southern Philippines and northern Sulawesi. Similar to water condition in Celebes Sea, Tawau is an area suitable for the cultivation of *E. cottonii*.

To the best of our knowledge, very little information is available on the physicochemical properties of κ-carrageenan isolated from *E. cottonii* in Tawau, Sabah. κ-carrageenan derived from particular seaweed species and geographic region may differ considerably in term of their solution and gelling properties. Therefore, the physicochemical analysis of κ-carrageenan is of great importance for industry to determine its values and applications in various food products. Furthermore, κ-carrageenan extracted from *E. cottonii* in Sabah has the potential to satisfy the world's increasing need for κ-carrageenan. The aim of this research study is thus to examine the physicochemical properties of κ-carrageenan extracted from *E cottonii* in Tawau, Sabah and to compare these properties with those of commercial κ-carrageenan samples. In addition, the microbiological properties of locally produced κ-carrageenan were investigated to ensure its safety for use in food products.

2. Materials and methods

2.1. Materials

κ-Carrageenan powder samples used in this study were obtained as gift from the indicated suppliers: TA150 (Tacara Sdn. Bhd., Tawau, Malaysia), SeaKem CM611, Gelcarin GP812, Gelcarin GP911 NF (FMC Biopolymer, Philadelphia PA, USA), and Grindsted®carrageenan CL220 (Danisco, Copenhagen K, Denmark). TA150 was produced from *E. cottonii* harvested in Tawau, Sabah. All the solvents and chemicals used for the analyses were of analytical grade. Deionized water used for the preparation of all the solutions was purified by Sartorius Atrium®611D1 (Sartorius Stedim Biotech, Germany).

2.2. Physical analysis

Five κ-carrageenan samples were stored in a cool and dark place before the analysis. κ-Carrageenan samples were subjected to various physical tests: colour, particle size, pH, texture, syneresis, and water holding capacity. All the results were reported as the average of triplicate measurements (mean ± standard deviation).

2.2.1. Sample preparation

κ-Carrageenan solution of defined concentration was prepared by dispersing required amount of κ-carrageenan sample powders into a hot (80 °C) deionized water. The solutions were magnetically stirred at 700 rpm for 1 h to ensure complete dissolution of the powder.

2.2.2. Colour

The colour of κ-carrageenan samples powder were measured using HunterLab UltraScan PRO colorimeter (Hunter Associate Laboratory Inc., Reston, USA) and values were expressed as L^*, a^*, and b^*.

2.2.3. Particle size

Particle size of κ-carrageenan samples were determined by using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK) with a dry powder dispersion accessory (Scirocco 2000). This machine can detect particle sizes ranging from 0.02 to 2000 μm. Pressure was set as 2 bar and a vibration feed of 30% was used. Refractive index of κ-carrageenan powder was set as 1.44. The particle size of κ-carrageenan powder was expressed in Sauter Diameter $D[3,2]$ $(=\sum n_i d_i^3 / \sum n_i d_i^2$, where n_i is number of particles with diameter d_i). Each sample was analyzed in triplicate and the results were reported as an average.

2.2.4. pH

1.5% (w/v) κ-carrageenan solutions were prepared and cooled to room temperature (25 ± 0.5 °C). pH meter was then used to measure the pH values of the κ-carrageenan solutions.

2.2.5. Texture

κ-Carrageenan solutions of 2.0–4.0% (w/v) were prepared for all the samples except SeaKem CM611. For SeaKem CM611, solutions of 4.0–6.0% (w/v) were prepared. The samples were allowed to set in the containers to form a gel of size 30 mm × 30 mm. Gel samples were stored overnight at room temperature (25 °C) prior to measurement. The TA.XT2i texture analyser (Stable Micro Systems, New York, USA) was used to determine the rupture strength and brittleness of the gel samples. A cylindrical probe of 0.5 inches diameter (P/0.5) was used. The cylindrical gel samples were underwent compression under a 5 kg load cell at a deformation rate of 20 mm/min.

2.2.6. Syneresis

κ-Carrageenan solutions 4% (w/v) were prepared, and the gels were set inside Petri dishes and cut into 30 mm × 8 mm sizes. The cylindrical gels formed were stored overnight in a container at room temperature (25 °C) for 2, 4, 6, 8, and 10 days. The initial weight of the gel samples (W_0) and the weight of the empty container were recorded. At certain time intervals (days), the water that condensed on the container walls was removed with tissue paper. After this water was removed, the weights of the gels were measured (W_t). The syneresis of the gels was calculated as the cumulative weight of the water collected divided by the weight of original sample and multiplied by 100:

$$\text{syneresis}(\%) = [(W_0 - W_t)/W_0] \times 100 \quad (1)$$

where W_0 is the initial weight of the gel and W_t is the weight of the gel on day t.

2.2.7. Water-holding capacity

Similar to the analysis of syneresis, 4% κ-carrageenan solutions were prepared. The gels were set inside a 15 ml centrifuge tube to form gels with heights of 100 mm. The initial weights of the gels were determined and recorded as W_0. The gels were stored overnight at room temperature (25 °C), 4 °C, and −18 °C for 24 h prior to centrifugation. The gels were centrifuged using a bench-top centrifuge (Sigma 3-18K, Osterode am Harz, Germany) at 4000 rpm for 30 min at 25 °C. After centrifugation, the supernatants were drained, and the weights of the gels were taken immediately after the centrifugation runs. The weight ratio (W_t/W_0) was used to characterise the water-holding capacity (WHC) of the gels.

$$\mathrm{WHC}(\%) = (W_t/W_0) \times 100 \quad (2)$$

where W_0 is the initial weight of the gel and W_t is the weight of the gel on day t.

2.2.8. Microscopy observation

The internal structures of κ-carrageenan gels stored at different temperature (25 °C, 4 °C, and −18 °C) were observed using a light microscope (Nikon eclipse 80i Binocular, USA) equipped with a CCD camera (Nikon 5 megapixel, Kanagawa, Japan) connected to digital image processing software (NIS-Elements Basic Research, Nikon Instruments). κ-Carrageenan gels were sliced into thin cross sections, placed on a microscope slide and covered with cover clip prior to observation under microscope. The samples were then analyzed at room temperature, using a magnification of 400×.

2.3. Chemical analysis

Moisture content, fat, protein, ash, acid-insoluble ash, crude fibre, acid-insoluble matter, sulphate, mineral contents and heavy metals of κ-carrageenan were determined using the AOAC International methods (AOAC, 2000) with slight modifications. All the results were reported as the average of triplicate measurements (mean ± standard deviation) and were expressed in percentage (% w/w) based on a dry weight basis.

2.3.1. Proximate analysis

Moisture content of different κ-carrageenan samples was determined using air drying oven method (AOAC 934.01). Fat content was determined by Soxhlet extraction method using petroleum ether (AOAC 991.36). Protein content was determined by the micro Kjeldahl distillation method (AOAC 981.10). A correction factor of 6.25 was applied to convert the % nitrogen to % protein. For ash determination, ashing was carried out in a muffle furnace at 550 °C (AOAC 930.05). For acid-insoluble ash, hydrochloric acid was used for digestion prior to ashing. The crude fibre content was determined based on acid–alkaline neutralization method. The κ-carrageenan samples were digested in sulphuric acid and then neutralized with sodium hydroxide (Aziah & Komathi, 2009).

2.3.2. Acid-insoluble matter and sulphate determination

κ-Carrageenan samples were treated with hydrochloric acid for an acid-insoluble matter determination. The sulphate content was determined based on the formation of white precipitates of barium sulphate. The sulphate content was calculated by multiplying the weight of barium sulphate by a conversion factor (0.4116).

2.4. Mineral and heavy metals analysis

Four minerals (sodium, potassium, calcium, and magnesium) and heavy metals (arsenic, lead, cadmium, and mercury) that commonly found in κ-carrageenan were analyzed using atomic absorption spectrophotometer (AAS) as described by Noel, Carl, Vastel, and Guerin (2008) with slight modifications. Results were expressed in g/kg or mg/kg of triplicate readings.

2.4.1. Test solution preparation

κ-Carrageenan samples were prepared using the wet-ashing method. 1.0 g of κ-carrageenan was dissolved in 10 ml of aqua regia (1:3 of concentrated HCl:HNO_3). The mixture was stirred well to ensure a complete dissolution of the sample. If the sample did not fully dissolve in the acid solution, heat was applied for at least 6 h to completely dissolve the sample. The dissolved sample was filtered through an ashless Whatman filter paper into a 100 ml volumetric flask to obtain a clear or yellowish solution. Volumetric flask was topped up to the 100 ml mark with deionised water and then mixed thoroughly. The test solutions were diluted according to the elements being analysed.

2.4.2. AAS determination

The digested samples were analysed for their mineral and heavy metal contents by AAS (Thermo Scientific S-series, Massachusetts, USA). All elements in the AAS analysis were assessed using an air–acetylene flame (fuel flow: 1.2 L/min), with the exception of As and Ca, which used a nitrous oxide–acetylene flame (fuel flow: 4.2 L/min). The standard curve was constructed for each element with an absorbance reading versus concentration in ppm. The results of mineral and heavy metal contents were expressed in g/kg (Eq. (3)) and mg/kg (Eq. (4)), respectively, using the following equations:

$$w = [(c \times v)/m \times 1000] \times f \quad (3)$$

$$w = [(c \times v)/m \times 1000] \times f \times 1000 \quad (4)$$

where w is the element concentration of the sample (g/kg or mg/kg), c is the element concentration (ppm) as read from the calibration curve, v is the volume (ml) of the flask to which the wet ashes were transferred, m is the sample mass (g), and f is the dilution factor of the test solution carried out during the preparation step.

2.5. Microbiological analysis

Determination of total plate count, yeast and mould, coliform and *Escherichia coli*, and *Salmonella* were conducted according to the methods in Joint FAO/WHO Expert Committee on Food Additives 2006 (JECFA, 2006).

2.5.1. Sample preparation

0.1 g of κ-carrageenan sample was mixed with 99 ml of sterile buffered peptone water in a stomacher bag. The mixture was homogenized in a stomacher for 2 min.

2.5.2. Microbiological test

Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were prepared by transferring 1 ml of previous dilution to 9 ml of 0.1% buffered peptone water. All dilutions were shook for 25 times and 1 ml of each dilution was pipetted into separate, duplicate, appropriately marked petri dishes. Total plate count determined using plate count agar, yeast and mould using potato dextrose agar, coliform and *E. coli* using Chromocult® coliform agar, and *Salmonella* using xylose lysine deoxycholate (XLD) agar. Plates for coliform and *E. coli* and *Salmonella* were incubated at 35 ± 1 °C for 24 h while plates for total plate count were incubated for 48 h at the same temperature. For yeast and mould, the plates were incubated at room temperature (25 °C) for 5 days. Plates for *Salmonella* were incubated for 24 h at 37 °C. After the incubation, all the colonies on the normal plates (25–250 colonies) were counted. Plates with less than 25 colonies were reported as too few to count (TFTC).

$$\text{Colony count}(\text{cfu/ml}) = \text{Number of colonies}/(\text{Volume plated} \times \text{dilution}) \quad (5)$$

2.6. Statistical analysis

The experimental results were analysed using Minitab software (Minitab Version 14.1). All data are expressed as the means ± standard deviations of triplicate measurements. One-way

analysis of variance (ANOVA) at the 5% significance level was used to determine significant differences ($p < 0.05$) between means.

3. Results and discussion

3.1. Physical properties

The physical properties of five different κ-carrageenan samples, TA150, SeaKem CM611, Gelcarin GP812, Gelcarin GP911 NF and Grindsted® carrageenan CL220, are presented in Tables 1 and 2 and Figs. 1 and 2. TA150 is the local κ-carrageenan produced from *E. cottonii* cultivated in Tawau, Sabah, while the others are commercial κ-carrageenan obtained from selected suppliers.

3.1.1. Colour, particle size, and pH

Hunterlab colourimetry results (Table 1) showed that κ-carrageenan samples were significantly different in terms of lightness (L^*), redness (a^*) and yellowness (b^*) ($p < 0.05$). L^* represents the degree of lightness, whereas 100 indicates white, and 0 indicates black. Redness is represented by $+a^*$, while $-a^*$ indicates greenness. Yellowness is represented by $+b^*$, while blueness is represented by $-b^*$ (Aziah & Komathi, 2009; Jamilah, Shu, Kharidah, Dzulkifly, & Noranizan, 2011). In general, κ-carrageenan powder appears white or light yellow to tan in colour. Thus, L^* and b^* values are more important in describing the colour of κ-carrageenan powder. In this study, the locally produced κ-carrageenan TA150 had the lowest L^* value (82.69) compared to the other commercial κ-carrageenan, indicating that TA150 has the darkest colour of all tested samples. By contrast, SeaKem CM611 exhibited the lightest colour, with the highest L^* value (88.87; $p < 0.05$). In addition, TA150 had the highest yellowness b^* value (17.16), but Seakem CM611 had the lowest b^* value (11.08). By combining L^* and b^*, TA150 has the darkest colour, while SeaKem CM611 has the lightest colour.

The results for particle size showed that the particle sizes of κ-carrageenan samples ranged from 57.93 μm to 96.08 μm; all fell below 100 μm in size. The pH of all κ-carrageenan sample solutions rose above 7 and varied from 8.04 to 10.09. This finding was in accordance with the specification set by JECFA (2006) that the pH of carrageenan must range from 8 to 11. The alkalinity of κ-carrageenan is mainly caused by the sulphate (SO_4^{2-}) group in the disaccharide unit and the variation is strongly dependent on its food applications (pH of foods) (Jones, Adamcik, Handschin, Bolisetty, & Mezzenga, 2010; Yaseen, Herald, Aramouni, & Alavi, 2005). For instance, Gelcarin GP812 which is specifically formulated for low pH gelatin-like water dessert gels had the lowest reported pH (8.04).

3.1.2. Textural properties

The addition of food additives, such as emulsifiers, thickeners, and gelling agents, may alter the textures of final products and therefore, studies of the textural properties of κ-carrageenan are very important for better control of final product textures. In this study, two textural properties of κ-carrageenan were examined: rupture strength and brittleness. According to Raina and Babbar (2011), gel strength is the amount of force required to rupture the gel. The rupture strength is an indication of the maximum force required to rupture the gel. The distance that the probe penetrates through the gel before the break occurs is expressed as gel brittleness (Raina & Babbar, 2011). Textural analysis is therefore more descriptive of the gel texture than simple gel strength measurements.

Textural measurements of κ-carrageenan samples were carried out at increasing gel concentrations. The concentrations of all κ-carrageenan samples varied from 2% to 4% (w/v), with the exception of SeaKem CM611. When TA150 and other κ-carrageenan samples were dissolved in hot water and cooled, they formed thermo-reversible gels at concentrations as low as 2% (w/v). At concentration higher than 4% (w/v), these samples started to clump and showed resistance to stirring. In the other hand, SeaKem CM611 did not form a gel at concentrations below 4% (w/v). A higher concentration was required for SeaKem CM611 to form a gel. SeaKem CM611 behaves differently from other carrageenan samples because it is a carrageenan product that specifically formulated for use in stabilizing chocolate milk instead of gelling and encapsulation functions as compared to other carrageenan sample. Thus, a range of 4–6% (w/v) was selected.

As tabulated in Table 2, the rupture strength of κ-carrageenan increased significantly with increasing concentration ($p < 0.05$). When the gels had higher water contents, they showed lower mechanical strength values. At the same concentration, Gelcarin GP812 showed the highest rupture strength, followed by Gelcarin GP911 NF, TA150, Grindsted® carrageenan CL220, and SeaKem CM611. Moreover, the increase in the rupture strength of Gelcarin GP812 was highest, from 9.89 N (at 2.0%) to 31.23 N (at 4.0%), giving a total increase of 215.77%. The brittleness of κ-carrageenan is presented in Table 2. A low brittleness value indicates a more brittle gel. Grindsted® carrageenan CL220 was the most brittle gel, as indicated by its lowest brittleness value. It was assumed that κ-carrageenan content in Grindsted® carrageenan CL220 is higher than other k-carrageenan samples.

3.1.3. Functional properties

κ-Carrageenan gel retains a large amount of water when undergoing a solution/gel transition. Due to intrinsic instability, water may be lost from κ-carrageenan via syneresis after an extensive storage time (Mao, Tang, & Swanson, 2001). Water loss may result in gel shrinkage, textural changes, and reductions in quality. These problems have become a challenge in the manufacturing of food gels. On the other hand, the water-holding capacity (WHC) is expressed as the capability of the gel to retain this water when subjected to external forces.

Table 1
Physical properties of commercial κ-carrageenan and κ-carrageenan isolated from Sabah *Eucheuma cottonii*.

Sample	Colour			Particle size (μm)	pH	WHC (%)		
	L^*	a^*	b^*			25 °C	4 °C	−18 °C
TA150	82.69 ± 0.23^{d}	2.10 ± 0.01^{a}	17.16 ± 0.15^{a}	86.50 ± 0.94^{b}	9.89 ± 0.08^{a}	99.74 ± 0.09^{a}	99.75 ± 0.10^{a}	98.56 ± 0.44^{a}
SeaKem CM611	88.87 ± 0.13^{a}	1.60 ± 0.05^{b}	11.08 ± 0.13^{c}	74.60 ± 3.36^{c}	10.09 ± 0.28^{c}	98.28 ± 1.62^{a}	99.02 ± 0.13^{b}	91.69 ± 3.85^{b}
Gelcarin GP812	83.84 ± 0.68^{c}	2.13 ± 0.02^{a}	13.13 ± 0.27^{b}	57.93 ± 2.36^{e}	8.04 ± 0.68^{c}	98.34 ± 0.33^{a}	98.79 ± 0.40^{b}	92.25 ± 1.18^{b}
Grindsted® carrageenan CL220	87.51 ± 0.42^{b}	0.27 ± 0.01^{c}	11.91 ± 0.57^{c}	96.08 ± 2.33^{a}	8.20 ± 0.42^{bc}	98.79 ± 0.28^{a}	98.83 ± 0.24^{b}	99.02 ± 0.05^{a}
Gelcarin GP911 NF	83.63 ± 0.29^{cd}	2.18 ± 0.09^{a}	12.37 ± 0.51^{bc}	62.47 ± 2.65^{d}	8.34 ± 0.39^{b}	98.75 ± 0.09^{a}	98.79 ± 0.27^{b}	95.31 ± 1.80^{ab}

Data were expressed as mean ± standard deviation ($n = 3$).
Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

Table 2
Textural properties of commercial κ-carrageenan and κ-carrageenan isolated from Sabah *Eucheuma cottonii*.

Sample	Concentration (%)	Rupture strength (N)	Brittleness (mm)
TA150	2.0	4.28 ± 0.47^{e}	14.32 ± 0.56^{a}
	2.5	8.14 ± 0.24^{d}	15.87 ± 0.33^{a}
	3.0	11.06 ± 0.36^{c}	15.09 ± 1.14^{a}
	3.5	12.93 ± 0.36^{b}	15.83 ± 0.92^{a}
	4.0	14.68 ± 0.14^{a}	16.29 ± 0.82^{a}
SeaKem CM611	4.0	0.64 ± 0.05^{e}	16.57 ± 0.41^{a}
	4.5	1.46 ± 0.11^{d}	18.13 ± 0.64^{a}
	5.0	2.51 ± 0.13^{c}	17.51 ± 0.89^{a}
	5.5	3.21 ± 0.01^{b}	19.16 ± 1.19^{a}
	6.0	3.98 ± 0.09^{a}	18.97 ± 0.97^{a}
Gelcarin GP812	2.0	9.89 ± 0.19^{e}	15.59 ± 0.58^{a}
	2.5	15.31 ± 0.64^{d}	16.16 ± 0.96^{a}
	3.0	19.67 ± 1.23^{c}	16.41 ± 0.83^{a}
	3.5	23.45 ± 0.25^{b}	16.20 ± 2.12^{a}
	4.0	31.23 ± 1.14^{a}	14.93 ± 0.41^{a}
Grindsted® carrageenan CL220	2.0	1.15 ± 0.01^{d}	8.90 ± 0.120^{b}
	2.5	5.15 ± 0.63^{c}	9.64 ± 0.39^{ab}
	3.0	6.29 ± 0.08^{c}	10.56 ± 0.58^{ab}
	3.5	10.79 ± 0.41^{b}	10.63 ± 0.93^{ab}
	4.0	14.94 ± 0.09^{a}	11.21 ± 0.23^{a}
Gelcarin GP911 NF	2.0	6.08 ± 0.03^{d}	18.44 ± 0.68^{a}
	2.5	8.13 ± 0.09^{d}	17.49 ± 0.08^{a}
	3.0	12.85 ± 0.28^{c}	16.92 ± 0.16^{a}
	3.5	17.50 ± 0.94^{b}	16.87 ± 0.90^{a}
	4.0	20.48 ± 0.88^{a}	16.80 ± 0.40^{a}

Data were expressed as mean ± standard deviation ($n = 3$).
Mean values with different superscripts in the same column are significantly different at $p < 0.05$.

The results for syneresis and WHC are summarised in Fig. 1 and Table 1. Syneresis of κ-carrageenan gels was significantly increased with increasing storage time ($p < 0.05$). The highest syneresis corresponded to TA150. In the first 2 days, 2.79–5.02% of water was extruded from the κ-carrageenan gels. Syneresis of κ-carrageenan gels reached 7.80–21.50% after 10 days of storage time. Lee et al. (2008) observed the same phenomenon, in which the water loss for κ-carrageenan gels increased with increasing storage time with up to 5% of water liberated from the κ-carrageenan gels.

The WHC of κ-carrageenan gels was observed at three different storage temperatures (25 °C, 4 °C, and −18 °C). Compared to 25 °C, the WHC of κ-carrageenan gels improved slightly when stored at 4 °C. Double helical structure of κ-carrageenan gel holds the water molecules firmer within the interstices of the three dimensional framework at lower temperature. However, this improvement was not significant ($p > 0.05$). The WHC of κ-carrageenan gels stored at −18 °C were found dropped significantly ($p < 0.05$). Fig. 2 showed that κ-carrageenan gel stored at −18 °C appeared porous, while κ-carrageenan gel stored at 25 °C and 4 °C appeared smooth. This suggests that formation of ice crystals in gels during freezing adversely affected the microstructure of κ-carrageenan gel, and

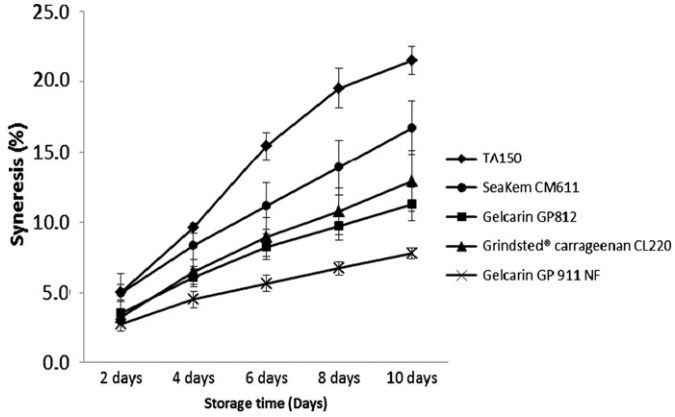

Fig. 1. Syneresis of commercial κ-carrageenan and κ-carrageenan isolated from Sabah *Eucheuma cottonii*. Data were expressed as mean ± standard deviation ($n = 3$).

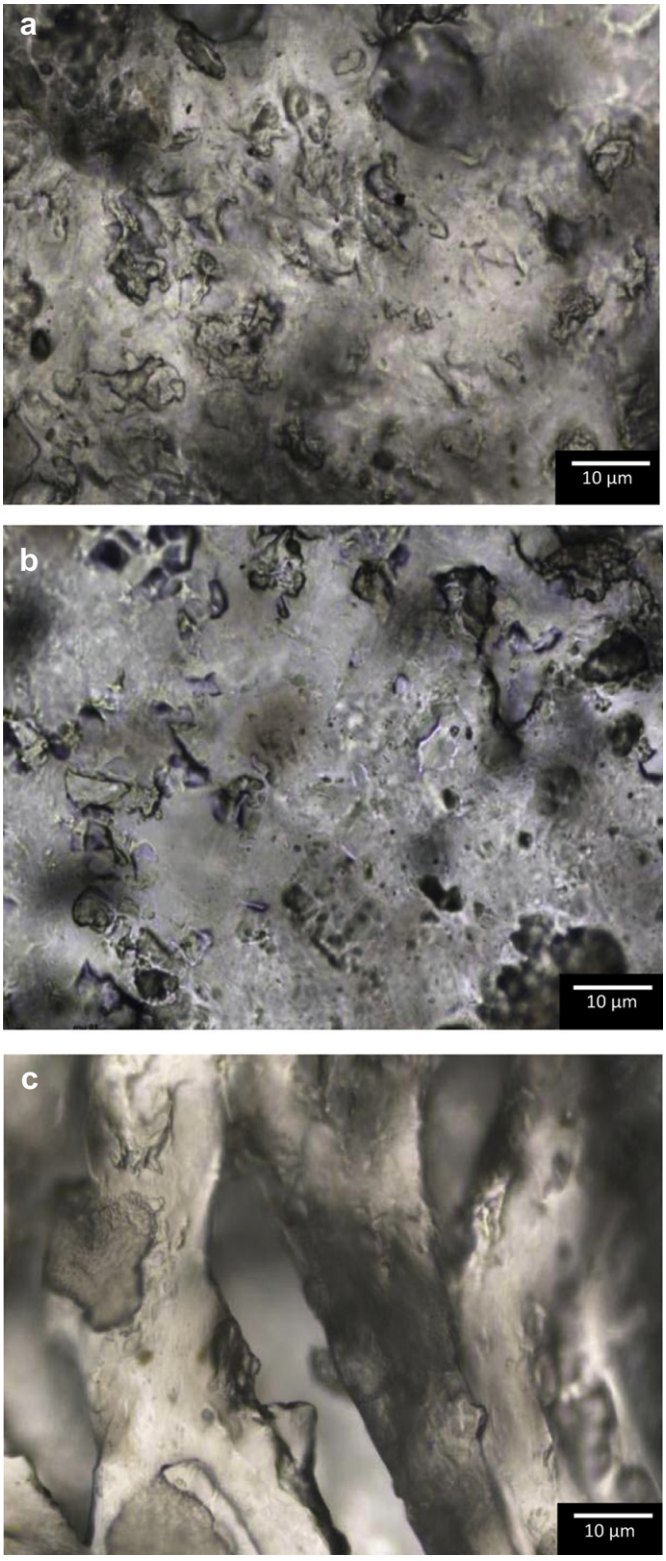

Fig. 2. Microscopic observation of κ-carrageenan gel stored at different temperature: (a) 25 °C, (b) 4 °C, and (c) −18 °C. Scale bar in all images represents 10 μm.

thus reduce their water holding capacity. Nevertheless, the overall WHC of κ-carrageenan was excellent (>90%) regardless of the storage temperature.

3.2. Chemical properties

The chemical analysis of the κ-carrageenan samples was divided into three major parts: (i) proximate analysis; (ii) acid insoluble matter and sulphate contents; and (iii) mineral and heavy metal contents. The results are presented in Table 3.

3.2.1. Proximate analysis

In food systems, proximate analysis allows the quantitative analysis of the different macronutrients that are found in a food product. Proximate analysis partitions food components into five main categories: moisture, ash, protein, fat and fibre. For κ-carrageenan, the moisture and ash contents are the most crucial components in determining the quality of the product. Other components, such as fat, protein and crude fibre, were present at negligible levels (<1%) in κ-carrageenan (Table 3). *E. cottonii*, raw material of κ-carrageenan, is a poor source of fat and protein and its crude fibre (cellulose and lignin) was break down through alkaline extraction to liberate the κ-carrageenan.

Water plays a crucial role in food products especially dry powdered products. The storage quality of powdered foods will be affected if the moisture content exceeds 14% because mould growth, insect infestation, and clumping will start to occur (Aziah & Komathi, 2009). According to the Food Chemicals Codex (FCC) (1981), the maximum moisture content allowed in carrageenan is 12%. κ-Carrageenan tends to absorb moisture over time due to its hygroscopic properties. Based on the results of the present study, the moisture content of the studied κ-carrageenan samples (3.65–11.41%) fell below the upper limit (12%); TA150 had the lowest moisture content. An earlier study by Al-Alawi et al. (2011) reported that the moisture content of κ-carrageenan extracted from *Hypnea bryoides* was slightly lower (4.07–9.46%) than that of TA150. The fluctuation in the moisture content of κ-carrageenan is thought to be affected by the drying temperature, drying time, and storage conditions.

Ash is the most abundant component in κ-carrageenan. The ash fraction of κ-carrageenan is mainly made up of macro-minerals, such as potassium, sodium, calcium and magnesium (Hurtado-Ponce, 1995). The international standard of ash levels in carrageenan is at least 15%, but not more than 40% (JECFA, 2006). The analysis showed that the ash content of κ-carrageenan was in the range of 17.75–33.18%, with the maximum level found in Gelcarin GP812 (33.18%) and the minimum level found in SeaKem CM611 (17.75%). This result was in accordance with Basmal, Sedayu, and Utomo (2009), who found that the ash content of κ-carrageenan ranged from 18.5 to 36.4% and varied with processing techniques and sanitation levels. Amimi, Mouradi, Bennasser, and Givernaud (2007) stated that the levels of minerals (ash content) in the thallus of seaweed were greatly affected by seasonal variations.

The acid-insoluble ash (AIA) measurement is designed to measure the amount of insoluble ash in diluted hydrochloric acid. The presence of AIA in carrageenan may due to processing contamination occurring as a result of such factors including water impurities, equipment contamination, chemical impurities, and original minerals found in seaweed. The results of the experiment showed that the AIA of κ-carrageenan ranged from 0.14 to 3.02%. The AIA of Gelcarin GP911 NF (3.02%) exceeded the international standard (1.0%). The present levels were much higher than the AIA contents (0.57–0.90%) in κ-carrageenan that were reported by Basmal et al. (2009).

3.2.2. Acid-insoluble matter and sulphate determination

Acid-insoluble matter (AIM) of κ-carrageenan consists of a network of cellulose that is normally present in algae cell walls (Imeson, 2000). AIM can modify the hydration, appearance and gel characteristics of the κ-carrageenan gel (Imeson, 2000). In the present study, the AIM of κ-carrageenan ranged between 0.22% and 3.74%. The AIM in TA150 was found to be 3.22%. In Philippine

Table 3
Chemical composition of commercial κ-carrageenan and κ-carrageenan isolated from Sabah *Eucheuma cottonii*.

Chemical composition	TA150	SeaKem CM611	Gelcarin GP812	Grindsted® carrageenan CL220	Gelcarin GP911 NF
Proximate					
Moisture content (% w/w DW)	3.65 ± 0.17^{d}	10.02 ± 0.20^{c}	10.98 ± 0.33^{b}	11.41 ± 0.20^{a}	9.95 ± 0.06^{c}
Fat (% w/w DW)	0.15 ± 0.01^{b}	0.05 ± 0.00^{c}	0.22 ± 0.03^{b}	0.50 ± 0.07^{a}	0.03 ± 0.01^{c}
Protein (% w/w DW)	0.14 ± 0.00^{c}	0.08 ± 0.02^{d}	0.31 ± 0.03^{a}	0.26 ± 0.04^{b}	0.29 ± 0.04^{ab}
Ash (% w/w DW)	18.72 ± 0.03^{c}	17.75 ± 0.78^{c}	33.18 ± 1.43^{a}	18.32 ± 1.00^{c}	26.48 ± 0.15^{b}
Acid-insoluble ash (% w/w DW)	0.14 ± 0.01^{c}	0.14 ± 0.03^{c}	0.35 ± 0.04^{b}	0.25 ± 0.02^{bc}	3.02 ± 0.22^{a}
Crude fibre (% w/w DW)	0.26 ± 0.02^{a}	0.12 ± 0.01^{b}	0.13 ± 0.03^{b}	0.30 ± 0.01^{a}	0.32 ± 0.05^{a}
Others					
Acid-insoluble matter (% w/w DW)	3.22 ± 0.20^{b}	0.22 ± 0.04^{d}	0.32 ± 0.06^{d}	1.01 ± 0.20^{c}	3.74 ± 0.36^{a}
Sulphate (% w/w DW)	14.10 ± 1.51^{b}	14.38 ± 1.0^{b}	12.00 ± 1.16^{b}	14.56 ± 0.92^{b}	19.71 ± 2.79^{a}
Minerals					
Sodium, Na (g/kg)	11.09 ± 0.01^{c}	39.70 ± 0.01^{a}	17.08 ± 0.03^{b}	1.66 ± 0.13^{e}	4.11 ± 0.03^{d}
Potassium, K (g/kg)	54.60 ± 2.83^{bc}	15.76 ± 1.47^{d}	100.42 ± 10.32^{a}	61.92 ± 1.70^{b}	40.90 ± 0.99^{c}
Calcium, Ca (g/kg)	4.24 ± 0.85^{c}	2.61 ± 0.64^{c}	28.55 ± 0.42^{b}	6.04 ± 0.45^{c}	36.47 ± 2.36^{a}
Magnesium, Mg (g/kg)	0.82 ± 0.09^{c}	1.02 ± 0.00^{b}	1.37 ± 0.06^{b}	2.25 ± 0.04^{a}	2.62 ± 0.18^{a}
Heavy metals					
Arsenic, As (mg/kg)	ND	ND	ND	ND	ND
Lead, Pb (mg/kg)	ND	ND	0.61 ± 0.00^{b}	3.35 ± 0.43^{a}	ND
Cadmium, Cd (mg/kg)	ND	ND	ND	ND	ND
Mercury, Hg (mg/kg)	ND	ND	ND	ND	ND

Data were expressed as mean ± standard deviation ($n = 3$).
Mean values with different superscripts in the same row are significantly different at $p < 0.05$.
DW = dry weight.
ND = not detected.

National Standard (PNS) (2007) specifications, the AIM of refined carrageenan must be less than 2%, while the AIM of semi-refined carrageenan must be less than 8%. Barium sulphate precipitation, as a rough estimation of the sulphate content in κ-carrageenan, was used in this study. Table 3 indicates that Gelcarin GP911 NF contained the highest amount of sulphate (19.71%), followed by Grindsted® carrageenan CL220 (14.56%), SeaKem CM611 (14.38%), TA150 (14.10%), and Gelcarin GP812 (12.00%). Sulphate is a typical component found in κ-carrageenan, as related to the high salt concentration of the seaweed growth environment as well as to specific aspects of ionic regulation (Manivannan, Devi, Thirumaran, & Anantharaman, 2008). The sulphate in κ-carrageenan is mainly derived from galactans in red algae and located at C-4 of the D-galactopyranose unit (Campo, Kawano, Silva Jr., & Carvalho, 2009; Manivannan et al., 2008).

3.3. Mineral and heavy metal analysis

Red algae (*E. cottonii*), the main raw material from which κ-carrageenan is extracted, is rich in vitamins and minerals that is important for human nutrition (Krishnaiah, Sarbatly, Prasad, & Bono, 2008). However, the mineral composition of *E. cottonii* is affected by the geographic area, seasonal year, and water temperature (Krishnaiah et al., 2008). In this study, four minerals (Na, K, Ca, and Mg) that are commonly found in κ-carrageenan were investigated. In addition, the levels of heavy metals (As, Pd, Cd, and Hg) in κ-carrageenan were studied as marine-based products may be contaminated with heavy metals.

κ-Carrageenan contains high amounts of macrominerals. The sodium contents in κ-carrageenan, from highest to lowest, were found in SeaKem CM611 (39.70 g/kg), Gelcarin GP812 (17.08 g/kg), TA150 (11.09 g/kg), Gelcarin GP911 NF (4.11 g/kg), and Grindsted® carrageenan CL220 (1.66 g/kg). Gelcarin GP812 had the highest potassium content (100.42 g/kg), followed by Grindsted® carrageenan CL220 (61.92 g/kg), TA150 (54.60 g/kg), Gelcarin GP911 NF (40.90 g/kg), and SeaKem CM611 (15.76 g/kg). For calcium and magnesium, the ranges were 2.61–36.47 g/kg and 0.82–2.62 g/kg, respectively. The ash contents parallelled the mineral contents. As mentioned previously, the ash in κ-carrageenan is made up of minerals such as sodium and potassium. Therefore, the κ-carrageenan (Gelcarin GP812) with the highest level of total minerals (147.42 g/kg) also had the highest reported ash level (33.18%). In addition, the mineral content had a profound effect on the textural properties of κ-carrageenan. The effectiveness of cation in enhancing gel strength of κ-carrageenan following the sequence of $K^+ > Ca^{2+} > Na^+$ (Kara, Tamerler, Bermek, & Pekcan, 2003; Lee et al., 2008). K^+ is more important than Na^+ in determining the gel strength of κ-carrageenan gels. κ-Carrageenan (Gelcarin GP812), which had the highest concentration of potassium, exhibited the strongest rupture strength and vice versa. For better comparison, standardization of ionic composition can be done to further understand the differences in textural performance due to κ-carrageenan itself.

Seaweeds are marine plants that are rich in nutrients, vitamins, and minerals. They are mostly used to extract carrageenan, alginates and some other hydrocolloids or are consumed directly after minor pre-processing (Besada, Andrade, Schultze, & Gonzalez, 2009). Unfortunately, some seaweeds exhibit a high affinity for heavy metals, and thus, they act as biomonitors for metal pollution in estuarine and coastal waters. The detection of heavy metals in κ-carrageenan is required to ensure its safety for human consumption. The JECFA (2006) limits for heavy metals in carrageenan are as follows: Arsenic <3 mg/kg; Lead <5 mg/kg; Cadmium <2 mg/kg; and Mercury <1 mg/kg (Table 4). Arsenic, Cadmium, and Mercury were not detected in any of the κ-carrageenan samples. Lead was detected in two κ-carrageenan samples: Gelcarin GP812 (0.61 mg/kg) and Grindsted® carrageenan CL220 (3.35 mg/kg). The level of lead fell within the acceptable range set by the JECFA.

Table 4
Standard specifications for carrageenan.

Chemical composition	JECFA	PNS
pH	8–11	8–11
Moisture content	<12%	<12%
Fat	–	–
Protein	–	–
Ash	15–40%	15–40%
Acid-insoluble ash	<1%	<1%
Crude fibre	–	–
Acid-insoluble matter	<2%	Refined: <2% Semi-refined: <8%
Sulphate	15–40%	15–40%
Sodium	–	–
Potassium	–	–
Calcium	–	–
Magnesium	–	–
Arsenic	<3 mg/kg	<3 mg/kg
Lead	<5 mg/kg	<2 mg/kg
Cadmium	<2 mg/kg	<2 mg/kg
Mercury	<1 mg/kg	<1 mg/kg

JECFA = Joint FAO/WHO Expert Committee on Food Additives.
PNS = Philippines National Standard.

3.4. Microbiological analysis

The microbial studies showed that there was no microbial growth in TA150, the κ-carrageenan extracted from Sabah *E. cottonii*. Coliform, *E. coli* and *Salmonella* were absent in 1.0 g of sample. The total plate counts of yeast and mould levels were reported as too few to count (TFTC) as there was only one colony found in the lowest dilution (10^{-3}) of each test. These results proved that the hygienic level of TA150 processing is satisfactory and that the product was stored appropriately in a clean location. Thus, TA150 is safe for incorporation into food products as a gelling agent or emulsifier for human consumption.

4. Conclusion

In this study, the physicochemical properties of κ-carrageenan (TA150) produced from *E. cottonii* in Tawau, Sabah were investigated and compared to the properties of commercial κ-carrageenan. TA150 and commercial κ-carrageenan showed a significant difference in terms of colour, particle size, texture, functional properties, and chemical composition ($p < 0.05$). These differences are mainly due to variations in seaweed species, geographical area, seasonal variation, and processing conditions. In general, κ-carrageenan samples are white to yellowish in colour. TA150 was reported to be the darkest powder with the lowest L^* value (82.69). The rupture strength of κ-carrageenan increased significantly with increasing concentration ($p < 0.05$). Freshly prepared κ-carrageenan gels showed excellent WHC (>90%) regardless of the storage temperature (25 °C, 4 °C and −18 °C). However, they experienced syneresis after 10 days of storage with highest syneresis (21.5%) reported in TA150. In the proximate analysis, ash and sulphate constituted the largest portion of κ-carrageenan. Fat, protein, and crude fibre were present at negligible levels. Furthermore, κ-carrageenan is a rich source of minerals, especially potassium (15.76–100.42 g/kg). According to the limits set by the JECFA, heavy metals in TA150 and other commercial κ-carrageenan fell below safe level limits. Further analyses such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR),

and gel permeation chromatography (GPC) can be carried out to relate the physicochemical properties of κ-carrageenan to their polysaccharide composition.

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