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Effect of lactic acid bacteria fermentation of *Pandanus tectorius* fruits on total phenolic content

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ABSTRACT

Pandanus tectorius fruit is a coastal plant rich in bioactive compounds, including phenolics, flavonoids, steroids, triterpenoids, saponins, and glycosides. Phenolic compounds possess antioxidant properties that may help prevent degenerative diseases. Fermentation using lactic acid bacteria (LAB) is a promising approach to enhance the availability of phenolic compounds through enzymatic degradation of plant cell walls. However, studies on the effect of LAB fermentation on the total phenolic content of sea pandan fruit remain limited. This study aimed to evaluate the effect of *Lactobacillus plantarum* fermentation on the total phenolic content and phytochemical profile of sea pandan fruit. An experimental method was employed by fermenting the fruit with *L. plantarum* for 96 hours in the presence of 2% glucose and comparing it with an unfermented control. Phytochemical screening and total phenolic determination were performed using the Folin–Ciocalteu method with UV–Vis spectrophotometry at 765 nm. The results showed that fermentation did not alter the qualitative phytochemical profile, as phenolic compounds and saponins were detected in both groups. However, total phenolic content increased from 2.11 ± 0.25 to 3.12 ± 0.50 mg GAE/g, representing an approximately 1.5-fold increase. These findings indicate that *L. plantarum* fermentation effectively enhances the functional value of sea pandan fruit.

Keywords: *Pandanus tectorius, Lactobacillus plantarum, lactic acid fermentation, total phenolics.*

INTRODUCTION

Indonesia is a tropical country that has very high biodiversity with various types of plants that have the potential to be used as traditional medicinal ingredients. Since ancient times, Indonesian people have used medicinal plants to maintain health and treat various diseases. The use of natural ingredients as traditional medicine continues to develop because it is considered to have relatively lower side effects than synthetic drugs and has been empirically proven to have beneficial pharmacological activity (Ningsih et al., 2020; Dewi et al., 2020; Indrawati, 2013). One of the plants that has the potential to be developed as a source of bioactive compounds is the sea pandanus fruit (Pandanus tectorius). This plant belongs to the Pandanaceae family, which has around 700 species and is spread across Africa, Madagascar, South Asia, Indochina, the Malaysian region including Indonesia, Australia, and the Pacific Islands (Rustamsyah et al., 2022). In Indonesia, Pandanus tectorius grows naturally along coastal areas, such as the north coast of Java Island, the Thousand Islands, Sumatra, and several other islands. Despite its abundant population, the use of sea pandan fruit as a food ingredient and raw material for traditional medicine is still relatively limited (Rustamsyah et al., 2022). Various studies report that sea pandan fruit contains various secondary metabolites, including phenolic compounds, flavonoids, steroids, triterpenoids, saponins, and glycosides. These compounds are known to have various biological activities, such as antioxidants, anti-inflammatory, anticancer, antitumor, antiviral, antidiabetic, and cholesterol-lowering activities (Budha Astawa et al., 2019; Puspasari et al., 2020; Rustamsyah et al., 2022). In addition to its secondary metabolite content, sea pandan fruit also contains nutrients in the form of protein, fat, carbohydrates, minerals, and β-carotene which allows this fruit to be used as a growth substrate for lactic acid bacteria during the fermentation process (Puspasari et al., 2020). Phenolic compounds are one of the secondary metabolite groups that receive a lot of attention because they have high antioxidant activity. The antioxidant activity of phenolic compounds comes from their ability to donate hydrogen atoms so that they are able to neutralize free radicals and inhibit oxidation reactions that can cause cell damage (Shahidi & Ambigaipalan, 2015; Singh et al., 2016). Therefore, various efforts are made to increase the content and availability of phenolic compounds in natural materials, one of which is through the fermentation process. Fermentation using lactic acid bacteria (LAB) is one of the biotechnology methods that are widely applied to improve food quality and bioactive compound content. Lactic acid bacteria are facultative anaerobic microorganisms that are able to convert carbohydrates into lactic acid and produce various extracellular enzymes that play a role in degrading the components that make up the plant cell wall (Rezaldi, 2022). One of the widely used species of LAB is Lactobacillus plantarum, which is a bacterium that is able to produce enzymes to break down bound phenolic components thereby increasing the release of free phenolic compounds and antioxidant activity of food (Ardilla et al., 2022; Ningsih et al., 2020). In addition, fermentation is also known to be able to increase food safety, improve nutritional value, produce useful secondary metabolites, and extend the shelf life of products through the formation of antimicrobial compounds (Suprihatin, 2010; Rezaldi, 2022). Research on fermentation using lactic acid bacteria has been widely carried out on various foods. However, studies on the effect of fermentation of Lactobacillus plantarum on the total phenolic content of sea pandan fruit (Pandanus tectorius) are still limited. Most previous studies have focused on the identification of phytochemical content and biological activity of sea pandan fruits, while information on changes in phenolic content after fermentation has not been widely reported (Puspasari et al., 2020; Rustamsyah et al., 2022). This condition shows that there is still a research gap that needs to be studied further. Based on this description, this study was conducted to answer the question of whether fermentation using lactic acid bacteria can affect the profile of secondary metabolites and increase the total phenolic content of sea pandan fruit (Pandanus tectorius). Therefore, the purpose of this study is to analyze the effect of fermentation using Lactobacillus plantarum on the results of phytochemical screening and the total phenolic content of sea pandan fruit. The results of the study are expected to provide scientific information about the potential of fermentation as a method to improve the phytochemical quality of sea pandan fruit so as to support its development as a raw material for functional food and herbal products.

METHODS

Research Design

This study is an experimental study that aims to evaluate the effect of fermentation using lactic acid bacteria on the total phenolic content of sea pandan fruit (*Pandanus tectorius*). The analysis was carried out through qualitative phytochemical screening and determination of total phenolic levels using the Folin–Ciocalteu method measured with a UV-Vis spectrophotometer. The data from the research results were analyzed descriptively in accordance with the research design used *(Sari & Ayuchecaria, 2017).*

Tools and Materials

The equipment used includes a set of laboratory glassware, blender, digital scale, incubator, UV-Vis spectrophotometer, pH meter, centrifuge, micropipette, water bath, filter paper, test tube, vial, stirring rod, ose, bunsen, and acid cabinet. The ingredients used include sea pandan fruit (*Pandanus tectorius*), *Lactobacillus plantarum culture*, glucose 2%, aquades, ethanol, sterile alcohol, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3) 20%, $FeCl_3$ 1%, HCl 2 N, concentrated HCl, glacial acetic acid, concentrated sulfuric acid, magnesium powder (Mg), potassium ferrisianide 1%, $MgSO_4$, ascorbic acid, oxalic acid, and gallic acid as the standard for determining total phenolic levels.

Sample Preparation

Ripe red or orange sea pandan fruit is obtained from the Pangandaran Beach area, Pangandaran Regency, West Java. The samples are first sorted, washed using running water, drained, then sprayed with sterile alcohol to reduce microbial contamination. The fruit is cut into small pieces, then as much as 50 g of samples are mashed using a blender with 200 mL of water and 2% glucose (4 g). The resulting suspension is put into a fermentation bottle, heated in the oven for one minute, then cooled before the fermentation process.

Fermentation Process

Fermentation is carried out using *the enzyme-assisted extraction* (EAE) method by utilizing enzyme activity produced by *Lactobacillus plantarum*. A total of 1 mL of *L. plantarum* starter culture is added to the suspension of sea pandan fruit that has been given 2% glucose, then fermented at 37°C for 96 hours.

As a control, samples were made without the addition of lactic acid bacteria with the same composition, namely 50 g of sea pandan fruit, 200 mL of water, and 2% glucose, but without the addition of *L. plantarum* cultures. Both treatment groups were processed under the same conditions until the analysis stage.

Phytochemical Screening

Phytochemical screening is performed qualitatively to identify the presence of several groups of secondary metabolites which include phenolics, flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids. The phenolic test was carried out by adding a 1% $FeCl_3$ solution to the extract, then the formation of a blackish-green color was observed as a positive indicator. The flavonoid test was carried out using magnesium powder and concentrated HCl, while the alkaloid test used the Dragendorff reagent. The saponin test was conducted based on the formation of a stable foam after shake, while the tannine test used $FeCl_3$ 1%. Steroid and triterpenoid tests were performed using the Liebermann–Burchard reagent through the addition of glacial acetic acid and concentrated sulfuric acid. The interpretation of the results is carried out based on the change in color or the formation of deposits according to the characteristics of each reagent *(Muthmainnah, 2017; Fajriaty et al., 2018).*

Determination of Total Phenolic Levels

Total phenolic levels were determined using the Folin–Ciocalteu method with galic acid as standard. Standard solutions are made at concentrations of 20, 40, 80, 120, and 160 ppm. A total of 0.2 mL of standard solution and sample solution were reacted with 1.8 mL of aqueduct and 0.2 mL of Folin–Ciocalteu reagent, then incubated for 6 minutes. Next, 2 mL of 20% Na_2CO_3 solution was added, homogenized, and re-incubated for 90 minutes at room temperature. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. The standard curve is created using a standard solution of gallic acid, then the total phenolic content of the sample is expressed as milligrams of Gallic Acid Equivalent per gram of sample (mg GAE/g) based on the standard curve regression equation *(Sari & Ayuchecaria, 2017).*

Data Analysis

The data from phytochemical screening were presented descriptively based on the presence or absence of secondary metabolites in each treatment. Meanwhile, the results of determining the total phenolic level were expressed as the average value of three times the repetition (average ± standard deviation) in units of mg GAE/g. The analysis was performed descriptively to compare the total phenolic content between samples fermented using *Lactobacillus plantarum* and unfermented samples.

RESULTS AND DISCUSSION

Fermentation of sea pandan fruit (*Pandanus tectorius*) was carried out using *Lactobacillus plantarum* as much as 1 mL with the addition of 2% glucose as a carbon source and incubated for 96 hours at a temperature of 37°C. As a comparison, samples were used without the addition of lactic acid bacteria with the same ingredient composition. The extraction process is carried out using *the enzyme-assisted extraction* (EAE) method, which is the use of enzymes produced by microorganisms to help the release of active compounds from plant tissues.

During fermentation, there is a change in the organoleptic characteristics of the sample. The extracts fermented using lactic acid bacteria had an orange color with a distinctive fermentation aroma and appeared clearer than the controls. Instead, samples without the addition of bacteria become cloudier and produce a more pungent odor. Nonetheless, the entire extract remains liquid and orange in color. These changes indicate the presence of metabolic activity during the fermentation process that results in changes in the physical properties of the extract.

Phytochemical screening was carried out to identify the presence of secondary metabolites in sea pandan fruit extract before and after fermentation. Testing includes flavonoids, phenolics, tannins, saponins, steroids, and alkaloids. The test results are presented in Table 1.

Table 1. Phytochemical screening of sea pandan fruit extract

Phytochemical Test	Result	
	fermented	Unfermented
Phenolic	+	+
Saponins	+	+
Alkaloids	-	-
Flavonoids	-	-
Steroids	-	-
Tannins	-	-
Alkaloids	-	-

Caption: (+) detected; (–) is not detected.

The screening results showed that fermentation using *Lactobacillus plantarum* did not change the secondary metabolite profile of sea pandan fruit extract. Both fermented and control samples showed positive results against phenolic compounds and saponins, while flavonoids, tannins, steroids, and alkaloids were not detected. The presence of phenolic compounds is characterized by the formation of a green color change after the addition of the $FeCl_3$ reagent. The color change occurs because the phenolic group reduces Fe^{3+} ions to Fe^{2+} resulting in a green complex, as explained by Fajriaty et al. (2018). The results of this study show that fermentation for 96 hours does not qualitatively change the presence of secondary metabolite groups, but rather affects the number of phenolic compounds that can be extracted.

In addition to phenolics, both treatments also showed positive results against saponins. The formation of stable foam in saponin testing indicates the presence of triterpenoid glycosides or steroids that have surfactant-like properties. Saponins are known to be able to lower the surface tension of the water so that it produces a stable foam after shake. These properties are in accordance with the characteristics of saponins described by Oleszek (2002) and Nurzaman et al. (2018). Overall, the results of phytochemical screening showed that fermentation using lactic acid bacteria did not cause the loss or emergence of new secondary metabolite groups. Thus, the effect of fermentation is more visible on changes in the levels of active compounds than on changes in the composition of secondary metabolites.

The determination of total phenolic levels was carried out using the Folin–Ciocalteu method with galic acid as the standard solution and absorbance measurements at a wavelength of 765 nm. The standard curve obtained has a regression equation $y = 0.0045x + 0.1208$ with a value of $R^2 = 0.9944$, which shows an excellent linear relationship between the galic acid concentration and the absorbance value.
The results of the measurement of total phenolic levels are presented in Table 2.

Table 2. Total phenolic content of sea pandan fruit extract

Samples	Total Phenolic Compounds (mg GAE/g)
Unfermented (K01)	2.11 ± 0.23
Fermented (K02)	3.12 ± 0.50

The results showed that fermentation using *Lactobacillus plantarum* increased the total phenolic content of sea pandan fruit extract. The mean value of total phenolic levels in K01 (unfermented) was 2.11 ± 0.23 mg GAE/g, while in K02 (fermented) increased to 3.12 ± 0.4969 mg GAE/g. The results showed that fermentation was able to increase the total phenolic content by about 47.9% or about 1.48 times compared to samples without fermentation. The increase indicates that the metabolic activity of *Lactobacillus plantarum* during fermentation plays a role in increasing the release of phenolic compounds previously bound to the plant cell wall matrix. The increase in phenolic content after fermentation is thought to be related to the activity of extracellular enzymes produced by lactic acid bacteria, such as β-glucosidase, esterase, and cell wall polysaccharide degrading enzymes. These enzymes hydrolyze the bonds between phenolic compounds and the structural components of the cell wall so that *bound phenolics* are transformed into *free phenolics* that are easier to extract using the Folin–Ciocalteu reactant. In addition, fermentation also produces a more acidic environment due to the formation of lactic acid, thereby increasing the solubility of some phenolic compounds. The mechanism is in line with the explanation of Suprihatin (2010) and Kunaepah (2008) regarding the role of fermentation in increasing the availability of bioactive compounds in food.

Phenolic compounds are secondary metabolites that have the ability to donate hydrogen atoms so that they are able to stop free radical chain reactions. Therefore, the increase in total phenolic levels after fermentation has the potential to increase the antioxidant activity of sea pandan fruit extract *(Alhabsyi et al., 2014).* However, this study only measured total phenolic content so the direct relationship between increased phenolic levels and antioxidant activity has not been ascertained and still requires further testing. Overall, the results showed that fermentation using *Lactobacillus plantarum* for 96 hours effectively increased the total phenolic content of sea pandan fruit without qualitatively changing its secondary metabolite profile. These findings support the use of fermentation as a simple approach to increase the functional value of sea pandan fruit as a functional food raw material and herbal products.

CONCLUSION

Fermentation of sea pandan fruit (*Pandanus tectorius*) using *Lactobacillus plantarum* for 96 hours increased the total phenolic content from 2.11 ± 0.23 mg GAE/g to 3.12 ± 0.50 mg GAE/g without changing its qualitative phytochemical profile. These findings indicate that fermentation is an effective method to improve the phenolic content of sea pandan fruit and support its potential use as a functional food ingredient and herbal raw material.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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