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Nutritional study of Kapparazii powder^{TM} as a food ingredient

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Abstract *Kappaphycus alvarezii* is one of the most important commercial sources of carrageenan. Red seaweeds are found in tropical areas, and *K. alvarezii* is famous for its high growth rate among other tropical red seaweeds. This study was conducted to produce Kapparazii powder^{TM}, a product comprised of high amount of carrageenan with valuable nutrients from *K. alvarezii* found in Sabah, Malaysia. Spray drying and an environmentally friendly process without using chemicals were employed to produce Kapparazii powder^{TM}. Physicochemical properties of Kapparazii powder^{TM} such as proximate composition (moisture, protein, lipid, ash, and crude fiber), mineral content, heavy metals, vitamins, amino acid, color, viscosity, gel strength, swelling capacity, and water and oil holding capacity were evaluated. Kapparazii powder^{TM} contained moisture (4.69±0.03 %), protein (5.11±0.02 %), lipid (1.00±0.02 %), ash (14.52±0.01 %), and crude fiber (0.93±0.02 %). Color analysis of Kapparazii powder^{TM} showed that lightness (L^*)=89.51±0.02, redness (a^*)=−1.27±0.03, and yellowness (b^*)=5.49±0.02. The value of viscosity, gel strength, swelling capacity, and water and oil holding capacity of the Kapparazii powder^{TM} were 0.06 ±0.00 Pa.s, 82.77±3.66 gf, 100±0.00 mL.g^{-1}, 4.67± 0.58 g.g^{-1}, and 5.11±0.36 g.g^{-1}, respectively. Moreover, Kapparazii powder^{TM} did not inhibit proliferation of L929 cells after 24 h of exposure at the highest concentration (2 mg.mL^{-1}). In conclusion, the Kapparazii powder^{TM} as a source of high nutrient hydrocolloid suggested on the point of healthy ingredient for food industry application.

Keywords Red seaweed · Nutritional content · Physicochemical properties · *Kappaphycus alvarezii*

Sjamsiah (✉) · N. Ramli · R. Daik · M. A. Yarmo
School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor Darul Ehsan, Malaysia
e-mail: sjamsiahuca@yahoo.com

Sjamsiah
Department of Chemistry, Faculty of Science and Technology, Alauddin State Islamic University Makassar,
Sultan Alauddin, No 63, Makassar, Indonesia

N. Ramli · Z. Ajdari
Innovation Center for Confectionery Technology (MANIS), Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor Darul Ehsan, Malaysia

Introduction

Application of seaweeds as a raw material in pharmaceutical, cosmeceutical, and nutraceutical industries play a huge role in boosting the economy of many countries (De Ruiter and Rudolph 1997; Marinho-Soriano et al. 2006; Webber et al. 2012). Seaweed has been used since ancient times as food, fodder, fertilizer, and as medicinal sources (Chapman and Chapman 1980). Seaweeds contain nutritional valuable components such as protein, lipids, vitamins, and minerals (Dawezynski et al. 2007; Venugopal 2009; Norziah and Ching 2000). However, nutrient contents of seaweeds vary with species, geographical location, season, and temperature (Jensen 1993; Kaehler and Kennish 1996). Currently seaweeds are the raw material for industrial production of agar, alginate, and carrageenan which are used for their gelling and viscosity functionality in many food products (Rupérez 2002; Marinho-Soriano et al. 2006; Chan et al. 2013). They are also widely consumed as fresh, dried, or ingredients in processed foods (Robledo and Pelegrin 1997).

Kappaphycus alvarezii is one of the most important commercial sources of carrageenan, and it has the fastest growth rate among tropical red seaweeds (De Barros-Barreto et al. 2013; Chan et al. 2013). Carrageenans are a family of linear sulfated polysaccharides, which are widely used in the food industry for their gelling, stabilizing,

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and thickening properties. There are various methods to extract this compound from seaweed (Muñoz et al. 2004; Webber et al. 2012)

Seaweed powder is a potential source to be used directly as an additive in food and beverages. This is due to the hydrocolloid of seaweed that has viscous characteristic, gel-forming (Černiková et al. 2008), and contains numerous nutrients such as carbohydrates, protein, fats, vitamins, minerals, and bioactive substances (Norziah and Ching 2000). The nutritional composition and physiochemical properties of seaweed powder depend on preparation techniques and drying methods used (Chan et al. 1997).

Due to the high demand in carrageenan and its economical and social benefits obtained from its production, recently, Malaysia started the plantation of this seaweed in Sabah. This study aimed to drive a high amount of carrageenan product from *K. alvarezii* planted in Sabah, Malaysia. Therefore, the first goal of the current study was to treat *K. alvarezii* to get a clean dried sample with an environmentally friendly method as primary processing. Then secondary processing was employed to produce white powder product, which was called Kapparazii powder^{TM}, by spray drying method.

In this study, a number of important properties of Kapparazii powder including color, pH, swelling capacity, water and oil holding capacity, viscosity, gel strength, proximate composition, vitamins, minerals, amino acid, and cytotoxicity were quantified in order to evaluate the potential use of Kapparazii powder^{TM} as food ingredient.

Materials and methods

Sample preparation

Primary processing Raw seaweed *K. alvarezii* was obtained from Sabah, Malaysia. Firstly, the seaweed was washed under running water to remove salt, dirt, and foreign particles. It then was soaked overnight (24 h) in distilled water to bleach the yellowish color , so it became colorless. After that, the sample was rinsed and dried under sunlight for 3 days. Afterward, the dried seaweed was chopped into small pieces before being blended by a hammer mill with 3-mm filter diameter. Finally, this product was stored until further processing (secondary processing). The dried seaweed reduced the storage space required and can be stored for a number of years without appreciable loss of the gelling property (Wong and Cheung 2001).

Secondary processing A 10-g sample from primary processing was dissolved in 2 L of boiling distilled water and boiled for 20 min. Then, the solution was dried by spray drying (Lab Plant SD-05, UK) to achieve a white powder which was called Kapparazii powder^{TM}.

Nutritional evaluation

Proximate analysis Proximate analysis including the determination of moisture, crude protein, crude lipid, ash, and crude fiber of Kapparazii powder^{TM} was carried out according to the Association of Official Analytical Chemists (AOAC 1990) methods.

Moisture content was determined by drying in an oven at 105 °C until a constant weight was obtained. Crude protein content was analyzed by the Kjeldahl method (Kjeltex System-Texator, Sweden) with a conversion factor of 6.25 to convert the total nitrogen into crude protein. Crude lipid content was determined using the Soxhlet method (Soxtec System-Texator, Sweden). The ash content was determined by heating the sample overnight at 550 °C, and the content was expressed as percentage of Kapparazii powder^{TM}. The crude fiber content was determined based on acid–alkaline neutralization using a Fiber Cap System 2022 (Foss, Denmark). One gram of sample was boiled in diluted 1.25 % H_2SO_4 for 30 min, then filtered and washed with 200 mL of boiling water. The sample was extracted again with boiling 1.25 % NaOH for 30 min and later washed with boiling water followed by 1 % HCl and once again with boiling water. The residue was dried at 105 °C for 5 h and finally heated at 550 °C for 4 h and the weight recorded.

Element analysis The element content of the sample were analyzed using X-Ray Fluorescence (Bruker S8 Tiger).

Vitamin analysis The measurement of vitamin content was carried out according to AOAC (1995a) for vitamin A, AOAC (1995b) for vitamin C, Kamman et al. (1980) for vitamin B1 and B2, and British Standards European Norm (BS EN 12822 2000) for vitamin E.

Amino acids analysis The amino acid composition was obtained by using a high-performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Fluorescence and AccQ Tag column (3.9×150 mm). AccQ Tag Eluent A and AccQ Tag Eluent B or 60 % acetonitrile acid was used as the mobile phase (flow rate=1 mL.min^{-1}).

Cytotoxicity (MTT assay)

Cytotoxicity test was carried out by using MTT assay to assess the cytotoxic potential of the Kapparazii powder^{TM} by determining the rate of cell proliferation in accordance with the requirements of the ISO 10993–12 (2009). A dose response cytotoxicity of Kapparazii powder^{TM} was evaluated in L929 mouse fibroblast cells. The cells were treated with concentration of 0.0625, 0.125, 0.25, 0.5, 1, and 2 mg L^{-1} of the test material extract for 24 h. The cytotoxicity was determined by assessing the rate of cell proliferation through the reduction of tetrazolium

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salts (MTT). Cell proliferation rate was obtained by dividing the mean optical density (OD) values of the test material with the mean OD of medium control and multiplied by 100.

Physicochemical properties

Color evaluation The color of sample powders was measured using Hunter Colorimeter (CR 300; Minolta Co., Japan) and values were expressed as $L*$, $a*$, and $b*$. This procedure was done in triplicate.

Determination of pH The pH of Kapparazii powder™ (1.5 % w.v^{-1}) was measured using pH meter (Eutech instruments, Singapore). This procedure was done in triplicate.

Swelling capacity (SWC) The SWC was measured using the bed volume technique after equilibrating it in excess solvent (Kuniak and Marchessault 1972). The swelling capacity was measured and expressed as milliliter swollen sample per gram sample.

Water holding capacity (WHC) WHC of sample was measured by the modified centrifugation method described by Suzuki et al. (1996). The results of WHC were expressed as g.g^{-1} DW (dry weight).

Oil holding capacity (OHC) OHC of samples was determined by the modified method of Caprez et al. (1986). The results of OHC were expressed as g oil.g^{-1} DW.

Gel strength For the gel strength measurement, Kapparazii powder™ solution was prepared by dissolving 0.45 g of Kapparazii powder™ in 30 mL of hot deionized distilled water with continuous magnetic stirring at 90 °C for 20 min. The solution was poured into a 50-mL glass bottle mold with an inside diameter of 4 cm and later stored in a refrigerator at 4 °C overnight (18 h). Gel strength was analyzed using Texture Analyser, Autograph, and Shimadzu with a fixture and analytical probe (1 cm) that were pressed on the sample to a depth of 70 % of its original height and speed of 1 mm s^{-1}. Each measurement was done in triplicate.

Viscosity The viscosity measurement was carried out according to Muñoz et al. (2004). Kapparazii powder™ (0.3 g) was dissolved in 20 mL of hot deionized distilled water (1.5 % w.v^{-1}) at a temperature of 90 °C and with vigorous stirring using a magnetic stirrer for 20 min. Viscosity was determined with a Physica MCR 301 (Anton Paar, Germany) rheometer using cone plate CP50-1-SN13463 at a temperature of 25 °C. Experiments were performed at constant shear rate (100 s^{-1}). Viscosity was expressed as Pascal-second (Pa.s). Each measurement was done in at least duplicate.

Results and discussion

A white powder product, known as Kapparazii powder™, was successfully produced from *K. alvarezii* using a simple environmentally friendly process described in the "Materials and methods."

Proximate composition

Table 1 shows the proximate composition of Kapparazii powder™ based on dry weight. The Kapparazii powder ™ contained: 5.11±0.02 % crude protein, 1.0±0.02 % crude lipid, 0.93±0.02 % crude fiber, 14.52±0.01 % ash, and 4.69 ±0.03 % moisture. Generally, the proximate composition values of Kapparazii powder ™ were higher than those of locally commercialized K-carrageenans as reported by Chan et al. (2013); however, the ash and almost moisture were less than commercial ones. The pattern of protein contents was Kapparazii powder™ (5.11 %) >Gelcarin GP812 (0.31 %) >Gelcarin GP911NF (0.29 %) >Grindsted Carrageenan C1220 (0.26 %) TA150 (0.14 %) >SeaKem CM611 (0.08 %). The protein content of Kapparazii powder™ is at least 4 % higher than the commercial products. Although the protein content of seaweeds is related to species, it is also strongly dependent on the availability of N where the seaweed is growing (Fluerence 1999; Galland-Irmouli et al. 1999; Martinez and Rico 2002).

The lipid content of Kapparazii powder™ was 1 % which lay in the range of lipid contents for tropical seaweeds, i.e.,

Table 1 Proximate composition (% w.w^{-1}, dry weight) of Kapparazii powder™ and commercial K-carrageenans

Components	Kapparazii powder™	TA150	SeaKem CM611	Gelcarin GP812	Grindsted® Carrageenan C1220	Gelcarin GP911NF
Protein	5.11±0.02	0.14±0.00	0.08±0.02	0.31±0.03	0.26±0.04	0.29±0.04
Lipid	1.00±0.02	0.15±0.01	0.05±0.00	0.22±0.03	0.50±0.07	0.03±0.01
Ash	14.52±0.01	18.72±0.03	17.75±0.78	33.18±1.43	18.32±1.00	26.48±0.15
Moisture	4.69±0.03	3.65±0.17	10.02±0.20	10.98±0.33	11.41±0.20	9.95±0.06
Crude fiber	0.93±0.02	0.26±0.02	0.12±0.01	0.13±0.03	0.30±0.01	0.32±0.05

Data are means of three determination ± standard deviation. Source of commercial carrageenan data: Chan et al. (2013)

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Table 2 Elements found in Kapparazii powder™

Components	Values (% g.g^{-1} dry weight)
K (Potassium)	9.64
Ca (Calcium)	1.22
Na (Sodium)	0.78
Mg (Manganese)	0.55
Cl (Chlorine)	0.28
P (Phosphorus)	0.01
Fe (Iron)	0.01
Mo (Molybdenum)	0.03
Si (Silicon)	0.02
Heavy metals	
As (Arsenic)	ND
Pb (Lead)	ND
Cd (Cadmium)	ND
Hg (Mercury)	ND

ND not detected

0.29–1.11 % dry weight as reported by Matanjun et al. (2009). This value was at least two times higher than in the several locally commercialized K-carrageenan and K-carrageenan isolated from Sabah *K. alvarezii* reported by Chan et al. (2013). The difference in lipid contents of products of the same genus can be due to geographical origin and season (Pazos et al. 1996; Marinho-Soriano et al., 2006) as well as the type of processing employed for producing these products.

The ash content of Kapparazii powder™ (14.52 %) was lower than that of local K-carrageenans (17.75–33.18 %) 10-2. The moisture content (4.69 %) was also lower than that of local K-carrageenans (9.95–11.41 %) except for TA150 (3.65 %). According to Nisizawa et al. (1987) and Rupérez (2002), the differences in ash contents depend on seaweed species, physiological factors, environmental factors, and type of product processing. Differences in moisture contents can be due to the drying methods (Chan et al. 1997; Wong and Cheung 2001). Furthermore, the crude fiber content of Kapparazii powder™ was higher (0.93 %) than local K-carrageenan (0.12–0.32 %) as reported by Chan et al. (2013). This difference might be due to the type of processing method employed in this study or different species.

The result of proximate analysis demonstrated that the Kapparazii powder™ is potentially a good source of carrageenan with high content of protein and lipid applicable as food ingredient compared to available local commercial product.

Mineral content

Seaweed is known as an excellent source of minerals (Chapman and Chapman 1980; Venugopal 2009). The mineral content of Kapparazii powder™ is shown in Table 2. The results revealed that Kapparazii powder™ contained high quantity of K with moderate amount of Ca, Na, Mg, and Cl, whereas P, Fe, Mo, and Si are present in small quantities. In addition, heavy metals such as As, Pb, Cd, and Hg were not detected in Kapparazii powder™. Rupérez (2002) explained that mineral content of seaweed vary according to species, wave exposure, seasonal, annual, environmental, and physiological factors and the type of processing and method of mineralization.

Table 3 Vitamins found in Kapparazii powder™

Vitamin, unit	Concentration
Vitamin A, µg.10-2 g^{-1}	3.5
Vitamin B1, mg.kg^{-1}	0.4
Vitamin B2, mg.kg^{-1}	0.5
Vitamin C, mg.10-2 g^{-1}	1.4
Vitamin E, mg.kg^{-1}	18.9

Vitamin content

The vitamin content of edible seaweeds makes them nutritionally valuable (Chapman and Chapman 1980; Venugopal 2009). Vitamins in seaweed are important due to their biochemical functions and antioxidant activity as well as health benefits such as decreasing of blood pressure (vitamin C),

Table 4 Amino acid content of Kapparazii powder™ and *K. alvarezii* seaweed

Amino acid	% Dry weight of Kapparazii powder™	% Dry weight of *K. alvarezii*
Asp (Aspartic acid)	0.33	0.66
Ser (Serine)	0.18	0.39
Glu (Glutamic acid)	0.33	0.73
Gly (Glycine)	0.16	0.28
His (Histidine)	ND	0.08
Arg (Arginine)	0.15	0.46
Thr (Threonine)	0.16	0.39
Ala (Alanine)	0.27	0.25
Pro (Proline)	0.15	0.17
Tyr (Tyrosine)	0.04	0.13
Val (Valine)	0.19	0.31
Lys (Lysine)	0.17	0.20
Ile (Isoleucine)	0.15	0.21
Leu (Leucine)	0.23	0.33
Phe (Phenylalanine)	0.15	0.24
Cys (Cysteine)	0.02	0.06
Met (Methionine)	0.06	0.19
Trp (Tryptophane)	ND	0.13
Total	2.74	5.21

Source Vinoj Kumar and Kaladhran (2007)

ND not detected

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Table 5 Optical density values and L929 cell viability obtained after 24 h exposure to the Kapparazii powder™ and controls

	Negative control	Positive control	Kapparazii powder™ ($mg.mL^{-1}$)					
			0.0625	0.125	0.25	0.5	1	2
Optical density (DO)	1.56±0.05	0.28±0.04	1.56±0.03	1.47±0.04	1.67±0.03	1.58±0.03	1.51±0.05	1.29±0.07
Viability (%)	100	20	100	92	107	101	97	83

Data are means of three determination ± standard deviation. Positive control=Zinc sulphate at 240 $\mu g.mL^{-1}$ and negative control=growth medium

prevention of cardiovascular diseases (β-carotene), or reducing the risk of cancer (vitamins E and C, carotenoids) (Škrovánková 2011). The vitamin content of Kapparazii powder™ is shown in Table 3. The concentration of vitamin E was the highest compared to vitamin A, B1, C, and B2, whereas the concentration of vitamin A was the lowest.

Amino acid composition

Table 4 shows the amino acids, which were identified in Kapparazii powder™. The Kapparazii powder™ contained essential amino acids (EAA): methionine, leucine, isoleucine, lysine, phenylalanine, tyrosine, arginine, threonine, valine, and cysteine. However, non-EAA was also found in Kapparazii powder™ namely aspartic acid, serine, glutamic acid, glycine, alanine, and proline. Aspartic acid and glutamic acid were the most abundant amino acids (0.33 %) found; cysteine (0.02 %), tyrosine (0.04 %), and methionine (0.06 %) were the lowest. The total amino acids content of Kapparazii powder™ was 2.74 % of dry weight. Vinoj Kumar and Kaladhran (2007) reported about 5.21 % total amino acid in *K. alvarezii*. Both, Kapparazii powder™ and *K. alvarezii* contained a large amount of aspartic and glutamic acid, which are responsible for the special flavor and taste (Wong and Cheung 2000).

Cytotoxicity

Kapparazii powder™ was evaluated for potential cytotoxic effects by determining the rate of cell proliferation using the MTT assay. The MTT assay indicated that Kapparazii powder™ did not inhibit the proliferation of L929 cells in different concentrations up to the highest concentration of 2 $mg.mL^{-1}$ of after 24 h exposure (Table 5).

Physicochemical properties

The color of Kapparazii powder™ was determined by measuring the lightness (L^*), redness (a*), and yellowness (b^*) values. L^* represents the degree of lightness, where 100 indicates white while 0 indicates black. Redness is represented by +a*, while –a* indicates greenness. Yellowness is represented by +b^*, while –b^*indicates blueness (Aziah and Komathi 2009). The color values of Kapparazii powder™ (Table 6) are lightness (L^*), 89.51±0.02; redness (a*), −1.27 ±0.03; and yellowness (b^*), 5.49±0.02.

Color is one the most important appearance attributes of food materials since it influences consumer's acceptance (Jim nez-Aguilar et al. 2011). The color values of Kapparazii powder™ in this study are lightness (L^*), 89.51±0.02; redness (a^*), −1.27±0.03; and yellowness (b^*), 5.49±0.02. These results indicated the color of Kapparazii powder ™ appears to be white. Kapparazii powder™ color compared to those of commercial K-carrageenan as reported by Chan et al. (2013) was whiter with commercial K-carrageenan powder appearing white or light yellow to tan in color. Furthermore, the pH value of Kapparazii powder™ was lower or nearly neutral (pH 6.41 ±0.01) as compared to those of commercial K-carrageenans (pH 8.04–10.09). The possible reason for the pH difference was the processing method employed in this study which does not involve usage of alkali.

Seaweed powder has unique physicochemical properties, i.e., it has the ability to swell, viscous, and jelly form at room temperature, therefore can be used as thickeners, gelling agents, texture modifiers, and stabilizers (Venugopal 2009).

Table 6 Color and pH of Kapparazii powder™ and commercial K-carrageenan

Samples	L^*	a^*	b^*	pH
Kapparazii powder™	89.51±0.02	−1.27±0.03	5.49±0.02	6.41±0.01
TA150	82.69±0.23	2.10±0.01	17.16±0.15	9.89±0.08
SeaKem CM611	88.87±0.13	1.60±0.05	11.08±0.13	10.09±0.28
Gelcarin GP812	83.84±0.68	2.13±0.02	13.13±0.27	8.04±0.68
Grindsted® Carrageenan C1220	87.51±0.42	0.27±0.01	11.91±0.57	8.20±0.42
Gelcarin GP911NF	83.63±0.29	2.18±0.09	12.37±0.51	8.34±0.39

Data are means of three determination ± standard deviation. Source of commercial carrageenan data: Chan et al. (2013)

L^* lightness, a* redness, b^* yellowness

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Table 7 Physicochemical properties of Kapparazii powder™

Parameter, unit	Values
SWC[a] ($mL.g^{-1}$)	100±0.00
WHC[b] ($g.g^{-1}$)	4.67±0.58
OHC[c] (g $oil.g^{-1}$)	5.11±0.36
Viscosity (Pa.s)	0.06±0.00
Gel strength (gf)	82.77±3.66

Data are means of three determination ± SD

[a] Swelling capacity

[b] Water holding capacity

[c] Oil holding capacity

Characterized physicochemical properties of Kapparazii powder™ were swelling capacity (SWC), 100±0.00 $mL.g^{-1}$; holding water capacity (WHC), 4.67±0.58 $g.g^{-1}$; oil holding capacity (OHC), 5.11±0.36 $g.g^{-1}$, viscosity, 0.06±0.00 Pa.s and gel strength, 82.77 gf (Table 7).

According to Robertson and Eastwood (1981) in Wong and Cheung (2001), water exists in fiber in three forms: it is bound to the hydrophilic polysaccharides; it is held within the fiber matrix; or it is trapped within the cell wall lumen. Wong and Cheung (2001) explained that higher SWC and WHC of the freeze-dried seaweed samples might be mainly related to the degree of damage of the polysaccharide cell wall. The degree of damage of the cell wall would be greater in the oven-dried seaweed than that in the freeze-dried samples. Besides that, the high temperature employed in oven-drying may cause the seaweed protein to denature, thus changing the protein conformations as well as the number and nature of the water-binding sites on the protein molecules, resulting in lower SWC and WHC (Chou and Morr 1979; Wong and Cheung 2001).

The SWC values of the Kapparazii powder™ were much higher (100 $mL.g^{-1}$) than the SWC of the freeze-dried seaweed sample reported by Wong and Cheung (2001). While, the WHC values of both samples were almost similar or slightly lower than in the freeze-dried seaweed. However, the OHC of Kapparazii powder™ was much higher than in the freeze-dried seaweed. The SWC, WHC, and OHC values of Kapparazii powder™ suggested that this product is potentialy useful for application as food ingredient.

Hydrocolloids are able to change their state from solid to liquid and liquid to solid easily. This reversible state change is one of the main properties of a hydrocolloid. This capability allows Kapparazii powder™ to be useful for the food and pharmaceutical industry. Gel strength of Kapparazii powder™ in this study was 82.77 gf (Table 7). Previous studies have found that the gel strength of carrageenan is highly dependent on saline solution, concentration, and type of ion present in the environment (Norziah et al. 2006; Almeida et al. 2010). Hence, further study would be needed to find out the effect of salts such as sodium, calcium, and potassium on Kapparazii powder™.

Viscosity is one of the most important properties of hydrocolloids. Viscosity testing was determined on a solution of 1.5 % seaweed at 25 °C. Results showed that the viscosity is 0.06 Pa.s.

In conclusion, based on Kapparazii powder's properties, it has a great potential as an alternative to local carrageenan products available in the market. Furthermore, Kapparazii powder™ is also a source of various nutrients. This suggests that Kapaparazi powder™ is potentially useful as an ingredient for the food industry.

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