J. Trop. Plant Physiol. 9 (2017):14-23

ANALYSIS OF CARRAGEENAN YIELD AND GEL STRENGTH OF *Kappaphycus* SPECIES IN SEMPORNA SABAH

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ABSTRACT

Kappaphycus sp. especially *K. alvarezii* is a major source of raw materials in the world for the production of *kappa*-carrageenan, which is important in the production of food products and various other applications. Since *kappa*-carrageenan extracted from different variants of *K. alvarezii* may be different with regards to their quantity and quality, this study has embarked upon the determination of carrageenan yield and gel strength from *Kappaphycus* species cultivated in Semporna located in the state of Sabah in Malaysia. A total of six samples have been selected for analysis which included *K. alvarezii* varieties locally known as *Tangan-tangan*, *Buaya*, *Tambalang Giant* and *Tambalang Green* as well as two *Kappaphycus* species, *K. striatum* var. *Green Flower* and *K. malesianus*. The alkaline extraction method was optimised where the best results for carrageenan yield and gel strength for semi-refined extraction was obtained from extraction performed for 4 hours whilst for refined carrageenan extraction, the best results were obtained from 3 hour extraction. The yield for semi-refined carrageenan extracted from these *Kappaphycus* species ranged from 30.3 to 39.6% whereas for refined carrageenan, the yield was in the range of 20.4 to 28.4%, in which *K. alvarezii* var. *Buaya* produced the highest yield. Meanwhile, the gel strength for the semi-refined carrageenan was in the range of 388 to 512 g cm^{-1} and for the refined carrageenan, the values ranged from 534 to 712 g cm^{-1}. For this analysis, carrageenan extracted from *K. alvarezii* var. *Tambalang Green* ehibited the highest gel strength. These results showed that the *Buaya* and *Tambalang Green* varieties have good potentials to be used in increasing the production of carrageenan in this industry.

Keywords: *Kappaphycus*, *kappa*-carrageenan, carrageenan yield, gel strength, Malaysia

INTRODUCTION

Kappaphycus alvarezii or commercially known as *Eucheuma cottonii* is an economically important tropical rhodophyta highly demanded for its cell wall polysaccharide, carrageenan, making it the most industrially important carrageenophyte in the world (Munoz et al. 2004; Bindu and Levine 2011). It is characteristically a spiny and bushy plant with numerous irregular smooth surface branches (Trono 1993; Imeson 2009). For the past four decades, *K. alvarezii* has become the most important source of *kappa*-carrageenan, a crucial resource for various products and applications (Munoz et al. 2004; Campo et al. 2009). It has been cultivated widely with the Southeast Asian countries contributing 96.5% of the total production of *kappa*-carrageenan where Philippines is the main contributor (55%) followed by Indonesia (38%) and Malaysia (2.5%) (Bono et al. 2011). *K. alvarezii* has also been introduced and cultivated in other countries such as Venezuela (Rincones and Rubio 1999), Mexico (Munoz et al. 2004), Brazil (Paula and Pereira 2003) and Fiji (Ask et al. 2003). This macroalga has shown its potential as a source of bioethanol (Khambhaty et al. 2012), antioxidant (Kumar et al. 2008) and bioabsorbent of heavy metals (Kang et al. 2011).

Carrageenans are sulfated polymers extracted from red algae that consist of alternating (1→3) α-ᴅ-galactose and (1→4)-β anhydro-ᴅ-galactose forming a linear chain. These polysaccharides are traditionally split into six basic forms which are *kappa*--, *iota*-, *lambda*-, *mu*- and *nu*-carrageenan with *kappa*-, *iota*- and *lambda*-carrageenan being the main classes of carrageenan of commercial interest (Campo et al. 2009). The extract of *E. cottonii* contains almost pure *kappa*-carrageenan, with less than 10% iota-carrageenan (Lee at al. 2008) which makes this species to be highly in demand.

Carrageenan is widely utilised in food industry because of its physical and functional properties, such as gelling, thickening, stabilising, water-binding agent and texturing variety of dairy-based and instant products such as chocolates, cheese, ice-creams, frozen desserts, sauces, yogurt, milk, pie and puddings (Bono et al. 2011). Furthermore, with its ability to interact with other food polymers such as proteins and starches, carrageenan has been applied in the confectionary and beer industries (Al-Alawi et al. 2011) as well as in the production of pet food (McHugh 2003). Aside from the food related products, carrageenan is also being applied in non-food industries such as in pharmaceuticals, cosmetics, printing and textile formulations (Campo et al. 2009). *Kappa*-carrageenan has also recently been studied for its ability to encapsulate bioactive compounds, aimed especially in the production of an effective controlled release carrier in matrix, bead, microcapsule and microgel forms (Ellis and Jacquier 2009; Keppeler et al. 2009).

The increasing demand of carrageenan due to its wide range of applications has led to rapid increase in the farming of *K. alvarezii* in the world. The carrageenan industry has grown rapidly in the past decades with 8% annual growth rate producing 28,000 metric tonnes of carrageenan with a value of US$ 270 million (Freile-Pelegrin and Robledo 2008). Therefore, Malaysia has taken a great interest in this multimillion-dollar growing market with focus on the production of *kappa*-carrageenan-producing alga. For the last 30 years, *E. cottonii* has been largely cultivated on the east coast of Sabah in Malaysia (Lee et al. 2008). The carrageenophytes were processed into semi-refined carrageenan chips or consumed as condiments by the locals (Tan et al. 2013).

Currently, several popular varieties of *K. alvarezii* locally named *Tangan-tangan*, *Buaya*, *Tambalang Giant* and *Tambalang Green* as well as *K. striatum* var. *Green Flower* and *K. malesianus* are being cultivated by farmers in Semporna which is a district in Sabah. All the varieties were named based on their external morphology and colour. The present study has evaluated carrageenan yield and gel strength from semi-refined and refined carrageenan extractions to identify the species that produced high quality carrageenan. Gel strength is one of the measurements for carrageenan quality since *kappa*-carrageenan is mainly used as gelling agent in food products and other applications. Different duration of alkaline extraction has been conducted on selected species in order to study the effect on carrageenan yield and gel strength of the seaweed.

MATERIALS AND METHODS

Sample collection and identification

Fresh *K. alvarezii* varieties locally known as *Tangan-tangan*, *Buaya*, *Tambalang Giant* and *Tambalang Green* together with *K. striatum* var. *Green Flower* and *K. malesianus* were collected from a farm in Semporna, Sabah, Malaysia. The algae were harvested after 30 days of cultivation time. The fresh *Kappaphycus* samples were examined based on their morphological properties and their unique features were identified.

Carrageenan extraction

The collected algae were sun-dried on site for 3 days before being processed in laboratory. The dried algae were then pretreated by removing visible foreign matters such as sand, stones and dried sea animals. The algae were further washed with running deionised water for 5 min to reduce salt content that might affect the final gelling property of the carrageenan. Then, the pretreated algae were dried in an oven at 60^{o}C until a constant weight was achieved to fully remove the excess moisture. The dried pretreated algae were kept in the clean, anhydrous state before carrageenan extraction was performed.

Two types of carrageenan extraction (semi-refined and refined) were conducted from equal amount of algae through alkaline extraction. In semi-refined carrageenan extraction, the algae (5 g) were extracted with 500 mL 1 M KOH based on earlier experiments by Mustapha et al. (2011) with some modifications. The extraction was conducted for 2 hours at 80^{o}C. The temperature was controlled to remain constant during the extraction procedure. The algae were later cooled down to room temperature and normalised with running deionised water until the pH of the algae solution became neutral. The wet algae were then further dried in the oven at 60^{o}C until constant weight. The alkaline extraction period was also conducted at 3, 4 and 5 hours to determine the best extraction duration.

Meanwhile, for refined carrageenan extraction, the modified hot alkaline extraction method by Hayashi et al. (2007) was employed. The dried algae (5 g) were treated in 500 mL of 1 M KOH for 2 hours at 80^{o}C in water bath followed by extraction in 500 mL deionised water at 80^{o}C for 1 hour. The extraction duration of alkaline extraction was also tested at 2, 3, 4 and 5 hours. The mixture was then homogenised and pressure filtered while still hot. The hot filtered solution was allowed to flow in 3 volumes of isopropanol, stirred and the precipitate was eventually collected through centrifugation. The precipitate was further dried in an oven at 60^{o}C. The refined carrageenan extraction was also conducted without using alkali using native water extraction method, for 3 hours.

Carrageenan yield determination

Dried carrageenans from both extraction processes were chopped and ground before they were kept in sealed container to protect from moisture content in the environment. The quantity of carrageenan extract for each variety was measured in controlled environment and the carrageenan yields (%) were determined according to the formula of Munoz et al. (2004):

$$\text{Yield} = (W_c/W_{ds}).100 \qquad (1)$$

where W_c is the carrageenan extract weight in grams and W_{ds} is dry algal weight (g) used for extraction. All the results were reported as the average of triplicate measurements (mean ± standard deviation) for comparison. The carrageenan extracts were used for gel strength measurement.

Gel strength determination

Carrageenan solution of 1.5% (w/v) was prepared in water and 0.2% potassium chloride. The solution was boiled at 85^{o}C with subsequent stirring to dissolve the carrageenan extract and salt. Water was added to obtain the initial carrageenan concentration and 100 mL of the hot solution was then transferred to three 250 mL beakers. The gel was allowed to set overnight at 4^{o}C and equilibrated for 20 min at 25^{o}C before measurement. Gel strength (g/cm^2 at 25^{o}C) was measured on discs of carrageenan (7 cm diameter, 3 cm height) using Texture Analyzer (Model CT3-1000, Brookfield, USA) with a 1 cm^2 probe area and operating at 1.0 mm s^{-1} descent rate. The analyses were carried out in triplicate. Gel strength was measured as the maximal penetration strength to break the gel.

Statistical analysis

Statistical analysis was performed using Minitab version 16 software package. All experiments were done in triplicate (biological sample) and results obtained were analysed using t-test to show any significant difference among the variants at 95% ($p<0.05$) significance level.

RESULTS

Sample identification

A total of six samples of *Kappaphycus* sp. (Figure. 1) were examined based on their morphological structures and unique characteristics.

K. alvarezii var. *Tambalang Green* (Figure 1a) is greenish in colour and 30 to 60 cm in length. This variety has smooth cylindrical primary thalli with irregular branching up to quaternary. The branch length and diameter decrease with each level of branching. The branch apex is dichotomous, from pointed to round.

K. alvarezii var. *Tambalang Giant* (Figure 1b) is dark brownish in colour with the largest size (60 to 120 cm) among the species collected. This variety was quite similar to *Tambalang Green* in terms of morphology apart from its larger size. Primary branch was dominant with unilateral, dense secondary branches. Terminal branches are mostly slender and pointed.

K. alvarezii var. *Tangan-tangan* (Figure 1c) is brownish in colour and 40 to 50 cm in length. It has smooth, cylindrical thalli with irregular primary and secondary branching. The secondary and tertiary branches bifurcate or multifurcate into hand-like structure. This feature is most probably the reason this variant is being named as *Tangan-tangan.*

K. alvarezii var. *Buaya* (Figure 1d) is brown in colour and 40 to 70 cm in length. This variant has a cylindrical rough crocodile scale-like thalli surface with irregular, indeterminate primary branch. The secondary and tertiary branches are small where they branch irregularly with pointed apex.

K. striatum var. *Green Flower* (Figure 1e) is greenish with the length of 20 to 40 cm. It has short, dense, smooth and robust thalli forming a cauliflower-like shape. The primary thalli were cylindrical, sturdy with irregular short branching up to quinary branches. The secondary branches were bifurcate or trifurcate with blunt-end or round apical tips.

K. malesianus (Figure 1f) is yellow brownish and 30 to 50 cm in length. It has slim and smooth thalli with diameter of less than 1 cm with irregular branching up to tertiary branches. The branches are the slimmest among the selected seaweeds.

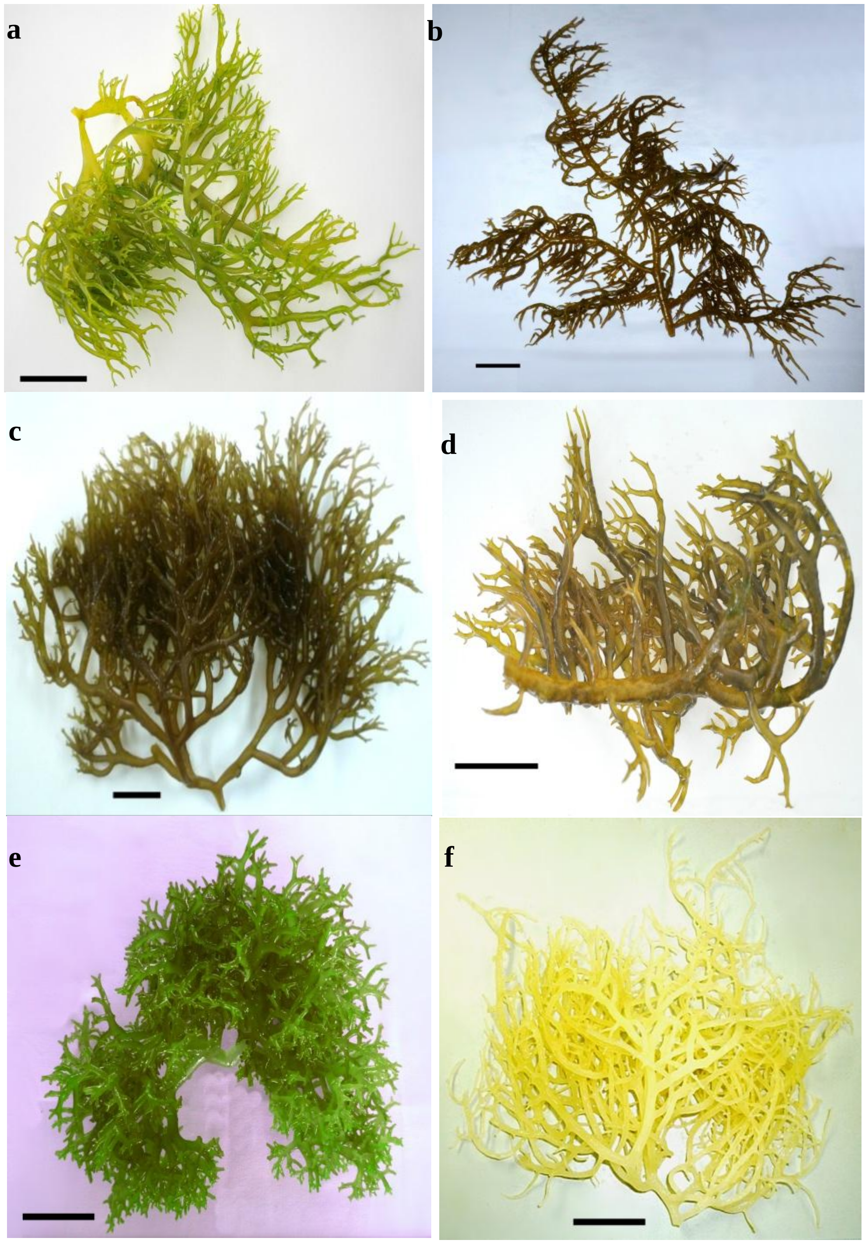


Figure 1. *Kappaphycus alvarezii* varieties and *Kappaphycus* sp. (a) *K. alvarezii* var. *Tambalang Green* (b) *K. alvarezii* var. *Tambalang Giant* (c) *K. alvarezii* var. *Tangan-tangan* (d) *K. alvarezii* var. *Buaya* (e) *K. striatum* var. *Green Flower* (f) *K.malesianus*. [a, b, c, d, e, f: scale bar=5 cm]

Carrageenan yield and gel strength

Semi-refined carrageenan (SRC) yield obtained from *Kappaphycus* species showed values ranging from 30.3 to 39.6% (Table 1). Slight differences in carrageenan yield were obtained among *K. alvarezii* varieties (35.5 to 39.6%) with *K. alvarezii* var. *Buaya* produced the highest yield while lower yields were obtained from *K. striatum* var. *Green Flower* (32.6%) and *K. malesianus* (30.3%). For refined carrageenan (RC) extraction, *Kappaphycus* species extracted without KOH (native carrageenan) showed higher yields (39.6 to 44.4%) compared to extraction with KOH (20.4 to 28.4%). In refined KOH extraction, the yield was lower than semi-refined carrageenan with *K. alvarezii* var. *Buaya* giving the highest yield (28.4%) while the lowest yield was obtained from *K. striatum* (20.4%).

Gel strength from semi-refined carrageenan showed results ranging from 388 to 512 g cm^{-1}. The highest gel strength from semi-refined carrageenan was obtained from *K. alvarezii* var. *Tambalang Green* (512 g cm^{-1}) with closer strength was recorded from *K. alvarezii* var. *Tangan-tangan* (494 g cm^{-1}) and *K. striatum* (484 g cm^{-1}) (Table 1). Refined carrageenan showed large differences in gel strength whereas the lowest gel strength was obtained from native carrageenan extracted without using KOH (124 to 202 g cm^{-1}). In refined carrageenan extraction using KOH, the highest gel strength was *K. alvarezii* var. *Tambalang Green* (712 g cm^{-1}) whereas the lowest strength was obtained from *K. malesianus* (534 g cm^{-1}) (Table 1).

Table 1. Yield and gel strength of *Kappaphycus* species determined using different carrageenan extraction methods.

Species	Yield			Gel Strength		
	Semi-refined	Refined		Semi-refined	Refined	
	KOH	Water	KOH	KOH	Water	KOH
K. alvarezii var. *Buaya*	39.6*	44.4	28.4	434	166*	647
K. alvarezii var. *Tambalang Green*	37.2*	43.6	26.6*	512	202*	712
K. alvarezii var. *Tambalang Giant*	37.4	41.5*	27.2	452*	186	615
K. alvarezii var. *Tangan-tangan*	35.5*	42.4	24.6*	494	181*	674
K. striatum	32.6	39.6*	20.4	484	178	586*
K. malesianus	30.3*	40.2	21.2	388*	124*	534

* significant at $p<0.05$, t-test on triplicate samples

Based on carrageenan yield and gel strength, *K. alvarezii* var. *Buaya* and *Tambalang Green* were selected for further analysis on carrageenan extraction period. Semi-refined carrageenan (SRC) extraction of the selected variants showed increase in yield with longer extraction period with the maximum yield of 44.5% obtained from extraction at 5 hours (Figure 2a). Refined carrageenan (RC) extraction also showed increase of carrageenan yield with longer extraction period for both variants. However there was not much difference in carrageenan yield (0.2%) for both variants between 4 and 5 hours of extraction time (Figure 2a). In this experiment, we found that *K. alvarezii* var. *Buaya* showed the highest yield for both types of extraction.

Gel strength from semi-refined carrageenan (SRC) extraction of *K. alvarezii* var. *Tambalang Green* recorded the highest (602 g cm^{-1}) at 4 hours of extraction time. The gel strength of semi-refined carrageenan for both variants increased from 2 to 4 hours of extraction and slightly decreased at 5 hours extraction period (Figure 2b). The highest gel strength for refined carrageenan (RC) was recorded at 3 hours of extraction (720 g cm^{-1}). Refined carrageenan gel strength increased from 2 to 3 hours of extraction periods but decreased at longer extraction duration (Figure 2b). Our results showed that *K. alvarezii* var. *Tambalang Green* had higher gel strength than *K. alvarezii* var. *Buaya* in both types of extraction.

DISCUSSION

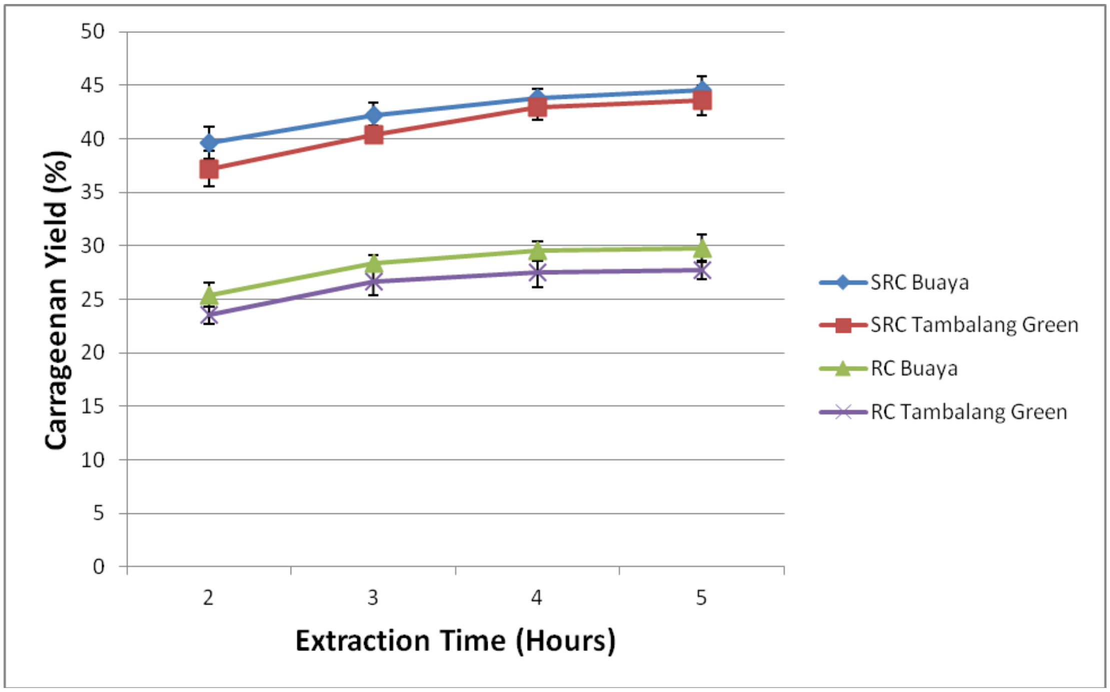
In this study, we have determined morphological properties, carrageenan yield and gel strength of *Kappaphycus* species cultivated at Semporna, Sabah, Malaysia. The differences in morphological characteristics of *K. alvarezii* varieties may be attributed to the interaction between light, water currents, water depth and availability of nutrients (Tan et al. 2012) which leads to different cultivars.

In this study, higher carrageenan yield was obtained from semi-refined carrageenan extraction compared to refined extraction. This might be due to the existence of residual cellulose from the cell walls after washing which only removes residual minerals, proteins and fats (McHugh 2003). Therefore, semi-refined carrageenan always contains cellulose residues which are not accepted by the industries such as cosmetics and pharmaceutical, while the refined extraction produces carrageenan extracts which are purer.

There have been many reports made on carrageenan yields of *K. alvarezii*. However, the quantitative and qualitative comparisons were difficult, since they vary depending on the extraction method (semi-refined or refined carrageenan) and the strain or variant of the species. In this study, refined carrageenan yield for *K. alvarezii* varieties (20.4 to 28.4%) was quite similar to the values reported by Istini et al. (1994) which was in the range of 21.8 to 31.5%. However, the varieties of *K. alvarezii* used in their study were not explained. Munoz et al. (2004) reported higher carrageenan yield (30.3 to 40.7%) of brown, green and red strains of *K. alvarezii* while lower yield was reported by Hurtado-Ponce (1995) (4.7 to 11.6%) and Hayashi et al. (2007) (15 to 20%). The difference in yield was usually due to the extraction methods used and in this study, excessive use of alkaline extraction method could possibly affect the final yield.

In this study, carrageenan extracted with alkaline solution showed higher yield with longer extraction period in both semi-refined and refined extraction methods. The hydroxide from alkaline reagent (KOH) penetrates into the algae and removes the sulphate groups in carrageenan through desulphation at 6-position of galactose unit of carrageenan to create recurring 3,6 anhydro-D-galactose (3,6-AG) while the anion (K^+) were able to neutralize the charges of removed sulphate group by combining together to form potassium sulphate (Mustapha et al. 2011). The longer period of extraction promotes better interaction of alkaline solution with the algae. However, extensive extraction period (>4 hours) promotes excessive destruction of carrageenan and thus, affecting the gel strength. It has also been reported that gel strength was influenced by the existence of 3,6-AG (Tuvikene et al. 2006). The highest gel strength obtained from *K. alvarezii* var. *Tambalang Green* was probably due to high 3,6-AG content or high ratio of *kappa*-carrageenan in the variant. Further studies need to be performed to confirm this hypothesis.

a



b

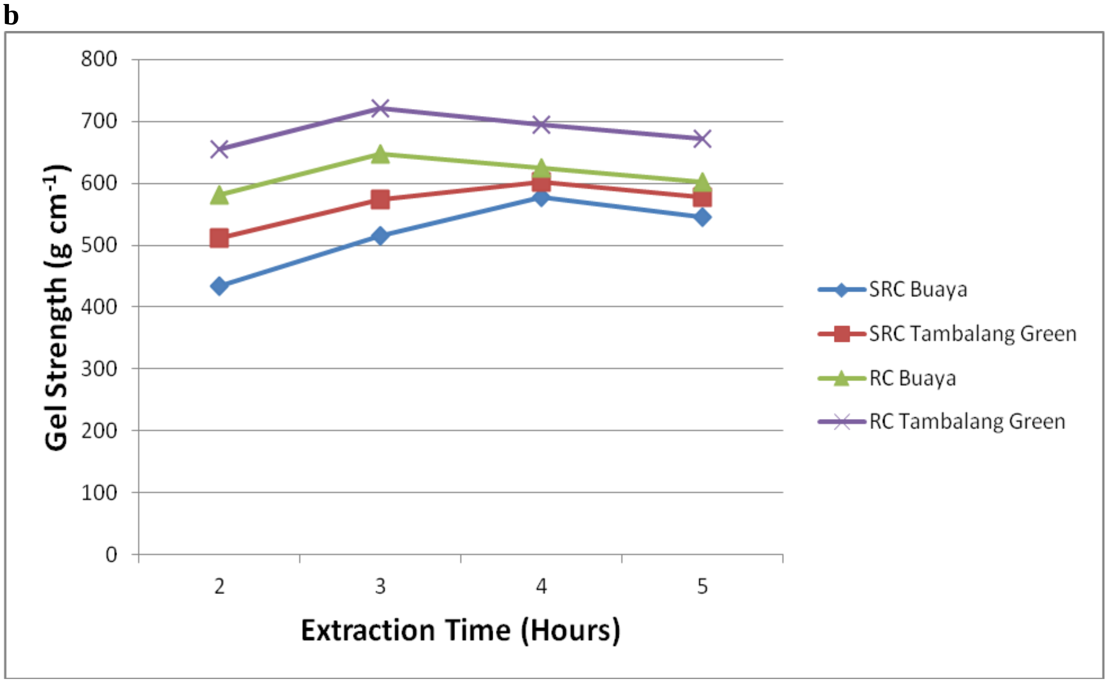


Figure 2. Carrageenan yield and gel strength of selected *K. alvarezii* varieties (*Buaya* and *Tambalang Green*) under different extraction period. (a) Semi-refined carrageenan (SRC) and refined carrageenan (RC) yield. (b) Gel strength of semi-refined and refined carrageenan.

CONCLUSION

In this study, we have examined carrageenan yield and gel strength from different *Kappaphycus* species cultivated in Semporna, Sabah. Carrageenan yield was varied among the species with *K. alvarezii* variants showing higher carrageenan yield compared to *K. striatum* and *K. malesianus*. Among *K. alvarezii* variants, *K. alvarezii* var. *Buaya* showed the highest yield whereas *K. alvarezii* var. *Tambalang Green* exhibited the highest gel strength in both semi-refined and refined carrageenan extractions. Our results showed that alkaline extraction at 4 hours for semi-refined and 3 hours for refined carrageenan produced better yield and gel strength. However, longer extraction period caused reduction in gel strength. The information generated here can be used to select better variants or species for cultivation and also to optimise the extraction method for improvement of local carrageenan productivity in future.

ACKNOWLEDGEMENTS

We would like to express our sincerest gratitude to the School of Chemical Sciences and Food Technology UKM especially to the late Associate Prof Dr Nazaruddin Ramli for his extensive help in carrageenan analysis. This study was financially supported by Sciencefund under the Ministry of Science, Technology and Innovation (MOSTI) (02-01-02-SF0586) and Department of Fisheries Malaysia (DOF) (STGL-007-2010/17).

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