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FULL PAPER

Bioprospecting brown algae (*Sargassum polycystum* C. Agardh) as a potential antioxidant additive in snack bar and its sensory evaluation

[1,*]Nurcahyanti, A.D.R., [2]Puika, V.I., [2]Pramitasari, R. and [1]Notario, D.

[1]*Department of Pharmacy, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya 2, 14440 Jakarta, Indonesia*
[2]*Department of Food Technology, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Raya Cisauk Lapan, Sampora, 15345 Banten, Indonesia*

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Abstract

Fucoxanthin from brown algae has potential as a promising functional food component. However, fucoxanthin is less stable under high temperature and light exposure. The purpose of this study was to analyse the effect of stevia and cacao butter in increasing the stability of *Sargassum polycystum* C. Agardh extract containing fucoxanthin and its application as a snack bar. Brown algae, *S. polycystum*, was extracted using 96% food-grade ethanol. Antioxidant activity and stability assay were performed using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method. Antioxidant activity of both, extract (E) and extract + stevia (ES), was found to decrease with time. Meanwhile, cacao butter was able to maintain extract's stability without any significant difference when compared to extract alone. A combination of extract, stevia, and cacao butter (ESC) were found to be able to maintain antioxidant stability. Formulation of a snack bar with cacao butter and 1% stevia were more preferred by the panellists when compared to the other formulations. This study reports that cacao butter and 1% stevia is able to maintain antioxidant stability and improve the economic value of *S. polycystum*, one of them through the formulations of the snack bar.

1. Introduction

Bioprospecting for sustainable antioxidant sources is promising opportunity since nature provides an abundant source of bioactive compounds. Utilization of the terrestrial resources has become the attention of major pharmaceutical and food industries worldwide (Dias *et al*., 2012). Limited application of marine resources is unfortunate as invertebrates, microalgae, and macroalgae that are usually found in these areas produce unique chemical substances with different biological activities (Montaser and Luesch, 2011; Martins *et al*., 2014). Macroalgae, such as *Sargassum*, has been used for ages in Traditional Chinese Medicine as seaweed medicine (Liu *et al*., 2012). Macroalgae are highly distributed and can be found in abundance. They can be easily harvested and huge potential lies in exploring and developing them as a commercial product. It is the potential to explore and develop as commercial product, especially for coastal communities (Loureiro *et al*., 2015; Kim *et al*., 2017).

Macroalgae or seaweed is categorized - based on their pigmentation properties - as brown algae (*Phaeophyceae*), red algae (*Rhodophyceae*), and green seaweed (*Chlorophyceae*) (Biris-Dorhoi *et al*., 2020). Brown algae have gained scientific interest due to carotenoids and phenolics contained that are beneficial for human health (Catarino *et al*., 2017; Hakim and Patel, 2020; Miyashita *et al*., 2020). The concentration of bioactive substances has been found to vary with geography and seasons, affecting not only metabolites production but also their biological activity (Wells *et al*., 2017). Examples of other algae that contains fucoxanthin are *Alaria esculenta* blade (0.87 mg/g) dry mass, *Fucus vesiculosus* (0.69 mg/g) and *Laminaria digitata* (0.65 mg/g) (Shannon and Abu-Ghannam, 2017).

Fucoxanthin is a major pigment present in brown algae (Xiao *et al*., 2012) and it has been well reported to possess a broad spectrum of pharmacological properties including antioxidant, anti-inflammatory, neuroprotector, and antiobesity activity (Peng *et al*., 2011; Gammone and D'Orazio, 2015; Mohibbullah *et al*., 2018). Carotenoids and phenolics in macroalgae have been proven to be associated with antioxidant activity, with

*Corresponding author.
Email: *adr.nurcahyanti@atmajaya.ac.id*

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fucoxanthin being the lead compound responsible for this (Foo *et al*., 2017). Fucoxanthin harbours alenic and 5,6-monoepoxide bonds (Miyashita *et al*., 2011; Shang *et al*., 2011) that is responsible for various pharmacological effects, including anti-oxidant (Yu *et al*., 2018) which is achieved by scavenging free radicals (Foo *et al*., 2017; Mohibbullah *et al*., 2018).

The aim of this study was the development of a snack bar enriched with brown algae extract from *Sargassum polycystum* C. Argardh, as a functional food. The different formulations used in this study were chosen while the low stability of fucoxanthin under acidic pH, temperature, and bright light led to its degradation (Zhao *et al*., 2019). A snack bar can be produced under a simple process and formulated in low-temperature settings. Due to the low stability of algal bioactive substances, such as fucoxanthin, cacao butter was tested as a stabilizing agent. Cacao butter is unique as it is brittle and melts quickly at room temperature. It also contains less polar bioactive substances - similar to fucoxanthin (a less polar carotenoid). Cacao butter contains triglycerides (triacylglycerols) as the main components along with other oils and fats. Triglycerides are esters of glycerol and fatty acids in which carbon atoms numbered l-3 of glycerol are most often interchangeable and very difficult to differentiate, and the 2-position is of special interest. This position of fatty acid is responsible for nutritional and functional values (Lipp and Anklam, 1998). Another important ingredient used in this study was stevia (Ahmad and Ahmad, 2018). The effect of cacao butter and stevia to retain antioxidant activity was assessed and sensory evaluation in snack bar formulation was discussed.

2. Materials and methods

2.1 Materials

Sargassum polycystum was collected from Sumbawa district, West Nusa Tenggara between April and May 2019. Morphological identification was performed by a senior marine biologist at the Research Center for Oceanography, Indonesian Institute of Sciences. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), methanol, DMSO, and fucoxanthin standard were purchased from Sigma-Aldrich USA. Ethanol and distilled water were obtained as an analytical grade. Cacao butter, stevia powder, and other snack bar ingredients like creamer, dried coconut, rolled oats, crispy rice, raisins, and almonds were purchased from a supermarket in South Jakarta.

2.2 Preparation of Sargassum polycystum and extraction

Sargassum polycystum was washed and air-dried for 5 days. *Sargassum polycystum* was then dried at 45°C for 8 hrs. Dried *S. polycystum* was pulverized using a food processor and filtered using a 40 mesh sieve. *Sargassum polycystum* powder was placed in a dark and well-sealed container at 25°C. *Sargassum polycycstum* is extracted following methods developed by Delbrut *et al*. (2018) with slight modification on duration (Delbrut *et al*., 2018). The extract was macerated in Ethanol absolute at 1:5 ratio and placed at 100 rpm orbital shaker. After 24 hrs, the solvent was evaporated using a vacuum rotary evaporator at 40°C, 60 rpm. The remaining solvent was evaporated by blowing using liquid nitrogen. The solvent-free extract was sealed and placed at 4°C and in dark until further analysis.

2.3 High-performance liquid chromatography-PDA

Analysis of fucoxanthin content was performed using High-Performance Liquid Chromatography (HPLC) Prominence Shimadzu and diode array SPD-M20A. Solid-phase used was C30 YMC (150 mm × 4.6 mm; Particle Size 5 µm), whereas the mobile phases used were methanol, methyl tert-butyl ether (MTBE), and distilled water HPLC grade (85:11:4). The flow rate was maintained at 1 mL/minute isocratic. The sample was filtered using 0.20 µm Minisart filters, then 20 µL was injected into the loop-injector. Detection of fucoxanthin was performed using a photodiode array (190-800 nm) at 446 nm. Fucoxanthin (Sigma Aldrich) was used to make a standard curve.

2.4 Antioxidant assay

Antioxidant activity of all ingredients was measured using the DPPH method as developed in a previous study by Brand-Williams *et al*. (1995). In brief, the sample was diluted in ethanol absolute and diluted in the same solvent for the assay. Serial dilution of the sample or extract standard was mixed with 1 mL DPPH 0.05 mM in Ethanol 96%. The mixture was incubated for 30 mins away from direct light exposure and the absorbance (A_1) was measured at 517 nm. Ethanol was used as the blank and measured at the same wavelength (A_0). Scavenging ability (%) was calculated by using the formula:

$$\text{Scavenging ability } (\%) = \left[\frac{(A0 - A1)}{A0}\right] \times 100$$

where A_0 was the absorbance of reaction control and A_1 was the absorbance of the sample

2.5 Effect of cacao butter, stevia, and its combination on antioxidant activity

Different formulations containing different amounts of *S. polycystum* extract, cacao butter, and stevia are described in Table 1. The combination was examined for their antioxidant activity using the DPPH method as described above at day-0, 1, and 6. Formulation and measurement of antioxidant activity were performed at room temperature (25°C) and low light exposure.

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Table 1. Different combination of *S. polycystum* extract, cacao butter, and stevia

Ingredient	E (mg)	EC (mg)	ES (mg)	ESC (mg)
S. polycystum extract	10	10	10	10
Cacao butter	0	100	0	100
Stevia	0	0	100	100

E = *S. polycystum* extract, EC = *S. polycystum* extract + cacao butter, ES = *S. polycystum* extract + stevia, ESC = *S. polycystum* extract + stevia + cacao butter

2.6 Snack bar formulation enriched with Sargassum polycystum extract

The snack bar was sized at 30 g apiece (Table 2). Tempering of cacao butter was performed using the double boiler method until the temperature reached 35° C. *Sargassum polycystum* extract was then added, followed by creamer and stevia. The mixture was stirred until it cooled down to 25°C. The mixture was maintained at 25°C for 10 mins. The remaining ingredients were then added, mixed thoroughly, and allowed to sit until they solidified. The snack bar was stored at 25°C, in a well-sealed food container and kept away from direct light.

Table 2. Formulation of snack bar

Ingredient	Formula (g)		
	1	2	3
Cacao butter	9.945	9.895	9.745
Stevia	0.05	0.1	0.25
S. polycystum extract	0.005	0.005	0.005
Creamer powder	5	5	5
Dried coconut	3	3	3
Rolled oats	3	3	3
Crispy rice	3	3	3
Raisin	3	3	3
Almond	3	3	3

2.7 Texture analysis of snack bar

Texture analysis was performed using a texturometer (Agrosta, France). Individual snack bar formulas were tested in triplicates following methods developed by (Arozarena *et al*., 2012). Hardness level was the parameter observed for this analysis and a commercial product (Fitbar Fruits, Kalbe) served as the control.

2.8 Sensory evaluation of snack bar

Sensory evaluation was performed using the hedonic assay (Meilgaard MC, Civille GV, 2016). The panellists consisted of 30 semi-trained individuals and the parameters tested were colour, appearance, aroma, texture, taste, aftertaste, and overall appearance. Each parameter was scored on a scale ranging from 1 (least favoured) to 9 (most favoured). Formulation with the highest score was used in further analysis.

2.9 Proximate analysis

Proximate analysis was performed to identify the presence of carbohydrates, protein, lipid, and fibre including micronutrients such as vitamin A, vitamin C, and vitamin E and carried out based on the International Standard guideline and the Weende proximate analysis system (Badan Standardisasi Nasional (BSN), 2008). Analysis was performed in the Laboratory of the Indonesian Ministry of Agriculture.

2.10 Shelf-life analysis

Shelf-life analysis of snack bar with formula 2 was performed for 28 days at 7-day intervals. Moisture or water content using the standard gravimetric method (Vera Zambrano *et al*., 2019), microbial contamination using the standard *total plate count* (TPC) method (Udayasoorian *et al*., 2017), and water activity using Aqua lab 4TE were measured during this period. All analysis was performed in triplicates.

2.11 Statistic analysis

Data from an organoleptic test, texture analysis, and self-life analysis was analyzed using one-way ANOVA followed by Duncan analysis to identify the presence of significant differences in treatment at a 95% confidence interval. Analysis of Dose-Response Curves was used in the antioxidant analysis.

3. Results and discussion

3.1 Extraction, identification, and antioxidant activity of fucoxanthin

The extraction yielded 0.70% of crude extract, slightly higher than what Gazali *et al*. (2018) reported, 0.56% (Gazali *et al*., 2018). Fucoxanthin peak was detected at a retention time of 3.954 minutes similar to the retention time of standard. The concentration of fucoxanthin obtained in this study, measured with HPLC, was 1.79 mg/g extract. Vieira *et al*. (2017) suggested fucoxanthin concentration in the carotenoid extract is in the range of 0.1 to 1.89 mg/g *Sargassum* sp dry mass (Martins *et al*., 2014).

Antioxidant activity of *S. polycystum* extract and individual ingredients was in descending order were as follows: ascorbic acid (positive control) > rice crispy > *S. polycystum* extract > cacao butter > raisin > stevia powder > almond > dried coconut > rolled oats > creamer powder (Table 3).

On its own, *S. polycystum* extract has an IC_{50} value of 0.16±0.03 mg/mL. A study on the algal, antioxidant activity showed that carotenoids and phenolics are significantly associated with antioxidant activity.

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Fucoxanthin is one of the dominant substances responsible for the activity (Foo *et al.*, 2017). In addition, the activity can be due to the synergistic effect between fucoxanthin and phenolics contained in the extract, one of them by preventing oxidative reaction and scavenging superoxide anions ($O2^{\bullet-}$) (Miyashita *et al*., 2011; Peng *et al*., 2011). Crispy rice showed profound antioxidant activity compared to other ingredients. This could be due to the presence of added antioxidant agents such as vitamin E and butylated hydroxytoluene (BHT) to maintain the stability and shelf-life of a food product. In addition, processing technology to produce crispy rice may also affect the active compounds in rice such as catechin and cyanidin 3-O-glucoside with potential antioxidant activity. A rice cooker and water bath are the best techniques to maintain losses of phenolics in rice. Other than that methods, processing rice can diminish active substances, such as boiling and removing the remaining cooking water, microwave, and pressure cooker (Fracassetti *et al*., 2020). Cacao butter contains fatty acids like oleic acid, palmitic acid, and stearic acid that are able to scavenge free radicals (Torres-Moreno *et al*., 2015). In addition, high phenolics content in cacao is also responsible for its antioxidant activity (Maleyki and Ismail, 2010; Katz *et al*., 2011). Other ingredients showed some degree of antioxidant activity, this may be due to the processing technologies causing partial or full degradation of bioactive substances (Al-juhaimi *et al*., 2018).

Table 3. IC_{50} DPPH of snack bar ingredients

Ingredient	IC_{50} (mg/mL)
Ascorbic acid	0.0018±0.00
Crispy rice	0.11±0.03
S. polycystum	0.16±0.03
Cacao butter	0.37±0.03
Raisin	1.84±0.08
Stevia powder	2.37±0.08
Almond	3.85±1.30
Dried coconut	11.78±0.91
Rolled oats	21.90±0.64
Powder creamer	46.92±0.21

3.2 Antioxidant activity combination of Sargassum polycystum extract, cacao butter, and stevia

Antioxidant activity of *S. polycystum* extract combined with cacao butter, stevia, or both of them was assessed and observed at day-0, 1, and 6 (Table 4). *S. polycystum* extract in combination with cacao butter showed greater antioxidant activity compared to its activity. This activity was maintained up until day-6. Synergistic effects can be explained due to the high content of fatty acids with low polarity improves the dissolution of the extract and thus improving the ability of active substances to scavenge free radicals. On the other hand, when the extract was combined with stevia, no improvement in antioxidant activity was observed (Table 4). The combination of three substances, extract, cacao butter, and stevia improved antioxidant activity when compared to the individual ingredients, however, a significant decrease is observed until day-6 ($p<0.05$, Table 4).

In this study, antioxidant activity acts as one of the stability parameters. Compounds with antioxidant activity in *S. polycystum* synergistically interact with polyphenols, phenolics, phytosterols, and α-tocopherol contained in cacao butter (Roiaini *et al*., 2016). Rao *et al.* (2007) observed that coconut oil, peanut oil, and mustard oil are able to maintain the stability of carotenoid astaxanthin esters due to the presence of polyphenols and α-tocopherol in the oils (Rao *et al*., 2007). In addition, cacao butter consists of triglycerides possessing carbon atoms numbered -3 of glycerol that is most often interchangeable. This property protects *S. polycystum* extract and its bioactive compounds from oxidative reaction (Boon *et al*., 2010; Ergönül *et al*., 2010). Analysis of stability using analytical approaches such as chromatography is required for further analysis.

3.3 Snack bar formulation enriched with Sargassum polycystum extract and texture analysis

Figure 1 shows snack bar formulation enriched with 5 mg *S. polycystum* extract. No visual difference is observed across the three formulations.

a b c

Figure 1. Snack bar formulation enriched with *S. polycystum* extract

Texture analysis was performed using a texturometer showing no significant difference was found among the three formulas when compared to a commercial snack

Table 4. Antioxidant activity of *S. polycystum* extract, cacao butter, stevia, and their combination

Day	*S. polycystum* extract	*S. polycystum* extract + Stevia	*S. polycystum* extract + Cacao butter	*S. polycystum* extract + Cacao butter + Stevia
0	2..65±0.66[a]	6.96±0.32[a]	1.54±0.17[a]	1.72±0.08[a]
1	5.67±0.79[b]	3.05±1.40[b]	1.21±0.07[b]	2.05±0.08[b]
6	12.62±0.89[c]	9.23±0.23[c]	1.56±0.05[a]	3.02±0.17[c]

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different at 95% confidence.

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bar (p≥0.05, Figure 2).

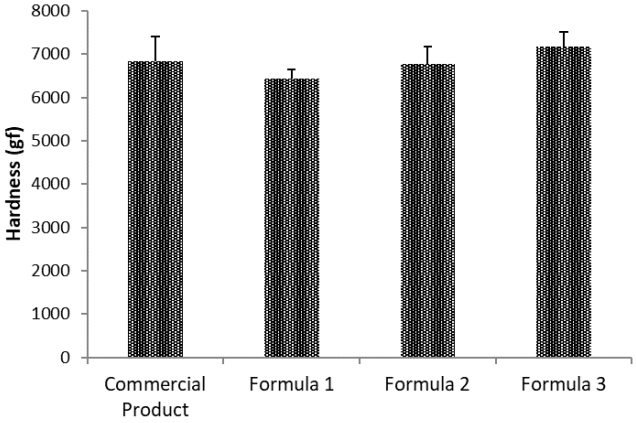

Figure 2. Texture of three snack bar formulas enriched with *S. polycystum* extract

3.4 Organoleptic evaluation of snack bar

Organoleptic evaluation is summarized in Table 5. Results obtained show that formula 2 is most favoured amongst the panellists. Based on the organoleptic evaluation, no significant difference was found in the colour, appearance, aroma, and texture of the three formulations, there were no significant differences among the three formulas. Based on taste and aftertaste, formula 3 was least favoured. Formula 3 contained the highest concentration of stevia that may have contributed to the bitter taste. Stevioside contained in stevia is responsible for the bitter taste and aftertaste. Other compounds such as volatile aromatics, tannins, and flavonoids may have also contributed to the bitter taste (Abou-arab *et al*., 2010). Formula 2 was chosen as the best formula from the organoleptic evaluation.

Table 5. Organoleptic analysis of three snack bar formulas

Criteria	Formula 1	Formula 2	Formula 3
Colour	7.27±0.72[a]	7.33±0.74[a]	7.17±0.76[a]
Appearance	6.93±1.47[a]	6.90±1.28[a]	6.90±1.22[a]
Aroma	6.40±1.47[a]	6.60±1.36[a]	6.33±1.41[a]
Texture	6.67±1.55[a]	7.00±1.43[a]	6.70±1.48[a]
Taste	5.43±1.96[a]	6.27±1.67[a]	3.60±1.63[b]
Aftertaste	5.17±2.12[a]	5.83±1.64[a]	3.60±1.69[b]
Overall	5.67±1.69[a]	6.50±1.31[b]	4.53±1.57[c]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different at 95% confidence.

3.5 Proximate analysis of snack bar

Proximate analysis of the best snack bar formulation, formula 2, is described in Table 6. The energy value of the 30 g snack bar was 144.33 kcal. A commercial snack bar used as a control in this study has an energy value of 132 kcal/30 g. A snack bar with formula 2 is categorized as an intermediate energy snack bar as the total energy value lies in between energy snack bar (280 kcal) and common snack bar (100 kcal) (Silva *et al*., 2013).

Table 6. Proximate analysis of formula 2

Component	Content (%)
Water content	5.83
Protein	6.22
Lipid	45.55
Fibre	5.48
Ash	1.28
Carbohydrate ready to use (available)	35.64
Total Carbohydrate	41.12

3.6 Snack bar shelf-life based on water content and water activity

There was no significant difference between water content on day-0 to day-28 (p≥0.05, Table 7). In addition, the water activity of snack bar formula 2 showed significant differences between day-0, 14, and 21 (p<0.05, Table 7), and no significant difference between day-21 and day-28 (p≥0.05, Table 7). Total plate count (TPC) evaluation showed no microbial growth from day-0 to day-28, indicating that snack bar formula 2 is safe for at least 28 days.

Table 7. Water content and water activity of formula 2 snack bar

Day	Water Content	Water Activity (A_w)
0	5.28±1.01[a]	0.49±0.00[a]
7	5.43±0.93[a]	0.54±0.00[b]
14	5.49±2.12[a]	0.52±0.00[c]
28	6.09±1.03[a]	0.50±0.00[c]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different at 95% confidence.

Water activity (A_w) is one of the factors affecting the growth of microbes in food. Microorganisms can grow only when water activity is greater than 0.61 (Rao *et al*., 2012). A_w of formula 2 was between 0.49 and 0.54 showing that the snack bar possessed low water activity and thus no microbial growth was observed.

4. Conclusion

Sargassum polycystum extract contains fucoxanthin and showed high antioxidant activity. In combination with cacao butter, antioxidant activity can be maintained, whilst in combination with stevia, the antioxidant activity was reduced. When applied onto a snack bar formula, concentration of stevia 1% and cacao butter was able to maintain antioxidant activity and was the most favoured formula based on organoleptic evaluation by semi-trained panellists. This study reported the application of *S. polycystum* extract in combination with cacao butter as a promising functional food with

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enhanced antioxidant activity. This application is simple enough to be performed by local communities in coastal areas. A further investigation employing broader organoleptic evaluation and lower temperature processing is warranted to improve the quality and shelf life of the product.

Conflict of interest

The authors declare no conflict of interest.

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