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# ALGINATE CHARACTERISTICS AND FUCOXANTHIN CONTENT FROM *SARGASSUM POLYCYSTUM* THROUGH ULTRASOUND-ASSISTED EXTRACTION AND THE RELATIONSHIP WITH WATER QUALITY OF HABITAT

***Siti Zachro Nurbani*[1], *I Ketut Sumandiarsa*[2], *Laely Nur Hidayah*[3], *Rezky Yunitasari Siregar*[4]**

[1] ORCID: 0009-0002-9022-2303
[2] ORCID: 0000-0002-0560-209X
[3] ORCID: 0009-0000-0384-1786
[4] ORCID: 0009-0002-9149-5928
[1–3] Jakarta Technical University of Fisheries, Indonesia
[4] PT Prima Indo Fish, Benoa, Bali

Key words: macroalgae, alginate, fucoxanthin, ultrasound-assisted extraction (UAE), water quality.

Abstract

The study attempted to obtain alginate and fucoxanthin from *Sargassum polycystum* C. Agardh using ultrasound-assisted extraction (UAE) and to correlate these with the water's environmental condition. The alginate was extracted using an acid pathway, the fucoxanthin was quantified using high-performance liquid chromatography, and the trace element was obtained using an atomic absorption spectrophotometer (AAS). The nutrients and micronutrient composition of *S. polycystum* were dominated by carbohydrates (32.8; 1.6%), with the main trace elements being selenium, iron, copper, and zinc. The alginate was attributed to 8.98±0.1 pH, 12.55±0.5% of water content, 22.63±0.9% of ash content, 70.28±0.5 Cps. of viscosity, and 37.24±0.8% of yield. The extract was also containing fucoxanthin, which was relatively high (0.41–0.59 mg $g^{-1}$). There was a favourable association between water quality and the content of alginate, fucoxanthin, and nutrition. According to the ultrasonication extraction process, *S. polycystum* could produce high-quality alginate with a high fucoxanthin content.

## Introduction

Seaweed as macroalgae is divided into three phyla based on the pigment colours, which are brown seaweed (Ochrophyta, class Phaeophyta),

Address: Siti Zachro Nurbani, Jakarta Technical University of Fisheries, Jakarta, Indonesia, e-mail: sitinurbani8@gmail.com

red (Rhodophyta), and green (Chlorophyta). More than 4400 species spread throughout the world, among which red species are the most abundant (DAWES 2016). Seaweed in Indonesia is an essential commodity, with 9.3 million tons of cultivated products in 2018 (FAO 2020). The study conducted by PUSPITA et al. (2020) stated the national production was still dominated by red seaweed species, specifically *Kappaphycus alvarezii*, *Eucheuma spinosum*, and *Gracilaria* sp., while *Gelidium* spp. and *Sargassum* spp., are still based on the natural harvested. *Sargassum* is the most diverse brown seaweed and has been reported to dominate Indonesian waters in terms of biomass and abundance, especially in the intertidal zone (WOUTHUYZEN et al. 2016, SETYAWIDATI et al. 2018, SUMANDIARSA et al. 2020b).

One types of *Sargassum* that is most often found in Indonesian waters is *Sargassum polycystum*. The study results by SUMANDIARSA et al. (2021a), found the distribution of this species varied between locations and seasons in Indonesian waters. The nutrients contained was also rich with carbohydrate is the highest, between 40–50% dry weight (KUMAR et al. 2015, PRAIBOON et al. 2018, SALOSSO 2019). In addition, the micronutrients contents such as iron (Fe), manganese (Mn), and iodine (I), were relatively high, which has potential to be used as additives in seaweed-based food and non-food products (FLORES et al. 2015, CIRCUNCISÃO et al. 2018, SUMANDIARSA et al. 2020a).

Alginate, as a polysaccharide, has been extracted from *Sargassum* seaweed, with the characteristics were comparable to those main resources. Currently, the largest alginate producers were *Laminaria* spp., *Lessonia* spp., and *Macrocystis*, accounting for 89% of world production (PORSE and RUDOLPH 2017). Several studies showed *Sargassum* produced a variation of alginate yields, such as about 8.5–45.54% from *S. cristaefolium* (SUGIONO et al. 2019), 23% from *S. natans,* and 17% from *S. vulgare* (RHEIN-KNUDSEN et al. 2017), 12.09–25.77% and 28.22% from *S. polycystum* (DHARMAYANTI et al. 2019, SUMANDIARSA et al. 2020a), and about 24% from *Sargassum* sp. (MOHAMMED et al. 2020).

Meanwhile, fucoxanthin as bioactive compound was also reported as valuable composited and found quite high in *Sargassum*, essentially in *S. horneri* (3.7 mg $g^{-1}$) and *S. cinereum* (0.179 mg $g^{-1}$), as well as *Sargassum* sp. (0.47 mg $g^{-1}$) (KUMAR et al. 2013, NURSID et al. 2015, RENHORAN et al. 2017, MIYASHITA et al. 2020). Thus, bioactive contents found varied depending on species, habitat temperature, depth, season, and the use of extraction method (PÁDUA et al. 2015, TERASAKI et al. 2017).

In response to the information stated above, the purposed of this study is to determine the effect of employing ultrasound-assisted extraction

(UAE) in the production of alginate and fucoxanthin from *S. polycystum*, as well as the influence of water quality on the habitat.

## Materials and Methods

### Sampling location

Brown *seaweed Sargassum polycystum* was taken from Tidung island waters in October 2019 (Figure 1). The location characteristics were flat and coral formed islands with classic sandy, muddy, and dead coral sediment. Anthropogenic pressure seems to be a big problem on Tidung Island, caused by the dense population (4,651 people km$^{-2}$) and tourism activities. The limitation of rainfall has also reduced the flow of organic matter to the coastline, in consequence, it was less fertile. However, *Sargassum* were still grow well and dominated as attached to the dead corals or any concrete substrate.

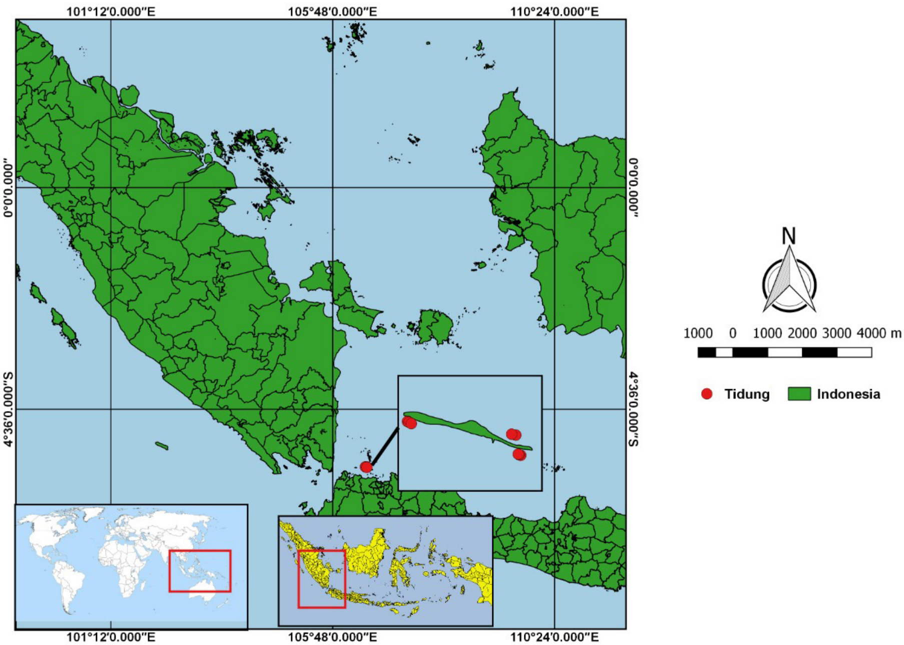


Fig. 1. Research location at Tidung Island, Seribu Island District, Jakarta

## Research procedures

### Sample collection and preparation

Seaweed samples were taken at optimal low tide with a depth between 15–30 cm. The distance between sampling points was approximately 50 meters parallel to the shoreline. Sample preparation was divide based on nutrition and alginate extraction purposes which were using dried samples, while fucoxanthin extraction from fresh pieces. Drying is done by dried the sample directly under the sunlight for two days, then stored dry until the next test. Fresh samples were prepared through a cleaning process with seawater, then stored in Ziplock plastic that had been given 90% ethanol. The samples were then stored in Styrofoam boxes provided ice to keep them cool and protected from direct sunlight. Furthermore, the samples were stored in cold storage with a temperature of -23°C before further analysis.

### Water quality

Parameters measured were dissolved oxygen (DO), pH, temperature, salinity, brightness, nitrate, phosphate, and ammonia. Physical chemistry parameters were measured directly at the research site (in situ) using the multi-parameter testing tool 1P67 Combo 8630. The nitrate, phosphate, and ammonia parameters of seawater were tested using a UV–VIS spectrophotometer using the APHA method (2017).

## Nutritional composition analysis

### Proximate composition

The Determination includes water, ash, protein, fat, crude fibre, and carbohydrate content. Water content (%) analysis conducted by 2 grams sample and placed in an oven at 105°C for 3 hours. The oven-dried sample was placed in a desiccator until it cooled and then weighed. Ash content was tested through an ashing process using a muffle furnace at a temperature of 550°C until it was white and free of carbon. Samples were weighed after cooling from the desiccator. Both tests refer to the AOAC method. The protein content was analysed using the Kjeldahl method with a conversion factor of 6.25, while the fat was tested by the gravimetric method, which refers to the AOAC standard. The crude fibre composition was tested using the $H_2SO_4$ destruction method followed by NaOH; then, the measurements were based on the weighing results after the heating process in the Furnace (AOAC 2005). The carbohydrate content of the sample is calculated based on the by difference, which is 100% – (moisture + ash + protein + fat + crude fibre).

### Trace elements content

The content of micronutrients in the form of Barium (Ba), Selenium (Se), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), and Molybdenum (Mo). 5 g of crushed dry samples were acidified using 5 ml of $HNO_3$, then stirred. The stirred sample was put in a 100 ml measuring cup, then added 5 ml of HCl and heated in a steam bath for 15 minutes. Then the sample was filtered using filter paper (polycarbonate) with a size of 0.40–0.45 m and added 100 ml of distilled water, stirred again, and analysed with AAS Pin Aacle 900 H with a detection limit of 0.001 mg $kg^{-1}$ with the APHA method, *23$^{rd}$ Edition* in the Proling laboratory of IPB.

### Alginate extraction and characterization

Alginate extraction using ultrasonication method (Ultrasound-assisted extraction) based on research by YOUSSOUF et al. (2017). A total of 25 grams of sample was immersed in 250 ml of 80% ethanol then filtered, then extracted on an ultrasonic device in $Na_2CO_3$ solvent. The Ultrasound was run at 35 kHz for 30 minutes. Furthermore, the precipitation was using a 99% technical isopropyl alcohol solution. All chemicals were purchase from Mallinckrodt chemical, USA.

Alginate yield. Alginate yield was calculated based on the extraction result after the drying process (sodium alginate flour) against the dry weight of the raw material, as figured out below.

$$\text{Yields [\%]} = \frac{\text{weight of alginate acid flour [g]}}{\text{weight of dry seaweed [g]}} \cdot 100\%$$

### Water content

The moisture content was obtained from the oven method, namely a 2 g sample was heated in an oven at 105°C for 4 hours, then the weighing was carried out after the sample in the desiccator had cooled. The percentage difference in weight produced is recorded as water content.

### Ash content

The ashing method done under a muffle furnace at a temperature of 550°C. A percentage difference between the sample that has become ash and the initial sample is recorded as ash content of alginate.

### Viscosity

Determination of alginate viscosity was carried out using a viscometer spindle (TV-10). alginate sample about 2.5 g dissolved in 50 mL of 20% $Na_2CO_3$ at a temperature of 80°C, then poured into a viscometer tube and set at a speed of 60 rpm. The resulting viscosity value is expressed in centiPoise (cP).

### Alginate pH

About 3 g of the samples was dissolved into 197 ml of distilled water. Then, the solution was heated at 80°C for 10 minutes until the alginate was completely dissolved. After the temperature dropped to 250°C, pH measurements were carried out using the IONIX pH5S Spear pH Tester.

### Extraction and determination of fucoxanthin content

Extraction using Ultrasound Assisted Extraction (UAE) LC60H made in Germany with 90% Acetone solvent from Mallinckrodt chemical, USA (Pro analyst) with a ratio of 1:6 weight/volume. The frequency of the Ultrasound water bath was 35 kHz with an extraction temperature of <30°C within 120 minutes, where the method was a modification from Kawee Ai et al. (2013). The resulting filtrate was then centrifuged at 4°C and a speed of 4,000 rpm for 20 minutes, then filtered with Whatman 2 paper and evaporated by using a Heidolph HB control rotary evaporator made in Germany. The evaporated solid was then diluted with 10 ml of ethanol and followed by centrifugation at a speed of 3000 rpm for 20 minutes at 4°C. Then the samples were freeze and stored at freezing temperatures (-20°C) before being quantified by HPLC. The fucoxanthin content was analysed using a Shimadzu LC-20A High-Performance Liquid chromatography (HPLC) equipped with a UV–Vis detector and separated using a C18 (20 mm x 250 mm) column (Luna Phenomenex). The mobile phase is HPLC grade water (solvent A) and acetonitrile (solvent B) with gradient elution system: 0 minutes, 80:20 (v/v) A: B, flow rate 15 mL/minute, for 30 minutes, and injection volume 2 mL. The detection wavelength was 254 nm, and the column temperature was 30°C. The amount of fucoxanthin in mg/g was obtained from converting the area of the active fraction of the sample to the standard fucoxanthin curve. The fucoxanthin standard was obtained from Sigma-Aldrich, Germany.

### Statistical analysis

All data are displayed with the mean and standard deviation. The response of the habitat environment to the content of nutrients, alginate, and fucoxanthin was analysed using Canonical Correspondence Analysis (CCA) multivariate statistics. Statistical analysis performed by Past Statistical Software V4.02 (HAMMER 2020).

## Results

### Proximate and trace element contents

The study found that carbohydrate content of *S. polycystum* was the most dominant, reaching 32.8%, followed by ash content (18.2%). The fat and protein contents showed the opposite, which was only 0.6 and 1.45%, respectively. Besides, the highest micronutrient was iron content of 0.219 mg $kg^{-1}$, followed by zinc, copper, and selenium. In contrast, three trace elements were not detected, which were barium, manganese, and molybdenum. Proximate composition and micronutrient contents are presented in Table 1.

Table 1

Proximate and micronutrient composition of *S. polycystum* ($n$ = 3; mean ±SD)

| Test parameters | Amount |
|---|---|
| Water [%] | 18.2 ±0.5 |
| Ash [%] | 26.9±0.9 |
| Proteins [%] | 1.45±0.1 |
| Fat [%] | 0.61±0.08 |
| Crude fibre [%] | 20.02±1.5 |
| Carbohydrates [%] | 32.8±1.6 |
| Barium [mg $kg^{-1}$] | 0 |
| Selenium [mg $kg^{-1}$] | 0.0011±0.0001 |
| Iron [mg $kg^{-1}$] | 0.219±0.07 |
| Manganese [mg $kg^{-1}$] | 0 |
| Copper [mg $kg^{-1}$] | 0.0083±0.0006 |
| Zinc [mg $kg^{-1}$] | 0.0.0143±0.003 |
| Molybdenum [mg $kg^{-1}$] | 0 |

## Characteristics of ultrasonication extracted alginate

The results obtained from alginate characterization is presented in Table 2, which showed a high yield that more than 37%. The ash and water content levels indicated that the alginate was still in compliance with commercial standards, as they only reached 12.55 and 22.63 percent, respectively. The viscosity value, which was 70 Cps in this study, is one of the most important parameters in determining alginate quality. It is indicated that *S. polycystum* has the potential to be a resource for alginate. This is also supported by a reasonably good pH value of 8.98.

Table 2

Characteristics of alginate (*n* = 6; mean±SD)

| Parameter | Value |
|---|---|
| Yield [%] | 37.24±0.8 |
| Water content [%] | 12.55±0.5 |
| Ash Content [%] | 22.63±0.9 |
| Viscosity [Cps] | 70.28±0.5 |
| pH | 8.98±0.1 |

## Fucoxanthin content of *S. polycystum*

The total fucoxanthin extracted from *S. polycystum* is presented in Table 3. Extraction of bioactive from macroalgae using Ultrasound is an environmentally friendly method (green extraction method/technology). The amount of fucoxanthin obtained was relatively high, and there was no statistically significant difference between extractions, with the highest being about 0.59 mg $g^{-1}$ and the lowest being about 0.41 mg $g^{-1}$.

Table 3

Fucoxanthin from *S. polycystum* seaweed. (*n* = 6; mean ±SD)

| Extraction | Total content [mg $g^{-1}$] |
|---|---|
| 1 | 0.436±0.006 |
| 2 | 0.413±0.02 |
| 3 | 0.59±0.02 |

## Sea-water quality

Table 4 shows the physical and chemical conditions of the waters at the time of sampling and the environment of sampling site showed in Figure 2. DO levels indicate a fairly good requirement of seawater quality, which is also supported by pH conditions that are favourable. Furthermore, the salinity is quite high (35.5‰), which corresponds to the average temperature of 27.5°C. Besides, the waters of the study site demonstrated good water fertility, with nitrate, phosphate, and ammonia levels of 0.34, 0.02, and 0.86 mg $L^{-1}$, respectively.

Table 4

Water conditions in Tidung Island ($n$ = 9; mean ± SD)

| Water quality parameters | Value |
|---|---|
| DO [mg $L^{-1}$] | 6.53±0.8 |
| pH | 7.35±0.3 |
| Temperature [°C] | 27.50±0.1 |
| Salinity [‰] | 35.45±0.5 |
| Brightness <1 meter [%] | 100±0.0 |
| Nitrate [mg $L^{-1}$] | 0.34±0.2 |
| Phosphate [mg $L^{-1}$] | 0.02±0.001 |
| Ammonia [mg $L^{-1}$] | 0.86±0.04 |

Fig. 2. Condition of sampling locations and samples of *S. polycystum* seaweed

## Environmental response to nutrient content, alginate, and fucoxanthin

Figure 3 depicts a biplot of canonical correspondence analysis results. According to the statistical analysis, there were variations in the data, with 70.1 percent and 29.9 percent contributions on axes 1 and 2, respectively. It demonstrates that environmental variables have a strong influence on the characteristics studied. Environmental variables such as phosphate, DO, salinity, pH, and temperature all had different effects on alginate yield and carbohydrate percentage. The ammonia content of seawater, on the other hand, influences the majority of the proximate composition of seaweed, including fat, protein, ash, and water content. Furthermore, the iron microminerals found in *S. polycystum* were linked to pH, salinity, DO, and temperature. Zinc, copper, and selenium, on the other hand, had a negative response to these variables. The content of fucoxanthin was only associated with phosphate and was negatively correlated with nitrate and ammonia variables.

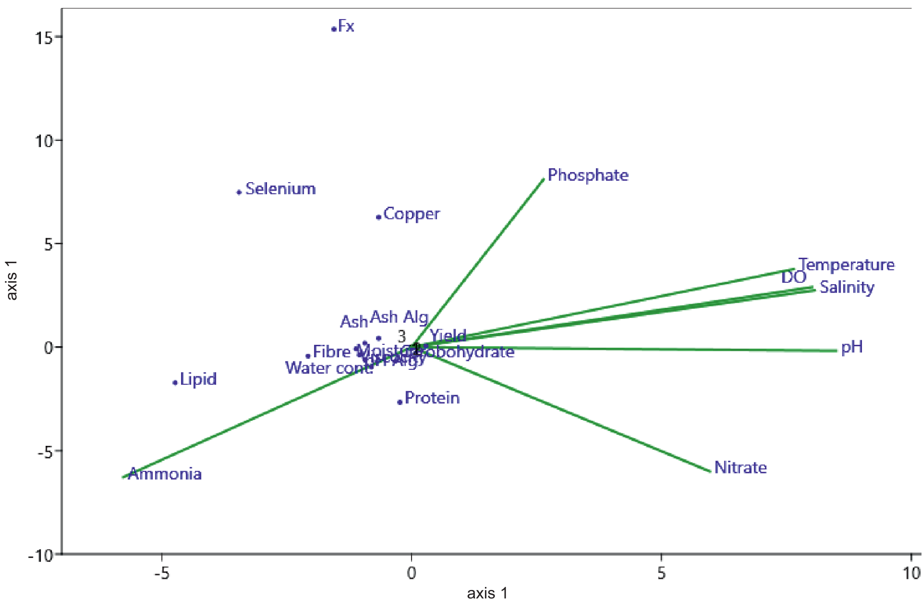


Fig. 3. Results of the canonical environmental correspondence analysis and nutritional characteristics, alginate, and fucoxanthin content of *S. polycystum*

## Discussion

### Proximate and trace element contents

Carbohydrate's content measured as general that were not differentiated based on their constituent monomers. The abundance of carbohydrates was related to photosynthesis process and food storage function in seaweed thallus. The high percentage was relatively similar with previous studies, such as 33.49% in *S. polycystum* from Malaysian waters (Matanjun et al. 2009) and 25% from Tamil Nadu, India (Perumal et al. 2019). The following essential nutrient was protein content with a lower concentration compared to other types of *Sargassum* such as *S. wightii* reaching 8–12% (Peng et al. 2015), while crude fibre is quite high at 20% when compared with *S. wightii* according to Syad et al. (2013) which reached 17%. On the other hand, the ash content showed a fairly high concentration and was comparable to studies on the types of *S. natans* and *S. vulgare* of 16 and 34%, respectively (Rhein-Knudsen et al. 2017).

The content of micronutrients shows promising potential, with a trend from the highest to the lowest, namely iron > copper > selenium > zinc > manganese > barium > molybdenum. The last three elements were not detected or below the detection limit of the AAS tool. The concentration of this nutrient varies according to species and growing location, so it is widely used as a bioindicator of the aquatic environment, such as the presence of Cu, Mn, and Zn (BRITO et al. 2012, MALEA and KEVREKIDIS 2014, SIREGAR et al. 2016). On the other hand, these micronutrients are useful for the human diet because they are needed in protein metabolism and enzyme formation (TSUJI et al. 2016). Adequate concentration and regular intake of seaweed products that contain trace elements can help fulfil the diet.

### Characteristics of ultrasonication extracted alginate

The yield of alginate extraction in yield characteristics is quite high compared to other *Sargassum* types, namely 13.7% of *S. muticum* (MAZUMDER et al. 2016) and *S. aquifolium* of 39.01% (SETYAWIDATI et al. 2018). The yield of the same species but with different methods was recorded at 12.09–25.77% from Banten waters, Indonesia (DHARMAYANTI et al. 2019) and 28.22% (SUMANDIARSA et al. 2020a) from the waters of Lampung, Indonesia. The amount of alginate in *Sargassum seaweed* is influenced by various factors, including age, fertility phase, and water depth, affecting adaptation to nutrient availability in seawater (PAUL et al. 2020). Alginate from *S. polycystum* is promising due to yield attributes; the utilisation of alginate also depends on the characteristics of viscosity, molecular weight, and monomer composition of mannuronic acid and guluronate (M/G). These rheological characteristics may vary due to biota conditions and habitat environment, such as the research results (SUMANDIARSA et al. 2021b), which found variations in viscosity and M/G ratio in alginate from *S. polycystum* that grows in various small islands in Indonesia. The viscosity obtained was not high, 70.22 Cps, but it was comparable to the results of previous studies. It found that *S. polycystum,* which grew in Banten, Indonesia, reached a viscosity of 35.00–81.33 Cps (DHARMAYANTI et al. 2019) but lower than the type found in the same species grew in Lampung waters was 195.7 Cps (SUMANDIARSA et al. 2020a).

The following characteristics are water content and alginate ash, which were detected at 12.55% and 22.62%. The composition of these two variables plays a role in determining the solubility of alginate required for its utilization. The water and ash content still followed the commercial alginate standard, namely 12% water content and 18–27% ash content (HERNÁNDEZ-CARMONA et al. 2013). On the other hand, the pH value of the alginate produced was relatively high. It was reaching 8.98, which is far above the commercial standard as found in research (KOK and WONG 2018) on the type of *S. polycystum* in Malaysian waters, which is 7.42 and higher than the research results by MAHARANI et al. (2017) of 7.2–7.77 in *S. fluitans* seaweed.

We found that the extraction of alginate from *S. polycystum* by ultrasonication was able to produce alginate characteristics comparable to those produced by conventional extraction. According to research (YOUSSOUF et al. 2017), the Ultrasound-assisted extraction (UAE) method can increase the yield and does not damage the rheological characteristics of the resulting alginate. In addition, this method can minimize the use of chemicals, temperature, and extraction time (FLÓREZ-FERNÁNDEZ et al. 2019). Based on these results, extraction using this method has promising potential. Thus, alginate utilization in the pharmaceutical and food world can be expanded due to supporting factors in physicochemical characteristics such as viscosity, gel properties, strong water solubility, high stability, non-toxicity, and high biocompatibility (ZHANG et al. 2020).

## Fucoxanthin content

Extraction of bioactive from macroalgae using Ultrasound is an environmentally friendly method (green extraction method/technology). It refers to various advantages, namely producing good yields, using fewer chemicals, environmentally friendly but capable solvent performance, and being more economical (CHEMAT et al. 2017, OKOLIE et al. 2019, TIWARI 2015). The total fucoxanthin extracted from *S. polycystum* is presented in Table 3. The fucoxanthin obtained was relatively high between 0.41–0.59 mg $g^{-1}$. These results are comparable with previous studies, including 0.47 mg $g^{-1}$ of *Sargassum* sp. taken from the waters of Lampung, Indonesia (RENHORAN et al. 2017) and around 0.155–0.587 mg/g of *S. polycystum,* which grows in western waters of Indonesia (RENHORAN et al. 2017, SUMANDIARSA et al. 2021b).

The amount of fucoxanthin contained in macroalgae generally varies and is influenced by various factors such as natural conditions, type of species, age, and part of the thallus. The study results (HEFFERNAN et al. 2016, TERASAKI et al. 2017) showed that habitat conditions and extraction methods resulted in varying amounts of fucoxanthin bioactive between species. This compound has excellent potential as a pharmaceutical ingredient because it can be used as an antioxidant, anticancer, anti-obesity, and anti-inflammatory (HEO et al. 2012, MOGHADAMTOUSI et al. 2014, GAMMONE and ORAZIO 2015, JOHNSON et al. 2019).

Fucoxanthin produced from seaweed *S. polycystum* in this study showed great potential. Utilisation as an ingredient in medicines, cosmetics, and other uses can be well explored due to the abundant biodiversity in Indonesian waters. One of the available potentials is the seaweed that grows on small islands such as Tidung Island, which has a large enough biomass and maintained habitat conditions. *Sargassum polycystum* is a brown seaweed that can grow in almost all parts of Indonesia which generally has hard substrates such as dead coral, coral reefs, and coral debris (Sumandiarsa et al. 2021a). This biota can grow well and dominate the waters because of its adaptability and high tolerance to drastic environmental changes (May-Lin and Ching-Lee 2013, Muta Harah et al. 2014, Hoang et al. 2016).

## Environmental condition

Tidung Island is a small island located in the Thousand Islands, Jakarta Bay, Indonesia, and is famous as a tourism destination. Anthropogenic pressure on this island is large enough to affect the condition of the waters on this island. The physical and chemical conditions of the waters at the time of sampling were determined. As an area with a dense population and the dominance of tourism activities, the waters around Tidung Island are under relatively high pressure, as happened in various other islands with high tourism activities (Kurniawan et al. 2016). The measurement results of environmental conditions show that the waters around the island are still quite good, characterised by high DO, good ratio of nitrate and phosphate, and controlled ammonia content. However, there are differences with other studies in the waters of the Thousand Islands with higher DO (13.4 mg $L^{-1}$) and lower salinity (30 permil) (Widiarti et al. 2021).

Anthropogenic factors significantly affect the waters' characteristics because organic materials can quickly enter the waters in large quantities. In addition to anthropogenic, climate change also significantly impacts coastal habitats, including macroalgae based on growth, distribution, and resilience (Harley et al. 2012). *Sargassum* can grow in extreme environmental conditions in the intertidal region, especially in limited nutritional conditions due to dynamic hydrodynamic processes (Lalegerie et al. 2020). The amount of biomass of this biota depends on the requirements of water fertility, namely the concentration of nitrate, phosphate, and ammonia (Hoang et al. 2016).

In general, the nutrients in the waters of Tidung Island are quite fertile, as can be seen from the water quality parameters in Table 4. High water quality can support the growth of various biota well, thus spurring intense competition. These conditions also encourage these biotas to produce specific chemical defences that can threaten between biotas. Species with high adaptive capacity will survive and be characterized by dominant biomass (Puglisi et al. 2014, Bui et al. 2018). The quality of the habitat may also be seen as an overview of the coastal conditions at the sample location shown in Figure 3.

## Environmental response to nutrient content, alginate, and fucoxanthin

Based on the canonical correspondence analysis results, there are variations in the data with contributions on axes 1 and 2 of 70.1% and 29.9%, respectively. It proves that there is a strong response from environmental variables to the characteristics studied. Environmental variables gave various responses, mostly in phosphate, DO, salinity, pH, and temperature, which affected the alginate yield and carbohydrate percentage. On the other hand, the ammonia content of seawater affects most of the proximate composition of seaweed, namely fat, protein, ash, and water content.

Furthermore, the micro minerals of iron contained in *S. polycystum* were associated with pH, salinity, DO, and temperature. On the other hand, zinc, copper, and selenium gave a negative response to these variables. Fucoxanthin content showed remarkable results, which was only associated with phosphate and negatively correlated with nitrate and ammonia variables. The quality of seawater influences the nutrient content of *Sargassum seaweed* as wild macroalgae in its habitat. The study results (Perumal et al. 2019, Praiboon et al. 2018) stated that the season affected the aquatic conditions of the *Sargassum* habitat and significantly affected its nutritional composition, including the proximate composition. Seasonal variations that have an impact on water quality also affect the metabolic processes of macroalgae. So, the accumulation of macronutrients can vary between species and between species that grow (Balboa et al. 2016, D'aRMAS et al. 2019).

Metabolites as critical chemical compounds produced by macroalgae also vary among the same species, such as in the production of alginate and fucoxanthin. Alginate characteristics of *Sargassum* are positively correlated with environmental conditions such as water depths that have variations in seawater nutrients. The depth of 10 meters resulted in the highest alginate yield due to low nutrition, which forced this biota to adapt molecularly to produce more food reserves through the production of polysaccharides (Paul et al. 2020). On the other hand, fucoxanthin as a secondary metabolite is more influenced by the availability of light and the period of exposure to direct sunlight. Fucoxanthin content in *S. polycystum* is strongly correlated with water fertility in nitrate, phosphate, and ammonia in the waters (Sumandiarsa et al. 2021b).

Based on the results obtained from this study, it was found that *Sargassum polycystum* seaweed has the potential as a source of nutrients, both trace elements and macronutrients. Furthermore, ultrasonication assisted extraction was able to produce better alginate and fucoxanthin than conventional extraction. Thus, extraction by ultrasonication can be suggested to have more prominent metabolites to be one of the best alternatives. This is supported by the results of previous studies by (Borazjani et al. 2017, Youssouf et al. 2017, Flórez-Fernández et al. 2019, Yuan et al. 2020), in which ultrasonication was able to produce alginate as a polysaccharide and fucoxanthin as part of bioactive carotenoids with high effectiveness.

## Conclusion

Ultrasound-assisted extraction method to obtain metabolites from *S. polycystum* seaweed, namely alginate and fucoxanthin, is encouraging. It is evident from the quantitatively high number of compounds compared to conventional methods. The proximate composition of this species is dominated by carbohydrates and fibre and small amounts of protein and fat. However, the composition of trace elements is quite high in the form of iron, zinc, copper, and selenium, which humans need in the diet process. A total of 37.24% alginate with a viscosity of 70 Cps and 0.41–0.56 mg $g^{-1}$ fucoxanthin was produced from ultrasonication extraction, proving that this method is effective and efficient. It also found that environmental conditions gave a strong response to the content of nutrients, alginate, and fucoxanthin, especially by variations in nitrate, phosphate, and ammonia. Thus, the extraction method and aquatic habitat conditions on Tidung Island affect the nutritional and metabolite characteristics of *S. plycystum*.

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