

Comparative Study of Drying Methods on Seaweeds (*Kappaphycus* sp. and *Padina* sp.) Based on Their Phytochemical and Polysaccharaide Content Located in Sabah

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ABSTRACT

Seaweed, one of the marine resources is known for their precious active compound. The dehydration process is required before the utilization of the seaweed. It helps to increase the shelf life and play a major role in the extraction of specific chemical components. This study was conducted to evaluate the effects of different drying treatments of two different seaweeds on its phytochemical contents and carrageenan properties. Seaweed used include edible seaweed which are *Kappaphycus* sp., and locally abundant seaweed *Padina* sp. Four (4) different drying methods used; namely sun-drying for five (5) days, air-drying for 14 days, freeze-drying for five (5) days, and oven drying with three different temperatures at 60 °C, 80 °C and 100 °C for six (6) h, respectively. The moisture content was measured, and air-dried seaweeds contain highest moisture content (19.32% - 16.21%). Methanol, MeOH was used as extraction solvent in the determination of phytochemicals content for total phenolic content (TPC) and total flavonoid content (TFC). Sodium hydroxide, was used to extract carrageenan from *Kappaphycus* sp., which was evaluated on their percentage yield. Oven dried at 100 °C extracts possessed lowest retention of phytochemicals content and carrageenan yield among all drying methods. This finding suggests that various drying methods applied significantly influenced the composition of seaweeds. Identifying the most effective post-harvest drying procedure for seaweed would be commercially advantageous.

Keywords: Carrageenan, drying, *Kappaphycus* sp., *Padina* sp., phytochemical.

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INTRODUCTION

In Malaysia, Sabah is notable for being a fertile ground for the growth and consolidation of several types of seaweed. It is due to geographical factor of Sabah compared to peninsular Malaysia, Sabah is geographically located below the monsoon and typhoon belt (Fudholi *et al.*, 2010; Hussin and Khoso, 2017). Been introduced since 1978, cultivation of seaweed in Sabah continues widely developed for several industries and became a job opportunity to the people (Ahemad *et al.*, 2010). Seaweed is a species of marine plants and algae that grows in the ocean, rivers, mangroves, and lakes. Seaweed has been utilised since the old times as food, grain, compost and as a wellspring of restorative medications (Mishra *et al.*, 1993). Seaweeds are categorised into three large groups

which are red algae, green algae, and brown algae, according to the pigment present. There are more than 1500 species of seaweed in each of the groups (Morais *et al.*, 2020). They also been studied for various applications, which can be incorporated into several value-added food products, animals feed, medicinal purpose, etc. as shown in Table 1.

Seaweed contains dietary fibres, vitamins, minerals, carotenoids and fatty acids. Other than that, high content of phenolics and flavonoids also reported in seaweed (Mei Ling *et al.*, 2013). Phenolic compound is a diverse group of molecules covers a wide range of aromatic secondary metabolite families. These phenolic compounds in seaweed are able to combat oxidative stress (Mei Ling *et al.*, 2013). Flavonoid is an essential antioxidant as it has

high redox potential. This content allowed seaweed to act as a great antioxidant. Several studies have conducted and reported the high

antioxidant activities of various seaweed (Yap *et al.*, 2019; Mei Ling *et al.*, 2013; Belattmania *et al.*, 2016).

Table 1. Applications of seaweed

Seaweed	Studies on Application	References
<i>Saccharina japonica</i>	Bio-oil production	Zeb <i>et al.</i> (2017)
<i>Gelidium robustum</i>	Biodegradable plastics	Freile-Pelegrín <i>et al.</i> (2007)
<i>Ascophyllum nodosum</i>	Fertilizer	Abdel-Mawgoud <i>et al.</i> (2010)
<i>Gracilaria cylindrica</i>		Yadav <i>et al.</i> (2023)
<i>Ulva rigida</i>		Latique <i>et al.</i> , (2013)
<i>Fucus spiralis</i>		
	Dermatological	Freitas <i>et al.</i> (2020)
<i>Kappaphycus alvarezii</i>	Edible film	Watt <i>et al.</i> (2014)
<i>Undaria pinnatifida</i>	Skincare	Jesumani <i>et al.</i> (2019)
<i>Fucus spiralis</i> Linnaeus	Anti-Inflammatory	Lopes <i>et al.</i> (2014)
Mixture of brown and red seaweed	Biogas production	Nkemka & Murto (2012)
<i>Gracilaria birdiae</i>	Biofilter	Marinho-Soriano <i>et al.</i> (2009)
<i>Hydrilla verticillata</i>	Adsorbent	Baral <i>et al.</i> (2009)
<i>Hypnea hippurodies</i> L.	Formulated shampoo	Tha (2012)
<i>Laminaria digitate</i>	Biofuel production	Vanegas <i>et al.</i> (2014)

Carrageenan is a polysaccharide that commonly extracted from red seaweeds (Rodophyta). It usually used in food, cosmetic and pharmaceutical industries (Moey *et al.*, 2014; Tha, 2012; Lopes *et al.*, 2014). According to Yong *et al.*, (2015), demand for carrageenan had increase. This has led to increase of cultivation of *Kappaphycus* sp. in Malaysia. Carrageenan is one of polysaccharides in seaweed other than agar and cellulose that contain galactose and glucose. Kappa-carrageenan (Figure 1) is used widely in food additives as it produces strong rigid gels (Ferdouse *et al.*, 2018). It is a colloid that used as stabilizer and thickening agent in food, cosmetic and pharmaceutical industries.

Carrageenan is an alternating copolymer of α -(1-3)-D-galactose and β -(1-4)-3,6-anhydro-D-galactose (Ili Balqis *et al.*, 2017). Two other classes of carrageenan are iota(ι)-carrageenan (Figure 2) and lambda(λ)-carrageenan (Figure 3). Iota-carrageenan comes mainly from *Eucheuma spinosum*. It gives a more elastic and soft structure while lambda-carrageenan is commonly from *Chondrus crispus* and it provides a creamy sensation in dairy products (Ferdouse *et al.*, 2018). The classification of carrageenan is according to the number of sulphate ester groups in each of them and the presence of 3,6-anhydro-D-galactose. Table 2 shows the difference of carrageenan.

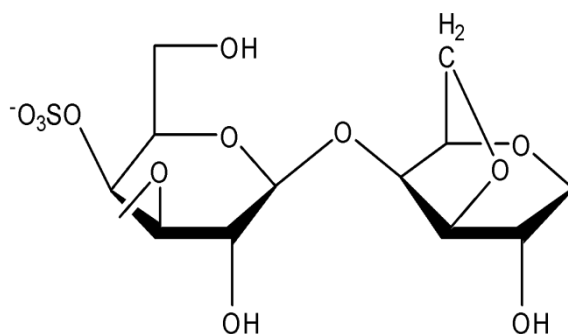


Figure 1. Kappa-carrageenan

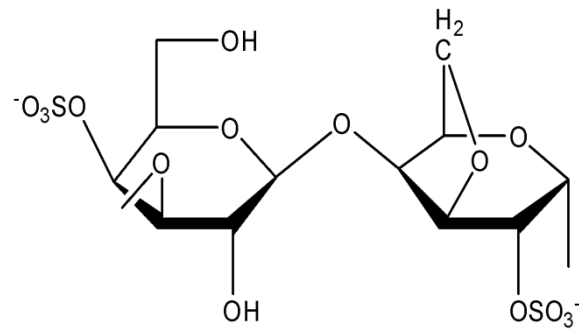


Figure 2. Iota-carrageenan

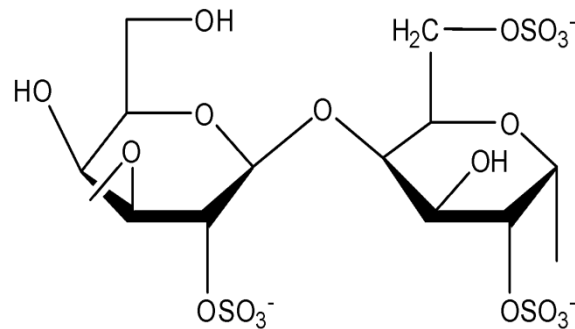


Figure 3. Lambda-carrageenan

Alginate is another type of polysaccharide extracted primarily from brown seaweeds (Phaeophyta). Commonly used in food, and actively studied on its pharmaceuticals, and biomedical applications for its gelling and binding properties (Salido *et al.*, 2024). Figure 4 shows the chemical structures of sodium alginate that is known for its ability to form gels in the presence of divalent cations like calcium. In this study, alginate is extracted from brown seaweed, *Padina* sp.

In tropical countries like Malaysia, sun drying is a common method used to maintain the root products. Drying temperature affect bioactive compound and will degrade when dried at high temperature (Ismail *et al.*, 2021). This study was conducted to investigate the impact of drying techniques on phytochemical content and polysaccharides extracted from *Kappaphycus* sp. and *Padina* sp. Methanol (80% v/v) was used for phytochemical content extraction and alkaline treatment for carrageenan extraction from *Kappaphycus* sp.

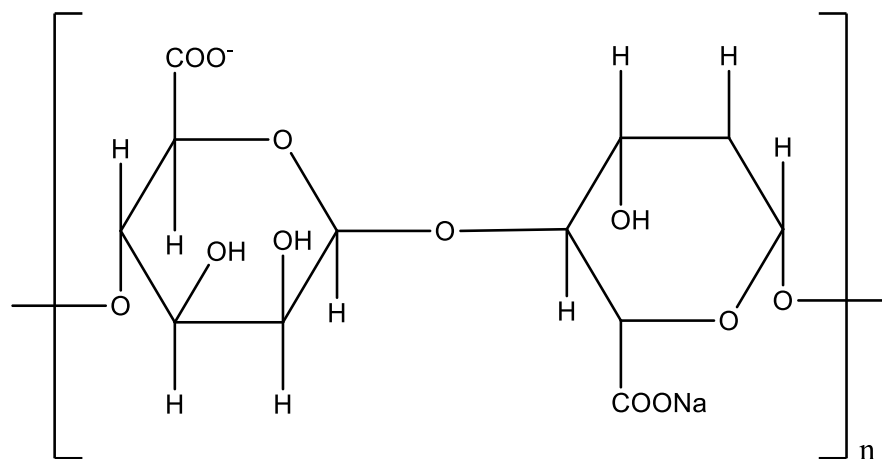


Figure 4. Sodium alginate structure

Table 2. Differences of carrageenan type (Shafie *et al.*, 2022)

Name	Structure	Main sources	Usage
Kappa-carrageenan		<i>Kappaphycus alvarezii</i>	<ul style="list-style-type: none"> • Thickening agent • Stabilising agent
Iota-carrageenan		<i>Eucheuma spinosum</i>	<ul style="list-style-type: none"> • Elastic and soft structure
Lambda-carrageenan		<i>Eucheuma gigartina</i>	<ul style="list-style-type: none"> • Thickening agent • Creamy product

MATERIALS & METHODS

Sample preparation

Both fresh *Kappaphycus* sp. and *Padina* sp. were collected from Semporna, Sabah. Seaweed was hand-picked from the cultivation locations. Harvests were occasionally cut by farmers using knives then followed by clean water wash and was sun-dried by laying on a horizontal platform before being delivered to Universiti Malaysia Sabah for experimental research purpose (Farhaduzzama *et al.*, 2023). *Kappaphycus* sp. labelled as (K) and *Padina* sp. labelled as (P). All seaweed was cleaned separately with tap water to remove epiphytes, salt and holdfast. Then, it was followed by washing with distilled water before continuing with drying process.

Drying process

Four different drying treatments were applied to both seaweeds under six different conditions. Washed seaweed was spread evenly in a single layer on trays except for freeze drying where the seaweeds were placed in a thermal container. Preliminary drying method was conducted to

achieve constant weight of dried seaweed. Sun dry and freeze dry was held for 5 days, while air dry were held for 14 days. All oven dry samples were held for 6 hours. All dried samples applied were labelled as shown in Table 3. The moisture content of dried seaweed was taken and calculated using the following formula Eq.(1):

$$\text{Moisture content (\%)} = \frac{w_1 - w_2}{w_1} \times 100 \quad \text{Eq.(1)}$$

Where,

W1: Weight (g) of sample before drying,

W2: Final weight (g) of sample after drying.

Extraction and determination of phytochemical content

Phytochemical content of dried seaweeds was extracted according to Neoh *et al.* (2021) with modification. Dried seaweed powders were extracted with 80% v/v of methanol with ratio sample: solvent of 1:20, w/v at room temperature. The mixture was sonicated for 30 minutes at 50 kHz in an Elmasonic S 180 H sonicator bath at 24°C, then shaken for 2 hours at room temperature in an incubator shaker. The

Table 3. Drying condition

Drying method	Condition for drying	Remark
Sun dry	5 days	SD
Freeze dry	-86 °C for 5 days	FD
Air dry	25 °C for 14 days	AD
Oven dry	100 °C for 6 hours	O1
Oven dry	80 °C for 6 hours	O2
Oven dry	60 °C for 6 hours	O3

extract was filtered through filter paper (Whatmann No. 1) before being stored at -20 °C for further analysis.

Total Phenolic Content (TPC)

Total phenolic content (TPC) from dried seaweed has been determined using Folin-Ciocalteu method by Ainsworth *et al.* (2007) with modification. About 100 µL of seaweed extract applied to 200 µL of Folin-Ciocalteu reagent. After five minutes, 800 µL of sodium carbonate applied to the mixture and allowed to stand in the dark room for 30 minutes. Gallic acid was used as standard. The results were expressed in the equivalent of 1 mg gallic acid equivalent per 1 g dried seaweed extract (mg GAE/g DS). A microplate spectrophotometer reader (Thermoscientific) against blank solution used to read absorbance at 765 nm. All measurements been performed in five replicates.

Total Flavonoid Content (TFC)

Total flavonoid content (TFC) been determined using the aluminium chloride calorimetric assay Neoh *et al.*, (2021) with modification. 120 µL of dried seaweed extract was used and added with 360 µL of methanol. After 5 minutes, 24 µL of aluminium chloride (10% w/v) was added to the mixture. 24 µL of potassium acetate was added to the mixture and the volume was made up to 1.2 mL with deionized water. The solution was well blended using vortex and sustained for 15 minutes in the dark room. Quercetin used as standard for standard calibration curve. Total flavonoid content was expressed as 1 mg quercetin equivalents in 1 g of dried seaweed (mg QUE/g DS). A microplate spectrophotometer reader against blank solution used to read absorbance at 510 nm. All measurements performed in five replicates.

Extraction and determination of carrageenan yield from *Kappaphycus* sp.

Carrageenan extraction for *Kappaphycus* sp. are according to Awalludin *et al.*, (2022) method with modification. five g of dried *Kappaphycus* sp. was heated in 200 mL of 1M NaOH and 200 mL of distilled water on a hot plate for 2 hours at 100°C. 100 mL of distilled water added, and the mixture then heated for another 2 hours. Yellowish-clear solution formed while heated. The mixture was filtered while hot and the solution was allowed to sustain in cold methanol overnight. The gel precipitate formed been centrifuge before filtered and oven dry at 60 °C for 6 hours. The yield of dried carrageenan was calculated using following formula; Eq. (2):

$$\text{Carrageenan yield \%} = \frac{W_c}{W_{ds}} \times 100 \text{ Eq.(2)}$$

W_c: Weight (g) of dried carrageenan,
W_{ds}: Weight (g) of dried seaweed used.

Extraction and determination of alginate yield from *Padina* sp.

Sodium alginate extraction from *Padina* sp. was performed following method of Rashedy *et al.* (2021) with slight modifications. Approximately 0.5 g of dried samples was immersed in a 1% CaCl solution overnight. The solution was then separated, and the soaked seaweed was rinsed with deionized water two to three times. Subsequently, 5% HCl solution was added and maintained at room temperature overnight. The solution was then filtered, and the residue was washed again with deionized water three times. Alginate extraction was achieved by adding 3% of Na₂CO₃ and shake for an hour. The extract was filtered through a cheesecloth filter, and the filtrate was subjected to bleaching with a 2.5% (v:v) sodium hypochlorite solution. The extract sodium alginate was precipitated in ethanol and dried at 60 °C overnight. Alginate yield was determined using following formula; Eq.(3):

$$\text{Polysaccharide yield \%} = \frac{\text{Weight (g) of dried alginate}}{\text{Weight (g) of dried seaweed}} \times 100 \quad \text{Eq.(3)}$$

RESULTS AND DISCUSSION

Moisture content

Table 4 shows the variation of moisture content for seaweeds at 6 drying methods ranged from 5.75% - 19.32%. The orders of moisture content for *Kappaphycus* sp. among different drying methods in descending order are as follows: oven dry 100 °C (15.94%) > oven dry 80 °C

(16.17%) > oven dry 100 °C (17.12%) > sun dry (18.29%) > air dry (19.32%). *Padina* sp. showed significantly lower moisture content than *Kappaphycus* sp., and this might be due to the difference in physical state of each selected seaweed. Physically, *Kappaphycus* sp. stored higher water content as it contained carrageenan, a hydrophilic compound (Farah Nurshahida *et al.*, 2020). In addition, early research had shown that drying seaweed at temperatures above 50 °C caused its colour to darken due to degradation of chlorophyll within 2 hours, which led to a loss in phytochemical content (Uribe *et al.*, 2018).

Table 4. Percentage yield of each seaweed

Seaweed	Drying techniques	Initial weight (g)	Final weight (g)	Yield (%)
<i>Kappahycus</i> sp.	SD	0.5030	0.4110	81.71
	AD	0.5113	0.4125	80.68
	FD	0.5001	0.4131	82.60
	O1	0.5140	0.4261	82.90
	O2	0.5073	0.4252	83.81
	O3	0.5021	0.4221	84.06
<i>Padina</i> sp.	SD	0.508	0.4562	89.82
	AD	0.5061	0.4243	83.83719
	FD	0.5013	0.448	89.36764
	O1	0.5011	0.465	92.79585
	O2	0.5021	0.472	94.00518
	O3	0.504	0.4753	94.30556

Phytochemical analysis

Total phenolic content (TPC) of *Kappaphycus* sp. ranged from 1.59 – 5.28 mg gallic acid equivalent (GAE) g⁻¹ dried seaweed (DS) (Table 5). It can be observed that TPC varied with different drying methods. The order of TPC for *Kappaphycus* sp. across various drying methods in descending order are as follows: oven dry at 100 °C (1.45 ± 0.68 mg GAE/g DS) > sun dry (1.59 ± 0.9 mg GAE/g DS) > oven dry 80 °C (1.61 ± 0.28 mg GAE/g DS) > oven dry 60 °C (1.65 ± 0.78 mg GAE/g DS) > freeze dry (2.31 ± 0.24 mg GAE/g DS) > air dry (5.28 ± 0.29 mg GAE/g DS).

Table 5 also shows total flavonoid content (TFC) of *Kappaphycus* sp. ranged from 0.05 – 0.12 mg quercetin equivalent (QUE) g⁻¹ dried seaweed (DS). The order of TFC for *Kappaphycus* sp. differ compared to its TPC. Order of extractability of flavonoids from

Kappaphycus sp. in descending order are as follows: sun dry (0.12 ± 0.04 mg QUE/g DS) > oven dry 60 °C (0.07 ± 0.01 mg QUE/g DS) > freeze dry and oven dry 80 °C (0.06 ± 0.005 mg QUE/g DS) > air dry and oven dry 100 °C (0.05 ± 0.006 mg QUE/g DS).

Low TPC in *Padina* sp. extracted and different order compared to *Kappaphycus* sp. with ranged from 0.89 – 1.63 mg GAE g⁻¹ dried seaweed (DS) as shown in Table 6. The order in descending order of TPC for *Padina* sp. are as follows: oven dry at 60 °C (0.89 ± 0.29 mg GAE/g DS) > freeze dry (0.97 ± 0.34 mg GAE/g DS) > oven dry 80 °C (1.25 ± 0.42 mg GAE/g DS) > oven dry 100 °C (1.34 ± 0.53 mg GAE/g DS) > air dry (1.58 ± 0.77 mg GAE/g DS) > sun dry (1.63 ± 0.31 mg GAE/g DS). Same table showed high TFC content in methanol extracts for *Padina* sp. It ranged from 0.31 – 0.95 mg quercetin equivalent (QUE) g⁻¹ dried seaweed (DS). In this study, the order in descending order

for TFC in *Padina* sp. is same as its TPC content with sun dry (0.95 ± 0.05 mg QUE/g DS) contain the highest TFC and oven dry 60°C (0.31 ± 0.005 mg QUE/g DS) contain the least TFC.

However, significant leaching of phenolic chemicals can be observed from sun drying and oven drying (100°C) in *Kappaphycus* sp. This may be due to disability of the degrading enzyme polyphenol oxidase. In contrast, *Padina* sp. shows sun dried extract exhibit the highest

phenolic content compared to other drying methods. Air drying still shows a higher TPC than other drying techniques. Oven dries of *Padina* sp. approved Gupta *et al.* (2011), where it stated that the percentage of total phenol and total flavonoid in dried seaweed will be reduced by 49-51% at temperatures below 40°C and the reduction would be decreased as the drying temperature increased more than 41°C . Both phenolics and flavonoids content in *Padina* sp. increase in oven dries ($60^\circ\text{C} > 80^\circ\text{C} > 100^\circ\text{C}$).

Table 5. Moisture content of *Kappaphycus* sp. and *Padina* sp. under different drying treatments.

Drying techniques	Moisture content (%)	
	<i>Kappaphycus</i> sp.	<i>Padina</i> sp.
SD	18.29	10.18
AD	19.32	16.21
FD	17.40	10.58
O1	17.12	7.19
O2	16.17	5.98
O3	15.94	5.75

Table 6. Phytochemical content of *Kappaphycus* sp.

Samples	Total Phenolics (mg GAE/g DS) ¹	Total Flavonoids (mg QUE/g DS) ²
KSD	1.59 ± 0.9	0.12 ± 0.04
KAD	5.28 ± 0.29	0.05 ± 0.01
KFD	2.31 ± 0.24	0.06 ± 0.01
KO1	1.65 ± 0.78	0.07 ± 0.01
KO2	1.61 ± 0.28	0.06 ± 0.005
KO3	1.45 ± 0.68	0.05 ± 0.006

¹Total phenolic content was expressed as mg gallic acid equivalents in 1g of dried seaweed (mg GAE/g DS)

²Total flavonoid content was expressed as mg quercetin equivalents in 1g of dried seaweed (mg QUE/g DS)

Carrageenan Yield

The drying process can alter both physical and chemical properties of the seaweed. The changes may impact the brittleness and texture which may influence the efficiency of subsequent carrageenan extraction processes. Figure 4 depicts the jelly-like carrageenan extracted from *Kappaphycus* sp. which is commonly utilised in food industries as thickening agent. Results show the percentage yield of carrageenan extracted from dried *Kappaphycus* sp. as shown in Figure 5. Carrageenan yield from *Kappaphycus* sp. varies depending on the drying techniques employed. These yields demonstrate variations attributed to the diverse drying methods applied to *Kappaphycus* sp. Carrageenan yield from *Kappaphycus* sp. treated with oven dry at 100°C were significantly lower than other drying treatments. Meanwhile, oven dry at 80°C yields higher carrageenan content

than others, followed by air dry, oven dry at 60°C , sun dry, freeze dry, and oven dry at 100°C . High temperatures can cause rapid evaporation of moisture from seaweed. This may cause oven dry of *Kappaphycus* sp. at 100°C yield low carrageenan as excessive moisture loss can hinder the extraction process and reduce carrageenan yield (de Faria *et al.*, 2014).

Alginate Yield

Different drying techniques influence the alginate yield from *Padina* sp. as shown in Figure 6. Significant differences in alginate yield were observed by employing different drying methods on *Padina* sp. The result shows freeze – drying *Padina* sp. gives high yield of alginate compared to other drying techniques. Freeze – drying method involves freezing materials and removing moisture through sublimation, which help in reducing the potential for thermal

degradation. As the result, this method helps preserve the structure and functionality of carrageenan molecules, potentially leading to higher alginate yields. In contrast, oven dry at 80 °C gives low alginate yields as the heat applied on *Padina* sp. can alter the structural properties of alginate. The heat may denature the proteins in alginate that affecting its gelling and stabilising capabilities (Kelishomi *et al.*, 2016).

CONCLUSION

In this study, different drying techniques on *Kappaphycus* sp. and *Padina* sp. were used to evaluate their phenolic and flavonoid content. The moisture content of seaweeds decreased along with the increase of temperature. Any type of processing and techniques applied such as high drying temperatures and extended drying may alter and lower certain phenol compounds in it (Li *et al.*, 2006). Moreover, in the least presence of moisture, all plant cell components adhere to another and potentially make the extraction difficult, resulting in lower total phenolic content. The results from this study showed that different drying techniques affect TPC and TFC indicating that they differ depending on the drying conditions. The moderate temperature (60°C) and short drying period (6 hours) used in this process, shows the least affected total phenolic content. High temperature used to both seaweeds has led to a decrease of TPC. Sun-dried samples had the lowest levels of phenolic content. This is due to the longer drying duration (4 to 5 days in direct sunshine) and dehydration throughout the drying process, the phenolic content in the drying technique was impacted. This experimental data suggests the suitability of air drying as the best drying method for seaweed to preserve phenolic and flavonoid. Oven drying (80°C) is the best drying method applied on *Kappaphycus* sp. to conserve the carrageenan content. While for *Padina* sp., freeze drying is the best drying method for high yield of alginate.

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