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Application of *Eucheuma spinosum* for Enhancing the Nutritional Value of Tempeh

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ABSTRACT

The research aims to enhance the nutritional value of tempeh, a traditional fermented food, by incorporating *Eucheuma spinosum*. Different concentrations of *E. spinosum* seaweed were added to tempeh to investigate its effect on nutritional composition and sensory attributes. With a 30% *E. spinosum* sp. addition, tempeh exhibited the highest dietary fiber content (17.99%) and significant changes in protein, carbohydrates, water, fat, and ash. However, no significant differences were observed in the hedonic test, indicating similar sensory preferences among the tempeh variations. Among the different concentrations tested, tempeh with a 20% *E. spinosum* addition was preferred in terms of sensory attributes. The findings suggest that adding *E. spinosum* seaweed can effectively increase the dietary fiber content of tempeh without compromising its overall acceptability. The research highlights the potential of *E. spinosum* seaweed as a supplementary ingredient for enhancing the nutritional value of traditional foods like tempeh.

Keywords: Dietary fiber, *Eucheuma spinosum*, nutritional value, tempeh

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INTRODUCTION

Tempeh, a popular food in Indonesia, has consistently increased consumption. In 2022, the frequency consumption of tempeh per individual reached 4–5 times per week (Astuti et al., 2023). Besides its delicious taste and affordability, tempeh is highly regarded for its nutritional value. Made from soybeans fermented with the help of *Rhizopus* sp., it is rich in vegetable protein, vitamins, minerals, isoflavones, and antioxidants. The fermentation process

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enhances its digestibility because the enzyme activity hydrolysis complex compounds into simpler ones, allowing for easier absorption in the body.

Tempeh is a nutrient-rich food containing various beneficial elements for body health. Every 100 g of tempeh provides 17.40 g of protein, 8.23 g of fat, 1.33 ash, 7.30 g of carbohydrate, and 2.5 g of fiber (Rizal et al., 2022; Tan et al., 2024). While tempeh is protein-rich, it has a relatively low fiber content. Fiber, an equally essential component for the body, is a complex carbohydrate usually found in plants. Its functions include optimizing food absorption, promoting intestinal health, aiding digestion, regulating blood sugar, reducing cholesterol and cardiovascular risks, preventing gastrointestinal issues, maintaining body weight, and strengthening the immune system (Mîndrican et al., 2022). Insufficient fiber intake can lead to constipation, unstable blood sugar levels, weight gain, fatigue, weakened immunity, and heightened inflammation risks (Tanes et al., 2021).

Seaweed is a nutrient-rich food source due to its abundant minerals, vitamins, essential minerals, dietary fiber, protein, essential amino acids, and polyphenols, which exhibit antioxidant and anti-inflammatory properties. Its high dietary fiber and low fat make it a healthy, nutritious, and low-calorie food, widely used as a substitute in various food products (Lomartire et al., 2021). Sofiana et al. (2020) found that seaweed has higher levels of soluble fiber compared to land plants like beans, fruits, and cereals, which primarily contain insoluble fiber. Seaweed typically contains 25%–70% total dietary fiber and 50%–80% dietary fiber. However, the fiber content may vary depending on the specific seaweed type, such as *Laminaria* sp. (36% food fiber), *C. racemosa* (33%–40%), *Ulva lactuca* (38%–43%), *E. cottonii* (15.5%) (Praveen et al., 2019), and *E. spinosum* (21.5%) (Diharmi et al., 2019).

Eucheuma spinosum, a type of red algae, is being utilized as an additional source of income by seaweed farmers. Its carrageenan content has applications in various industries, including cosmetics, pharmaceuticals, textiles, and food (Kurniawan & Managi, 2018). Studies regarding the application of *E. spinosum* are still limited. Sagita et al. (2023) applied *E. spinosum* in pudding to improve the texture and be organoleptically acceptable. Iwada et al. (2021) stated that *E. spinosum* could improve the texture and increase the fiber and protein content in local Riau Regency food called "pekdos." There has yet to be any research regarding the application of *E. spinosum* in making tempeh, so this is the first time this research has been performed. Therefore, the research aimed to enhance the nutritional value of tempeh by incorporating *E. spinosum* seaweed.

METHODS

Materials

The study utilized half-dried *E. spinosum* sourced from seaweed farmers in Southern Lampung, Indonesia, as well as soybeans and Raprima brand tempeh yeast obtained from Bandarjo Traditional Market, Semarang, Indonesia.

Seaweed Preparation

The initial step involved preparing the *E. spinosum* seaweed by washing it three times to remove any remaining salt and dirt. Subsequently, the soaking process followed the method used in the research by Siah et al. (2014), employing a ratio of 1:15 (dry seaweed to water) and a soaking time of 117 min. The research required 200 g of dried seaweed using 3,000 ml of water. The next stage involved blanching, which aimed to deactivate the enzymes in the seaweed (Xiao et al., 2017) and was accomplished by placing the seaweed in boiling water for 4 min before draining and allowing it to cool. Finally, the seaweed underwent size reduction by blending until it achieved a slightly smooth consistency.

Tempeh Production with *Eucheuma spinosum* Seaweed Addition

The tempeh-making process follows the guidelines set by the Rizal et al. (2022) with some modifications. The modification was on boiled time, where a traditional processor just boiled once, whereas in the study, boiling was done two times to clean the seeds. The initial step involved sorting and washing the soybean seeds under running water. The soybeans were then boiled for 30 min using a 1:2 ratio of soybeans to water. After boiling, the soybeans were soaked overnight using the remaining cooking water to create an acidic environment. The following day, the soybean skin was peeled off by submerging the soybeans in water and squeezing them until the skin separated, leaving skinless pieces. The soybean pieces were washed again and steamed for 30 min until cooked. Once cooked, the soybeans were lifted and placed in a wide container with a perforated bottom to allow water to drain. The next step involved adding 1 g of tempeh yeast per kilogram of soybeans and thoroughly mixing until evenly distributed.

Subsequently, the seaweed substitution was blended into concentrations of 0% (K), 10% (A), 20% (B), and 30% (C). The seaweed was thoroughly mixed with the other ingredients, wrapped in plastic, and sealed. Toothpick holes were then made approximately 1 cm apart for gas space movement. The raw tempeh was left to ferment on shelves with perforated bottoms. Typically, mold growth occurs within 48 h at room temperature; after this period, the tempeh was ready for analysis.

Crude Protein Content

The protein content was analyzed using the Kjeldahl method (Association of Official Analytical Chemists, 1995), consisting of three main stages: destruction, distillation, and titration. The analysis started by preparing a 0.5 g sample, which was then combined with 0.5 g of selenium catalyst and 10 ml of concentrated sulfuric acid (H_2SO_4) (Sigma Aldrich, USA). The destruction stage was carried out until the solution became clear green. Subsequently, distillation was conducted by adding 100 ml of distilled water, 40 ml of 45% NaOH, 5 ml of 4% H_3BO_3 trap, and two drops of Methyl Red and Methylene

Blue indicators (Sigma Aldrich, USA). The distillation results were titrated using 0.1 N Hydrogen chloride until the solution changed to purple.

$$\text{Crude Protein Content (\%)} = \frac{(mL\ HCl\ sample - mL\ HCl\ blanko) \times N\ HCl \times 6.25 \times 14}{mg\ sample} \times 100\%$$

Crude Fat Content

First, 10 ml of H_2SO_4 (SigmaAldrich, USA) was placed into a Gerber tube. Then, 11 ml of the sample was poured into the same Gerber tube, and 1 ml of isoamyl alcohol was added. The Gerber tube was capped tightly and flipped to thoroughly mix the solution. Next, the tube was centrifuged for 4 min and placed in a water bath at 60°C–63°C for 5 min. The fat content was then determined by reading the results (Association of Official Analytical Chemists, 1995).

Total Moisture Content

An empty cup was dried in an oven at 105°C for 15 min and then weighed to determine the water content. Next, 5 g of tempeh samples were placed in the cup and dried in an oven at 105°C for 6 h or until a constant weight was achieved. The cup was then placed in a desiccator for 30 min before being weighed again. The water content can be calculated by dividing the weight of the sample after drying with the initial weight of the sample multiplied by 100% (Association of Official Analytical Chemists, 1995).

Total Ash Content

A clean cup was heated in a furnace at 400°C for 1 h to determine the ash content (Association of Official Analytical Chemists, 1995). The cup was carefully removed from the furnace using pliers and allowed to cool in a desiccator for 1 h. The weight of the cup after cooling (a) was recorded. A 5 g tempeh sample with a known weight (b) was placed into the porcelain cup. The cup with the sample was then placed back into the furnace for 1 h. Afterward, the cup was taken out of the furnace and allowed to cool in a desiccator. Finally, the cup's weight with the sample residue (c) was recorded. The ash content can be calculated by dividing the difference between c and a with b multiplied by 100%.

Total Carbohydrate Content

The carbohydrate analysis procedure follows the carbohydrate content analysis method (Association of Official Analytical Chemists, 1995). Carbohydrate content differs from 100% minus the total water, ash, protein, and fat content.

Total Dietary Fiber Content

The Association of Official Analytical Chemists (1995) measured dietary fiber. A sample weighing 0.5 g was placed in an Erlenmeyer glass and mixed with 50 ml of phosphate buffer and 0.1 ml of alpha-amylase enzyme and heated on a magnetic stirrer at 100°C for 30 min with occasional stirring. After cooling, 20 ml of distilled water, 5 ml of 1N HCl, and 1% pepsin enzyme (1 ml) were added (Sigma Aldrich, USA). The mixture was heated again at 100°C for 30 min. Then, 5 ml of 1N NaOH and 0.1 ml of beta-amylase enzyme were added. The Erlenmeyer glass was sealed and heated at 100°C for 1 h. The solution was then cooled and filtered using pre-weighed constant filter paper, and the residue was washed with 10 ml of ethanol (twice) and 10 ml of acetone (twice). The filtrate was dried overnight in an oven at 105°C, cooled in a desiccator, and weighed to determine the insoluble food fiber. The filtrate was weighed to 100 ml, and 400 ml of 95% ethanol was added. After settling for 1 h, the solution was filtered, washed with 10 ml of ethanol (twice) and 10 ml of acetone (twice), and dried overnight in an oven at 105°C. The final weight was obtained by weighing the dried sample in a desiccator, representing the dissolved fiber. The total dietary fiber was calculated by adding insoluble fiber and soluble fiber.

Sensory Acceptance

The sensory acceptance was analyzed using the hedonic test. The hedonic aimed to determine consumer preference test was conducted following the methodology outlined by (García-Gomez et al., 2022). A total of 35 semi-trained panelists participated in the evaluation of tempeh substituted with seaweed. The panelists assessed the level of acceptance for appearance, texture, flavor, and aroma using a 9-point scale, where 1 represented "very strongly dislike" and 9 represented "very strongly like." Panelists were instructed to cleanse their mouths with water to neutralize lingering tastes and ensure the taste test's accuracy.

Fatty Acid Analysis

Fatty acid analysis was performed using a Gas Chromatography-Mass Spectrometer (GCMS-QP2010S Shimadzu, Japan) based on Purnamayati and Kurniasih (2020). It used an Rtx column with dimensions of 30 m x 0.25 mm and 0.25 μm film thickness. A total of 1 μl of sample was injected into a column whose temperature had been set to 70°C with a temperature increase of 5°C per minute. The temperature increase is regulated until it reaches 300°C. Fatty acid analysis used a detector set to a temperature of 250°C with a pressure to separate methyl esters of 13.7 kPa—the fatty acid data obtained then identified using the mass spectra database in the Willey 229 dictionary.

Microstructure with Scanning Electron Microscopy

Microstructural analysis was performed using scanning electron microscopy (SEM JEOL JSM 5310 LV, USA), based on Kustyawati et al. (2018). Several samples were placed in the tub and then coated with gold using a generator equipped with a vacuum pump for 20 min. The microstructure was observed at 5000x magnification.

Statistical Analysis

The study used a completely randomized design with triplicates. The data were then analyzed using Analysis of Variance (ANOVA) and processed with the IBM SPSS Statistics application (version 23). Significant differences were further tested using the Tukey test.

RESULTS AND DISCUSSION

Table 1
Chemical characteristics of Eucheuma spinosum *tempeh*

Sample	Crude protein content (%)	Crude fat content (%)	Total moisture content (%)	Total ash content (%)	Total carbohydrate content (%)	Total dietary fiber content (g/100g Carbohydrate)
K	20.19 ± 0.67^{d}	7.38 ± 0.35^{d}	64.93 ± 0.24^{a}	0.94 ± 0.01^{a}	6.55 ± 0.06^{a}	10.87 ± 0.06^{a}
A	16.71 ± 0.24^{c}	6.61 ± 0.13^{c}	67.60 ± 0.08^{b}	1.02 ± 0.01^{b}	8.04 ± 0.17^{b}	14.40 ± 0.34^{b}
B	14.60 ± 0.74^{b}	5.62 ± 0.13^{b}	69.24 ± 0.06^{c}	1.08 ± 0.01^{c}	9.44 ± 0.19^{bc}	17.27 ± 0.07^{c}
C	11.57 ± 0.18^{a}	4.34 ± 0.20^{a}	74.74 ± 0.46^{d}	1.22 ± 0.01^{d}	10.12 ± 0.86^{cd}	17.99 ± 0.17^{c}

Note. Data in triplications ± deviation standard. Data followed by a different number in the same column show a significant difference (p <0.05). K= tempeh with no *E. spinosum*; A= tempeh with *E. spinosum* 10%; B= tempeh with *E. spinosum* 20%; C= tempeh with *E. spinosum* 30%

Crude Protein Content

Protein is a source of amino acids with C, H, and O elements, which are not present in fats or carbohydrates. While fishery products like fish and shrimp are known to have relatively high protein content, seaweed differs in its protein content. Biancarosa et al. (2017) stated that the protein content in seaweed can vary depending on the species. The red algae species *P. dioca* has a relatively high protein content of 20.6%, whereas the brown algae species *A. nodosum* tends to have a lower percent protein content of 3%. *E. spinosum,* which belongs to the red algae category, has a very low protein content compared to *P. Dioca*, which has only 1.67% protein content. Generally, red algae have higher protein content compared to green and brown algae. The protein concentrations in red algae typically range from 5.2% to 40% (dry weight), as Vega et al. (2020) noted. However, the digestibility of protein in algae is lower than animal protein. It may be attributed to the high concentration of dietary fiber present in algae, which limits the accessibility of digestive and proteolytic enzymes to the protein. The presence of dietary fiber in seaweed can affect protein digestion and absorption in the human body.

Tempeh is a food product known for its high protein content. The primary source of protein in tempeh is soybeans, which have one of the highest protein levels among crops. Soybeans have a protein content of approximately 34.95% (Tahir et al., 2018). However, during the cooking process of tempeh, which involves heat treatment, the protein content decreases to around 20.19%. The decrease in protein content can also be influenced by other additives used in the tempeh-making process.

In the study mentioned, adding seaweed to tempeh significantly affected the final product's protein content (Table 1). Tempeh had the highest protein content of 20.19% without any additional seaweed. On the other hand, tempeh, with the highest addition of *E. spinosum* seaweed, at 30% concentration, had the lowest protein content of 11.57%. Tempeh with 10% and 20% seaweed additions had 16.71% and 14.60% protein levels, respectively. These results were higher than Cornelia and Kartika (2022), who added 50% seaweed flour to tempeh and achieved protein levels of 12.36%. The decrease in protein levels in tempeh with seaweed addition can be attributed to the relatively low protein content. The protein content of seaweed was influenced by factors such as organic compounds in the marine water, current, and salinity of the water where seaweed grew. Fresh seaweed mainly consists of water, accounting for about 80%–90% of its composition, while the protein and fat content is relatively small, as noted by (Sofiana et al., 2020).

Crude Fat Content

Seaweed generally tends to have a low-fat content, and the fatty acid composition of seaweed is significant for health. Seaweed fats are rich in omega-3 and omega-6 fatty acids, which play crucial roles in various bodily functions, such as forming brain tissue membranes, nerves, eye retina, blood plasma, and reproductive organs. Seaweed contains omega-3 fatty acids such as eicosatetraenoic acid (EPA), which amounts to around 24% of the fatty acids in *Eucheuma* sp. (Mohamed et al., 2012). In line with the research of Vega et al. (2020), all types of algae have a low-fat content; however, the fat content is of high quality in terms of nutritional value. Belghit et al. (2017) state that red algae have a higher concentration of amino acids such as glutamate, ornithine, citrulline, serine, and glycine than green and brown algae. Amino acids derived from red algae protein digestion can be affected by various antinutritional agents such as polyphenols, polysaccharides, and glycoproteins.

Soybean tempeh has low fat, dietary fiber, and carbohydrate content (Romulo & Surya, 2021). Compared to beef, tempeh generally contains higher amounts of nutrients, except for fat. During the fermentation process of tempeh, there is an increase in the unsaturation of fats, leading to an increase in polyunsaturated fatty acids (PUFAs), such as oleic and linolenic acids (Damanik et al., 2018). According to Table 1, it can be observed that tempeh, without the addition of Spinosum seaweed, had the highest fat content of 7.38%, and the addition of Spinosum seaweed significantly reduced it. A concentration of 10% Spinosum seaweed resulted in a fat content of 6.61%, 20% *E. spinosum* had a fat content of 5.62%, and 30% Spinosum had the lowest fat content of 4.34%. The higher concentrations of *E. spinosum* seaweed led to lower fat content in the tempeh. The fat content from adding seaweed in this study was lower than in Cornelia and Kartika (2022), who used *K. alvarezii* seaweed, resulting in a fat content of 14.68% in tempeh. The low-fat content in tempeh, with the addition of *E. spinosum* seaweed, makes it a suitable and healthy alternative for consumption. Furthermore, it can be included in a balanced diet menu, contributing to a well-rounded and nutritious meal plan (Vital et al., 2018).

Total Moisture Content

Seaweed is known to have a high-water content, typically around 90% in its fresh form (Djaeni & Sari, 2015). However, some types of seaweed are distributed in dry form. The use of spinosum seaweed in this study is one such example. According to research by Ahmad et al. (2012), the water content in fresh seaweed generally ranges from 76% to 96%. Different types of seaweed may have varying water contents, with *K. alvarezii* typically having a lower water content compared to Caulerpa and Laurencia. Tempeh itself also has a relatively high-water content. Without the addition of Spinosum seaweed, the water content in tempeh is reported to be 64.93% (Table 1). As the concentration of Spinosum seaweed increases, the water content in tempeh also increases. The addition of 10% Spinosum seaweed resulted in a water content of 67.60%, 20% Spinosum had a water content of 69.24%, and 30% *E. spinosum* had the highest water content of 74.74%. The increase in water content in tempeh is attributed to the inclusion of wet seaweed, which contributes to the overall water content of the tempeh. It is worth noting that this study reports lower water content compared to the research conducted by Yulia et al. (2019), who stated a high-water content of 82.96% in tempeh. The difference in water content could be attributed to variations in processing methods, such as boiling, steaming, soaking, and adding ingredients with high water content. High water content in tempeh can create favorable conditions for the growth of microorganisms, including bacteria, which can potentially lead to food spoilage, as mentioned by Kustyawati et al. (2018).

Total Ash Content

Ash content refers to the total amount of minerals or inorganic components in food. Although present in small quantities, these minerals are very useful (Afify et al., 2017). The ash content in seaweed typically ranges from 17.3% to 44.5%, varying among species and depending on the habitat of the seaweed (Bikker et al., 2020). The high ash content can be attributed to the passive absorption of ions through charged polysaccharides in the seaweed cell wall, active absorption, and residual salts associated with the biomass (Olsson et al., 2020). In the case of Spinosum seaweed, it has an ash content of 22.82%.

In this study, the ash content in tempeh was relatively low. Without adding *Spinosum* sp, tempeh had the lowest ash content at 0.94% (Table 1). With the addition of Spinosum, the ash content increased. The ash content was 1.02% with 10% addition, 1.08% with 20% *E. spinosum* addition, and the highest ash content of 1.22% was observed with 30% Spinosum addition. The data indicates that higher concentrations of *E. spinosum* added result in higher ash content in tempeh. The ash content in this study is lower than that in the research conducted by Tahir et al. (2018), where tempeh had an ash content of 4.12%. The difference can be attributed to the processing of soybeans, as raw soybeans have a higher ash content of 4.48%, which decreases during processing into tofu (0.8%) and tempeh (0.78%). The decrease in ash content during processing is due to the loss of solids during soaking and cooking (Damanik et al., 2018). Adding *E. spinosum* seaweed to tempeh increases ash content due to the high mineral content present in seaweed.

Total Carbohydrate Content

Seaweed content is high in polysaccharide compounds. Polysaccharides are extensively used in the food industry as thickeners and gelling agents. Incorporating seaweed into food products can enhance their functionality and texture (Rioux & Turgeon, 2015). Polysaccharides derived from seaweed, such as agar, carrageenan, and alginate, have been widely commercialized in food, biotechnology, biomedicine, and textiles. The carbohydrate content of seaweed has gained attention as a functional food ingredient, and these carbohydrates exhibit various biological activities, including anti-implantation, anticoagulant, antioxidant, anti-proliferative, and immunostimulant properties. Moreover, these polysaccharides are also considered dietary fiber, and the consumption of dietary fiber has been associated with positive impacts on human health (Lafarga et al., 2020). The carbohydrate content contained in *E. spinosum* seaweed is 40%.

Total carbohydrates are carbohydrate complexes of starch, sugar and dietary fiber (Compaore-Sereme et al., 2022). Tempeh is known for its high nutritional composition, and its carbohydrate content typically ranges from 6 to 10% (Rizal et al., 2022). The fermentation process involved in tempeh production enhances the digestion of soy carbohydrates (Syida et al., 2018). In the case of tempeh with the addition of *Spinosum* sp. seaweed, differences in carbohydrate levels were observed in the carbohydrate levels. Without adding *E. spinosum*, tempeh had a carbohydrate content of 6.55%, which increased with the addition of *E. spinosum* (Table 1). The concentrations of 10%, 20%, and 30% *E. spinosum* resulted in carbohydrate levels of 8.04%, 9.44%, and 10.12%, respectively. The increase in carbohydrate content can be attributed to the enzyme activity produced by the microbes involved in the fermentation process. These enzymes have catabolic properties, meaning they can break down complex compounds into simpler ones easily digested. The microbes utilize the chemical components in the substrate as an energy source for their growth and reproduction. The increase in carbohydrates was due to the addition of *E. spinosum*. Sofiana et al. (2020) stated that fresh *E. spinosum* contains 6% carbohydrates on a wet basis or 41.18% on a dry basis. Based on the analysis, it is evident that the highest carbohydrate content was achieved by adding 30% Spinosum, while tempeh had the lowest carbohydrate content without adding *E. spinosum*. The data indicates that the concentration of *E. spinosum* added influences the carbohydrate levels in *E. spinosum* tempeh, with higher concentrations resulting in higher carbohydrate content.

Total Dietary Fiber Content

The addition of *E. spinosum* seaweed concentration significantly affects the fiber content of the produced tempeh, as described in Table 1. Dietary fiber is crucial for the digestive system, influencing water absorption. Dietary fiber can help lower blood sugar, insulin, triglyceride, and cholesterol levels. There are two types of dietary fiber: insoluble and soluble (Raposo et al., 2016). Insoluble fiber, like cellulose, hemicellulose, and lignin, cannot be digested and is insoluble in water. Soluble fiber, such as pectin, Arabic gum, biological polysaccharides, and synthetic polysaccharides, cannot be digested but mostly dissolves in water (Yang et al., 2017). The consumption of high-fiber foods, including seaweed, has positive effects on the body, reducing the risk of colon cancer, constipation, hypercholesterolemia, obesity, and diabetes (Lafarga et al., 2020).

Dietary fiber is part of carbohydrates that cannot be digested in the small intestine (Compaore-Sereme et al., 2022). Therefore, the results show that dietary fiber is part of the total carbohydrates. The dietary fiber content of ordinary tempeh is 10.87% and increases with the addition of *E. spinosum* seaweed. The higher the concentration of seaweed added, the higher the dietary fiber content in the tempeh. For example, adding 30% *E. spinosum* resulted in the highest dietary fiber content of 17.99%. The finding aligns with a study by Fauzi et al. (2023), which demonstrated that adding seaweed to rice improved the dietary fiber content of seaweed rice. Furthermore, the dietary fiber content in the research was higher than in another study by Cornelia and Kartika (2022), who added 50% seaweed to tempeh and achieved a dietary fiber content of 9.88%. Seaweed is rich in soluble fiber, such as agar, alginate, and carrageenan, constituting about 50%–70% of the fiber content in seaweed (Stévant et al., 2020). These soluble fibers can help reduce cholesterol levels in the blood and lower the risk of heart disease. Additionally, seaweed contains pigments, often referred to as "green food," which serve as functional food or supplements rich in natural fiber nutrients. These pigments have various health benefits, including their potential as anti-cancer agents and detoxifiers and aid in wound healing and digestion (Okolie et al., 2017). Seaweed also contains bioactive compounds that contribute to its health benefits. These compounds are formed through secondary metabolic processes, including alkaloids, flavonoids, tannins, terpenoids, and steroids (Safia et al., 2020). These bioactive components further enhance the nutritional and therapeutic value of seaweed.

Sensory Acceptance of *Eucheuma spinosum* Tempeh

Based on the data in Table 2, the overall evaluation of the tempeh samples' appearance, aroma, texture, and flavor indicated a positive response, falling within the "like" category.

Table 2
Sensory acceptance of Eucheuma spinosum *tempeh*

Sample	Appearance	Aroma	Texture	Flavor
K	7.48 ± 1.28[a]	6.81 ± 1.64[a]	6.81 ± 1.35[a]	7.65 ± 1.01[d]
A	7.06 ± 1.48[a]	6.74 ± 1.69[a]	6.68 ± 1.62[a]	6.69 ± 1.46[b]
B	7.29 ± 1.29[a]	6.84 ± 1.26[a]	6.97 ± 1.49[a]	7.26 ± 0.96[c]
C	6.81 ± 1.44[a]	6.19 ± 1.72[a]	6.13 ± 1.45[a]	6.03 ± 1.40[a]

Note. Data in triplications ± deviation standard. Data followed by a different number in the same column show a significant difference (p <0.05). K= tempeh with no *E. spinosum*; A= tempeh with *E. spinosum* 10%; B= tempeh with *E. spinosum* 20%; C= tempeh with *E. spinosum* 30%

The tempeh without adding Spinosum was preferred in terms of appearance, as it resembled regular tempeh (Table 2). The most favorable aroma was found in tempeh with a 20% *E. spinosum* addition, as it retained the typical tempeh aroma while also having a distinctive aroma. The texture of tempeh with a 20% *E. spinosum* concentration was preferred due to its denser and chewier texture. The most preferred flavor was observed in tempeh without *E. spinosum* addition, followed by tempeh with a 20% *E. spinosum* addition. The 20% seaweed tempeh had a more desirable flavor compared to the 10% *E. spinosum* tempeh, which was perceived as less flavorful. It should be noted that the appearance, aroma, texture, and flavor of the tempeh can vary depending on the amount of *E. spinosum* added. Tempeh with a 30% *E. spinosum* addition tended to be less favorable (although still falling within the "like" category) compared to lower additions. The attribute was a less attractive appearance, less dominant soybean tempeh aroma, a mushier texture, and less desirable flavor. Despite these differences, the overall liking level of tempeh products with different *E. spinosum* additions was relatively similar as they all fell within the "like" category.

Fatty Acid of *Eucheuma spinosum* Tempeh

Table 3
Fatty acid of Eucheuma spinosum *tempeh*

Type	Parameters	K	A	B	C
Saturated fatty acid	Myristic acid	0.33	0.28	0.29	0.33
	Palmitic acid	12.76	12.3	12.55	12.24
	Heptadecanoic acid	0.72	0.67	0.66	0.27
	Arachidic acid	0.61	0.58	0.52	0.45
	heneicosanoate acid	0.25	0.18	0.25	0.25
Unsaturated fatty acid	Palmitoleic acid	0.12	0.11	0.11	0.76
	Linolelaidate acid	25.64	25.03	27.09	26.8
	Linolenic acid	49.1	50.38	48.36	48.74
	Eicosenoic acid	5.00	5.14	4.42	4.49
	Docosahexaenoic acid	0.13	0.16	0.14	0.13

Note. K= tempeh with no *E. spinosum*; A= tempeh with *E. spinosum* 10%; B= tempeh with *E. spinosum* 20%; C= tempeh with *E. spinosum* 30%

The most dominant saturated fatty acid was palmitic acid, followed by heptadecanoic and arachidic acid (Table 3). Tempeh without the addition of *E. spinosum* seaweed had more palmitic acid compared to tempeh with the addition of *E. spinosum*. Palmitate acid is a saturated fatty acid found in food and synthesized endogenously. Although often thought to be involved in adult chronic disease, palmitic acid is an essential component of membrane lipids, secretion, and transport, which plays a role in forming proteins and molecules (Innis, 2016). Heptadecanoic acid and Arachidic acid have the highest values for tempeh without adding seaweed, while the lowest was for tempeh with a 30% *E. spinosum* addition. The heptadecanoic acid in the control tempeh had quite a lot of differences from the tempeh with the addition of 30% spinosum. The control tempeh had a Heptadecanoic acid value of 0.72%, while tempeh, adding 30% spinosum, was 0.27%. Arachidic acid in tempeh decreased with the addition of spinosum seaweed to tempeh.

An analysis of unsaturated fatty acids in tempeh shows that the most dominant is linolenic acid, followed by linolelaidate acid and eicosenoic acid. Damanik et al. (2018) stated that soybeans are a source of linoleic, oleic, and linolenic acids. Soybeans usually contain 25-64% linolenic acid, 1-12% linoleic acid, and 11-16% oleic acid. The linolenic acid in tempeh in this study was lower than in soybeans. It is due to the processing of tempeh, such as soaking, boiling, and fermenting. Heat heating can convert fatty acid components into volatile compounds such as aldehydes, ketones, acids, and hydrocarbons. The compounds evaporate if given heat treatment, reducing fatty acids (Nguju et al., 2018). The highest linolenic acid content in this study was in tempeh, with the addition of 10% spinosum of 50.38%.

In comparison, the highest linolelaidate acid was found in Spinosum tempeh 20%, namely 27.09%. Eicosenoic acid and Docosahexaenoic acid had the highest values in tempeh with the addition of 10% *E. spinosum*. Linolenic acid has many benefits for the body. Linolenic acid is more effective than linoleic acid for lowering triglycerides in the blood. The linolenic acid is usually most commonly found in fish oil; the linolenic acid content in fish oil is very high, so it needs dosage and doctor's supervision to consume it. Linolenic acid is not as high as fish oil in tempeh, so it can be more freely consumed in large quantities without reducing its benefits (Klek, 2016).

Microstructure of *Eucheuma* s*pinosum* Tempeh

SEM (Scanning Electron Microscopy) is an analysis used to determine microscopic magnification by showing detailed visual images of particles with high quality and spatial resolution of the appearance of a material (Akhtar et al., 2018). The results of SEM analysis with the addition *of E. spinosum* to tempeh are shown in Figure 1.

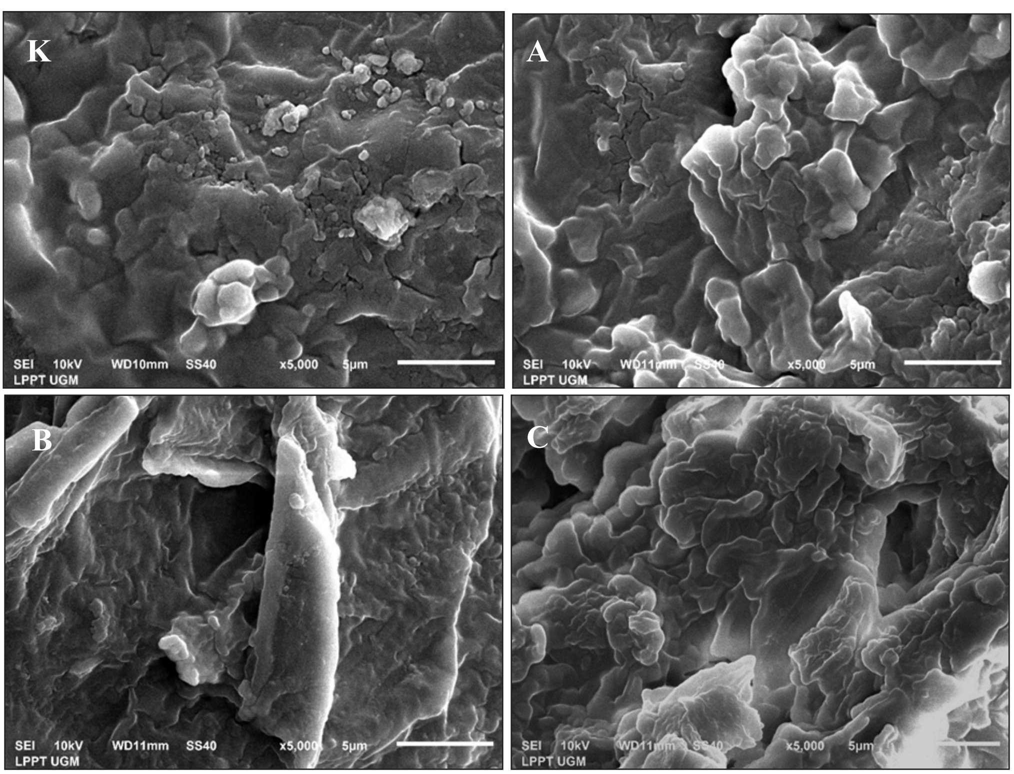

Figure 1. Microstructure of *E. Spinosum* Tempeh. K= tempeh with no *E. spinosum*; A= tempeh with *E. spinosum* 10%; B= tempeh with *E. spinosum* 20%; C= tempeh with *E. spinosum* 30%

The results show that the higher the addition of the *E. spinosum* concentration, the more in amount and more significant the cavities formed between the tissues. The phenomena are caused by adding *E. spinosum* seaweed, which causes the formed tempeh to have more cavities than regular tempeh. Kustyawati et al. (2014) stated that tempeh, without treatment, usually has smaller inter-tissue cavities than treated tempeh. The cavity will affect the texture of a product; the smaller the cavity, the denser the texture will be.

CONCLUSION

The research showed that the addition of *E. spinosum* seaweed to tempeh has a significant impact on its nutritional composition. With a 30% addition, tempeh has the highest dietary fiber content at 17.99%, while the protein content decreases to 11.57%. The fat content is low at 4.34%, and the water content increases to 74.74%. The carbohydrate content increases to 10.12%, and the ash content increases to 1.22%. The addition of *E. spinosum* seaweed significantly affects the levels of food fiber, protein, carbohydrates, water, fat, and ash in tempeh. However, no significant differences were observed in the hedonic test, indicating that the overall liking for tempeh with different *E. spinosum* additions was similar. Furthermore, among the different concentrations of *E. spinosum* seaweed added to tempeh, the 20% addition was preferred in terms of sensory attributes compared to the 10% and 30% additions. It suggests that the 20% Spinosum addition balanced sensory appeal and nutritional benefits. Overall, incorporating Spinosum seaweed into tempeh offers the potential for enhancing its nutritional composition, particularly in terms of fiber content, without compromising sensory acceptance.

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