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THE EFFECT OF SEAWEED FLOUR (*Gracilaria verrucosa*) SUBSTITUTION ON THE QUALITY CHARACTERISTICS AND FIBER CONTENT OF BISCUITS

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ABSTRACT

Biscuits with specific treatments can become functional foods made from wheat flour and are often consumed as snacks. Biscuits usually contain high carbohydrates and fats, but low protein and fiber. G. verrucosa is a red algae rich in dietary fiber (53.57%). Adding G. verrucosa flour to biscuits can increase the natural fiber content, and be an alternative nutritious and affordable product. This study aims to determine the effect of seaweed flour substitution as a reference for high-fiber biscuits, quality characteristics and nutritional content, and the best concentration based on sensory tests. The research method for this biscuit was carried out experimentally in the Laboratory using a completely randomized design (CRD) consisting of 4 treatments and 3 replications of RL (0%), RL (2%), RL (4%) and RL (6%). Research parameters observed include : protein content, fat content, moisture content, ash content, food fibre, texture, color test and sensory analysis. Data analysis were carried out using SPSS 16 software. Parametric data were analyzed using ANOVA, Duncan and Independent Sample T-test (food fibre, while non-parametric data were analyzed using Kruskall-Wallis and Mann-Whitney tests. The results of the study showed that the higher the concentration of G. verrucosa flour substituted into biscuits, the more it would increase the nutritional content, such as protein, fat, moisture, ash, fiber and texture that met the requirements of SNI 2973:2022. In addition, it reduces the carbohydrate value and sensory characteristics such as a darker color and distinctive odor. The best results were the RL (4%) treatment with dietary fiber (3.52%), protein (8.58%), water (3.54%), fat (22.34%), ash (1.71%), carbohydrates (63.84%), crispness (629.42 gf - more cryspy), and color with L* values 53.28, a* (-10.35), b* 19.21, and panelist sensory had a 95% confidence interval of 7.83 <μ <8.26.

Keywords: Biscuits; Gracilaria verrucosa; Crispyness; Dietary Fiber; Substitution

INTRODUCTION

Seaweed as a rapidly growing marine resource shows great potential in the nutritious food industry. According to data from the FAO, global seaweed production in 2021 was 36.3 million tons. Seaweed production is dominated by the *Laminaria* sp. species, contributing 36.7%, followed by *Kappaphycus* sp. (17.2%), *Gracilaria* sp. (16.5%), *Undaria* (7.6%), *Euchema* sp. (6.8%), and other species (15.2%). One of Indonesia's seaweed productions is dominated by *Gracilaria* with a volume of 1.91 million tons (32.1% of global production) (KKP, 2023). This situation provides innovation to increase the supply of fiber derived from seaweed into food products. *Gracilaria verrucosa* seaweed is known to be rich in dietary fiber content. The fiber found in *G. verrucosa* is important in maintaining digestive health, particularly soluble fiber. *G. verrucosa* belongs to the red seaweed group, rich in polysaccharides, minerals, vitamins, and high fiber content (Darmanto *et al.,* 2022). In the study by Cirik *et al*. (2010), the proximate composition of *G. verrucosa* showed that the moisture content was 11.71%, ash content 12.8%, fat content 2.66%, crude fiber content 0.82%, protein content 20.28%, and carbohydrate content 67%. The use of seaweed as an additive can be maximized by turning it into seaweed flour, an alternative for diversifying nutrient-rich food products. One such product has been developed as a snack, contributing to the development of seaweed in the processed food sector and improving the quality of processed products.

Developed in line with public awareness of the importance of nutritious food, one example is biscuits, especially to develop healthier snacks, such as biscuits. In Indonesia, biscuit consumption is quite high. However, this is not accompanied by balanced biscuit content. Biscuits are functional food made from wheat flour and are often consumed as snacks by people of all ages. The low fiber and protein content is believed to be addressed by adding or substituting wheat flour with other flour types rich in dietary fiber. According to Hoang *et al*. (2022), dietary fiber in biscuits has received significant attention due to its numerous health benefits. The nutritional content of biscuits includes protein (8.1%), ash (1%), fat (25.6%), soluble fiber (0.7%), and total fiber (1.8%).

Biscuits are also one of the staple foods that are popular worldwide because they are ready to eat, affordable, and have a long shelf life. The average fiber consumption for children is 3.91 g/day. This value is still below the recommended nutritional adequacy. The Recommended Daily Allowance (RDA) for children and adults generally ranges between 26-35 g daily (Septiana *et al*. 2018). This study aims to analyze the effect of substituting *G. verrucosa* flour on biscuit products' quality characteristics and nutritional content. The parameters observed include sensory testing, color, crispness, fiber content, and proximate composition. These biscuits are expected to serve as an alternative functional food product derived from marine resources, offering economic value, high fiber, and health benefits.

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RESEARCH METHODS

Time and Place

This research was conducted from November 2024 to March 2025. Biscuit production occurred at the Processing Laboratory, Department of Fisheries Product Technology, Diponegoro University. Proximate analysis has been conducted at the Functional Food Laboratory of the Fisheries Product Technology Department, Diponegoro University, while fiber testing has been performed at the Chemix-Pratama Laboratory in Bantul, Yogyakarta.

Method

The study was conducted experimentally in a laboratory using a completely randomized design (CRD), consisting of 4 treatments: RL0, RL2, RL4, and RL6, each with three replications. The primary method employed involved varying the concentration of *G. verrucosa* seaweed flour. The analyses used in this study included sensory testing, dietary fiber content, protein content, fat content, ash content, moisture content, carbohydrate content, color, and texture (*crispness*). Data analysis in the biscuit study was conducted using parametric and non-parametric methods. Parametric data analysis included proximate content, dietary fiber, crispness, and color testing. Based on ANOVA, when, treatments were significantly different, further test was conducted (Duncan test). Non-parametric data, namely sensory test results, were analyzed using the *Kruskal-Wallis test* and followed by the *Mann-Whitney test*.

Preparation for Making *G. verrucosa* Seaweed Flour

The raw material is dry seaweed (*G. verrucosa* seaweed) obtained from the city of Brebes, Central Java. Production of *G. verrucosa* flour using the modified method of Hudi *et al*. (2023), *G. verrucosa* seaweed is washed, then soaked in clean water and followed by soaking in CaO for 24 hours each, dried in an oven at 60°C for 24 hours, then ground and sieved to 100 mesh.

Preparation biscuits substituted with *G. verrucosa* flour

The raw material for making biscuits in this study was G. verrucosa seaweed obtained from Brebes, Central Java. Other ingredients used in making biscuits were wheat flour, cornstarch, tapioca flour, margarine, sugar, eggs, salt, powdered milk, and baking powder. The biscuit formulation can be seen in Table 1.

Table 1. Formulation of *G. verrucosa* Seaweed Biscuits

Materials	Treatment			
	RL0	RL2	RL4	RL6
G. verrucosa flour	0	8	16	24
Wheat flour	140	132	124	116
Tapioca flour	30	30	30	30
Cornstarch	20	20	20	20
Powder milk	10	10	10	10
Sugar	64	64	64	64
Margarine	90	90	90	90
Eggs	36	36	36	36
Vanilla	4	4	4	4
Baking powder	2	2	2	2
Salt	2	2	2	2
Water	6	6	6	6
Total	404	404	404	404

The biscuit-making process was conducted by the modified method of Kusumawardani *et al*. (2018). The first weighing the ingredients, followed by mixing 1, which involves adding margarine, sugar, and milk to a container and stirring until homogeneous. Then mixing 2 is wheat flour, cornstarch, tapioca flour, salt, baking powder, 3 mL of egg white, and *G. verrucosa* flour at 2%, 4%, and 6% concentrations. The ingredients are then kneaded until the dough is smooth. After all ingredients are mixed, the dough is shaped and placed in a baking tray lined with baking paper. The dough is baked in an oven at 150°C for 40 minutes. After that, the biscuits are cooled at room temperature (29C) for approximately 5 minutes before analyzing their quality characteristics and nutritional content.

Sensory Test

Sensory testing is the testing of product characteristics conducted by 40 trained panelists to assess texture, aroma, taste, and appearance using a 1–9 hedonic scale. Each panelist received 4 samples (control, 2%, 4%, and 6%) with random codes. The best formula is selected based on the highest hedonic score and acceptance level for further studies, namely total dietary fiber content analysis.

Color Test (MATLAB)

Digital image processing in color testing is a computer vision technology used as a research object. Images were captured using an LED-lit box (24×27.5×33 cm) with a white background, employing a Samsung A6+ camera with a 16 MP resolution from a distance of 30 cm. The results obtained were converted into the L* (Lightness) a* (Redness) b* (Yellowness) color model.

Crispness Test (TA-TX, Lloyd TAPlus

Texture profile analysis (TPA) testing is a quantitative method for measuring texture properties such as hardness, chewiness, crispness, and elasticity of a food product. The test is conducted using a mechanical device with a 0.5-inch probe with trigger force : 0,5 gf and Test speed : 100 mm/min, depression limit : 15 mm, probe : spherical probe. The device presses or pulls the food sample to measure several texture parameters gradually and record the initial response of the sample to pressure.

Proximate Analysis

Protein content using the titration method (SNI 01-2354.4-2006), moisture content using the gravimetric method (SNI 2354.2-2015), ash content using the direct combustion method (SNI 2354.1-2010), fat content using the Soxhlet extraction method (SNI 2354-3:2017), carbohydrate content using by difference method and total dietary fiber content using the multienzyme method (AOAC, 1995).

RESULT AND DISCUSSION

Sensory Test

The hedonic test is one of the stages in product development, particularly in evaluating the quality of seaweed crackers. Sensory analysis parameters include appearance, smell, taste, and texture using human senses. Sensory evaluation is conducted subjectively based on the preferences of each panelist, which are recorded on a sensory evaluation sheet. Each parameter (taste, texture, aroma, appearance) can be evaluated using a hedonic scale of 1-9, where a score of 1 indicates "very dislike" and a score of 9 indicates "very like".

Appearance

The appearance score ranged from 6.91 (like)to 8.48 (very like). The results indicate that panelists preferred biscuits with a bright green appearance from treatment RL4. The appearance of green color can be observed based on the composition of the ingredients used, such as seaweed, which is predominantly green in color. The substitution of *G. verrucosa* flour causes a change in the color of the biscuit, which initially was yellowish, becoming increasingly greenish. This aligns with the L*a*b* color test, which shows that the more *G. verrucosa* is substituted, the darker, greener, and yellower the biscuit becomes. According to Ariyanto *et al*. (2022), color in a product is one of the parameters that emphasize appearance. Panelists disliked darker-colored biscuits because they appeared less appealing. Consumers' first impression of a product is based on its attractive appearance.

The appearance of biscuits generally has a neutral color ranging from yellow to brown. Additionally, biscuits made with *G. verrucosa* flour substitution exhibit a greenish-brown color change. Factors influencing color in biscuit appearance parameters include uneven biscuit thickness, baking time and temperature, and the occurrence of the Maillard reaction. Biscuits subjected to prolonged baking tend to be darker in color. According to Kamilah *et al.* (2022), the color of cookies is influenced by the type of ingredients used and the occurrence of the Maillard reaction during baking. High temperatures and relatively long baking times can trigger browning reactions (*Maillard*). The *Maillard* reaction occurs due to the interaction between the amino groups of proteins and the carbonyl groups of sugars.

Aroma

Based on the average aroma parameters of biscuits with *G. verrucosa* flour substitution, the results were 6.83 (like)-8.04. (very like) The aroma characteristics of the biscuits were specific to milk and sweetness, while biscuits with added seaweed had a pungent aroma, namely a fishy smell. This caused the panelists to dislike the aroma of the RL6-treated biscuits The aroma significantly differed between control and the treatments, but there was no difference between treatement RL2 and RL3. However, as the amount of seaweed added increased, the product's aroma became more specific and pungent. The aroma originated from the combination of food ingredients used. According to Ethasari *et al*. (2024), the substitution of seaweed flour causes a fishy aroma, which is less preferred by the panelists. The more seaweed flour used, the stronger the fishy aroma from the seaweed, leading to a decrease in aroma acceptability.

Taste

Taste is one of the parameters in sensory testing conducted using human senses, which can influence consumer product assessment. The average taste parameter values for *G. verrucosa*-substituted biscuits ranged from 6.57 (like) to 8.13 (very like). The results indicate that taste decreases as the substitution level of *G. verrucosa* increases. The panelists' lowest and least preferred result by the panelists was the biscuit with 6% seaweed substitution, with an average score of 6.57.. According to Laili *et al.* (2023), as the concentration of seaweed flour in the biscuits increases, the results indicate that biscuits with higher seaweed substitution have a sweeter taste but a noticeable seaweed aftertaste. This causes the panelists' preference for the biscuit's taste to decrease as the seaweed concentration increases.

Texture

The texture results were influenced by the high substitution of *G. verrucosa* flour added to the biscuits. The highest result was obtained in the RL4 treatment with a value of 8.57 (very like), and the lowest result was obtained from the RL6 treatment, which was 7.52 (like where the panelists still preferred the biscuit texture. This indicates that the RL(6%) treatment value is not as high as the other treatments, despite having the same composition, because the texture is harder and denser. The more *G. verrucosa* flour is substituted, the harder and sandier the biscuit texture becomes. This aligns with the texture crispness test, which shows increasing values of 545.21 *gf* for the control, 607.27 *gf* for RL2, 629.42 *gf* for RL4, and 642.98 *gf* for RL6. The texture the panelists prefer is not too hard but breaks apart in the mouth when chewed According to Mondong and Sulistijowati (2023), the texture criteria indicate that the harder the cookies become, the more seaweed flour is substituted. This is due to the seaweed's high water-binding capacity. Generally, hard-textured biscuits are not preferred by consumers. Additionally, the composition of other ingredients can also influence the texture of the biscuits. According to Mandik *et al*. (2024), texture refers to the external and internal characteristics of biscuits that can be perceived using the mouth, such as whether the biscuit is crispy. A crispy texture can be achieved by using ingredient compositions containing fat.

Table 2. Sensory Analysis of *Gracilaria verrucosa* Seaweed Biscuits

Sample	Specifications				Confidence Interval
	Appearance	Aroma	Taste	Texture	
RL0	7.96±1.02^b	8.04±1.02^c	8.13±1.01^b	7.87±1.01^a	7.78<μ<8.22
RL2	8.13±1.01^b	7.52±0.90^b	7.78±1.00^b	8.48±0.90^b	7.78<μ<8.17
RL4	8.48±0.90^b	7.43±0.84^b	7.70±0.97^b	8.57±0.84^b	7.83<μ<8.26
RL6	6.91±0.73^a	6.83±0.58^a	6.57±0.84^a	7.52±0.90^a	6.81<μ<7.10

Table 3. Proximate Analysis of Biscuit Substituted with *Gracilaria verrucos*

Parameters (%)	RL0	RL2	RL4	RL6
Protein	6.14 ± 0.20^{a}	7.38 ± 0.14^{b}	8.58 ± 0.24^{c}	10.12 ± 0.07^{d}
Moisture	3.35 ± 0.12^{a}	3.44 ± 0.20^{ab}	3.54 ± 0.19^{ab}	373 ± 0.11^{b}
Fat	18.65 ± 0.44^{a}	21.34 ± 0.14^{b}	22.34 ± 0.20^{c}	23.32 ± 0.21^{d}
Ash	1.31 ± 0.01^{a}	1.34 ± 0.01^{b}	1.71 ± 0.01^{c}	1.93 ± 0.02^{d}
Carbohydrate*	70.55 ± 0.20^{d}	66.51 ± 0.15^{c}	63.84 ± 0.34^{b}	60.90 ± 0.29^{a}

Note: Data are the means of 3 replicates. Data followed by different lowercase letters in the same column indicate significant differences (P<0.05). *By difference

Protein Content

Protein content measurements were conducted to determine the important components in increasing the nutritional content of biscuits by substituting of *G. verrucosa* flour. Statistical results show that the substitution of *G. verrucosa* flour has a significant effect on the protein content of biscuits. Table 3 shows that the protein content of biscuits increased from 6.14% to 10.12% with the substitution of *G. verrucosa* flour. The RL4 treatment biscuits with 4% *G. verrucosa* substitution had a protein content of 8.58%. The increase in protein content in the biscuits is due to the added concentration of seaweed substitution, as the protein content of *Gracilaria* sp. flour is 12.78% (Purwaningsih and Deskawati, 2020). It can be concluded that the higher the concentration of seaweed substitution in the biscuits, the higher the protein content obtained in the biscuits. This aligns with the findings of Henggu (2024), which showed that the protein content of biscuits with *Ulva reticulata* addition reached 10.62%, while the control biscuits had a lower value of 9.83%. The higher protein content in biscuits with the addition of *U. reticulata* compared to biscuits without seaweed addition is likely due to the protein content in the seaweed itself.

The results of this study were compared with commercial biscuits, and the protein content of the biscuits in this study was relatively high. Some seaweeds of the same type also sometimes have different proteins. The protein content of biscuits with *C. racemosa* (7.69%-9.01%) (Kumar *et al*. 2018) and crackers with the addition of *G. tenuistipitata* yielded protein content values of (7.47%-12.01%) (Raiyan *et al*., 2024). The higher the percentage of seaweed substitution, the higher the protein content in the biscuits. According to Sandrasari & Chusna (2020), the higher the amount of seaweed added to cookies, the higher the protein content.

Moisture Content

Moisture content can influence the shelf life and resistance of biscuit products. Moisture content can affect the final result, such as texture and the growth of microorganisms in the product. The results of the moisture content of biscuits can be seen in Table 3. Statistical analysis results indicate that the moisture content of high-fiber biscuits with *G. verrucosa* substitution does not show a significant different effect as the seaweed concentration increases. Based on Table 3, the moisture content of biscuits with *G. verrucosa* flour substitution ranges from 3.35% to 3.73%. The average moisture content obtained was below 4%. This result meets the requirements of SNI 2973:2022, where the maximum moisture content for biscuit products is 5%. Increasing the addition of seaweed flour causes the moisture content in the biscuits to increase. According to Pari *et al.* (2024), the relatively high moisture content in cookies is due to the components of the materials used. This occurs because seaweed contains hydrophilic hydrocolloid compounds, thereby increasing the moisture content. The results of this study are higher than those of Yusup *et al*. (2022), where biscuits enriched with *E. cottoni* had a moisture content ranging from 1.40% to 2.04%, and lower than those of Raiyan *et al*. (2024), where *G. tenuistipitata* crackers had a value of 4.46%.*Kappaphycus alvarezii* cookies had a value of 8.02% (Nie *et al*., 2024). The moisture content of raw materials used also influences the moisture content of final product.. The moisture content of *Gracilaria* sp. seaweed flour is 10.72%. Moisture content is the most important component in biscuit products. According to Lobo *et al*. (2024), low moisture content in products can cause the texture to become more brittle and reduce viscosity. High moisture increases the risk of microbiological damage, such as mold growth.

Furthermore, higher moisture content in biscuits is due to wheat flour being substituted with seaweed flour, which has a relatively high moisture content. Seaweed naturally contains hydrophilic compounds, such as polysaccharides and hydrocolloids, one of which is agar. Hydrocolloids like agar allow them to bind and retain water, thereby increasing moisture content. As a result, the moisture content in the resulting biscuits increases as the seaweed flour percentage increases. High relative humidity causes seaweed to absorb water from the air easily. Additionally, seaweed containing hygroscopic salt crystals facilitates water absorption (Aisah, 2021). According to Mamat *et al*. (2023), adding *E. cottonii* powder to a food dough can increase moisture content in seaweed biscuits. Furthermore, hydrocolloid properties can also inhibit the amount of vapor produced. An increase in the water content of the biscuits can also occur when the biscuit product is stored in humid conditions or not tightly sealed, so that the seaweed biscuits will reabsorb the water vapor in the surrounding environment.

Fat Content

Fat is one of the important nutritional components that serves as the main energy source besides protein and carbohydrates. Fat content in biscuits has an important influence because fat content in biscuits can change food properties. Data on the fat content of biscuits substituted *with G. verrucosa* can be seen in Table 3. Statistical test results showed that the substitution of *G. verrucosa* flour significantly affected the fat content of biscuits. The results showed an increase in fat content along with the increase of *G. verrucosa* flour substitution in biscuits. This is due to the content of *G. verrucosa* flour,which has a fat content of 1.18%. In addition, the high increase in fat content can also be caused by other raw materials in making biscuits such as margarine, milk and eggs. Based on the Ministry of Health's Food Composition table (2018), wheat flour fat is 1 g, milk powder is 30 g and margarine is 81 g per 100 g. One egg consisting of a mixture of white and yolk has a fat content of 10.8 g (Goestjahjanti *et al,* 2024). According to Warkey *et al.* (2023), the high fat in biscuits is a contribution from high fat-containing ingredients used in the processing process such as margarine, eggs and milk. Fat functions as an emulsifier and shaper of flavor and texture. In addition, the addition of fat proportion can produce savoury and crispy products. The fat content of biscuits substituted with *G. verrucosa* flour in this study was higher than that of *E. cottoni* substitution *cookies* with an average fat content of 22.71% (Ethasari *et al.*, 2024), and lower when compared to *U. lactuca* and Sargassum sp. kastengel *cookies*, which were 31.94%-35.98% (Nusaibah *et al*., 2024), and brown seaweed *cookies* (*Turbinaria decurrens*) which were 41.47% (Pitrianingsih *et al.,* 2024). According to Fajariyanti *et al.* (2022), fat content is also influenced by margarine raw materials. Margarine can be bound as a lipoprotein, so when margarine when added to the dough, the dough will have a high fat content.

Ash Content

Statistical test results presented in Table 3 show that the substitution of *G. verrucosa* flour in biscuits influenced the ash content. The average obtained in this study ranged from 1.31% - 1.93%. As the concentration of *G. verrucosa* flour increases, it will have a different effect. It is suspected that the more seaweed added, the ash content of the biscuits will increase. The ash content of *G. verrucosa* flour used as a reference is 10.19% (Purwaningsih & Deskawati, 2020), while the ash content of wheat flour is 1%. According to Lolopayung & Asnani (2019), adding of seaweed to a product can increase its ash content. The more seaweed added, the higher the ash content. This is because seaweed contains quite high mineral substances.

Biscuits treated with RL4 and RL6 with *G. verrucosa* flour substitution (4%) had ash content of 1.71% and (6%) of 1.93%. This is not much different from the research of Kumar *et al.* (2018), biscuits with the addition of 5% seaweed had an ash content of 1.68% and Sandrasari's research (2020), *Gracilaria* sp. *cookies* obtained a value of 1.95%. This can be caused by the ingredients used and the ash content of the seaweed flour. If the ash content in food exceeds the permissible limit, hurt the health of the body. According to Smith *et al.* (2023), explained that measuring ash content is one of the important parameters for evaluating nutrients and composition in a sample. In addition, measuring ash content is important for assessing the mineral content, quality, and purity of food ingredients and can also indicate contamination if the ash content is too high. These minerals are important because they play a role in bodily functions, such as enzyme cofactors and metabolic processes.

Carbohydrate Content

Carbohydrate content in a food is an important parameter because it is the body's main energy source. In addition, carbohydrates are organic compounds consisting of crude fiber and free matter without nitrogen. The biscuit proximate test calculated Carbohydrate content using the by difference method. Statistical test results showed that the substitution of *G. verrucosa* flour significantly affected biscuit products. Based on Table 3 biscuits with *G. verrucosa* flour substitution have carbohydrate content ranging from 60.90%-70.55%. The average test result of the carbohydrate content *of G. verrucosa* biscuits is 65.45%. This value is lower when compared to the research of Ethasari *et al.* (2024), the carbohydrate content of *E. cottoni* substitution *cookies* obtained an average of 65.05%, research by Kusumawardani *et al.* (2018), carbohydrate biscuits adding seaweed composite flour amounted to 62.31%. Research by Muhammad *et al.* (2024), the carbohydrates produced from *E. cottoni* biscuits amounted to 87.19%. According to Pari *et al*. (2024), the combination of spirulina with seaweed decreased the percentage of carbohydrate content in the product. The decrease in carbohydrates is due to increased in protein which shifts the carbohydrate composition in sago cookies.

The carbohydrate content of *G. verrucosa* substitute biscuits was obtained using the *by difference* method. The carbohydrate content calculation method involves other quality components such as protein, fat, water and ash. The basic ingredient in making biscuits is wheat flour, the main carbohydrate source in biscuits. The *Gracilaria* flour mixed in the biscuits has a carbohydrate content of 65.13%. The wheat flour used is the type with medium protein content. The nutritional content in 100 g of wheat flour is 333 kcal energy, 9.0 g protein, 1.0 g fat, 77.2 g carbohydrate, 22 mg calcium, 150 mg phosphorus, and 1.3 mg iron (Indonesian Food Composition, 2017). The composition of wheat flour is 67%-70% carbohydrates, 10%-14% protein, and 1% fat (Amertaningtyas *et al.*, 2021). According to Mamat *et al.* (2023), the reduction in the carbohydrate content contained in bread is due to a decrease in the amount of wheat flour in the formulation. Although, dried red seaweed consists of 38.3% carbohydrates, all are not a good source as they contain a high proportion of soluble dietary fibre. Therefore, adding of red seaweed powder to the bread will reduce the carbohydrate content.

Dietary Fiber

Dietary fiber is a polysaccharide that digestive enzymes cannot digest. Total dietary fiber is the sum of soluble and insoluble dietary fiber. Food fiber testing was conducted on the RL0 treatment and RL4 as a comparator. The selection of RL4 treatment as a comparison was based on the best results based on hedonic and texture test parameters. The results of total dietary fiber *G. verrucosa* biscuits can be seen Table 4. Statistical test results of *G. verrucosa* flour substitution biscuits a significant difference in the content of dietary fiber in treatments RL0 and RL4 (4% *G. verrucosa* substitution). The test results of dietary fiber content increased between the RL0 treatment by 1.55% and RL4 by 3.52%. This is due to the effect of *G. verrucosa* flour substitution on biscuits. This high fiber content comes from polysaccharides contained in seaweed cells. According to Prita *et al*. (2021), adding seaweed to the product can increase its food fiber content. *Cookies are* much loved by the public and can be enjoyed by all ages can be processed with the addition of seaweed which is rich in dietary fiber so as to reduce the risk of fiber deficiency diseases more enjoyablyThe addition of seaweed as much as 2.5%-6% can increase the fiber content in serabi with a value of 5.32%. This makes seaweed potentially used as an additional ingredient in healthier food products (Yani *et al*., 2023).

Table 4. Dietary Fiber of Biscuits with Substitution of *G. verrucosa*

Concentration (%)	Mean	p	Total Dietary Fiber (%)
RL0	1.5433±0.098	0,000	1.55 ± 0.04^{a}
RL4	3.5167±0.081		3.52 ± 0.02^{b}

Note: Data followed by different lowercase letters in the same column indicate significant differences (P<0.05)

The weight of the biscuit in the RL4 treatment per piece is 2.54 g. Therefore, RL4 biscuits, weighing 2.54 g per piece, contain approximately 0.09 g of dietary fiber. The total dietary fiber content of biscuits is 3.52%.Biscuits containing *G. verrucosa* can be consumed in quantities of 8–10 pieces, assuming each biscuit weighs 2.54 g, serving as an alternative source of dietary fiber. Based on the Nutrition Adequacy Rate (AKG) and the Ministry of Health guidelines (2018), adult men need about 34-37 g of fiber, while women need 28-32 g per day. According to BPOM (2016), adult fiber needs are generally around 30 g daily. A food product can be said to be a source of high fiber if it contains at least 3 g of fiber per 100 g of product. This proves that *G. verrucosa* flour can be used as an effective functional food ingredient to increase fiber content, adding nutritional value to the biscuit products produced.

Table 5. Physical Parameter of *Gracilaria verrucosa* Substitution Biscuits

Parameters		RL0	RL2	RL4	RL6
Hunter color coordinates	L*	61.12 ± 0.67^{d}	55.56 ± 0.45^{c}	53.28 ± 0.18^{b}	52.45 ± 0.32^{a}
	a*	(-9.17) ± 0.13^{c}	(-9.92) ± 0.11^{b}	(-10.35) ± 0.20^{a}	(-10.60) ± 0.09^{a}
	b*	30.02 ± 0.41^{d}	21.35 ± 0.71^{c}	19.21 ± 0.17^{b}	9.05 ± 0.68^{a}
Crispness (*gf*)		545.21 ± 34.19^{a}	607.27 ± 51.91^{ab}	629.42 ± 14.79^{b}	642.98 ± 37.19^{b}

Note: Data are the means of 3 replicates. Data followed by different lowercase letters in the same column indicate significant differences (P<0.05)

Texture Crispness

Texture test on food products is one of the methods used to measure the quality and properties of texture related to the sensation of crispness when consumed. The instrumental crispness texture test uses a tool such as a *probe-based Texture Analyzer* to apply a compressive force to the biscuit. The crunchiness values of seaweed biscuits are presented in Table 5. The results of statistical tests of biscuits substituted *with G. verrucosa* flour showed significant differences in crispness texture. The change in the increased crispness value is most likely due to the high levels of dietary fibre in seaweed flour. The crispness value of the biscuits increased as the substitution of *G. verrucosa* increased. The lowest value of treatment RL0 was 545.21 *gf*, while treatment RL6 had a crispness level of 642.98 *gf*. The food fibre content of this seaweed plays a role in providing a crispier texture. It iseasily destroyed, so the biscuits become more easily broken and cause a crunchy sensation when chewed. The difference in the level of crispness of the biscuits is due to the fibre and polysaccharide content of *G. verrucosa* seaweed which plays a role in forming the biscuit matrix structure, thus affecting the breakability and crispness of the product. According to Kesuma & Muniroh (2015), seaweed substitution greatly affects the texture of biscuits. The more seaweed, the harder the biscuit texture. This is thought to be due to the large particle size of seaweed flour, which tends to be coarse and grained like sand with a high fibre content and affects texture.

The increase in crispness value of biscuits with different *G. verrucosa* flour substitutions showed similar results up to a certain level of addition. This is in line with the results of the sensory assessment, where the panelists' level of liking for texture decreased in the RL6 treatment with a higher concentration. The increase in crunchiness occurred due to the interaction between the hydrocolloid components in seaweed with starch and protein in the dough, resultingin a denser texture but still crunchy after baking. In addition, the crispness of the biscuits is also influenced by the high fibre and fat content in the ingredients used. According to Laili *et al.* (2023), the crispness of *cookies* is also influenced by the high fibre content contained in seaweed flour. Increasing the concentration of seaweed flour in the dough causes changes in texture that resemble a gel. This occurs due to the properties of seaweed flour similar to starch, which acts as a gelling, thickener, stabilizer, and emulsifier in the product.

Color Test

Color analysis is important for products to determine the quality of the color produced. Generally, biscuit products are yellow to brown in color, so they lack a distinctive appeal to consumers. Biscuit products with striking colors in the market usually attract more consumer interest. Color analysis of biscuits in this study was carried out using MATLAB *software* which then obtained a scale of L* (*Lightness*), a* (*Redness*), b* (*Yellowness*) values. Data on the results of color analysis for each treatment are presented in Table 5. Statistical test results showed that the substitution of *G. verrucosai* flour affected the color of biscuits. It can be concluded that the higher the concentration of *G. verrucosa* flour can produce a darker product color. This is indicated by decreased L* value in each treatment. The greater the L* (*lightness*) value indicates that the sample color will be lighter and the smaller the L* value, the darker the sample color. Factors that influence the difference in results include *G. verrucosa* flour with a green color and the reduced concentration of wheat flour added, so the resulting color is darker. In addition, in baking biscuits, the *Maillard* reaction occurs which makes the biscuits brownish (melanoid). According to Muhardina *et al.* (2024), *Maillard* reaction is a non-enzymatic chemical reaction between reducing sugars and amino acids or proteins. Both of these occur during the baking process at high temperatures. Baking biscuits in the oven will make the surface turn brown due to the *Maillard* reaction.

Based on Table 5, the decrease in a* value in biscuits is influenced by the higher concentration of *G. verrucosa* substitution. The overall result of the biscuit color profile of the *G. verrucosa* flour substitution a* parameter shows negative, sothe biscuit color profile is dominated by green. This is also in line with the results of the appearance sensory test where the higher the substitution, the greener the color. The increase in the greenish color level of seaweed biscuits is due to chlorophyll degradation from the greenish color of *G. verrucosa* to brownish. According to Raiyan *et al.* (2024), the a* value which indicates the level of *redness* of the biscuit increases significantly along with the increase in the *percentage of* seaweed used. This is due to the presence of pink pigments in the seaweed. In the research of Munandar *et al.* (2019), stated that the color of *gracilaria* spp. seaweed flour is dark green with a white degree value of 49.90%. *Gracilaria* spp. is blackish green so the color of seaweed flour is less than maximum. The main pigment in *Gracilaria* is pink ficocyanin, which is sensitive to high temperatures so it changes color to brownish during the roasting process.

Based on the b* color test results, the average value ranged from 9.05 to 30.02. The results showed that the biscuits had a dominant yellow color, although there was a decrease in the b* value as the substitution of *G. verrucosa* increased. The decrease was attributed to the baking process using high temperatures and a long time. According to Orilda (2022), the yellow color can be caused by the high content of damaged phycoerythrin in seaweed during drying, so that the red degree value decreases and the yellow degree increases in the treatment without adding seaweed. Phycoerythrin is very sensitive to heat, so that it undergoes degradation and polymerization, which causes a brown color change. In general, the longer the drying and the higher the temperature used, the greater the loss of pigments in the material.

CONCLUSION

Biscuits with *G. verrucosa* flour substitution influenced product characteristics. This study proved that the higher the substitution of *G. verrucosa* flour in biscuits can increase the value of protein, fat, ash, moisture, crispness and dietary fiber content in biscuits. In the contrary, it reduce the sensory value on the parameters of color which becomes darker and the distinctive aroma on the biscuits. Based on the sensory test, the best result was treatment RL4 with seaweed substitution (4%). RL4 treatment biscuits are still accepted by panelists with dietary fiber (3.52%), protein (8.58%), moisture (3.54%), fat (22.34%), ash (1.71%), carbohydrates (63.84%), crispness (629.42 *gf*), and color with L* values of 53.28, a* (-10.35), b* 19.21, and sensory panelists have a 95% confidence interval of $7.83<\mu<8.26$.

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