Agriculture and Food

AIMS Agriculture and Food, 11(1): 274–289.
DOI: 10.3934/agrfood.2026014
Received: 12 December 2025
Revised: 17 March 2026
Accepted: 31 March 2026
Published: 31 March 2026

https://www.aimspress.com/journal/agriculture

Research article

In vitro antidiabetic activity and consumer acceptance level of fermented brown seaweed *Sargassum hystrix* tea using *Lactobacillus plantarum*

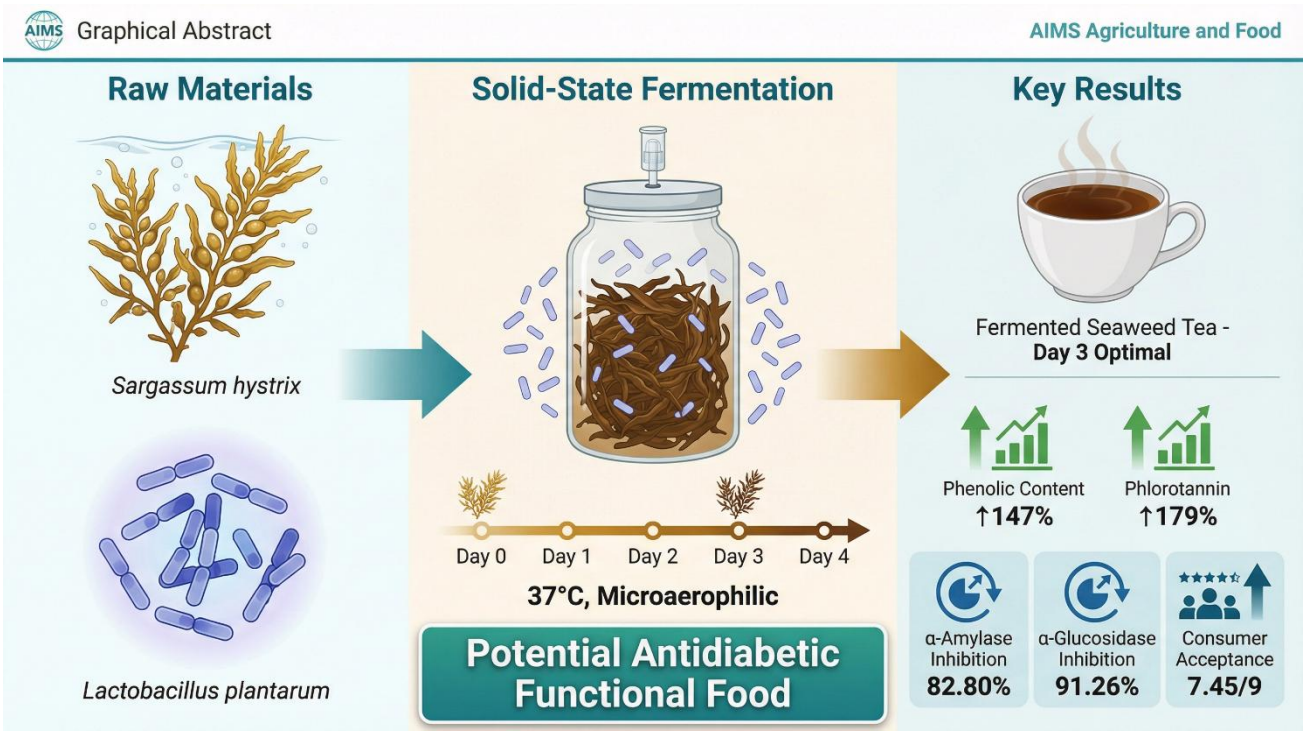
Khalishah Jasmine Putri Abdillah[1], Amir Husni[1,2,*], Masagus Muhammad Prima Putra[1,2] and Siti Ari Budhiyanti[1,2]

[1] Fisheries Science Postgraduate Program, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia
[2] Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia

* **Correspondence:** Email: a-husni@ugm.ac.id; Tel: +62274551218.

Abstract: *Sargassum hystrix* is a brown seaweed that shows potential antidiabetic activity through its phenolic and phlorotannin compounds. The use of *S. hystrix* as a functional food is constrained by the stability of bioactive compounds and a fishy odor that reduces consumer acceptance. Fermentation is a processing method that plays a role in improving the sensory quality, functional characteristics, and biological activity of products. This study aimed to evaluate the effect of fermentation duration using *Lactobacillus plantarum* on antidiabetic activity and consumer acceptance level of *S. hystrix* tea. The fermentation process was carried out for 0, 1, 2, 3, and 4 days under microaerophilic conditions at 37 °C. The results showed that the highest increase in functional characteristics was obtained on the third day of fermentation, marked by higher phenol content (12.94 ± 0.68 mg GAE/g), phlorotannin (14.60 ± 0.95 mg PGE/g), and inhibition activity of α-amylase (82.80% ± 1.70%) and α-glucosidase (91.26% ± 0.44%) than those of other experimental treatments. Sensory evaluation showed that longer fermentation duration reduced the taste and fishy odor of *S. hystrix* tea. The third day of fermentation treatment yielded the highest acceptance scores for aroma, taste, and overall product. Therefore, a 3-day fermentation duration is recommended as the optimal condition to produce *S. hystrix* tea with enhanced functional and sensory qualities.

Keywords: α-amylase; diabetes; fishy odor; lactic acid bacteria; *Sargassum hystrix*



1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion or action [1]. The global number of affected individuals reached 537 million in 2021 and is projected to increase to 784 million by 2045 [2]. These data indicate an urgent need for safe and effective glucose-control strategies that can be integrated into daily dietary patterns. Glucose regulation largely depends on the activity of carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase. Inhibition of these enzymes can slow the hydrolysis of polysaccharides into glucose, thereby reducing carbohydrate absorption in the small intestine. This mechanism is the primary target of oral antidiabetic agents such as acarbose [3,4]. However, the use of synthetic antidiabetic drugs is known to cause gastrointestinal side effects, prompting the exploration of natural enzyme inhibitors derived from functional foods [5]. Numerous molecular studies have reported that polyphenols, flavonoids, and phlorotannins from brown seaweeds can interact with enzyme active sites through hydrogen bonding, hydrophobic interactions, and competitive binding, resulting in significant reductions in α-amylase and α-glucosidase activities [6,7].

In recent years, there has been a growing interest in the utilization of seaweed as a food source. Seaweeds are increasingly recognized not only for their unique sensory properties, such as umami and salinity, but also for their rich nutritional profile and potential health benefits [8]. Furthermore, clinical studies have demonstrated that seaweed extracts can improve carbohydrate metabolism, making them particularly beneficial for overweight and obese individuals [9]. Alongside the interest in novel food sources, fermentation technology has gained significant attention in the development of functional foods and beverages. Fermentation is a traditional processing method that has been revitalized to enhance the bioavailability of nutrients, produce bioactive compounds, and improve the sensory characteristics of food products, thereby offering promising avenues for functional food innovation [10].

Sargassum hystrix is a brown seaweed that shows potential antioxidant and antidiabetic activity.

However, the bioactive compounds in seaweed are susceptible to processing. Total phenolic content has been reported to degrade during high-temperature drying [11] and storage [12]. In addition, the characteristic fishy odor of seaweed reduces consumer acceptability, thereby limiting its application as a functional food [13]. This has driven the development of processing alternatives that not only maintain the stability of bioactive compounds but also improve sensory attributes. Fermentation by lactic acid bacteria (LAB), such as *Lactobacillus plantarum*, is a biochemical approach that produces various enzymes capable of releasing bound polyphenols into their aglycone forms, which are more biologically active. This leads to enhanced bioactivity, including antidiabetic effects [14,15]. From a sensory standpoint, fermentation can modify volatile compounds and produce organic acids that reduce fishy odors and improve flavor [16], thereby increasing consumer acceptance. Tea is used as a matrix because it enables the release of bioactive compounds without significant degradation. The brewing process using boiling water can better preserve polyphenol stability [17], ensuring that the bioactivity of the fermented product remains optimal upon consumption. Moreover, tea is familiar, convenient, and easily integrated into daily dietary habits, offering more realistic potential for development as a functional antidiabetic food product [18]. Therefore, this study aims to explore the effect of fermentation with LAB on the antidiabetic activity and consumer acceptance of brown seaweed *S. hystrix* tea.

2. Materials and methods

2.1. Material

Sargassum hystrix was obtained from Teluk Awur Beach (Jepara, Indonesia) on 25 June 2025. *Lactobacillus plantarum* FNCC 0026 isolate was purchased from the Center for Food and Nutrition Studies, Universitas Gadjah Mada (Indonesia). Media for culture and enrichment were purchased from Merck (Germany). Chemicals were obtained from several companies, including Sigma-Aldrich (Germany) and Merck (USA).

2.2. Inoculum preparation and fermentation of S. hystrix *tea*

An isolate of *L. plantarum* was cultured in MRS broth at 37 °C for 24 h. A total of 10% (v/v) inoculum from the first culture was sub-cultured at 37 °C for 24 h before being used for experiments [19]. The seaweed was cleaned before being dried using an oven at 60 °C for 4 h, and the yield was calculated based on the ratio of dry to fresh weight. The dried *S. hystrix* was then crushed and filtered using a 10-mesh screen. Solid-state fermentation of seaweed was carried out by adding 60 mL of sterile distilled water, 10% (v/w) *L. plantarum* starter, and 1.5 mL of 20% glucose stock as additional nutrients. A total of 20 g of seaweed powder was added gradually, and the mixture was homogenized. Fermentation took place at 37 °C under microaerophilic conditions for 0, 1, 2, 3, and 4 days. This duration was selected based on preliminary studies indicating optimal bioactive compound release within this time frame [20]. After fermentation, the pH value, total titratable acidity, total LAB, and total bacteria were monitored. The fermentation samples were then inactivated at −30 °C. The fermentation product was moist fermented seaweed substrate (post-fermentation), and some samples were re-dried at 60 °C for 90 min to produce dry fermented seaweed substrate (post-drying) [21]. Unfermented *S. hystrix* powder (TF) and commercial black tea (KK) were also used in the test as comparison samples.

2.3. Phenolic content analysis

The phenolic content was determined using the Folin–Ciocalteu method [22]. Briefly, a 1 mL sample (prepared by dissolving 50 mg of sample in 5 mL of distilled water) was added with 1 mL of ethanol 96%, 5 mL of distilled water, and 0.5 mL of 50% Folin–Ciocalteu reagent. The mixture was homogenized and left to stand for 5 min. Then, 1 mL of 5% Na_2CO_3 was added and homogenized again. The reaction mixture was incubated at room temperature in the dark for 1 h. Then, the absorbance was measured at 725 nm using a UV-Vis spectrophotometer. The phenolic concentration was quantified as gallic acid equivalents (GAE).

2.4. Phlorotannin content analysis

The phlorotannin content was determined using the method of Koivikko et al. [23]. A phloroglucinol standard curve was prepared using serial dilutions of 6.25, 12.5, 25, 50, and 100 mg/L. A total of 50 mg of the sample was dissolved in 5 mL of ethanol. From each standard and sample solution, 1 mL was transferred into a test tube. Then, 1 mL of Folin–Ciocalteu reagent and 2 mL of 20% Na_2CO_3 solution were added. The mixture was incubated in the dark for 45 min and then centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 730 nm using a UV-Vis spectrophotometer. The phlorotannin content is expressed in mg PGE/g extract.

2.5. Inhibitory activity of α-glucosidase

The inhibitory activity of α-glucosidase was assessed by referring to the method of Gazali et al. [24]. The S1 solution was prepared by adding 50 µL of phosphate buffer (pH 7) to a microplate, followed by the addition of 25 µL of 0.5 mM p-Nitrophenyl-α-D-glucopyranoside (p-NPG) substrate. The mixture was then supplemented with 25 µL of the sample dissolved in dimethyl sulfoxide (DMSO) at 10,000 mg/L and 25 µL of α-glucosidase 0.2 U/mL. The mixture was incubated for 30 min at 37 °C. The reaction was stopped by adding 100 µL of 0.2 M Na_2CO_3.

The S0 solution was prepared using the same procedure, but the enzyme was replaced with 75 µL of phosphate buffer (pH 7). Meanwhile, the K solution was prepared by mixing 75 µL of phosphate buffer (pH 7), 25 µL of p-NPG, and 25 µL of enzyme, while the B solution was prepared with the same volume, but the enzyme was replaced with buffer. Acarbose (10,000 mg/L) was used as a positive control. Inhibition activity was determined based on the amount of p-nitrophenol formed, measured by absorbance at a wavelength of 405 nm using an ELISA microplate reader. The percentage of inhibition was calculated using the formula:

$$\text{Inhibitory activity of α-glucosidase (\%)} = \frac{(\mathrm{K}-\mathrm{B})-(\mathrm{S1}-\mathrm{S0})}{\mathrm{K}-\mathrm{B}} \times 100 \quad (1)$$

where *K* = control with enzyme addition; *B* = control without enzyme addition; *S1* = sample with addition of enzyme; and *S0* = sample without addition of enzyme.

2.6. Inhibitory activity of α-amylase

The inhibitory activity of α-amylase was tested based on the method described by Gazali et al. [24].

Solution S1 was prepared by adding 25 µL of the sample dissolved in DMSO at a concentration of 10,000 mg/L, then adding 25 µL of α-amylase with a concentration of 13 U/mL. The mixture was incubated for 10 min at 37 °C. Next, 25 µL of 1% starch was added to the mixture as a substrate and incubated again at 37 °C for 10 min. After incubation, 50 µL of 3,5-dinitrosalicylic acid (DNS) color reagent was added to the mixture and heated in boiling water for 10 min to stop the reaction. The mixture was then cooled to room temperature and transferred to a cuvette containing 500 µL of distilled water. The S0 solution was prepared using the same procedure, but the addition of α-amylase was replaced with 25 µL of phosphate buffer (pH 7). Meanwhile, solutions K and B were also prepared in the same way as S1 and S0, but the use of samples was replaced with phosphate buffer (pH 7). Acarbose (10,000 mg/L) was used as a positive control. Absorbance was measured using a spectrophotometer at a wavelength of 540 nm. The percentage of inhibition was calculated using the formula:

$$\text{Inhibitory activity of α-amylase (\%)} = \frac{(\text{K}-\text{B})-(\text{S1}-\text{S0})}{\text{K}-\text{B}} \times 100 \quad (2)$$

where *K* = control with enzyme addition; *B* = control without enzyme addition; *S1* = sample with addition of enzyme; and *S0* = sample without addition of enzyme.

2.7. Consumer acceptance analysis

Consumer acceptance analysis was conducted in line with the method described by Pratiwi and Husni [25], with modifications to the hedonic scale. A total of 80 untrained panelists aged 16–54 years with diverse occupational backgrounds were involved in the hedonic test. Fermented *S. hystrix* tea was prepared by brewing 1 g of tea in 100 mL of boiling water for 6 min, with the tea bag moved up and down 5 times and stirred 2–3 times before removal. Approximately 25 mL of tea solution was served in randomly coded cups. Panelists rated the color, aroma, taste, and overall acceptance using a 6-point hedonic scale (1 = strongly dislike to 6 = strongly like).

2.8. Data analysis

Statistical analyses were conducted using Microsoft Excel and IBM SPSS Statistics 25, with a significance level set at 5% probability ($p < 0.05$). All experiments, including microbial analysis, enzyme inhibition, phenolic assays, and sensory analysis, were performed in triplicate (n = 3). Data were analyzed using the one-sample Kolmogorov–Smirnov test to determine data distribution. If the data were normally distributed ($p > 0.05$), further testing was performed using analysis of variance (ANOVA). If the data were not normally distributed, Kruskal–Wallis testing was performed. If the ANOVA results showed significant differences between treatments overall, a Tukey test was performed to determine which treatment had the most significant differences. In the figures and tables, different superscript letters (a, b, c, d, e, f) indicate statistically significant differences between treatments at $p < 0.05$. Specifically, treatments sharing the same letter are not significantly different from each other, while treatments with different letters are significantly different. For example, in Figure 1, if day 0 is marked with superscript *a*, and day 1 is marked with *b*, this indicates that the pH values between these two days are significantly different. Conversely, if both day 3 and day 4 are marked with the same letter *d*, this indicates no significant difference between these two fermentation durations.

3. Results and discussion

3.1. pH and total titratable acidity

pH and total titratable acidity are parameters used to assess the success of the fermentation process [19]. During fermentation, lactic acid bacteria (LAB) metabolize sugars and carbohydrates into organic acids, particularly lactic acid [26]. The accumulation of these organic acids causes a decrease in pH and an increase in total titratable acidity as the fermentation duration increases.

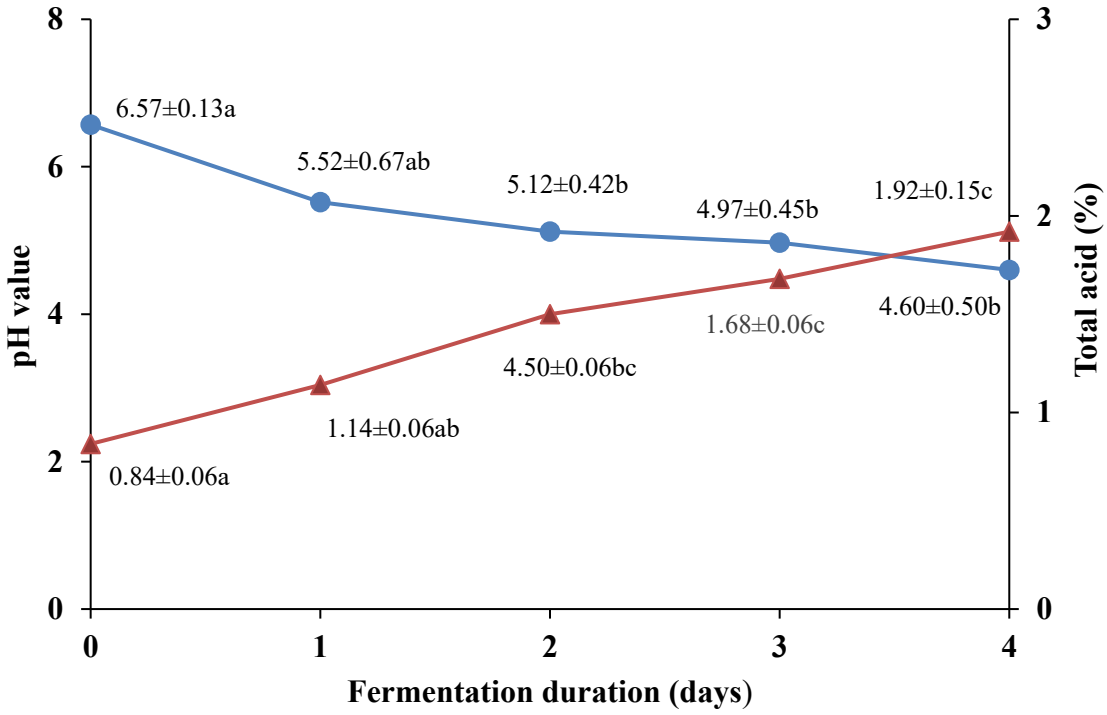


Figure 1. Effect of fermentation duration of *Sargassum hystrix* tea fermented by *Lactobacillus plantarum* on the pH (●) and total acid content (▲). Note: Different letter notations indicate a significant difference ($p < 0.05$).

The results show that fermentation duration has a significant effect on pH and total acid (TA) values ($p < 0.05$). As shown in Figure 1, the pH of *S. hystrix* tea gradually decreased from 6.57 ± 0.13 on day 0 to 4.60 ± 0.50 on day 4. This trend is inversely proportional to TA, which increased from 0.84% ± 0.06% at the start (day 0) of fermentation to 1.92% ± 0.15% on day 4. This pattern of change reflects an increase in organic acid production during fermentation. The decrease in pH and accumulation of acid during fermentation affect the modification of the structure of bioactive compounds in the raw material [14]. Excessively low pH and excessive acid accumulation can suppress microbial growth and reduce the stability of phenolic compounds, which can potentially reduce their bioactivity [27].

These findings are consistent with previous research [28], which showed a decrease in pH and an increase in TA during the fermentation of *Sargassum cristaefolium* kombucha. Another study reported

a similar pattern in the fermentation of mangrove tea, where total acid increased while pH decreased over the course of fermentation. The consistency of these findings reinforces that organic acid production by LAB is a common mechanism in plant-based fermentation, including seaweed.

3.2. Total lactic acid bacteria and total viable bacteria

The total number of bacteria and LAB are key indicators that describe the microbiological dynamics during the fermentation process. The results showed that fermentation duration had a significant effect on the number of LAB and total bacteria ($p < 0.05$). Figure 2 shows an increase in the number of colonies on the first day of fermentation. The LAB population increased from 6.97 ± 0.38 log CFU/mL on day 0 to 8.17 ± 1.06 log CFU/mL on day 1. This increase indicates that the bacteria are in the exponential phase. In line with this, the formation of primary metabolites, such as enzymes, also occurs optimally at the end of the exponential phase to the beginning of the stationary phase [29]. An upward trend was also observed in total bacteria, with colony counts increasing from 7.19 ± 0.16 log CFU/mL on day 0 to 8.29 ± 0.97 log CFU/mL on day 1, before decreasing to 6.27 ± 0.07 log CFU/mL on day 4. This decrease was likely caused by increased acidity and decreased nutrient availability, which inhibited the growth of microorganisms [30].

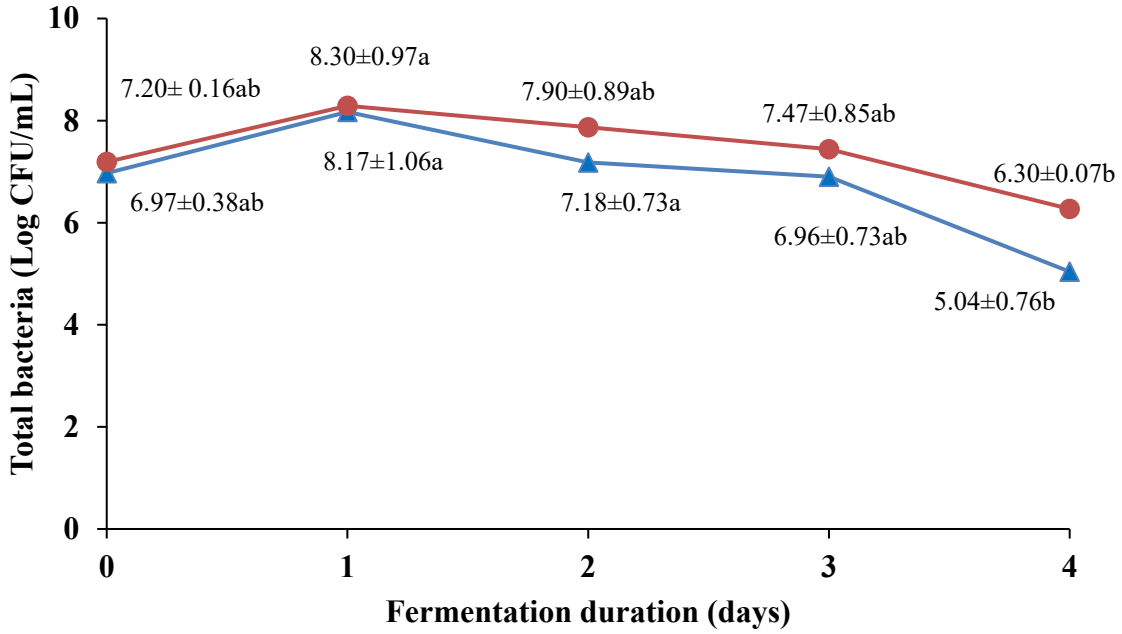


Figure 2. Total number of lactic acid bacteria (▲) and viable bacteria (●) during fermentation of *Sargassum hystrix* tea using *Lactobacillus plantarum*. Note: Different letter notations indicate a significant difference ($p < 0.05$).

Although the total number of viable bacterial colonies was slightly higher than LAB in all observations, both parameters showed consistent trends, indicating that LAB was the dominant bacterial species in the total bacterial population during fermentation and that changes in the total

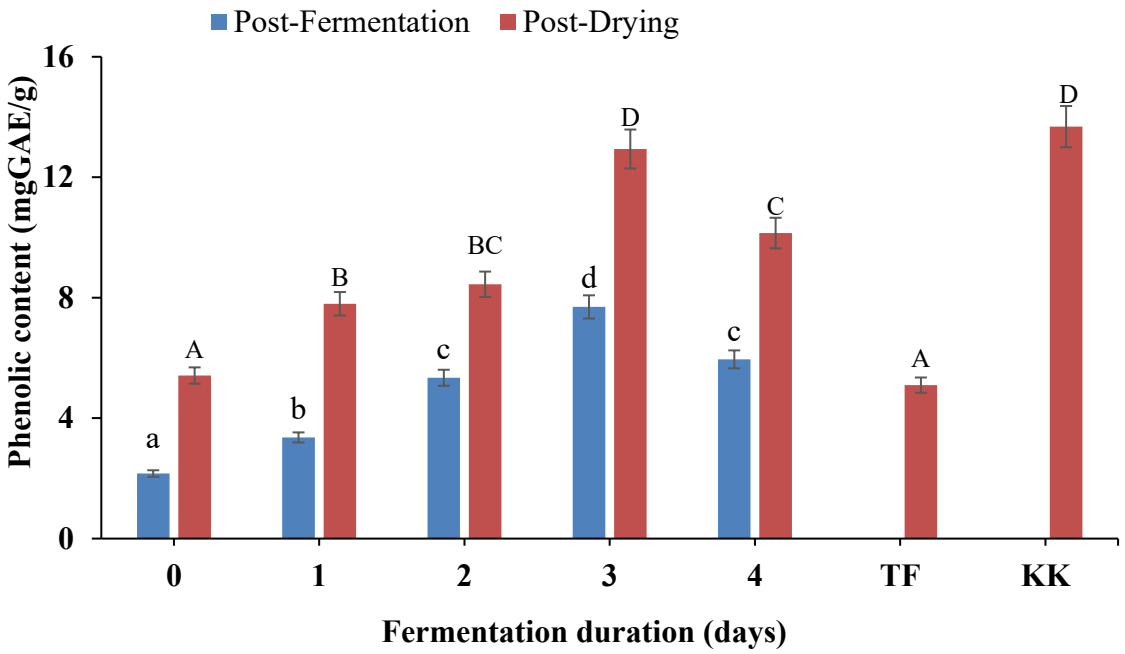
bacteria were largely influenced by the growth dynamics of LAB. The difference in the total number of bacterial colonies and total LAB was likely due to the presence of other microorganisms originating from seaweed or the environment at the start of fermentation. However, as LAB activity increases, the fermentation environment becomes more acidic, inhibiting the growth of non-LAB microorganisms [31]. Nevertheless, some microorganisms can still survive and grow because they are tolerant to acidic conditions, allowing them to dominate until the end of fermentation and potentially be used as starters [32].

3.3. Phenolic and phlorotannin content

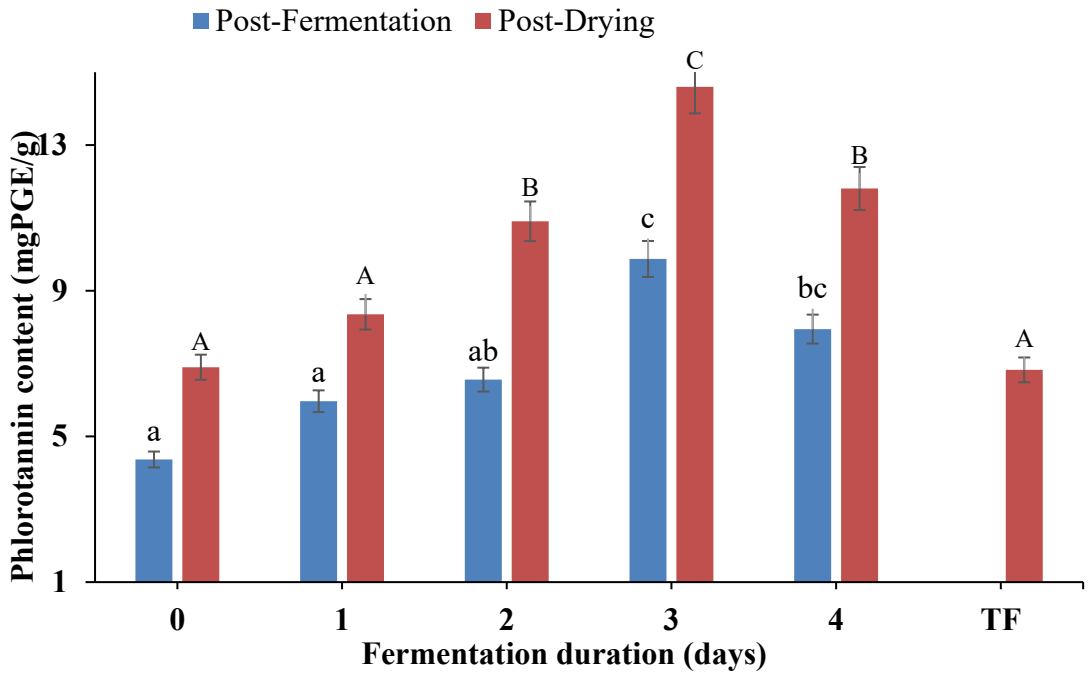
The fermentation mechanism increases the levels of phenols and phlorotannin (Figure 3a and 3b), which can be explained by the biochemical activity of *L. plantarum*, which has been reported in the literature to produce various enzymes like amylase, β-glucosidase, and tannase [14]. However, specific enzyme activities were not measured in this study, and further phytochemical profiling (e.g., HPLC or LC-MS) is needed to confirm the responsible compounds. The β-glucosidase enzyme plays an important role in releasing bound phenols by breaking β-glycoside bonds, thereby producing more reactive phenolic aglycones. Meanwhile, the amylase, peptidase, and proteinase enzymes contribute to the degradation of cell wall polysaccharides and proteins, which accelerates the release of phenols previously trapped in the cell matrix. This mechanism was demonstrated by measuring free phenol levels, which showed a consistent increase in *Betaphycus gelatinum* fermented by *L. brevis* from 0 to 60 h, while bound phenol levels decreased significantly.

On the other hand, the decrease in total phenols and phlorotannin on day 4 is thought to be due to further degradation by enzymes such as phenolic acid decarboxylase and phenol reductase, which are capable of converting complex phenols into simpler or even non-phenolic forms that are undetectable [14]. In addition, increasingly acidic fermentation conditions can inhibit phenol release while accelerating phenolic structure degradation. These findings are consistent with the fermentation of *S. cristaefolium* kombucha, which shows an increase in total phenols up to a certain optimum point before declining in the final phase of fermentation. The decrease in total phenols at the end of fermentation occurs because the environment becomes very acidic due to the accumulation of organic acids, causing phenolic compounds to begin to degrade and inhibiting the activity of microbial enzymes that release phenols from the cell matrix. Low pH conditions also make phenols more stable in their bound form [28].

The increase in total phenol and total flavanol values after drying, especially dry heating, may occur because previously bound phenolic compounds become easier to release, as well as due to the formation of Maillard reaction products. These Maillard products have the characteristics of phenolic compounds and reductants, which arise due to partial damage to the cell structure during the heating process, making them detectable in total phenol testing [33].



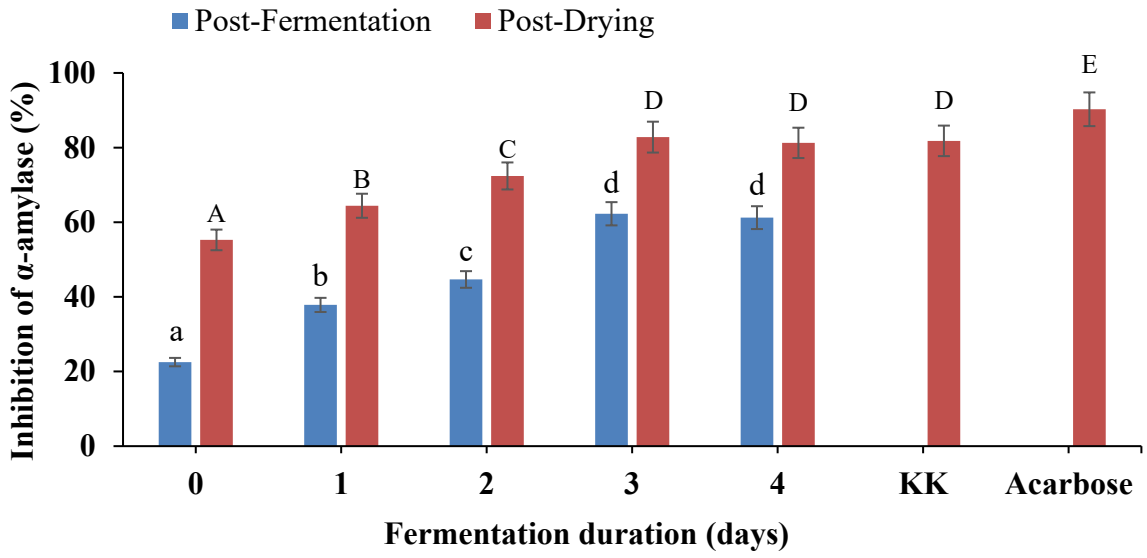
(a)



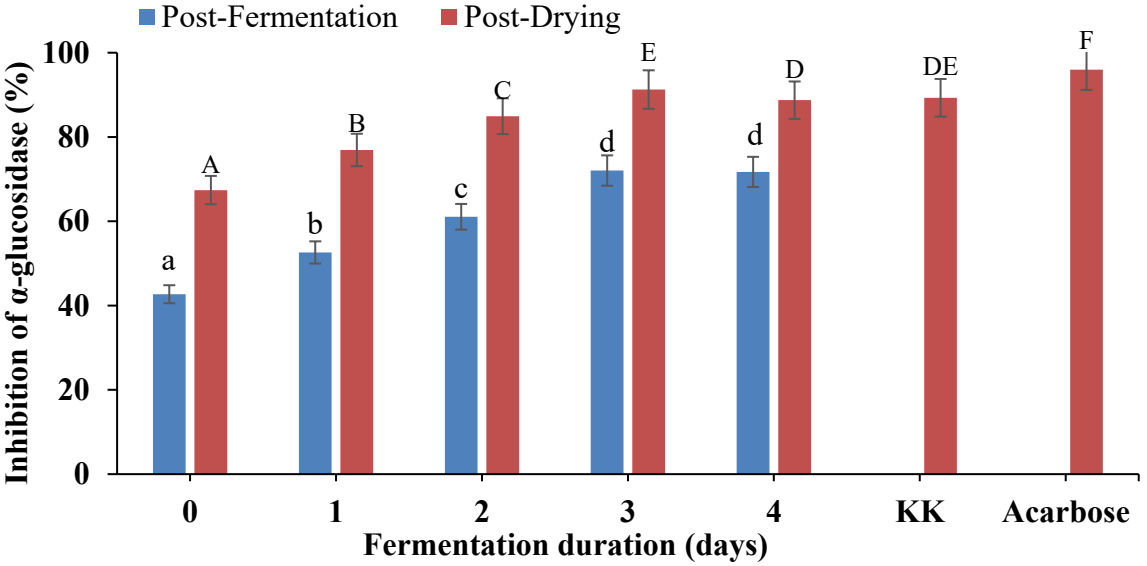
(b)

Figure 3. Effect of fermentation duration of *Sargassum hystrix* tea fermented by *Lactobacillus plantarum* on the phenolic (a) and phlorotannin content (b). Note: TF = Unfermented *S. hystrix* powder. KK = Commercial black tea. Different letter notations indicate a significant difference ($p < 0.05$).

3.4. Antidiabetic activity of fermented seaweed tea



(a)



(b)

Figure 4. Effect of fermentation duration of *Sargassum hystrix* tea fermented by *Lactobacillus plantarum* on inhibitory activity of α-amylase (a) and α-glucosidase (b). Note: KK = Commercial black tea. Different letter notations indicate a significant difference ($p < 0.05$).

The duration of fermentation of *S. hystrix* tea using *L. plantarum* had a significant effect on α-amylase and α-glucosidase inhibition activity ($p < 0.05$). In the α-amylase test (Figure 4a), the percentage of inhibition increased with longer fermentation duration. In post-fermentation samples, inhibition activity increased from 22.52% ± 0.50% on day 0 to 62.27% ± 0.50% on day 3, before decreasing slightly to 61.23% ± 1.27% on day 4. In post-drying samples, inhibition values were higher, increasing from 55.26% ± 1.02% (day 0) to a peak of 82.80% ± 1.70% on day 3, then decreasing slightly to 81.26% ± 0.75% on day 4. The commercial black tea (KK) showed an inhibition activity of 81.80% ± 1.10%, which was equivalent to the values on days 3 and 4 post-drying. Meanwhile, acarbose, as a standard drug, had the highest inhibition value, namely 90.27% ± 1.53%.

A consistent pattern was also observed in α-glucosidase inhibition activity (Figure 4b). In post-fermentation samples, inhibition activity increased from 42.69% ± 1.66% (day 0) to 72.04% ± 1.75% on day 3. It then decreased slightly on day 4 to 71.71% ± 1.43%. The peak activity indicates that the optimum fermentation point occurred on day 3. A similar result was found in the post-drying sample, where activity increased from 67.39% ± 1.10% (day 0) to a peak of 91.26% ± 0.44% (day 3), then decreased slightly on day 4 (88.74% ± 1.01%). This value is comparable to the commercial black tea (KK), which had an activity of 89.30% ± 0.35%. Acarbose, as a standard drug, showed the highest inhibition value of 95.97% ± 0.84%, but the difference with day 3 post-drying was relatively small, indicating that fermentation was able to increase α-glucosidase inhibition activity at the tested concentration (10,000 mg/L). However, further studies determining IC_{50} values are required for a more accurate comparison with pharmaceutical inhibitors.

This study shows a positive correlation between phenol, phlorotannin content, and antidiabetic activity, with day 3 showing the highest levels. These findings show that the increased α-amylase and α-glucosidase inhibitory activity are in line with the increased total phenol content in fermented *Saccharina japonica* and *Undaria pinnatifida* [34].

The hydrolysis of starch by α-amylase and the absorption of glucose by α-glucosidase cause an increase in blood sugar levels in people with type 2 diabetes. Inhibiting these two enzymes is one method of controlling diabetes. Phenolic compounds and phlorotannin in *S. hystrix* tea, which are enhanced through fermentation, produce increased inhibitory activity against α-amylase and α-glucosidase. Mechanistically, phenolic compounds work in several ways. Phenolic compounds can compete with substrates to bind to the active site of the enzyme, thereby blocking substrate access and reducing its catalytic activity. Some phenolic compounds that do not bind to the active site can interact with other parts of the enzyme through hydrogen bonding and π–π interactions. These π–π interactions are strengthened by the delocalization of electrons in double bonds (C=C or C–O) and aromatic rings in the phenolic structure, thereby increasing the stability of the bond between phenolic compounds and enzymes. These bonds can cause changes in enzyme conformation or interfere with the efficiency of the active site, even though the substrate remains available [35,36].

3.5. Consumer acceptance level of seaweed tea

The hedonic test was designed to measure the level of preference for a product [37]. The hedonic test in this study aimed to determine consumer acceptance of *S. hystrix* fermented tea products with different fermentation durations. Table 1 indicates that there is a significant effect of fermentation duration on the aroma, taste, and overall acceptability of *S. hystrix* tea. In contrast, no significant effect was observed for color attributes ($p > 0.05$). Table 1 also shows no significant differences ($p > 0.05$)

between the 3- and 4-day fermentation treatments for any of the evaluated hedonic parameters. The tea color became progressively lighter with increasing fermentation duration. This phenomenon may be associated with the activity *of L. plantarum*, which is known to influence color changes during seaweed fermentation [38]. The decrease in pH during fermentation can also promote the degradation of brown seaweed pigments such as fucoxanthin, resulting in a brighter appearance [39].

Table 1. Effect of fermentation duration of *Sargassum hystrix* tea fermented by *Lactobacillus plantarum* on consumer acceptance level.

Parameters	Fermentation duration (days)				
	0	1	2	3	4
Color	3.41 ± 1.79^{a}	3.43 ± 1.18^{a}	3.41 ± 0.92^{a}	3.64 ± 1.25a	3.69 ± 1.51^{a}
Aroma	1.69 ± 1.03^{a}	2.41 ± 1.07^{b}	2.91 ± 1.02^{c}	4.05 ± 1.25^{d}	3.89 ± 1.24^{d}
Taste	2.26 ± 1.39^{a}	2.64 ± 1.11^{b}	3.14 ± 1.05^{c}	4.14 ± 1.23^{d}	4.08 ± 1.17^{d}
Overall	2.18 ± 1.19^{a}	2.79 ± 1.03^{b}	3.24 ± 0.93^{c}	4.29 ± 1.06^{d}	4.18 ± 1.10^{d}

Note: Different superscript letters in the same line indicate a significant difference ($p < 0.05$).

The Mann–Whitney test results for the aroma parameter showed a significant effect ($p < 0.05$) between each treatment except for the 3- and 4-day fermentation treatments ($p > 0.05$) (Table 1). The highest value was obtained in the 3-day fermentation, while the lowest value was obtained in the 0-day fermentation. Tea with 0-day fermentation had a strong fishy aroma from *S. hystrix* seaweed, which was less preferred by the panelists. The intensity of the fishy aroma decreased as the fermentation duration increased. This fishy aroma is generally caused by the presence of amine compounds in seaweed [40]. The reduction in fishy aroma in tea is due to the formation of volatiles during fermentation through carbohydrate and lipid metabolism, polysaccharide degradation, proteolysis, and fatty acid breakdown [41]. This process produces volatile compounds such as esters and alcohols, as well as lactic acid and acetic acid, resulting in a more acceptable aroma [41]. This is reinforced by similar research [42], which reported a decrease in the intensity of the fishy aroma in red seaweed after fermentation.

The different fermentation durations also had a significant effect ($p < 0.05$) on tea taste (Table 1). Each treatment interaction had a significant effect ($p < 0.05$) except for the 3- and 4-day fermentation treatments ($p > 0.05$). The highest value for the taste parameter was obtained by the 3-day fermentation. Meanwhile, the lowest hedonic taste value was in the 0-day fermentation. The fishy taste decreased as the fermentation duration increased, accompanied by the emergence of a faint sour taste. This sour taste was caused by the formation of volatile compounds from the lactic acid fermentation process, such as esters, alcohols, and organic acids [41]. The panelists preferred this sour fermentation taste to the fishy taste of seaweed.

The overall parameter contains all tested attributes, including color, aroma, and taste. Different fermentation durations had a significant effect ($p < 0.05$) on the overall hedonic value (Table 1). The highest overall hedonic value was found in tea with a fermentation period of 3 days, while the lowest value was found in the 0-day fermentation. Although the highest overall hedonic value in the 3-day fermentation treatment was not significantly different from the 4-day fermentation treatment ($p > 0.05$), the 3-day fermentation was selected as the optimal condition because it achieved the highest functional characteristics (phenol content, phlorotannin, and enzyme inhibition activity) while maintaining comparable sensory acceptance to the 4-day treatment. This approach balances both functional and sensory optimization while minimizing unnecessary fermentation time and potential resource consumption.

4. Conclusions

This study demonstrates that solid-state fermentation of brown seaweed *S. hystrix* using *L. plantarum* significantly enhances its in vitro antidiabetic potential and consumer acceptance. During the fermentation process, pH gradually decreased while total titratable acidity increased, indicating successful microbial metabolism. The optimal results were achieved on the third day of fermentation, which yielded the highest functional characteristics, including total phenolic content (12.94 ± 0.68 mg GAE/g), total phlorotannin (14.60 ± 0.95 mg PGE/g), and inhibitory activities against α-amylase (82.80% ± 1.70%) and α-glucosidase (91.26% ± 0.44%). Although statistical analysis showed no significant difference in overall sensory acceptance between the 3- and 4-day fermentation treatments, the 3-day duration is recommended as the optimal condition. This selection is based on its superior functional profile and greater practical efficiency, as it minimizes unnecessary fermentation time and resource consumption while achieving maximum bioactive compound release.

These findings suggest that lactic acid fermentation is an effective processing strategy to improve both the bioactivity and sensory profile of seaweed-based products, effectively reducing the characteristic fishy odor. The implications of this study highlight the potential of fermented *S. hystrix* tea as a natural, functional beverage that could assist in managing blood glucose levels, offering a promising dietary complement to conventional diabetes management. However, the interpretation of these results must consider several limitations. The enzyme inhibition assays were conducted at a single concentration (10,000 mg/L) without determining the half-maximal inhibitory concentration (IC_{50}), which limits direct potency comparisons with pharmaceutical inhibitors like acarbose. Furthermore, the current evidence is restricted to in vitro biochemical assays and preliminary sensory evaluations. Future in vivo studies, bioavailability analyses, and clinical trials are essential to confirm the physiological antidiabetic efficacy, determine appropriate dosages, and establish the safety profile of the fermented tea in human subjects.

Use of AI tools declaration

The authors acknowledge the use of Manus for language editing and sentence rephrasing in the preparation of this manuscript. All AI-generated content has been carefully reviewed, edited, and validated to ensure its accuracy and originality. Full responsibility for the final content of the manuscript remains with the authors.

Acknowledgments

We would like to thank the Directorate of Research and Community Services, Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, for funding this research through the 2025 Regular Fundamental Research program [2415/ UN1/DITLIT/Dit-Lit/PT.01.03/2025]. This article is part of the first author's thesis.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Khalishah Jasmine Putri Abdillah: Conceptualization, methodology design, data curation, investigation, formal analysis, validation, writing the original draft, and editing. Amir Husni: Conceptualization, methodology design, supervision, validation, writing the original draft, review, and editing. Masagus Muhammad Prima Putra: Conceptualization, methodology design, supervision, validation, review, and editing. Siti Ari Budhiyanti: Supervision, investigation, validation, review, and editing.

References

1. Mustofa EE, Purwono J, Ludiana L (2021) Penerapan senam kaki terhadap kadar glukosa darah pada pasien diabetes melitus di wilayah kerja Puskesmas Purwosari Kecamatan Metro Utara. *J Cendikia Muda* 2: 78–86. https://doi.org/10.30787/asjn.v2i1.831
2. International Diabetes Federation, IDF Diabetes Atlas 10th edition (2021) Available from: https://diabetesatlas.org/.
3. Paredes SM, de Abia NA (2008) Antidiabéticos orales. Actualización [Oral anti-diabetes medicines, an update]. *Rev Enferm* 31: 7–10.
4. Shahzad A, Liu W, Hussain S, et al. (2025) Integrated in vitro, in silico, and in vivo approaches to elucidate the antidiabetic mechanisms of *Cicer arietinum* and *Hordeum vulgare* extract and secondary metabolites. *Sci Rep* 15: 6620. https://doi.org/10.1038/s41598-025-89642-5
5. Panamuan A, Untari EK, Rizkifan S (2021) Pengaruh usia pasien dan dosis terhadap efek samping metformin pada pasien diabetes tipe 2. *J Farmasi Komunitas* 8: 51–58. https://doi.org/10.20473/jfk.v8i2.27117
6. Lee SH, Jeon YJ (2013) Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. *Fitoterapia* 86: 129–136. https://doi.org/10.1016/j.fitote.2013.02.013
7. Azizi W, Ekantari N, Husni A (2019) Inhibitory activity of *Sargassum hystrix* extract and its methanolic fractions on inhibiting α-glucosidase activity. *Indones J Pharm* 30: 35–42. https://doi.org/10.14499/indonesianjpharm30iss1pp36
8. Dangal A, Rai K, Timsina P, et al. (2026) Algae and seaweeds: Oceanic treasures for umami and salinity. In: Sarkar, T, Smaoui S (Eds.), *Dietary, Sensory and Gastronomic Applications,* New York: Academic Press, 271–291. https://doi.org/10.1016/B978-0-443-33345-3.00019-5
9. Elidottir AS, Sveinsdottir K, Ingadottir B, et al. (2021) Seaweed extract improves carbohydrate metabolism in overweight and obese adults. *Curr Nutr Food Sci* 17: 216–224. https://doi.org/10.2174/1573401316999200706012619
10. Giuffrè D, Giuffrè AM (2024) Fermentation technology and functional foods. *Front Biosci* 16: 8. https://doi.org/10.31083/j.fbe1601008
11. Husni A, Putra DR, Lelana IYB (2014) Aktivitas antioksidan *Padina* sp. pada berbagai suhu dan lama pengeringan. *J Pascapanen Bioteknol Kelaut Perikan* 9: 165–173. https://doi.org/10.15578/jpbkp.v9i2.109
12. Afgatiani PM, Husni A, Budhiyanti SA (2020) Aktivitas antioksidan bubuk *Sargassum hystrix* selama penyimpanan pada suhu berbeda. *Agritech* 40: 175–181. https://doi.org/10.22146/agritech.18134
13. Setiyawan A, Husni A (2022) Effect of cinnamon powder substitution on antioxidant activity, antidiabetic, and sensory properties of *Sargassum hystrix* tea. *Food Res* 6: 159–165. https://doi.org/10.26656/fr.2017.6(2).226

14. Hur SJ, Lee SY, Kim YC, et al. (2014) Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem* 160: 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112
15. Zhou L, Zhao L, Su Y, et al. (2019) In vitro hypoglycemic potential of dietary fiber from fermented seaweed. *IOP Conf Ser: Mater Sci Eng* 612: 022072. https://doi.org/10.1088/1757-899X/612/2/022072
16. Zhu W, Jiang B, Zhong F, et al. (2021) Effect of microbial fermentation on the fishy-odor compounds in kelp (*Laminaria japonica*). *Foods* 10: 25–32. https://doi.org/10.3390/foods10112532
17. Cheng Y, Xue F, Yang Y (2023) Hot water extraction of antioxidants from tea leaves: Optimization of brewing conditions for preparing antioxidant-rich tea drinks. *Molecules* 28: 3030. https://doi.org/10.3390/molecules28073030
18. Meliala D (2020) Penetapan kadar kafein pada bubuk teh hitam yang beredar di Pasar Deli Tua menggunakan spektrofotometri UV. *J Penelitian Farm Herb* 3: 21–28. https://doi.org/10.36656/jpfh.v3i1.313
19. Lein MA, Desniar, Hardiningtyas SD (2025) Fermentasi daun mangrove *Rhizophora mucronata* sebagai teh herbal. *JPHPI* 28: 170–186. https://doi.org/10.17844/jphpi.v28i2.61684
20. Admantin CY, Turner M, Lo R, et al. (2023) Fermentation of green seaweed *Ulva fasciata* using six different strain of lactic acid bacteria (LAB). *Proceedings of the 3rd International Conference on Smart and Innovative Agriculture (ICoSIA 2022),* 611–624. https://doi.org/10.2991/978-94-6463-122-7_58
21. Ulandari D, Nocianitri KA, Arihantana NM (2019) Pengaruh suhu pengeringan terhadap kandungan komponen bioaktif dan karakteristik sensoris teh white peony. *ITEPA* 8: 36–47. https://doi.org/10.24843/itepa.2019.v08.i01.p05
22. Sinurat E, Suryaningrum TD (2019) The effect of blanching time on antioxidant activity and sensory characteristic of brown seaweed *Sargassum* sp. tea. *JPHPI* 22: 581–588. https://doi.org/10.17844/jphpi.v22i3.29228
23. Koivikko R, Loponen J, Honkanen T, et al. (2005) Content of soluble cell wall-bound and exuded phlorotannins in the brown alga *Fucus Vesicolosus*, with implications on their ecological functions. *J Chem Ecol* 31: 195–212. https://doi.org/10.1007/s10886-005-0984-2
24. Gazali M, Jolanda O, Husni A, et al. (2023). In vitro α-Amylase and α-Glucosidase inhibitory activity of green seaweed *Halimeda tuna* extract from the Coast of Lhok Bubon, Aceh. *Plants* 12: 393. https://doi.org/10.3390/plants12020393
25. Pratiwi DR, Husni A (2021) The impact of immersion time in lime solution on antioxidant and antidiabetic properties and consumer evaluation of *Sargassum hystrix* seaweed tea. *AACL Bioflux* 14: 2354–2366.
26. Dalié DKD, Deschamps AM, Richard F (2010) Lactic acid bacteria potential for control of *mould* growth and mycotoxins: A review. *Food Cont* 21: 370–380. https://doi.org/10.1016/j.foodcont.2009.07.011
27. Zhang J, Bing W, Hu T, et al. (2023) Enhanced biodegradation of phenol by microbial collaboration: Resistance, metabolite utilization, and pH stabilization. *Environm Res* 238: 117269. https://doi.org/10.1016/j.envres.2023.117269
28. Lestari S, Husni A, Nurjanah N, et al. (2025) Chemical characteristic, antioxidant activity, and consumer acceptance level of kombucha from *Sargassum cristaefolium* seaweed tea. *J Multidiscipl Appl Nat Sci* 5: 949–968. https://doi.org/10.47352/jmans.2774-3047.293
29. Sonia NMO, Kusnadi J (2015) Isolasi dan karakterisasi parsial enzim selulase dari isolat bakteri OS-16 asal Padang Pasir Tengger-Bromo. *J Pangan Agroind* 3: 11–19.

30. Silitonga P, Setyati W, Susanto A, et al. (2022) Pengaruh fermentasi *Gracilaria verrucosa* dengan penambahan starter *Lactobacillus plantarum* pada profil metabolit dan aktivitas biologisnya. *J Mar Res* 11: 309–319. https://doi.org/10.14710/jmr.v11i2.33262
31. Pswarayi F, Gänzle MG (2019) Composition and origin of the fermentation microbiota of mahewu, a Zimbabwean fermented cereal beverage. *Appl Environm Microbiol* 85: 11. https://doi.org/10.1128/AEM.03130-18
32. Zhang X, Zhang C, Gong M, et al. (2025) Dynamics of lactic acid bacteria dominance in sour bamboo shoot fermentation: Roles of interspecies interactions and organic acid stress. *Foods* 14: 3481. https://doi.org/10.3390/foods14203481
33. Sharma S, Kataria A, Singh B (2022) Effect of thermal processing on the bioactive compounds, antioxidative, antinutritional and functional characteristics of quinoa (*Chenopodium quinoa*). *LWT* 160: 113256. https://doi.org/10.1016/j.lwt.2022.113256
34. Suraiya S, Lee M, Cho J, et al. (2018) *Monascus* spp. fermented brown seaweeds extracts enhance bio-functional activities. *Food Biosci* 21: 90–99. https://doi.org/10.1016/j.fbio.2017.12.005
35. Lee SH, Jeon J (2013) Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. *Fitoterapia* 86: 129–136. https://doi.org/10.1016/j.fitote.2013.02.013
36. de Paulo Farias D, de Araújo F, Neri-Numa A, et al. (2021) Antidiabetic potential of dietary polyphenols: A mechanistic review. *Food Res Intern* 145: 110383. https://doi.org/10.1016/j.foodres.2021.110383
37. Mareta DT (2019) Hedonic test method for measuring instant pindang seasoning powder preferences. *J Sci Applic Technol* 3: 34–36. https://doi.org/10.35472/jsat.v3i1.195
38. Singh S (2023) Optimization of solid-state fermentation process for brown seaweed using LAB and characterization of the products. Lunds Universitet. Master's Thesis Report.
39. Zhao D, Yu D, Kim M, et al. (2019) Effects of temperature, light, and pH on the stability of fucoxanthin in an oil-in-water emulsion. *Food Chem* 291: 87–93. https://doi.org/10.1016/j.foodchem.2019.04.002
40. Ramasari DW, Evi L, Atikah N, et al. (2019) Nori preference level based on the condition of the raw material *Eucheuma cottonii* seaweed. *Asian Food Sci J* 11: 1–10. https://doi.org/10.9734/afsj/2019/v11i430070
41. Ji J, Jiang X, Song P, et al. (2025) Multi-omics insights into microbial interactions and fermented food quality. *Microorganisms* 13: 2679. https://doi.org/10.3390/microorganisms13122679
42. Du P, Li D, Wang A, et al. (2021) A systematic review and meta-analysis of risk factors associated with severity and death in COVID-19 patients. *Can J Infect Dis Med Microbiol* 10: 6660930. https://doi.org/10.1155/2021/6660930

AIMS Press