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The Use of Natural Solution for Odour Elimination in Red Seaweed (*Kappaphycus* spp.) and Its Effect on Antioxidant Activity

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Abstract

Kappaphycus spp. is highly demanded as raw material for kappa-carrageenan. However, the strong fishy odour of this species may limit its application in the industry. The use of chemical to remove the odour might possess hazard to the human and environment. The use of natural solution such as lemon, tamarind, rice flour and vinegar might be an interesting alternative since it is simple and environmental-friendly. In this study, different varieties of *Kappaphycus spp.* (*K.alvarezii var. Giant* (WG), *K.alvarezii var. Giant* (PG) and *K.striatum var. Green flower* (GF)) were treated with different solutions (lemon, tamarind extract, rice flour and vinegar) in order to investigate its effect on the odour and antioxidant activity. Based on the findings obtained, the highest Total Phenolic Content value (1.311 mg GAE/g), DPPH radical scavenging activity (63.322%) and FRAP value (1.285 mM/g) were observed in GF sample treated with 5% tamarind extract. Sensory analysis showed that samples treated with lemon and tamarind extract obtained the lowest score for fishy odour. The findings suggested that the treatment using 5% and 3% tamarind extract shows a promising benefit in reducing the fishy odour and at the same times, increasing the antioxidant activity.

Keyword: *Kappaphycus* spp, DPPH radical scavenging activity, FRAP value

INTRODUCTION

Seaweed is a large and diverse group of macroorganisms that play important ecological roles. It refers to multicellular, macroscopic marine algae that come in a variety of colors, including red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta), and grows in the ocean, rivers, lakes, and other bodies of water. Seaweed cultivation was brought to East Malaysia, contributing to an increase in global carrageenan production. It is now one of Malaysia's primary priority development sectors for the agriculture sector (Farah et al., 2015).

Certain seaweed species are useful for nutrition, biomedicine, bioremediation, and other purposes because they contain organic and inorganic compounds that are beneficial to human health. The seaweed is said to have a high content of fibers, vitamins, and minerals, as well as anti-inflammatory, anti-carcinogenic, and anti-microbial properties. The antioxidants, on the other hand, are the most sought-after component (Maria & Narendra, 2018). According to Datu Razali et al. (2015) the most common seaweed cultivated in Malaysia is *Kappaphycus* spp., also known as elkhorn sea moss, which can be found abundantly in tropical and temperate oceans and has been produced commercially in Malaysia.

According to Norhidayu & Aminah (2016), *Kappaphycus* spp. is one of the most important commercial sources of carrageenan which has been used as emulsifier, thickener and stabilizing agents in food processing, fertilizers and animal feeds ingredients. However, *K.alvarezii* are characterized by strong fishy odour which limits its utilization. Therefore, some sort of pre-treatment is required to remove the unpleasant odour, which can subsequently be used in small and large-scale industries like food and pharmaceuticals (Sharifah Habibah et al., 2017). Previous research looked into the usage of natural solutions such as rice water, tamarind extract, vinegar, and lemon juice in removing odours and preventing microbiological decomposition in fish (Keyimu & Aminah, 2014). Therefore, the potential of a few natural solutions to eliminate the strong fishy odor of *Kappaphycus* spp. while without reducing or affecting its antioxidant activity was explored in this work. The objective of this study was to determine and compare the antioxidants activity in raw and treated samples of *Kappaphycus* spp.

METHODOLOGY

Sample preparation

Samples used in this study were obtained from Tawau, Sabah, Malaysia which is *Kappaphycus alvarezii var. Giant* white (WG), *Kappaphycus alvarezii var. Giant* purple (PG) and *Kappaphycus striatum var. Green flower* (GF) seaweed. Samples preparation was done according to the method of Siah et al. (2014).

Odour elimination treatment (immersion method)

Odour removal treatment was performed according to the approach by of Keyimu and Aminah, (2014). Approximately 10 g of wet seaweed was soaked with 100 ml of 1%, 3% and 5% each of rice water, tamarind extract, lemon and 1% of vinegar in 250 mL conical flask respectively and distilled water was used as control in 250 mL conical flask. Rotary shaker was used to place the immersed samples for 24 hours at room temperature and 200 rpm. pH for each treatment was taken before and after 24 hours treatment using pH meter.

Extraction of phenolic compound

The extraction of the phenolic compound was performed according to Zuhair et al. (2013) with some modifications. The universal bottle was filled with one gram of sample. The samples were homogenized in a homogenizer at 24,000 rpm for 1 minute after being combined with 10 mL of 50% acetone. After centrifuging the material for 10 minutes at 1,000 rpm, the supernatant was collected for further analysis.

Total phenolic content (TPC) analysis

TPC analysis was performed according to the method stated by Ganesan et al. (2008) with modifications where each sample extract (100 µL) was mixed with 2 mL of 2% sodium carbonate and then was incubated for at least 2 minutes before 100 µL of 50% folin-ciocalteu reagent was added. The mixture was incubated at room temperature in darkness for 30 minutes. The absorbance reading was taken at 720 nm wavelength against blank solution. Calibration curve using gallic acid solution was prepared. The result was expressed as mg of gallic acid equivalents per gram sample (mg GAE/g) using the following equation (1):

$$\text{TPC value} = \frac{\text{TPC per mL} \times \text{dilution factor} \times \text{solvent volume (100 mL)}}{\text{seaweed weight}}$$

Equation (1)

DPPH free radical scavenging activity determination

DPPH free radical scavenging activity determination was performed according to the method stated by Norakma et al. (2018) with modifications where approximately 1 mL of 0.1 mM methanolic DPPH was mixed with 1 mL of seaweed extracts. The sample then was incubated in darkness for 30 minutes at room temperature. Control was prepared by mixing 1 mL of methanol and 1 mL of DPPH. The absorbance reading was taken at 517 nm wavelength against blank solution. Calibration curve using ascorbic acid solution was prepared. The radical scavenging activity was expressed as radical scavenging activity percentage using the following equation (2):

$$\%\ \text{scavenging} = \left(\frac{\text{Ac}-\text{As}}{\text{Ac}}\right) \times 100\%$$

Equation (2)

Where A_c = absorbance of control and A_s = absorbance of sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP analysis was conducted according to the method described by Benzie and Strain (1996) with modifications. Calibration curve using $FeSO_4.7H_2O$ aqueous solution was prepared. FRAP assay was performed by mixing 150 µl of seaweed extracts with 2,850 µl of FRAP reagent. Then, the mixture was incubated at 37°C in water bath for 4 mins. The absorbance was determined at 593 nm against blank using distilled water by using UV spectrophotometer. The value of FRAP was calculated using equation (3) as follow:

$$\text{FRAP value} = \frac{\text{raw FRAP value} \times \text{dilution factor} \times \text{solvent volume (100 mL)}}{\text{seaweed weight}}$$

Equation (3)

Sensory analysis

Sensory analysis was conducted according to Le et al. (2002) with modifications. Hedonic scale was used to tabulate the scoring data. The experiments were carried out in triplicates and One-way ANOVA using SPSS ver. 20 was used to analyse the experimental data.

RESULTS AND DISCUSSION

Total phenolic content (TPC) analysis

Table 1 shows the TPC values for all samples. According to Table 1, the 3 % rice flour treatment had the highest TPC value (1.249 mg GAE/g), whereas the PG treated with 5 % lemon juice had the lowest value (0.630 mg GAE/g). The maximum TPC value was obtained from WG treated with 5% tamarind extract (1.308 mg GAE/g), and the lowest TPC value was obtained from WG treated with 1% lemon juice (0.600 mg GAE/g). The maximum TPC value in GF was 1.311 mg GAE/g in the 5% tamarind extract treatment, while the lowest value was 0.659 mg GAE/g in the 1% lemon juice treatment.

When examined with TPC analysis, Bhuyar et al. (2019); Babak and Arezoo (2018) found that the seaweed possessed increased antioxidant activity due to the presence of phenolic compounds. The total phenolic content of treated samples was substantially higher than that of untreated samples, according to these data. According to Aminah and Keyimu (2014), the higher the TPC value, the greater the antioxidant activity.

Table 1 TPC value of *Kappaphycus* spp. in different concentrations of natural solution.

Seaweed Solution		PG	WG	GF
Vinegar	1%	$1.016^{a} \pm 0.030$	$0.977^{a} \pm 0.023$	$0.712^{a} \pm 0.002$
	Control	$1.059^{a} \pm 0.007$	$0.898^{a} \pm 0.009$	$0.970^{a} \pm 0.029$
Lemon juice	1%	$0.640^{a} \pm 0.002$	$0.600^{a} \pm 0.004$	$0.659^{a} \pm 0.008$
	3%	$0.685^{a} \pm 0.010$	$0.613^{a} \pm 0.005$	$0.685^{a} \pm 0.007$
	5%	$0.630^{a} \pm 0.005$	$0.656^{a} \pm 0.012$	$0.666^{a} \pm 0.004$
	Control	$1.059^{a} \pm 0.007$	$0.898^{a} \pm 0.009$	$0.970^{a} \pm 0.029$
Tamarind extract	1%	$0.836^{a} \pm 0.007$	$0.823^{a} \pm 0.010$	$0.924^{a} \pm 0.016$
	3%	$1.049^{a} \pm 0.020$	$1.069^{a} \pm 0.013$	$1.072^{a} \pm 0.015$
	5%	$1.167^{a} \pm 0.005$	$1.308^{a} \pm 0.012$	$1.311^{a} \pm 0.007$
	Control	$1.059^{a} \pm 0.007$	$0.898^{a} \pm 0.009$	$0.970^{a} \pm 0.029$
Rice flour	1%	$1.069^{ab} \pm 0.009$	$0.941^{a} \pm 0.002$	$1.213^{b} \pm 0.012$
	3%	$1.249^{b} \pm 0.015$	$0.879^{a} \pm 0.006$	$1.124^{ab} \pm 0.008$
	5%	$0.983^{a} \pm 0.003$	$1.082^{ab} \pm 0.010$	$1.294^{b} \pm 0.011$
	Control	$1.059^{a} \pm 0.007$	$0.898^{a} \pm 0.009$	$0.970^{a} \pm 0.029$

The TPC value (mg GAE/g) are expressed as mean ± SD of triplicates samples (n = 3). (Horizontal) The values with different superscript letter (a and b) denotes with significantly different ($p<0.05$). Note: PG (*K. alvarezii var. Giant* (purple seaweed)), WG (*K.alvarezii var. Giant* (white seaweed)),and GF (*K. striatum var. Green Flower)*

DPPH radical scavenging activity

The DPPH radical scavenging activity of all samples was tabulated in Table 2. Referring to Table 2, the result for the highest DPPH value of PG was 3% tamarind extract (61.451%) while the lowest value was control (15.986%). The highest value of DPPH for WG can be seen in 5% tamarind extract which was 62.358% while the lowest value was 3% rice flour which is 32.120%. For GF sample, the highest DPPH value (63.322%) was found in sample treated with 5% tamarind extract and the lowest value (11.054%) in sample treated with 5% rice flour.

The finding suggested that 5% and 3% tamarind extracts were capable of retaining or increasing the antioxidant activity when tested with DPPH analysis. Through previous study conducted by Aminah and Keyimu (2014) reported that samples treated with 5% of lemon and rice flour showed higher antioxidant activity as compared to the untreated samples. Aminah and Keyimu (2014) also reported that the highest antioxidant activity was found in sample treated with 5% rice flour solution. It is suggested that the antioxidant capability of sample might be contributed by the presence of phenolic compound in the sample and other antioxidant compound present in the natural solution treatment that being used.

Table 2 DPPH radical scavenging activity (%) of *Kappaphycus* spp. in different concentrations of natural solution.

Seaweed	Solution	PG	WG	GF
Vinegar	1%	$31.519^{a} \pm 0.140$	$37.302^{a} \pm 0.035$	$41.609^{a} \pm 0.055$
	Control	$15.986^{a} \pm 0.108$	$35.374^{a} \pm 0.008$	$46.088^{a} \pm 0.072$
Lemon juice	1%	$46.372^{a} \pm 0.077$	$43.481^{a} \pm 0.057$	$46.882^{a} \pm 0.020$
	3%	$51.417^{a} \pm 0.031$	$43.481^{a} \pm 0.046$	$40.420^{a} \pm 0.024$
	5%	$43.594^{a} \pm 0.047$	$48.243^{a} \pm 0.017$	$42.120^{a} \pm 0.006$
	Control	$15.986^{a} \pm 0.108$	$35.374^{a} \pm 0.008$	$46.088^{a} \pm 0.072$
Tamarind extract	1%	$47.676^{a} \pm 0.030$	$47.109^{a} \pm 0.060$	$52.268^{a} \pm 0.024$
	3%	$61.451^{a} \pm 0.016$	$54.308^{ab} \pm 0.030$	$52.041^{b} \pm 0.012$
	5%	$46.088^{b} \pm 0.011$	$62.358^{a} \pm 0.050$	$63.322^{a} \pm 0.030$
	Control	$15.986^{a} \pm 0.108$	$35.374^{a} \pm 0.008$	$46.088^{a} \pm 0.072$
Rice flour	1%	$18.821^{b} \pm 0.041$	$39.683^{a} \pm 0.020$	$31.576^{a} \pm 0.021$
	3%	$22.959^{b} \pm 0.0191$	$32.120^{b} \pm 0.038$	$52.721^{a} \pm 0.024$
	5%	$10.658^{b} \pm 0.051$	$50.680^{a} \pm 0.015$	$11.054^{b} \pm 0.020$
	Control	$15.986^{a} \pm 0.108$	$35.374^{a} \pm 0.008$	$46.088^{a} \pm 0.072$

The scavenging value (%) are expressed as mean ± SD of triplicates samples (n = 3). (Horizontal) The values with different superscript letter ([a] and [b]) denotes with significantly different ($p<0.05$). Note: PG (*K. alvarezii var. Giant* (purple seaweed)), WG (*K. alvarezii var. Giant* (white seaweed)), and GF (*K. striatum var. Green Flower*).

FRAP value

Based on the present study conducted (Table 3), The highest result of FRAP for PG was 0.900 mM/g which was treated with 3% tamarind extract and lowest values was 3% rice flour which is 0.858 mM/g. The highest FRAP value of WG can be seen in the treatment with 3% rice flour (0.872mM/g), while the lowest value was the control (0.844 mM/g). The highest FRAP values of GF are 5% tamarind extract which was 1.285 mM/g and the lowest value are 1.231mM/g (control).

Table 3 FRAP value (mM/g) of *Kappaphycus* spp. in different concentrations of natural solution.

Seaweed	Solution	PG	WG	GF
Vinegar	1%	$0.860^{a} \pm 0.010$	$0.871^{a} \pm 0.028$	$1.252^{b} \pm 0.020$
	Control	$0.863^{b} \pm 0.009$	$0.844^{a} \pm 0.007$	$1.231^{c} \pm 0.005$
Lemon juice	1%	$0.863^{a} \pm 0.010$	$0.865^{a} \pm 0.005$	$1.248^{b} \pm 0.007$
	3%	$0.862^{a} \pm 0.005$	$0.868^{a} \pm 0.007$	$1.252^{b} \pm 0.005$
	5%	$0.860^{a} \pm 0.010$	$0.864^{a} \pm 0.008$	$1.231^{b} \pm 0.007$
	Control	$0.863^{b} \pm 0.009$	$0.844^{a} \pm 0.007$	$1.231^{c} \pm 0.005$
Tamarind extract	1%	$0.861^{a} \pm 0.001$	$0.863^{a} \pm 0.005$	$1.244^{b} \pm 0.035$
	3%	$0.900^{a} \pm 0.058$	$0.866^{a} \pm 0.005$	$1.239^{b} \pm 0.010$
	5%	$0.882^{a} \pm 0.028$	$0.871^{a} \pm 0.019$	$1.285^{b} \pm 0.010$
	Control	$0.863^{b} \pm 0.009$	$0.844^{a} \pm 0.007$	$1.231^{c} \pm 0.005$
Rice flour	1%	$0.863^{a} \pm 0.010$	$0.869^{a} \pm 0.008$	$1.279^{b} \pm 0.008$
	3%	$0.858^{a} \pm 0.001$	$0.872^{a} \pm 0.013$	$1.234^{b} \pm 0.024$
	5%	$0.861^{a} \pm 0.003$	$0.867^{a} \pm 0.008$	$1.266^{b} \pm 0.009$
	Control	$0.863^{b} \pm 0.009$	$0.844^{a} \pm 0.007$	$1.231^{c} \pm 0.005$

The FRAP value (mM/g) was expressed as mean ± SD of triplicates samples (n = 3). (Horizontal) The values with different superscript letter ([a], [b] and [c]) denotes with significantly different ($p<0.05$). Note: PG (*K. alvarezii var. Giant* (purple seaweed)), WG (*K. alvarezii var. Giant* (white seaweed)), and GF (*K. striatum var. Green Flower*).

The present finding shows GF seaweed demonstrated higher reducing power of 1.285 mM/g when being treated with 5% tamarind extract. The usage of 5% and 3% tamarind extract in treating seaweed samples that capable to reduce power of antioxidant activity. Previous research has shown that the K. alvarezii var. green flower seaweed demonstrated the highest capabilities of reducing power which contributed by the presence of phenolic compound in the sample and other antioxidant compounds present in the natural solution treatment that being used. Nur Shahirah (2016) stated that the highest the value of FRAP, the higher the antioxidant activity.

Sensory analysis

The sensory analysis was conducted in empty room provided with table and chair. The sample was placed on the tray and 35 panellists were asked to smell the sample given in the designated box. The panellist was asked to smell the coffee bean provided to neutralize the smell every time before smell the samples. The questionnaires were assessed using hedonic scale on the odour only.

Based on Table 4, using 1% lemon juices on PG, WG treated with 1% lemon juices and GF treated with 5% lemon had the lowest score for the fishy odour while the untreated (control) retain the highest score for the fishy odour. Then, PG treated with 5% tamarind extract, WG treated with 5% lemon juices and GF seaweed treated with 3% tamarind extract presented with medium score for fishy odour while the untreated (control) retain the highest score for the fishy odour. Lemon juice and tamarind extract usage were able to reduce the odour of the seaweed due to the amine compound and had a pleasant odour compared to the untreated (control) samples. Moreover, both treatment solutions which are acidic in nature, able to neutralize the alkaline based amine effect thus eliminate the possible odour produced. The seaweed treatment with rice flour producing off-flavour due to free fatty acid in the rice itself as stated by Keyimu and Aminah (2014).

Table 4 Sensory score for fishy odour of control and treated samples

Seaweed Solution		PG	WG	GF
Control		5.000 ± 4.320	4.735 ± 2.670	4.735 ± 2.504
Vinegar	1%	3.889 ± 2.848	4.735 ± 2.120	3.889 ± 2.088
Lemon juices	1%	3.889 ± 1.900	3.889 ± 1.364	4.375 ± 2.560
	3%	4.735 ± 1.408	4.375 ± 1.598	3.889 ± 2.205
	5%	3.889 ± 2.315	4.375 ± 1.506	3.889 ± 1.833
Tamarind extract	1%	5.833 ± 3.971	3.889 ± 2.315	7.000 ± 3.082
	3%	3.889 ± 2.088	3.889 ± 2.147	4.375 ± 2.446
	5%	4.111 ± 2.977	3.889 ± 1.537	4.375 ± 2.925
Rice flour	1%	5.833 ± 3.189	3.889 ± 1.764	5.000 ± 5.164
	3%	5.833 ± 4.262	3.889 ± 2.088	5.000 ± 5.228
	5%	5.000 ± 4.397	4.500 ± 2.070	5.833 ± 3.869

CONCLUSION

Overall finding showed in term of TPC value, the phenolic contents in each seaweed sample where GF treated with 5% tamarind extract displayed higher TPC value (1.311 mg GAE/g) with higher percentage of DPPH by 63.322% with the same treatment. The other laboratory finding based on FRAP assay where GF seaweed treated with 5% tamarind extract showed the highest value of 1.285 mM/g respectively. Based on the sensory evaluation, it showed that the usage of the 5%, 3% and 1% lemon juice and tamarind extract produced the lowest score which suggested a great potential to eliminate the fishy odour besides producing pleasant smell compared to the untreated (control) samples and rice flour treatment.

In conclusion, the application of 5% and 3% tamarind extract can be considered as odour eliminating agent and at the same times, enhancing the antioxidant activity of seaweed. Further analysis should be carried out using qualitative method to determine the specific phytochemical that being altered during treatment. Further sensory analysis should be carried out in proper and specific conditions to get more precise and reliable sensory result.

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