Food Research 8 (Suppl. 2) : 22 - 29 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Nutritional, physicochemical and sensory evaluation of biscuits enriched with *Ulva* sp. from Gunungkidul coast

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Article history:
Received: 5 March 2023
Received in revised form: 27 September 2023
Accepted: 20 January 2024
Available Online: 30 March 2024

Keywords:
Biscuits,
Ulva sp.
Physicochemical,
Antioxidant,
Dietary fiber,
Sensory evaluation

DOI:
https://doi.org/10.26656/fr.2017.8(S2).34

Abstract

Ulva sp. a seaweed commonly found on Gunungkidul coast, had the potential as a source of functional food that contained high antioxidants, protein and dietary fiber. *Ulva* sp. was developed into biscuits, one of the most popular foods in Indonesia. This study aimed to investigate the physicochemical and sensory evaluation of biscuits enriched with *Ulva* sp. The nutritional value, antioxidant activity, dietary fiber, texture, color, appearance and sensory evaluation were studied. *Ulva* sp. was collected from Gunungkidul coast and treated using 5% lime for fishy-smell removal, then dried to produce *Ulva* sp. powder. The *Ulva* sp. powder was used for biscuit enrichment with 0% powder (STD), 3% powder (BC3), 5% powder (BC5) and 7% powder (BC7) substitution. Biscuits enriched with *Ulva* sp. increased the protein, fat, and ash content, while the carbohydrate content decreased ($p<0.05$). The more *Ulva* sp. was substituted in biscuits, the higher antioxidant activities and dietary fibers were measured ($p<0.05$). The crunchiness of BC5 (139.95±28.84 Nmm) was high, and no significant difference with STD (393.05±34.11 Nmm), while the crispiness and the hardness of enriched biscuits were lower than STD. The lightness (L*), redness (a*), and yellowness (b*) of enriched biscuits were lower than STD because the *Ulva* sp. powder contributed to giving dark-greenish colors. Sensory evaluation showed that BC5 was the most well-liked by the panellist in terms of its appearance, aroma, taste, flavor, texture, aftertaste, and overall (there was no difference with STD). Thus, biscuits enriched with *Ulva* sp. were confirmed to improve nutritional and physicochemical, and it has sensory evaluation acceptance.

1. Introduction

The development of functional food increased through the awareness and importance of healthy food. Moreover, the COVID-19 pandemic required us to increase our healthy habits and consume healthier food. Consuming functional food has many beneficial effects such as increased immunity, a healthy body and prevent degenerative disease. Every year, market demand for functional food increase by around 15-20% (Bagchi and Nair, 2017; Bayomy, 2022). There are many functional food sources to be developed, including edible macroalgae, also known as seaweed. Based on photosynthetic pigment, seaweeds can be classified as Chlorophyceae (green seaweeds), Phaeophyceae (brown seaweeds), and Rhodophyceae (red seaweeds) (Pangestuti *et al*., 2021). Seaweeds have bioactive compounds such as antioxidant, dietary fiber, carotenoid, tocopherol, phenolic, amino acid, and fatty acid, which have potential as a functional food (Ortiz *et al*., 2006; Hossain *et al*., 2017; Bayomy, 2022). Gunungkidul coast has various macroalgae, especially *Ulva sp.* or sea lettuce, which include Chlorophyceae algae. *Ulva sp.* has high growth and productivity in various geo-climatic and potentially used bioactive profiles (Angell *et al*., 2014; Magnusson *et al*., 2016; Mata *et al*., 2016). *Ulva sp.* widespread from Baron, Drini, Sepanjang, and Wediombo on Gunungkidul coast (Suryandari, 2017; Jatmiko *et al*., 2019; Wibowo *et al*., 2022).

Ulva sp. has the potential to be used for functional food by its nutritional value and bioactive compounds. *Ulva lactuca* from Sepanjang has carbohydrate, protein, fat, moisture, ash and dietary fiber contents of 49.09%, 9.24%, 0.38%, 9.89%, 31.4% and 3.68%, respectively (Jatmiko *et al*., 2019). *Ulva* sp. contained carotenoids such as β-carotene, neoxanthin,

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eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

β-cryptoxanthin, violaxanthin, antheraxanthin and zeaxanthin; which are related to biomass growth (Eismann *et al*., 2020). *Ulva* sp. was appropriate for a low-calorie diet because it is low in fat and high in protein and polysaccharides (Pangestuti *et al*., 2021). *Ulva lactuca,* from Tabuk coast of Red sea Saudi Arabia, has been studied for culinary treatments, including boiled, steamed, and sous vide, affect the water activity, pH, moisture, ash, protein and lipid (Bayomy, 2022). *Ulva* sp. can be used as a bioactive source cause its high contents of dietary fiber, antioxidants, sulphated polysaccharides, phenolics (gallic acid and vanillic acid), dyes (chlorophyll and carotenoids), minerals (K, Ca, Mg), fatty acids, tocopherol, protein, and sugar (Ortiz *et al*., 2006; Jatmiko *et al*., 2019; Kidgell *et al*., 2019; Eismann *et al*., 2020; Pangestuti *et al*., 2021; Bayomy, 2022). Various bioactive in *Ulva* sp. reveal a high potential to be developed as a food that has a functional effect.

Previous studies examined the utilization of seaweed for bakery products, and developments such as gluten-free chestnut cookies enriched with brown seaweed have high antioxidant activity (Arufe *et al*., 2019) cookies containing astaxanthin-rich microalgae and wholemeal flours incorporation improved their bioactive compounds and lowered the glycaemic response (Hossain *et al*., 2017); the red seaweed (*Kappaphycus alvarezii*) in dough and bread affected the textural properties (Mamat *et al*., 2014). The development of *Ulva* sp. from Indonesia, especially Gunungkidul coast, as a substitute for cookies was rarely studied and has yet to clear. Therefore, it became a potential gap to develop cookies enriched with *Ulva* sp. seaweed as a functional food. Seaweed substitution in powder form can be applied to bakery products such as biscuits, cakes, bread, and cookies (Lu and Chen, 2022). This study aimed to investigate the physicochemical and sensory evaluation of biscuits enriched with *Ulva* sp. powder for functional food. The benefit of the sustainability of this research was to develop the local potential of *Ulva* sp. seaweed in Gunungkidul Regency to improve the economy of coastal communities and create functional food from local ingredients for future food.

2. Materials and methods

2.1 Materials

Ulva sp. was collected from Gunungkidul coast, Yogyakarta, Indonesia. The *Ulva* sp. was collected and sorted in dry conditions. High-protein wheat flour (14%) and other raw materials for biscuit production were purchased from the local market.

2.2 Ulva sp. powder production

Ulva sp. was soaked in 5% lime solution for 24 hrs to remove the fishy taste, then washed and blanched for 2 mins. The seaweed was drained and dried in a cabinet drying at 50°C for 24 hrs. *Ulva* sp. was ground into powder. Dried ground seaweed was substituted for wheat flour to produce biscuits.

2.3 Biscuits production

The recipe and materials of biscuits enriched with 0% (STD), 3% (BC3), 5% (BC5), 7% (BC6) *Ulva* sp. are shown in Table 1. Mix the ingredients of the biscuit to become a smooth dough. The dough was then set aside for 30 mins. The dough was flattened with a pasta maker; before flattening using a pasta maker, roll it using a rolling pin to a thickness of 5 mm or until it is easily flattened with a pasta maker. After the dough was flattened with a rolling pin, it was flattened with a pasta maker with sizes 1, 2, 3 and 4. After flattening to size 4, sprinkle dust filling with a dose of 3 g. The dust filling was sprinkled on the surface and flattened. The dough was covered by folding and flattened again to sizes 3 and 4. Repeat the steps for giving dust filling, folding, and milling up to 4 times. The biscuits dough was molded using the prepared mold, then a hole was made so that the biscuits did not expand in the oven. Biscuits that have been molded and arranged were heated in an oven at 160°C for 20 mins. Biscuits are allowed to stand and ready to be packed.

Table 1. Recipe of biscuits enriched with *Ulva* sp.

Ingredients	STD (g)	BC3 (g)	BC5 (g)	BC7 (g)
Flour	100	97	95	93
Baking soda	1	1	1	1
Baking powder	1	1	1	1
Salt	3	3	3	3
Margarine	25	25	25	25
Maize	20	20	20	20
Skim milk	0.5	0.5	0.5	0.5
Water	41.5	41.5	41.5	41.5
Ulva sp.	0	3	5	7

2.4 Nutritional value

The nutritional values including fat, protein, carbohydrate, moisture and ash content were estimated using the AOAC (2002) method. The fat content was measured using Soxhlet (Weibull method). The protein content was measured by Kjeldahl method using Kjeltec protein analyzer (Foss–Tecator AB, Sweden). The carbohydrate was calculated by difference. The moisture content and ash content were determined by the gravimetric method.

https://doi.org/10.26656/fr.2017.8(S2).34

2.5 Determination of antioxidant activity using DPPH

The antioxidant activity of biscuits was performed using DPPH according to Ningrum *et al.* (2021), with modification. First, the biscuits were ground, mixed with methanol (99%), and stirred for 5 mins. Next, the samples were centrifuged at 2,700 rpm for 10 mins at room temperature. Then, biscuits (1.5 mL) supernatant was added with 1.5 mL 0.1 mM DPPH solution and incubated in the dark room at room temperature for 30 mins. The absorbance was measured at a wavelength of 517 nm. Antioxidant activity that was presented by radical scavenging activity (RSA) was calculated by the following Equation (1):

$$RSA\% = 1 - \frac{(Abs_{sample})}{(Abs_{blanko})} \times 100 \quad (1)$$

2.6 Determination of dietary fiber

The dietary fibers of biscuits were measured using enzymatic gravimetry (AOAC, 2002; Garbelotti *et al.*, 2003). The method includes enzymatic hydrolysis with α -amylase, protease and amyloglucosidase. 1 g sample was suspended in 40 mL MES-TRIS buffer, and a series of enzymes were added in the following sequence in a water bath: 50 µL of thermo-resistant α-amylase at 95-100°C for 35 mins, 100 µL of protease at 60°C for 30 mins, and then following pH correction to 4.0-4.7, and 300 µL of amyloglucosidase at 60°C for 30 mins. After soluble fiber precipitation using ethanol (95% v/v) at 60°C, the sample was filtered. The crucibles containing the residues were dried at 105°C, cooled in a desiccator, and weighed.

2.7 Texture

The texture of biscuits was carried out using a texture analyzer (Brookfield LFRA texture analyzer, USA) (Purwantari *et al*., 2016). Texture analysis was carried out on 5×5×0.65 cm biscuits using an LFRA texture analyzer with preload 1 N, stress speed 300 mm/min, test speed of 0.5 mm/s and wait time of 0.5 s to obtain hardness, gumminess, crispiness, crunchiness values.

2.8 Color

The color determination of biscuits was performed by a Minolta Chromameter (Model CR400, Osaka, Japan) according to Ningrum *et al.* (2021), with modification. Lightness (L), redness (a), and yellowness (b) systems were set on the chromameter. The biscuits were measured in triplicate and averaged.

2.9 Organoleptic test for sensory evaluation

An organoleptic test for sensory evaluation was carried out by hedonic test using 30 half-trained panelists, according to Noviatri *et al.* (2020), with modification. The panelists tested the biscuit's color, taste, aroma, texture and overall value. These biscuits were provided to the panelist's desk in the following manners: biscuits enriched with *Ulva* sp. (STD, BC3, BC5, BC7), a bottle of mineral water for mouth cleansing and neutralizing, and a questionnaire form. A questionnaire with a five-point facial hedonic scale (1 = dislike extremely; 5 = like extremely). Panelists need to fill out the questionnaire forms to assess the biscuits.

2.10 Statistical analysis

The experiments were performed in triplicate to express mean and standard deviation (SD) values. The experimental plan is full randomized trial. The statistical analysis was performed by analysis of variance (ANOVA) using IBM SPSS Statistics Software (Version 21.0, IBM SPSSS Inc, USA). Duncan's post hoc multiple comparison test at 5% significant level was performed for this study. The test was applied to determine the differences in the mean values of samples. Data with $p< 0.05$ were showed considered significant.

3. Results and discussion

3.1 Nutritional value

Nutritional values of the biscuits with their proximate values such as protein, fat, carbohydrate, moisture, and ash content are shown in Table 2. This study demonstrated that biscuits enriched with *Ulva* sp. significantly increased protein, fat and ash content ($p<0.05$), while the carbohydrate decreased significantly ($p<0.05$). It showed that moisture content was no significant change. *Ulva* sp. has higher ash, protein and fat content than wheat, so it has an increasing effect. *Ulva* sp. also had lower carbohydrates than wheat, so the carbohydrate content on biscuits decreased along with the addition of *Ulva* sp. *Ulva* sp. had a protein content of 9.24%, fat content of 0.38%, carbohydrate content of 49.09%, moisture content of 9.89% and ash content of 31.40% (Jatmiko *et al*., 2019). *Ulva sp.* has high protein that can be developed as a functional food source (Jatmiko *et al*., 2019; Pangestuti *et al*., 2021). Previous studies of biscuits enriched with other ingredients showed a change in proximate value. Biscuits incorporated from the *Gelidium spinosum* reduced moisture and carbohydrate content, while the crude fiber, fat and protein content increased (Poulose *et al*., 2021). Biscuits supplemented with sea grapes increased the protein and ash content (Kumar *et al*., 2018).

3.2 Antioxidant activity

The antioxidant activity of biscuits enriched with *Ulva* sp. presented as radical scavenging activity

Table 2. Nutritional value of biscuits enriched with *Ulva* sp.

Biscuits	Protein (%)	Fat (%)	Carbohydrate (%)	Moisture (%)	Ash (%)
STD	9.11±0.01[a]	9.44±0.25[a]	67.99±0.19[d]	9.01±0.13[a]	4.46±0.08[a]
BC3	12.23±0.02[b]	9.59±0.05[b]	64.68±0.13[b]	8.88±0.04[a]	4.64±0.02[b]
BC5	13.43±0.19[c]	9.89±0.15[bc]	63.08±0.06[c]	8.82±0.05[a]	4.80±0.03[c]
BC7	13.84±0.13[d]	10.12±0.18[c]	62.01±0.13[a]	8.77±0.12[a]	5.28±0.04[d]

Values are presented as mean±SD. Values with different superscripts within the same columns are statistically significantly different ($p<0.05$).

(RSA) is shown in Figure 1. RSA presented the antioxidant from the substance to stabilize and prevent the damage caused by free radicals (Rahman *et al*., 2015). BC5 and BC7 had RSA higher than STD ($p<0.05$), which showed more effective antioxidant activity. Biscuits enriched with *Ulva* sp. were obtained with the increase in seaweed concentration. *Ulva* sp. contained bioactive antioxidant compounds such as carotenoid (β-carotene, neoxanthin, β-cryptoxanthin, violaxanthin, antheraxanthin and zeaxanthin), melatonin, phenolic, protein and bioactive peptide (Tal *et al*., 2011; Kazir *et al*., 2019; Eismann *et al*., 2020). Oligosaccharides from *Ulva lactuca* enhanced the catalase, superoxide dismutase, telomerase, and glutathione levels, and total antioxidant capacity decreased malondialdehyde levels and advanced glycation end products (Liu *et al*., 2019). *Ulva* sp. might have augmented the hydrogen donating ability of the biscuits, thereby increasing the radical scavenging activity. Heat treatment was often encountered during food processing, such as in making biscuits, while antioxidant was not heat resistant (Wu *et al*., 2018; Sari *et al*., 2022). It caused the increase of antioxidant activity of biscuits enriched with *Ulva* sp. to be not too high. Previous studies showed that added seaweed could increase antioxidant activity. Biscuits supplemented with *C. racemose* showed significantly ($p<0.05$) higher antioxidants than control, caused by high phenolic content (Kumar *et al*., 2018). Gluten-free chestnut cookies enriched with brown seaweed have high antioxidant activity (Arufe *et al*., 2019)

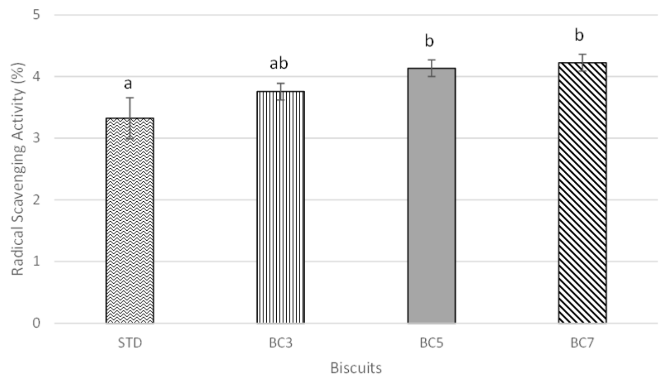

Figure 1. Antioxidant activity enriched with *Ulva* sp. Bars with different notations are statistically significantly different ($p<0.05$).

3.3 Dietary fiber

The dietary fiber of biscuits enriched with *Ulva* sp. is displayed in Figure 2. It showed that adding *Ulva* sp. to biscuits significantly increased the dietary fiber content ($p<0.05$). The more *Ulva* sp. was substituted in biscuits, the higher dietary fiber was measured. *Ulva lactuca* has a high contain fiber, such as 60.5% total dietary fiber, 27.2% soluble dietary fiber, and 33.3% insoluble dietary fiber (Ortiz *et al*., 2006). The consumption of fiber was considered to help alleviate the risk of various diseases like diabetes, obesity, hypertension, cancer, heart disease and gastrointestinal disorders (Ortiz *et al*., 2006; Anderson *et al*., 2009; Kumar *et al*., 2018). Increasing fiber consumption led to decreasing blood pressure and serum cholesterol levels, improving insulin sensitivity, enhancing weight loss and reducing gastrointestinal diseases (Anderson *et al*., 2009).

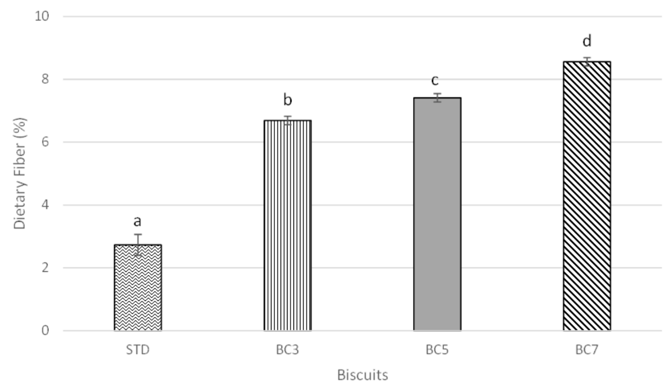

Figure 2. Dietary fiber of biscuits enriched with *Ulva* sp. Bars with different notations are statistically significantly different ($p<0.05$).

3.4 Texture

The quality of bakery products depends on various sensory attributes, including texture. The texture of biscuits enriched with *Ulva* sp. including hardness, gumminess, crispiness and crunchiness are shown in Table 3. The crunchiness of BC5 (321.77±32.84 Nmm) was high, and no significant difference with STD (393.05±34.11 Nmm), while the crispiness and the hardness of enriched biscuits were lower than STD. Hardness was defined as the force required to break the biscuits (Razack *et al*., 2020). Gumminess was defined as stickiness or the denseness throughout mastication or

Table 3. The texture of biscuits enriched with *Ulva* sp.

Biscuits	Hardness (N)	Gumminess (N)	Crispiness	Crunchiness (Nmm)
STD	761.71±3.68[b]	546.97±4.04[a]	3080±10[b]	393.05±34.11[c]
BC3	546.74±20.85[a]	391.23±20.42[a]	2198±19[a]	228.01±10.10[b]
BC5	587.32±14.31[a]	394.04±190.10[a]	2429±45[a]	321.77±32.84[c]
BC7	535.47±66.02[a]	439.46±59.77[a]	2161±327[a]	139.95±28.84[a]

Values are presented as mean±SD. Values with different superscripts within the same columns are statistically significantly different ($p<0.05$).

energy required to break down semisolid food to a ready-to-swallow state (Bourne, 1982). Crunchiness and crispiness are textural attributes often associated with the firmness and freshness of foods (Tunick *et al*., 2013). The crunchiness was associated with pleasure and fun, while crispiness was associated with the most versatile single-texture characteristics. The addition of *Ulva* sp. affects the texture, but BC5 has the same crunchiness as the standard. It indicated that 5% *Ulva* sp. has good crunchiness, the same as the standard. The quality of biscuits can be affected by enriched components. *Ulva* sp. provides dietary fiber that imparts specific functional properties. Seaweed was able to improve food texture, retard starch retrogradation, improve moisture retention, and enhance overall quality (Mamat *et al*., 2014; Salehi, 2019).

3.5 Color and appearance

Color became an essential parameter for consumer preference. The color of biscuits enriched with *Ulva* sp. are showed in Table 4. The lightness/darkness (L*), redness/greenness (a*), and yellowness/blueness (b*) of enriched biscuits were affected by *Ulva* sp. concentration. Increasing *Ulva* sp. concentration, the L* value decreased significantly ($p<0.05$). The a* value of enriched biscuits is lower than the standard, meaning greener. It caused *Ulva* sp. powder to have a green color and affected the biscuit's color into greenness. The b* value of biscuits enriched with *Ulva* sp. was lower than the standard. It indicated that the yellowness biscuits enriched with *Ulva* sp. decreased. Thus, *Ulva* sp. powder contributed to giving a dark-greenish appearance. The appearance of biscuits is displayed in Figure 3. *Ulva* sp. classified as a Phaeophyta group or green seaweed, which has a dominant green pigment called chlorophyll (Pangestuti *et al*., 2021; Bayomy, 2022). This pigment was responsible for the color and appearance of biscuits. In a previous study, another seaweed, *C. racemosa,* affected the organoleptic evaluation of biscuits (Kumar *et al*., 2018).

Table 4. Color of biscuits enriched with *Ulva* sp.

Biscuits	L*	a*	b*
STD	69.65±0.48[c]	2.52±0.47[b]	31.51±0.35[b]
BC3	69.01±0.03[c]	-1.33±0.31[a]	27.53±0.49[a]
BC5	67.30±0.50[b]	-2.16±0.19[a]	26.24±0.30[a]
BC7	60.74±0.04[a]	-1.12±0.93[a]	26.99±0.95[a]

Values are presented as mean±SD. Values with different superscripts within the same columns are statistically significantly different ($p<0.05$).

Figure 3. Appearance of biscuits enriched with *Ulva* sp.

3.6 Sensory evaluation

The sensory evaluation of biscuits enriched with *Ulva* sp. is shown in Table 5. The sensory evaluation included appearance, aroma, taste, flavor, texture, aftertaste, and overall. The appearance, aroma, taste and overall showed that BC5 and standard were the most like than others. At the same time, the flavor had no significant difference, and the texture parameter showed that BC5 and BC7 became favorites. Sensory evaluation was crucial for developing new food products. The color was related to the appearance of biscuits. Consumers preferred biscuits with a green color but did not like colors that were too dark, so BC7 had a lower score on appearance. Consumers also preferred crunchy biscuits, so BC5 and BC7 have higher organoleptic texture scores.

Table 5. Organoleptic test of biscuits enriched with *Ulva* sp.

Biscuits	Appearance	Aroma	Taste	Flavor	Texture	Aftertaste	Overall
STD	3.77±0.77[b]	3.50±0.68[b]	3.23±0.82[b]	3.30±0.92[a]	2.90±0.88[ab]	3.27±0.64[a]	3.37±0.72[b]
BC3	3.63±0.76[ab]	3.03±0.72[a]	2.70±0.79[a]	2.97±0.72[a]	2.63±0.89[a]	2.97±0.61[a]	2.93±0.74[a]
BC5	3.80±0.71[b]	3.50±0.57[b]	3.27±1.01[b]	3.33±0.88[a]	3.17±0.70[bc]	3.17±0.95[a]	3.43±0.73[b]
BC7	3.27±1.23[a]	3.17±0.91[ab]	3.07±0.87[ab]	3.17±0.83[a]	3.37±0.81[c]	3.07±0.87[a]	3.27±0.78[ab]

Values are presented as mean±SD. Values with different superscripts within the same columns are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.8(S2).34

4. Conclusion

Biscuits enriched with *Ulva* sp. are innovation and development of a new product with potential as a functional food. They had a high content of protein, fat, ash, dietary fiber, and antioxidants. The texture and color of biscuits enriched with *Ulva* sp. affected the sensory evaluation of biscuits. Thus, it was confirmed that *Ulva* sp. in biscuits could improve nutritional, physicochemical, and sensory evaluation acceptance. This study could give rise to a new segment of seaweed food products. It uncovered the functional food benefits of marine resources. The utilization of marine resources can strengthen healthy food for humans.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors appreciated the funding from the Research Group the Faculty of Engineering, Yogyakarta State University.

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