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CHARACTERISTICS OF TEMPE MADE FROM SEAGRASS SEEDS (*Enhalus acoroides*) USING A COMBINATION OF RICE FLOUR, WHEAT FLOUR, AND SOYBEAN TEMPE STARTER

Karakteristik Tempe Dari Biji Lamun (*Enhalus acoroides*) Menggunakan Kombinasi Starter Tepung Beras, Terigu, dan Tempe Kedelai

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ABSTRACT

The raw materials used for tempe production are highly diverse. In addition to soybeans, tempe can also be produced from seagrass seeds (*Enhalus acoroides*) using tempe starter culture. To accelerate fermentation, additional starters in the form of rice flour and wheat flour were used. This study aimed to determine the characteristics of tempe made from Enhalus acoroides seeds using rice flour and wheat flour starters in combination with soybean tempe flour. The ratio of soybean tempe flour to wheat flour (A1) and rice flour (A2) was 1:3. The combined starter was added at 20% of the total weight of seagrass seeds. The research employed an experimental method. The parameters analyzed included appearance, taste, aroma, and texture for subjective evaluation, and moisture content, protein, fat, ash, carbohydrate, calcium (Ca), iron (Fe), and lead (Pb) for objective analysis. The results showed that the use of wheat flour and rice flour starters combined with soybean tempe flour produced tempe with varied sensory values and chemical compositions. The sensory evaluation of Enhalus acoroides tempe yielded appearance scores ranging from 3,12 to 3,87 (like to very like), taste scores of 3,18-3,20 (like), aroma scores of 3,0-3,3 (like), and texture scores of 3,7-3,8 (very like). The chemical composition included moisture content ranging from 56,04-59,73%, ash 0,52-0,96%, fat 0,85-1,3%, protein 1,52-2,92%, carbohydrate 35,53-40,63%, calcium (Ca) 0,15-0,77 mg/L, iron (Fe) 8,22-8,73 mg/L, and lead (Pb) 0,002-0,125 mg/L. The use of rice flour starter contributed the most to the sensory characteristics, moisture, fat, protein, and iron content of tempeh, while the wheat flour starter was more dominant in increasing ash, carbohydrate, and calcium levels. The lead (Pb) content was also found to be below the maximum permissible limit, indicating that the tempeh produced in this study is safe for consumption.

Keywords: *Enhalus acoroides*, Tempe, Rice Flour, Wheat Flour

ABSTRAK

Bahan baku pembuatan tempe sangat beragam. Selain kedelai, tempe juga dapat dibuat dari biji lamun *Enhalus acoroides* menggunakan ragi tempe. Untuk mempercepat fermentasi, starter yang ditambahkan berupa tepung beras dan tepung terigu. Penelitian ini bertujuan untuk mengetahui karakteristik tempe yang dihasilkan dari biji lamun *Enhalus acoroides* mengunakan starter tepung beras dan tepung terigu, dikombinasikan dengan tepung tempe kedelai. Perbandingan tepung tempe dengan: tepung terigu (A_1) dan tepung beras (A_2) adalah 1:3. Kombinasi starter tersebut ditambahkan sebanyak 20% dari berat total biji lamun. Metode yang digunakan dalam penelitian ini adalah metode eksperimen. Parameter subjektif yang dianalisa meliputi kenampakan, rasa, bau dan tekstur. Parameter objektif meliputi kadar air, protein, lemak, abu, karbohidrat, Ca, Fe dan Pb. Hasil penelitian menunjukkan bahwa penggunaan starter tepung terigu dan tepung beras yang dikombinasikan dengan tepung tempe kedelai dapat menghasilkan tempe dengan karakteristik sensoris dan komposisi kimia yang bervariasi. Tempe lamun *Enhalus acoroides* memiliki nilai kenampakan berkisar antara 3,12-3,87 (suka sampai sangat suka), rasa 3,18-3,2 (suka), bau 3,0-3,3 (suka) dan tekstur 3,7-3,8 (sangat suka). Memiliki kadar air berkisar antara 56,04-59,73%, abu 0,52-0,96%, lemak 0,85-1,3%, protein 1,52-2,92%, karbohidrat 35,53-40,63%, kalsium (Ca) 0,15 mg/L-0,77 mg/L, besi (Fe) 8,22-8,73 mg/L dan timbal (Pb) 0,002-0,125 mg/L. Penggunaan starter tepung beras memberikan kontribusi tertinggi terhadap karakteristik sensoris, kadar air, lemak, protein, serta kandungan besi pada tempe sedangkan starter tepung terigu lebih dominan meningkatkan kadar abu, karbohidrat, dan kalsium. Kandungan logam berat Pb (timbal) juga masih berada di bawah batas maksimum yang diperbolehkan, sehingga produk tempe hasil penelitian ini dinyatakan aman untuk konsumsi.

Kata Kunci: Lamun *Enhalus acoroides*, Tempe, Tepung Beras, Tepung Terigu

INTRODUCTION

Seagrass fruit has not yet had any economic value, but has been utilized by some coastal communities as a food source to replace rice and side dishes. This practice has been carried out by coastal communities in the Maluku, Lombok, and Kupang regions. Seagrass fruit (Enhalus acoroides) is made into rice, processed by removing the seeds, then boiling, drying, and making flour. When used as a side dish, seagrass fruit is processed in coconut milk sauce or stir-fried with shellfish (Tapotubun et al., 2024). Seagrass seeds contain various components such as protein, fat, fiber, and carbohydrates. The protein content is 8.1%, fat 0.2%, water 9.8%, ash 6.4%, carbohydrates 2.4%, and fiber 2.4% (Kole et al., 2020). In addition, seagrass seeds also contain quite high mineral content so they can be used as a source of health food and medicine (Dita & Subhan, 2022). To increase the added value of seagrass as a food source, several efforts have been made, including snacks such as pancakes and sticks made from seagrass seed flour (Tapotubun et al., 2024), seagrass fillus (Dita & Subhan, 2022), and side dishes such as tempeh made from seagrass seeds (Kole et al., 2020). Utilizing seagrass seeds is one way to replace soybeans in tempeh production.

In Indonesia, tempeh is made from a wide variety of raw materials. Besides soybeans, tempeh can also be made from other legumes such as long beans, winged beans, black soybeans, lamtoro, mung beans, red beans (Faizah & Gazali, 2022); (Suknia, 2020), pigeon peas (Lebui), komak beans (Pinasti et al., 2020), and cowpeas (Naisali & Wulan, 2020). There are also grains such as melinjo seeds (Yulia et al., 2019), corn (Lestari & Mayasari, 2016), and seagrass (Kole et al., 2020). Tempeh can even be made from fermented tofu (tempe gembus), from coconut (tempe bongkrek), and from fermented jack bean (tempe benguk) (Alvina et al., 2019). The length of fermentation time determines tempeh classifications, such as fresh tempeh, semangit tempeh, and rotten tempeh, each with varying nutritional content (Sari & Mardhiyyah, 2020). The various ingredients used in tempeh production serve different purposes, such as increasing nutritional value, enhancing functional value, and enhancing taste (Khanifah, 2018).

Temeh is a traditional food product made from soybeans, fermented using yeast as a starter. Conventionally, starter production can be done in several ways, such as utilizing existing tempeh, using leftover tempeh packaging, or using dried tempeh. This tempeh can be either whole, dried tempeh, or thinly sliced and then dried in the sun. According to Ellent et al. (2022), during fermentation, the fungi Rhizopus oryzae or Rhizopus oligosporus form a white mycelium of fine threads. These fungi produce protease, lipase, and amylase enzymes, which function to break down proteins, fats, and carbohydrates into simple compounds. Widiany et al. (2023) state that tempeh quality is influenced by factors such as the type of soybean used, the type of microorganism or tempeh mold, processing stages such as soaking, boiling, and fermentation, and environmental conditions such as temperature, pH, and humidity.

Tempeh can be made from seagrass seeds using a yeast starter (Kole et al., 2020). Even using yeast, tempeh formation also takes a long time, so additional starter is needed to speed up the fermentation process. Starters that can be used include rice flour, tapioca flour, or wheat flour because the carbohydrate content is sufficient to serve as a substrate (Qohir & Fajri, 2020). This study aims to determine the characteristics of tempeh produced from Enhalus acoroides seagrass seeds using rice flour and wheat flour starters combined with soybean tempeh flour.

RESEARCH METHOD

This research took place in September 2024. Sampling was conducted in the coastal waters of Negeri Suli, Salahutu District, Central Maluku Regency. Proximate and sensory analyses were conducted at the Fisheries Product Technology Laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University, Ambon. Mineral and heavy metal analyses were conducted at the Maluku Provincial Health Laboratory.

The materials used in this study were: young Enhalus acoroides seagrass seeds, soybean tempeh, rice flour, wheat flour, water, tissue paper, PE plastic, and toothpicks. The tools used were: knives, basins, blenders, sieves, frying pans, pots, stoves, straws, plates, spoons, ovens, analytical scales, and several other analytical tools.

This research was conducted using an experimental method in three stages: starter preparation, seagrass seed preparation, and tempeh production. Two types of starter flour were used: wheat flour (A1) and rice flour (A2). This stage used a modified method of Kole et al. (2020). The first stage begins with making a starter, soybean tempeh is cut into small pieces, left for 12 hours until mold or fine white fibers appear, then dried for 2 days until dry. The dried tempeh is mashed, filtered and stored in a container and is ready to be used as a starter mixture. Each treatment: wheat flour (A1) and rice flour (A2) are dried in an oven at 400C for 1 hour until dry. The treatment is then mixed with soybean tempeh flour until evenly distributed. The ratio of soybean tempeh flour to wheat flour (A1): soybean tempeh flour to rice flour (A2) is 1:3. The mixture is ready to be used as a starter in making tempeh. The second stage is making seagrass seeds, starting with taking young seagrass seeds that have been removed from the fruit skin, washed and cleaned, then boiled for 3 minutes at 900C, then removed and dried on tissue. The third stage is making tempeh, starting with weighing 100 grams of boiled young Enhalus acoroides seagrass seeds and then mixing them with a starter of 20% of the total weight of the seagrass seeds until coated. The mixture is put into a 13x8 cm plastic bag and perforated with a toothpick. This manufacturing stage is repeated three times, then fermented in dry and dark conditions at room temperature of 270C for 24 hours. The finished fermented tempeh is cut into 1 cm thickness for analysis. For the taste test before being served to the panelists, the tempeh is fried in hot oil for about 1 minute until browned. The analysis carried out is a subjective and objective analysis. Subjective analysis includes appearance, taste, smell and texture using a heidonic test with a scale of 1 to 4 where 1 = Very Dislike, 2 = Dislike, 3 = Like, 4 = Very Like using a panel of 30 people. Objective analysis included water, protein, fat, and ash content using the AOAC method (2005), carbohydrates using the by-difference method, and minerals Ca, Fe, and heavy metal Pb using the AAS (Atomic Absorption Spectrophotometry) method. The analyzed samples were repeated three times. The data obtained were then tabulated and analyzed using Microsoft Office Excel and analyzed descriptively. The results obtained were compared with the literature and then presented in tabular form.

RESULT

Proximate, Minerals and Heavy Metals from Seagrass Seed Tempe Enhalus acoroides

The results of the analysis (Table 1) show that seagrass tempeh Enhalus acoroides produces water content ranging between 56.04-59.73%, ash 0.52-0.96%, fat 0.85-1.3%, protein 1.52-2.92% and carbohydrate 35.53-40.63% where the highest water, fat and protein content is achieved by seagrass tempeh with rice flour while the highest ash and carbohydrate content is achieved by seagrass tempeh with wheat flour. Calcium (Ca) levels ranged from 0.15 mg/L to 0.77 mg/L, iron (Fe) from 8.22 to 8.73 mg/L, and lead (Pb) from 0.002 to 0.125 mg/L. The highest calcium (Ca) levels were found in seagrass tempeh made with rice flour, the highest iron (Fe) levels were found in seagrass tempeh made with wheat flour, and the lowest lead (Pb) levels were found in seagrass tempeh made with wheat flour, at 0.002 mg/L.

Table 1. Proximate values of tempeh made from seagrass seeds (Enhalus acoroides)

Proximate (%)	Flour type starter	
	Flour (A_1)	Rice flour (A_2)
Water	56.04±0.90	59.73±0.92
Ash	0.96±0.02	0.52±0.04
Fat	0.85±0.01	1.3±0.01
Protein	1.52± 0.05	2.92±0.05
Carbohydrate (by difference)	40.63± 0.89	35.53±0.93
Mineral (mg/L)		
Calsium (Ca)	0.15±0.02	0.77±0.02
Fe (besi)	8.73±0.04	8.22±0.03
Heavy metal		
Pb (timbal)	0.002±0.01	0.125±0.01

Sensory Characteristics of Seagrass Seed Tempeh Enhalus acoroides

Observations show that the use of wheat flour and rice flour starters can affect the sensory characteristics of seagrass tempeh, such as the appearance, taste, odor, and texture. The heidonic test results (Table 2) show that the average appearance score for seagrass tempeh ranged from 3.87 to 3.12 (like to very like), taste 3.18 to 3.2 (like), odor 3.0 to 3.3 (like), and texture 3.7 to 3.8 (very like), with the highest appearance, taste, odor, and texture scores obtained with the rice flour treatment. Seagrass tempeh has the following characteristics: an attractive appearance, a regular and uniform rectangular shape, an even, clean white color, with the characteristic white color of tempeh; a specific odor of fresh tempeh; a dense, compact texture, easy to cut into uniform shapes, and a delicious, characteristic tempeh flavor after frying. The sensory characteristics of seagrass seed tempeh are shown in Figure 1.

Table 2. Results of the heidonic test for tempeh from seagrass seeds (Enhalus acoroides).

Sensory attributes	Flour type starter	
	Flour (A_1)	Rice flour (A_2)
Appearance	3.12	3.87
Taste	3.18	3.23
Odor	3.0	3.3
Texture	3.7	3.8

Figure 1. Sensory characteristics of seagrass seed tempeh: before (a), after frying (b)

DISCUSSION

Proximate, Minerals and Heavy Metals from Seagrass Seed Tempe Enhalus acoroides

The proximate composition, minerals, and heavy metals of seagrass tempeh are influenced by the type of flour used as a starter during the fermentation process. Using a rice flour starter can increase the water, fat, and protein content of seagrass tempeh. Rice flour contains starch and is hydrophilic. Rice flour has a higher capacity to bind free water than wheat flour. Rice flour has a starch content of 78.3%, while wheat flour has a starch content of around 68-78% (Susanti, 2023). The water content of tempeh is also influenced by mold activity during the fermentation process. According to Ellent et al. (2022), mold activity affects water content because during the fermentation process, mold digests the substrate and produces water and energy. If the substrate requirement is relatively low, the mold will utilize the available substrate for metabolic processes and produce water in an amount proportional to the proportion of the substrate.

The use of a rice flour starter can increase the fat content of seagrass tempeh. The fat content of rice flour is 4.52%, while the ash content of teringu flour is 1.0% (Susanti, 2023). The resulting ash content will increase along with the high fat content of the raw materials. The fat content of seagrass tempeh from this study is lower than the fat content of tempeh from the study by Kole et al., (2020), which is 1.83-2.74%. The use of rice flour can increase the protein content of seagrass tempeh. This is because the protein characteristics of rice flour are more easily digested by microorganisms that ferment soybeans and other ingredients. Rice flour also has a different protein composition, which can support the growth of tempeh mold better than wheat flour, which contains gluten and has a protein structure that is more difficult to break down during the fermentation process. According to Azzahra et al., (2024), rice flour has a simpler carbohydrate composition, so that tempeh mold can quickly use energy from rice starch for its growth. The availability of this energy supports the activity of microorganisms in breaking down proteins during fermentation, resulting in increased protein. The water content of this study met the maximum tempeh quality standard of 65%. However, the fat and protein content were still below the established standard of 7% and 15%, respectively (BSN, 2015). Therefore, the tempeh produced in this study cannot yet be used as a source of marine vegetable protein; therefore, to meet the established standards, other food ingredients are needed to meet adequate protein requirements.

The use of wheat flour can increase the ash and carbohydrate content of seagrass tempeh. The high ash content is due to the addition of wheat flour, which increases the inorganic content of the product (Mumtazah et al., 2021). The use of wheat flour can also increase the carbohydrate content of seagrass tempeh. The carbohydrate content of a product is influenced by its nutritional composition. If the water, ash, protein, and fat content are relatively low, the carbohydrate content tends to increase. Conversely, if the water, ash, protein, and fat content are high, the carbohydrate content will decrease (Silaban, 2024). The ash content of this study meets the established tempeh quality standard of 1.5% (BSN, 2015). The high calcium and iron levels are due to an increase in the proportion of inorganic compounds and a decrease in organic compounds during the fermentation process, resulting in increased mineral concentrations (Astawan et al., 2016). Tempeh mold produces the enzyme phytase, which breaks down phytic acid and binds several minerals to form phosphorus and inositol. This phytic acid breakdown process makes minerals such as iron, calcium, magnesium, and zinc more easily absorbed by the body (Sine & Soetarto, 2018). The calcium and iron levels of seagrass tempeh in this study were lower than those of pigeon pea tempeh, found in Sine & Soetarto (2018), which had 14.083 mg/L for iron and 202.478 mg/L for calcium. Lead levels still met the 2015 standard set by the National Standardization Agency (BSN) of 0.25 mg/kg, making the tempeh product safe for consumption.

Sensory Characteristics of Seagrass Seed Tempeh Enhalus acoroides

Acceptance of food ingredients varies from person to person according to the social aspects of the recipient community (Ellent et al., 2022). This acceptance is greatly influenced by its appearance, especially attractive and natural-looking colors (Naisali & Wulan, 2020). From the results of observations, researchers prefer seagrass tempeh with rice flour starter (A2) because this tempeh has attractive appearance characteristics, regular and uniform square shape, even clean white color, with the typical white color of tempeh, while seagrass tempeh with wheat flour starter (A1) although its appearance is attractive, regular square shape, but the resulting color is uniform creamy white. According to Azzahra et al., (2024) quality tempeh is characterized by an even white color on its entire surface. This color appears due to the abundant growth of mold mycelium after the inoculation process on rice flour media. The appearance of the color of tempeh is also greatly influenced by the character of the mold mycelium that plays a role in fermentation. The most commonly used molds in tempeh starter cultures are Rhizopus oligosporus, with its white to yellowish-gray mycelium, and Rhizopus oryzae, which produces a grayish-white mycelium. This combination of growths results in an even white color in the tempeh.

Panelists' preference for the taste of seagrass tempeh fell within the typical tempeh characteristics: delicious, savory, and distinctive. This preference is influenced by the type of flour used as the starter, the fermentation process, and the frying process. During the fermentation process, the mold utilizes the fat, protein, and carbohydrates in the ingredients, creating the distinctive aroma of tempeh. Furthermore, during frying, physical and chemical changes occur, resulting in various elements transforming into a distinctive, delicious flavor (Naisali & Wulan, 2020). According to Hastian & Basir (2022), different types of flour produce different taste sensations.

The aroma of a food is influenced by the sensitivity of each person's sense of smell (Ellent et al., 2022). Researchers preferred seagrass tempeh with a rice flour starter (A2) because it had a very strong odor characteristic of fresh tempeh. While the odor value of the wheat flour starter (A1) had a characteristic odor characteristic of fresh tempeh, its strong, distinctive odor was somewhat reduced. The odor or aroma of tempeh is also influenced by the fermentation process and the raw materials used. The characteristic aroma of tempeh results from the growth of mold and the degradation of components in the beans into volatile compounds, such as ketones (Azzahra et al., 2024). This aroma is a combination of the characteristic odor of mold mycelium, savory aromas derived from free amino acids, and additional aromas formed from the breakdown of fat compounds (Naisali & Wulan, 2020).

Based on the texture values obtained, panelists' preference for the texture of seagrass tempeh showed relatively little difference. The texture values of the seagrass tempeh with these two flour starters were still within the range of tempeh characteristics: dense, compact, easy to cut with a uniform shape. This difference in values was influenced by the type of flour starter used. According to (Azzahra et al., 2024) and (Naisali & Wulan, 2020), quality tempeh has a dense texture and holds together compactly. This is due to abundant mycelium growth, so the tempeh structure appears tight and solid. The presence of mycelium plays a role in increasing the density of the tempeh mass, forming a unified structure, and reducing the presence of air cavities within it. As a result, tempeh does not easily crumble when pressed. Conversely, tempeh that is not successful experiences suboptimal and uneven mycelium growth, resulting in a loose texture and is easily crumbled because the mycelium network is unable to firmly bond the tempeh components.

CONCLUSION

The use of wheat flour and rice flour starter combined with soybean tempeh can produce tempeh with varying sensory characteristics and chemical composition. Enhalus acoroides seagrass tempeh has an appearance value ranging from 3.12-3.87 (like to very like), taste 3.18-3.2 (like), odor 3.0-3.3 (like) and texture 3.7-3.8 (very like). It has a water content ranging from 56.04-59.73%, ash 0.52-0.96%, fat 0.85-1.3%, protein 1.52-2.92%, carbohydrate 35.53-40.63%, calcium (Ca) 0.15 mg/L-0.77 mg/L, iron (Fe) 8.22-8.73 mg/L and lead (Pb) 0.002-0.125 mg/L. The use of a rice flour starter significantly improves the sensory characteristics, moisture content, fat content, protein content, and iron content of tempeh, while the wheat flour starter significantly increases the ash content, carbohydrate content, and calcium content. The lead content is also below the maximum permissible limit, making this tempeh product safe for consumption.

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