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IMPACT OF INITIAL DRYING TECHNIQUES AND FERMENTATION DURATION ON THE MICROBIOLOGICAL AND CHEMICAL PROPERTIES OF FERMENTED *Sargassum* sp.

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Abstract

Sargassum sp. is seaweed as source of phenolic compounds with antioxidant properties and has potential as a raw material for herbal tea production. This study aimed to determine the effect of drying methods and fermentation duration on the microbiological and chemical characteristics of Sargassum sp. during fermentation, and to assess changes in total phenol and tannin contents after fermentation. The study employed a completely randomized factorial design with two factors: drying method (sun-dried, air-dried, and oven-dried) and fermentation duration (0, 1, 2, 3, and 4 days), each with three replications. Data were analysed using factorial ANOVA followed by Duncan's multiple range test. The results showed that the sun-drying method produced the best raw material characteristics, with a moisture content of 7.38%, total phenol content of 32.15 mg GAE/g, total tannin of 0.27%, and pH of 4.90. During fermentation, the air-dried treatment for two days yielded higher values of pH, total titratable acidity (TTA), total lactic acid bacteria (LAB), total phenol, and total tannin compared to the sun-dried and oven-dried treatments (p<0.05), with values of 4.75, 0.32%, 7.6 $\log_{10}$ CFU/g, 39.16 mg GAE/g, and 0.12%, respectively. These findings indicate that air-drying is a promising method for processing Sargassum sp. herbal tea as a functional beverage with high bioactive potential.

Keywords: bioactive compounds, herbal drink, lactic acid bacteria, phenol, tannin

Pengaruh Metode Pengeringan Awal dan Lama Fermentasi terhadap Karakteristik Mikrobiologis dan Kimia Rumput Laut *Sargassum* sp. Terfermentasi

Abstrak

Rumput laut *Sargassum* sp. merupakan sumber senyawa fenolik yang berperan sebagai antioksidan dan berpotensi dikembangkan sebagai bahan baku teh herbal. Penelitian bertujuan menentukan pengaruh metode pengeringan terhadap karakteristik bahan baku, menentukan pengaruh metode pengeringan dan lama fermentasi terhadap karakteristik mikrobiologis dan kimiawi Sargassum sp. selama fermentasi dan menentukan pengaruh total fenol dan tanin setelah fermentasi. Penelitian menggunakan rancangan acak lengkap faktorial dengan dua faktor, yaitu metode pengeringan (kering matahari, kering angin, dan kering oven) serta lama fermentasi (0, 1, 2, 3, dan 4 hari), dengan tiga kali ulangan. Hasil penelitian menunjukkan bahwa metode kering matahari memberikan karakteristik bahan baku terbaik dengan kadar air 7,38%, total fenol 32,15 mg GAE/g, total tanin 0,27%, dan pH 4,90. Selama fermentasi, perlakuan kering angin selama dua hari menghasilkan nilai pH, total asam tertitrasi (TAT), total bakteri asam laktat (BAL), total fenol, dan total tanin yang lebih tinggi dibandingkan metode kering matahari dan kering oven (p<0,05), masing-masing sebesar 4,75; 0,32%; 7,6 $\log_{10}$ CFU/g; 39,16 mg GAE/g; dan 0,12%. Hasil ini menunjukkan

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bahwa metode pengeringan angin berpotensi diterapkan dalam proses pengolahan teh herbal Sargassum sp. sebagai minuman fungsional dengan aktivitas bioaktif yang tinggi.

Kata kunci: asam laktat, fenol, minuman herbal, senyawa bioaktif, tanin

INTRODUCTION

Sargassum sp. is a brown seaweed that is widely used as a raw material for alginate production. This seaweed contains various bioactive compounds, including fucoxanthin (Gazali *et al.,* 2022; Susanto *et al.,* 2016), phlorotannins (Barbosa *et al.,* 2021), polyphenols (Rushdi *et al.,* 2020), and flavonoids (Mulyadi *et al.,* 2019; Neoh *et al.,* 2021). Phenolic compounds in seaweed act as antioxidants by neutralizing the free radicals. The total phenol content in *Sargassum* sp. has been reported to be 149.04 mg GAE/g (Dharmawan *et al.,* 2023). Excessive free radicals in the body can cause oxidative stress, which triggers various degenerative diseases (Sanjeewa *et al.,* 2016, 2018). The antioxidant activity of *Sargassum* sp. 2,2-difenil-1-pikrilhidrazil (DPPH) method with methanol extract yielded an IC_{50} value of 10,93 mg/L (Henri *et al.,* 2023). The use of seaweed in Indonesia is growing through the innovation of functional food and beverage products, including its use as a raw material for herbal tea.

Tea is generally prepared using the leaves of Camellia sinensis. Along with the development of functional food innovations, various other natural ingredients have been used as raw materials for herbal tea, including butterfly pea (*Clitoria ternatea*) (Putri & Baharza, 2022; Ansori *et al.,* 2023), cogon grass (*Imperata cylindrica*) (Dhyanaputri *et al.,* 2022; Afriannisa *et al*., 2025), and God's crown (*Phaleria macrocarpa*) (Mubarok *et al*., 2022; Hartisyah *et al*., 2024). Herbal teas contain various bioactive compounds, including alkaloids (Jeszka-Skowron *et al.,* 2019) and phenolic compounds (Alexander *et al.,* 2019). This product is also a natural source of antioxidants and can be used as an alternative to fruits and vegetables. The use of *Sargassum* sp. as a raw material for herbal tea has been reported in several studies (Kartikaningsih *et al.,* 2019; Sinurat & Suryaningrum, 2019; Larasati & Husni, 2021; Silva *et al.,* 2022). However, the processing of seaweed-based herbal tea still faces obstacles, namely the high moisture content in seaweed (±90% wet weight), which makes it easily damaged and quickly degraded after harvest (Wang *et al.,* 2011). One of the commonly used solutions is drying.

Drying is an essential method for reducing water activity to inhibit microbial growth, maintain the quality of raw materials, and minimize the storage volume (Gupta *et al.,* 2011). An improper drying process can reduce the quality of raw materials and decrease the bioactive compound content. The extent of degradation of nutrients and functional compounds largely depends on the drying method and conditions used (Akdaş & Başlar, 2015). Various drying methods can be applied, including sun, air, and oven drying. Sun drying is practical and economical, although quality control is limited (Paga *et al.,* 2022). Air drying is reported to be optimal for maintaining phenol content (Masduqi *et al.,* 2014). Oven drying at 35–40 °C can increase the total phenol levels (Gupta *et al.,* 2011). Therefore, applying the proper drying method is vital for processing *Sargassum* sp. as an herbal tea, as it has the added benefit of extending shelf life and determining the content of functional compounds that act as natural antioxidants. In addition, the manufacture of herbal tea made from seaweed also has another obstacle, namely the fishy aroma typical of seaweed, which is not liked by consumers (Sinurat & Suryaningrum, 2019). Fermentation is one approach that can be used to reduce undesirable odors while improving the sensory characteristics of the material.

Fermentation of S*argassum* sp. can reduce the fishy aroma while improving the quality of the health drinks. Fermentation is crucial for enhancing the taste, organoleptic profile, and bioactive compounds of products, making them more appealing to consumers (Song *et al.,* 2021; Healy *et al.,* 2023). This process also has the potential to increase the

bioactivity of biomass and is considered a promising treatment approach, although it is still rarely applied to seaweed-based products (Reboleira *et al.,* 2021). Fermented seaweed will change its sensory properties to a milder flavor and reduce the iodine content (Bruhn *et al.,* 2019). Fermentation generally uses lactic acid bacteria (LAB), which can easily break down complex compounds into simpler forms that can be easily absorbed by the body (Rianingsih & Sumardianto, 2020). LAB is practical for fermenting brown seaweed because it can utilize carbohydrates such as mannitol and oligosaccharides (Allahgholi *et al.,* 2023). One potential strain is *Lactobacillus plantarum* SK (5), which can be used as a starter culture.

Lactobacillus plantarum SK (5) is a lactic acid bacterium isolated from shellfish in Kayu Agung, South Sumatra. This strain exhibits antibacterial activity against several pathogen-indicator bacteria, including *Escherichia coli, Salmonella typhimurium* ATCC 14028, *Bacillus cereus, Staphylococcus aureus*, and *Listeria monocytogenes* (Desniar *et al.,* 2013). *Lactobacillus plantarum* SK (5) has potential as a probiotic because it is tolerant to acid and bile salts and produces antimicrobial compounds (Syafiqoh, 2016). This strain is also known to produce proteolytic enzymes (Anggrahini, 2016). Its applications have been further developed, including the hydrolysis of seaweed polysaccharides from *Caulerpa racemosa*(Sudibyo *et al.,* 2024) and the fermentation of mangrove leaves (*Rhizophora mucronata*) for herbal tea production (Lein *et al.,* 2025). The quality of *Sargassum* sp. herbal tea is strongly influenced by its processing methods. The drying process determines the extent to which bioactive compounds are retained, whereas the fermentation duration can affect the taste and aroma and enhance bioactive compounds with potential biological activity. Differences in drying methods and fermentation duration are likely to produce varying results in herbal tea quality. However, research on this topic remains limited. This study aimed to determine the effect of drying methods on the characteristics of the raw material, evaluate the influence of drying methods and fermentation duration on the microbiological and chemical characteristics of *Sargassum* sp. during fermentation, and assess the changes in total phenol and tannin contents after fermentation.

MATERIALS AND METHODS

Preparation of Raw Materials

Sargassum sp. seaweed was collected from the waters of Lhok Bubon, West Aceh. The seaweed was soaked for 6 h (in two repetitions), rinsed with running water, and weighed 10,000 g for each treatment. The samples were dried using three different methods: (1) sun drying (SD) from 08:00 to 17:00 WIB (Western Indonesian Time) at a temperature of approximately 30 °C for 4 days; (2) air drying (AD) from 08:00 to 17:00 WIB at approximately 24 °C for 8 days; and (3) oven drying (OD) at 40 °C for 48 h (Larasati & Husni, 2021) with modifications. The dried samples were analyzed for moisture content (AOAC, 2012), total phenol, total tannin, and pH using a pH meter.

Starter Culture Preparation

The starter culture used was *Lactobacillus plantarum* SK (5), isolated from *bekasam* of *seluang* fish originating from Kayu Agung, South Sumatra, and obtained from the Microbiology Laboratory, Department of Fisheries Product Technology, IPB University. A loopful of *L. plantarum* SK (5) from the glycerol stock culture was inoculated onto de Man Rogosa and Sharpe Agar (MRS-Agar) (Oxoid, United Kingdom) slants and incubated under semi-anaerobic conditions using a Thermocline type 4200 incubator at 37 °C for 48 h (Desniar *et al.,* 2023). Cultures grown on MRS-Agar were verified through Gram staining, motility testing, catalase testing, and glucose fermentation tests.

The starter inoculum was prepared by transferring one loopful of culture from MRS-Agar to MRS-Broth (*de Man Rogosa and Sharpe Broth*) (Oxoid, United Kingdom), followed by incubation at 37 °C for 24 h under semi-anaerobic conditions. The inoculum was then analyzed for total lactic acid bacteria (LAB) count using the pour plate method (Total Plate Count) (Badan Standardisasi Nasional [BSN] 2009).

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Fermentation of Seaweed *Sargassum* sp.

The dried *Sargassum* sp. from the three drying methods was blended into pieces of approximately ±0.5–1 cm in size. A total of 10 g of *Sargassum* sp. was mixed with approximately ±30 mL of water in a 100 mL Schott bottle. The samples were sterilized using an autoclave (Yamato SM 52, Japan) at 121 °C for 15 min. After sterilization, each sample was inoculated with 1,5 mL of *L. plantarum* SK (5). Fermentation was performed at 37 °C for 4 days (Healy *et al.,* 2023, modified). Observations were conducted on days 0, 1, 2, 3, and 4 of fermentation, with parameters including total lactic acid bacteria (LAB) count, total titratable acidity (TTA), and pH.

The fermented samples were dried using a dehydrator at 50 °C for 5 h, followed by brewing. One gram of dried fermented seaweed was placed into an empty tea bag and infused with 100 mL of hot water (80 °C) for 6 min (Sinurat & Suryaningrum, 2019). The resulting tea solution was analyzed to determine the total phenol and tannin contents.

Total Titratable Acidity

Total titratable acidity (TAT) measurement refers to BSN (2009). A 5 g seaweed fermentation sample was weighed, and 45 mL of aquades was added and homogenized using a homogenizer. The homogeneous sample was then placed in a volumetric flask (Iwaki, Pyrex) and diluted with aquades to a 50 mL mark. The sample solution was filtered using filter paper, and 5 mL of the filtrate was transferred into an Erlenmeyer flask (Iwaki, Pyrex). Then, 1–2 drops of 1% phenolphthalein (PP) (Merck) indicator were added, and the sample was titrated with NaOH 0.1 N (Merck) until the color changed to pink.

Total Lactic Acid Bacteria (LAB)

The total LAB count was determined according to BSN (2015). Fermented seaweed (10 g) was weighed, ground in a mortar, and diluted in 90 mL of sterile physiological solution to obtain a 10^{-1} dilution. Serial dilutions were prepared up to 10^{-7}. The last three dilution levels were plated (in single replicates) on MRS Agar medium supplemented with 0.5% sterile $CaCO_3$ (Merck). The plates were incubated at 37°C for 48 h under microaerophilic conditions. The colonies were counted using a colony counter.

Total Phenolic Content

The total phenolic content was determined according to Ramamoorthy and Bono (2007). One gram of the fermented seaweed sample was dissolved in 100 mL of hot demineralized water (80°C). The diluted solution was 1 mL, then 0.5 mL of 96% ethanol (Merck), 2.5 mL of aquadest, and 0.5 mL of 50% Folin-Ciocalteau (Merck) reagent. The mixture was allowed to sit for 5 min, and then 2 mL of 5% Na_2CO_3 (Merck) was added. The mixture was homogenized and incubated in the dark conditions for one hour. Standard gallic acid (Merck, Germany) was used at concentrations of 0, 5, 10, 15, 20, 30, and 40 ppm. The results are expressed in milligrams of gallic acid equivalent per gram (mg GAE/g). The absorbance of the standard solution and sample was measured using a UV-Vis spectrophotometer at a wavelength of 725 nm.

Total Tannin Content

The total tannin test was performed as described by Maulida *et al.* (2020). One gram of the fermented seaweed samples was dissolved in 100 mL of hot demineralized water at 80 °C. Next, 50 mL of the sample solution was placed into a 100 mL measuring flask, and 2.5 mL of indigocarmine (Merck) and water were added until it reached the impression mark. The solution was titrated with $KMnO_4$ (Merck) until golden yellow. The same procedure was used to prepare the blank solution without adding the samples. It is known that 1 mL of $KMnO_4$ 0.1 N is equivalent to 0.004157 g of tannins.

Data Analysis

This study consisted of two stages. The first stage used a completely randomized design (CRD) to test the characteristics of raw materials with different drying method treatments (sun-dry, air-dry, and oven-dry),

each carried out three times. The second stage used a completely randomized factorial design (CRFD) with two factors. The first factor was the drying method, which consisted of three stages, and the second factor was the fermentation time, which consisted of four stages (0, 1, 2, 3, and 4 days). Each treatment was performed in triplicate. Observational data were first tested for normality and homogeneity, and then analyzed using one-way analysis of variance (ANOVA) at a confidence level of 95% (α=0.05). If there was a significant effect ($p< 0.05$), a follow-up test was performed using Duncan's Multiple Range Test (DMRT) to determine the difference between treatments. All data processing was performed using Microsoft Excel 2021 and SPSS version 25.0.

RESULTS AND DISCUSSION

Characteristics of Lactic Acid Bacterial Starter

The study utilized a single-strain lactic acid bacteria starter, *L. plantarum* SK (5), with a cell count of 9.07 log CFU/mL (1.1×10^9 CFU/mL). The number of starter cells in this study met the required standard, aligning with the Indonesian National Standard (SNI), which stipulates that probiotic bacteria must have cell counts exceeding 10^7 CFU/mL to exert health benefits (BSN, 2009).

Characteristics of Seaweed Raw Materials *Sargassum* sp.

The characteristics of *Sargassum* sp. as a herbal tea raw material, including moisture content, total phenols, total tannins, and pH, are presented in Table 1. Based on the ANOVA test, differences in drying methods during the extraction of *Sargassum* sp. showed a significant effect ($p<0.05$) on the moisture content, total phenol, and pH of the raw material, but no significant effect ($p>0.05$) on total tannin. The lowest moisture content was obtained from seaweed dried using the sun-drying method (7.38%), compared to the air-drying and oven-drying methods. The moisture content obtained in this study was lower than that reported by Gazali *et al.* (2018), which was 10.54%. A lower moisture level reflects better material quality (Tamaheang *et al.,* 2017). In addition, dried Sargassum sp. simplicia can have a longer shelf life if its moisture content is below 10% (Gazali *et al.,* 2018). All three drying methods produced moisture content values that complied with the SNI 2690:2018 standard, which sets a maximum limit of 18%.

The highest total phenol content was obtained from air drying, reaching 33.97 mg GAE/g, compared to oven drying at 31.24 mg GAE/g. This finding is consistent with that of Dhakal *et al.* (2024), who reported that high temperatures can significantly reduce phenolic content. Sedjati *et al.* (2017) reported that the total phenol content in seaweed dried by air and extracted with infused hot water was 0.95 mg GAE/g. Masduqi *et al.* (2014) reported total phenolic contents obtained through sun-drying, air-drying, and oven-drying methods, with respective values of 1,179.7, 1,656.3, and 1,274.4 ppm. The extraction of phenolic compounds from plants or fruits is strongly influenced by the polarity of the solvent and solubility of the extracted compounds. Highly polar solvents are more effective in dissolving phenolic compounds in polar media (Dip *et al.,* 2024). Water-based solvents can extract more phenolic compounds than alcohol-based solvents can (Sedjati *et al.,* 2017).

The results also showed that variations in the drying methods did not significantly affect the total tannin content. The total tannin values obtained in this study were lower than

Table 1 Characteristics of dried seaweed raw materials

Drying method	Moisture (%)	Total phenolic (mg GAE/g)	Total tannin (% db)	pH
Sun-dried	7.38 ± 0.38^{a}	32.15 ± 0.8^{ab}	0.27 ± 0.02^{a}	4.90 ± 0.02^{a}
Air-dried	14.44 ± 0.22^{b}	33.97 ± 1.0^{b}	0.29 ± 0.00^{a}	4.91 ± 0.02^{a}
Oven-dried	9.82 ± 0.09^{c}	31.24 ± 1.0^{a}	0.29 ± 0.00^{a}	5.26 ± 0.01^{b}

Significant differences ($p<0.05$) within the same column are indicated by different superscripts ($^{a, b}$)

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those reported by Paga *et al.* (2021), which were 0.77% for oven-dried drying and 0.89% for sun-dried samples. The pH values of the dried seaweed differed across the various drying methods. Sun- and air-dried samples showed pH values of 4.90 and 4.91, respectively, whereas oven drying produced a higher pH of 5.26. These findings indicate that, regardless of the drying technique applied, the dried seaweed remained within an acidic pH range. Kumesan *et al.* (2017) similarly observed variations in pH resulting from two different drying approaches, namely sun drying and cabinet drying, which yielded pH values of 5.58 and 4.93, respectively. The increase in pH may be associated with the microbial production of nitrogenous compounds and the formation of peptides and amines derived from protein degradation during the drying process (Delbarre-Ladrat *et al.,* 2006).

Characteristic of Herbal Tea during Fermentation

Total lactic acid bacteria

Lactic acid bacteria are natural microorganisms commonly used as fermentation agents in the food industry. The verification results showed that *L. plantarum* SK (5) belongs to the homofermentative group of bacteria. The total lactic acid bacteria (LAB) count of fermented *Sargassum* sp. is presented in Figure 1.

The analysis of variance, the drying method, fermentation duration, and their interaction had a significant effect ($p< 0.05$) on the total count of lactic acid bacteria. During fermentation, the total LAB in the sun-drying and air-drying treatments remained relatively stable until day 2, with counts of 7.7 and 7.6 $\log_{10}$ CFU/g, respectively. This suggests that these two drying methods can maintain conditions that support microbial activity throughout fermentation. In contrast, the oven-drying treatment decreased the total LAB count to 6.8 $\log_{10}$ CFU/g on the same day, which was significantly different ($p<0.05$) from the other two methods. High temperatures during oven drying may degrade bioactive compounds that play an important role in support the growth of lactic acid bacteria during fermentation.

The significant interaction ($p<0.05$) between the drying method and fermentation duration indicates that the pattern of LAB growth over time was influenced by the drying method used. Accordingly, natural drying at lower temperatures tends to maintain LAB growth during fermentation more effectively. Lein *et al.* (2025) stated that simple compounds' environmental conditions and nutrient availability determine bacterial growth phases. These findings contrast with those of Ambarsari *et al.* (2018), who reported that during the fermentation of dried *Ulva lactuca* seaweed, LAB counts increased over fermentation time, from 8.31 to 9.10 $\log_{10}$ CFU/g.

Air- and sun-drying tended to maintain more stable LAB counts than oven drying. This difference is likely not solely

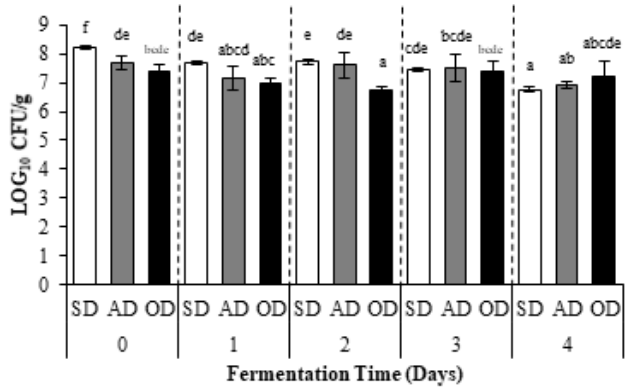

Figure 1 Total LAB during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference ($p<0.05$).

related to the heating temperature, as all three methods can, in principle, be set to similar temperature ranges. Other factors, such as temperature and humidity fluctuations during sun-drying, as well as the lower thermal stability of the raw material, may influence the availability of bioactive compounds that support LAB growth during fermentation. Meanwhile, the more consistent heating conditions during oven-drying may accelerate the degradation of heat-sensitive compounds, leading to a different response during fermentation. LAB may enter the stationary phase due to nutrient limitation in the medium or a decrease in pH caused by lactate accumulation during fermentation (Allahgoli *et al.,* 2023). Lactic acid fermentation is an anaerobic respiration process in which glucose is fermented to produce lactic acid as an end product (Carr *et al.,* 2002). LAB consortia can utilize most monosaccharides that make up brown seaweed polysaccharides, including glucose, mannitol, galactose, xylose, and mannose, except for fucose from fucoidans. Glucose and mannitol are the preferred substrates, serving as the primary sources of carbohydrates and energy in the fermentation medium (Allahgoli *et al.,* 2023).

Total titratable acidity

Total titratable acidity (TTA) is a parameter used to determine the overall concentration of acids in materials. The TTA levels during fermentation are shown in Figure 2. Based on the ANOVA results, the total titratable acidity (TTA) of the fermented seaweed was significantly influenced by the drying method, fermentation duration, and their interaction (p<0.05). The TTA values after four days of fermentation increased significantly compared to the initial values (day 0) in the sun and air-drying treatments. In the sun-drying treatment, there was no significant difference between days 3 and 4 (p>0.05). Similarly, the air-drying treatment on day 4 did not differ significantly from sun-drying, indicating that both methods had similar effects on acid formation during fermentation (Figure 3).

During fermentation, the TTA values of the seaweed ranged from 0.09% to 0.37%. The highest TTA was observed in the air-drying treatment on day 4 (0.37%), while the lowest was found in the oven-drying treatment on the same day (0.09%). The increase in total acidity was influenced by the activity of lactic acid bacteria, which break down substrates into organic acids, primarily lactic acid, as a metabolic product of glucose utilization during fermentation (Lengkey & Belia, 2014; Nuraini *et al.,* 2014). Lactic acid bacteria ferment glucose into organic acids, contributing to increased TTA and decreased pH (Desniar *et al.,* 2023).

These results indicate that the drying method and fermentation duration affect this process. *Air-* or sun-dried Sargassum sp. was more effective as a fermentation medium than oven-dried Sargassum sp. This is likely because natural heat drying better preserves bioactive compounds, whereas oven drying can accelerate the degradation

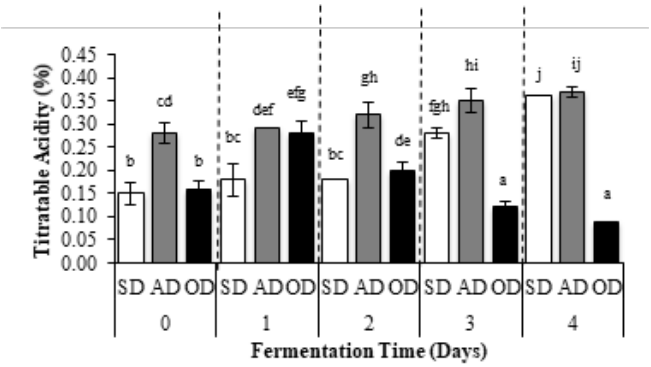

Figure 2 Total TTA during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference (p<0.05).

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of essential compounds owing to continuous heat exposure. Kadam *et al.* (2015) reported that oven-drying typically involves high temperatures for extended periods, which can damage heat-sensitive bioactive compounds and reduce nutrient content. The higher total titratable acidity in fermented seaweed may be attributed to the accumulation of organic acids during *L. plantarum* fermentation (Yue *et al.,* 2021).

pH Value

The pH value is an important parameter for indicating the acidity level of a material and serves as a key indicator for assessing the success of fermentation. An increase in the acid concentration leads to a decrease in pH. Generally, pH values are inversely related to total titratable acidity (TTA), where an increase in TTA is accompanied by a decrease in pH (Desniar *et al*., 2023), and vice versa. The changes in pH during fermentation are shown in Figure 3.

Based on the ANOVA results, the pH of the fermented seaweed was significantly influenced by the drying method, fermentation duration, and their interaction ($p<0.05$). In the air-drying and sun-drying treatments, no significant differences were observed between days 3 and 4 ($p>0.05$), indicating that both methods produced relatively similar fermentation conditions regarding acid production. The pH decreased from day 0 to day 4, ranging from 5.25 to 4.4 (except for the oven-drying treatment). The lowest pH was observed in the air-dried seaweed (4.4) on day 4, whereas the highest was found in the oven-dried seaweed (5.47) on the same day. This decrease in pH indicates that fermentation proceeded effectively in seaweed.

The pH reduction observed during air drying reflects a more efficient fermentation process. This decrease was caused by the accumulation of organic acids produced by bacteria during fermentation. Desniar (2012) reported that *L. plantarum* SK (5) produces lactic and acetic acids as the main organic acids responsible for lowering the environmental pH during fermentation. The greater the lactic acid production during fermentation, the higher the concentration of hydrogen ions (H^+) accumulated in the medium, resulting in a further decrease in pH (Silitonga e*t al*., 2022). Pratomo *et al*. (2020) also stated that the increase in titratable acidity and the decrease in pH during fermentation are due to the acidification activity of lactic acid bacteria.

A low pH is also important for inhibiting the growth of contaminating microbes and enhancing the stability of the fermented product. A pH <4.6 is ideal for fermented beverages, as it inhibits the growth of pathogens such as *Clostridium botulinum*, whereas a pH >5 is considered less safe because it can still support the growth of proteolytic *Clostridium botulinum* and *Listeria monocytogenes* (Sørensen *et al*., 2021). In this study, sun-drying and air-drying treatments achieved a pH < 4.6, while the oven-drying treatment remained >5.

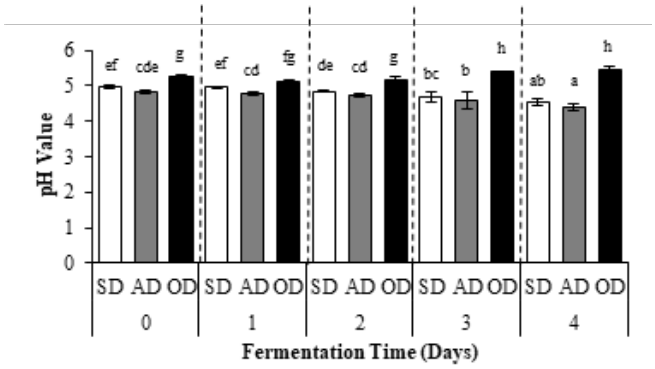

Figure 3 pH values during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference ($p<0.05$).

Characteristics of *Sargassum* sp. Tea

Total phenolic content

Phenolic compounds are antioxidants owing to their ability to donate hydrogen atoms (Thirumurugan *et al.*, 2018). A total phenol test was conducted to assess the effects of drying method and fermentation time on the phenolic compound levels in fermented seaweed. Total phenol content was determined using spectrophotometry with the Folin-Ciocalteu reagent. The results of the total phenol measurements in the fermented seaweed samples are presented in Figure 4.

The ANOVA results showed that the drying method, fermentation duration, and their interaction had a significant effect (p<0.05) on the total phenol content of the fermented seaweed. On day 2, the total phenol content in the air-drying treatment differed from that in the sun and oven drying treatments. The highest total phenol content was observed in the air-drying treatment, reaching 39.16 mg GAE/g on day 2 of fermentation, which was not significantly different from that on day 1 (38.25 mg GAE/g). In the oven-drying treatment, total phenol increased to 31.24 mg GAE/g on day 1, but gradually decreased until day 4 of fermentation. The increase in total phenol content in the air-drying treatment is likely related to enzymatic activity and the breakdown of complex compounds into simpler, more measurable phenols during fermentation. Conversely, the decrease in total phenol content in the oven-drying treatment may be due to the thermal degradation and partial destruction of phenolic compounds caused by high temperatures during drying, which reduced the measurable phenol content over time.

Fermentation can disrupt seaweed cell walls, increasing the polyphenol content of the fermented samples. Fermentation is a bioconversion process driven by diverse microbial activities. During this process, microorganisms produce a range of hydrolytic enzymes that modify the structural integrity of algal cell walls, thereby facilitating the release of bioactive compounds embedded within the cellular matrix (Eom *et al.*, 2011; Rafiquzzaman *et al.*, 2015). Hung *et al.* (2025) reported that fermentation of *Laminaria japonica* with *Bacillus subtilis* resulted in an increase in total phenolic content from 176.71±6.07 mg/mL in the non-fermented sample to 204.58±11.90 mg/mL after 72 h of fermentation. *Sargassum siliquanstrum* fermented with *Lactobacillus* sp. yielded a total polyphenol content of 344.23 µg/mL (Lee *et al.*, 2015).

The increase in phenol content occurs because bacteria ferment sugars in the sample, producing primary metabolites such as lactic acid and secondary metabolites, including polyphenols, which contribute to higher phenol levels (Ambarsari *et al.*, 2018). During fermentation, microorganisms produce soluble and sugar- or organic acid-conjugated phenolic compounds, which can be transformed into free, more bioactive phenolic forms (Gan *et al.*, 2016). Total phenol was measured by brewing fermented seaweed

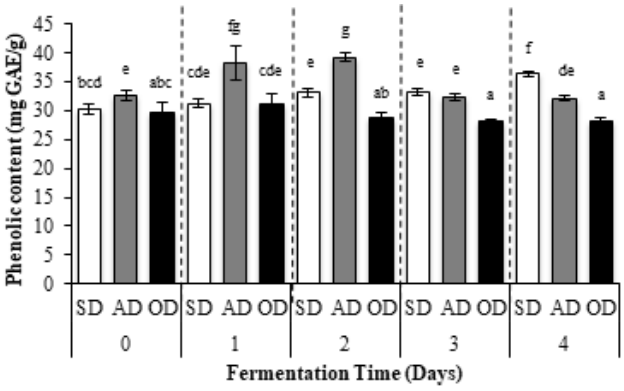

Figure 4 Total phenols during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference (p<0.05).

http://dx.doi.org/10.17844/n3h3eb98

using hot water (infused). Water-based solvents are more effective in extracting phenolic compounds than alcohol-based solvents (Sedjati *et al*., 2017). Fermentation also breaks down cell wall structures, promoting the release and synthesis of phenolic compounds and enhancing phytochemical activity that is beneficial to health (Hur *et al*., 2014; Shobharani *et al*., 2013). The phenol content of *Sargassum* sp. tea in this study meets the Indonesian National Standard (SNI) for packaged dry tea, which requires a minimum polyphenol content of 5.2 mg GAE/g (BSN, 2013).

Total tannin content

The total tannin content was determined using the permanganometric titration method with indigo carmine sulfonate solution as an indicator. The addition of this indicator caused the sample color to change from blue to yellow. Tannins are known to contribute to a bitter taste, but some condensed tannins also possess antioxidant activity that benefits health (AwadElkareem & Taylor, 2011). The results of the total tannin measurements in the fermented seaweed are presented in Figures 5 and 6.

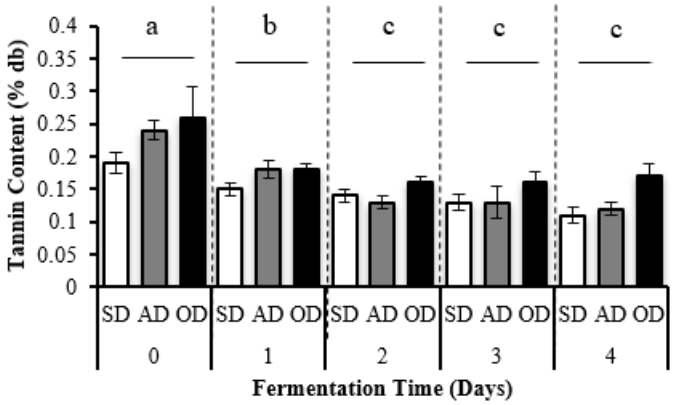

Figure 5 Total tannins during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference (p<0.05).

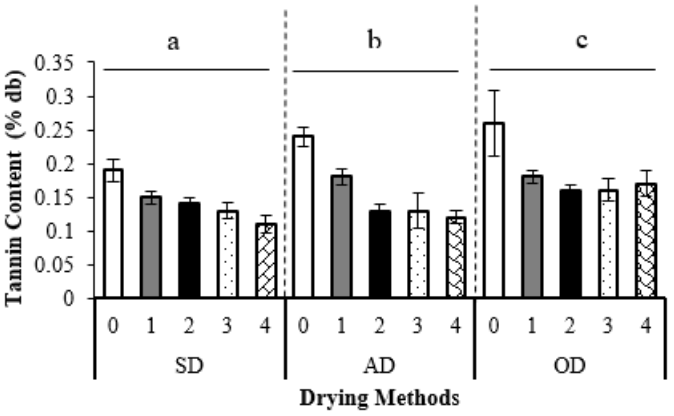

Figure 6 Total amount of tannins during fermentation of *Sargassum* sp. with different drying methods: sun-dried (□), air-dried (■), and oven-dried (■). Different superscripts showed a significant difference (p<0.05).

Based on the ANOVA results, the drying method and fermentation duration had a significant effect (p<0.05) on the total tannin content, whereas their interaction did not show a significant effect (p>0.05). Fermentation on days 0 and 1 differed significantly from that on days 2–4 across all drying methods. Significant differences (p<0.05) were also observed among the sun, air, and oven drying treatments.

Sun-drying and air-drying treatments showed a decrease in total tannin content until day 4, from 0.24% to 0.11%. This decline indicates the degradation of tannin compounds during fermentation, which is concomitant with increased enzymatic activity from lactic acid bacteria and fermentation conditions. However, the pattern of change was slightly different in the oven-drying treatment. The tannin content decreased from 0.24% on day 0 to 0.14% on days 2 and 3, but then showed a slight increase to 0.16% on day 4. Jiménez *et al*. (2014) stated that the reduction in total tannin during fermentation reflects the conversion of complex phenolic compounds into simpler forms through enzymatic activity, for example, by tannase produced by fermentative microorganisms such as *Lactobacillus plantarum*. Tannase is an enzyme that catalyzes the hydrolysis of tannins and is produced by bacteria, yeast, and fungi. Microorganisms such as *Lactobacillus plantarum* and *Saccharomyces cerevisiae* produce extracellular tannase, which can break ester bonds in tannin compounds, yielding glucose and gallic acid (Aguilar-Zarate *et al*., 2014).

CONCLUSION

The air-drying treatment of *Sargassum* sp. yielded the highest phenolic content, indicating that this method is effective for preserving total phenolic levels. Air-drying for four days of fermentation produced the highest total titratable acidity (TTA) and the lowest pH, indicating the most intensive fermentation activity. Meanwhile, air-drying for two days of fermentation resulted in the highest total phenol content, suggesting an increase in bioactive compounds due to optimal fermentation at this duration. Overall, air-drying provided better fermentation outcomes by supporting microbiological and chemical activity while effectively preserving bioactive compounds in *Sargassum* sp.

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