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Antioxidant activity and characterization of arrowroot (*Maranta arundinacea*) tuber yogurt

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Abstract. *Yuningtyas S, Roswiem AP, Azahra D, Alfarabi M. 2023. Antioxidant activity and characterization of arrowroot* (Maranta arundinacea) *tuber yogurt. Biodiversitas 24: 2850-2854.* The main benefit of yogurt is its ability to improve the digestive health tract by enhancing the condition of the intestinal flora and its environment. Nowadays, adding natural ingredients to yogurt is a widely practiced trend towards further increasing its efficacy as a healthy drink. Arrowroot tuber (*Maranta arundinacea* L.) is a food source with many health benefits and is not yet widely used in Indonesia. Fermentation can increase the health value of a food product compared to existing products. Therefore, the novelty and objective of this study are to produce and characterize arrowroot tuber yogurt using two species of Lactic Acid Bacteria (LAB), *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum*. These LAB species are used for yogurt starter and arrowroot tuber as a substrate in the fermentation process. The fermentation process was carried out using a combination of *L. plantarum* and *L. casei* (1:1 % (v / v)), *L. plantarum* 2% (v / v), and *L. casei* 2% (v / v). The characterization involved determining the pH value, total lactic acid level, total lactic acid bacteria level, and the antioxidant activity of the yogurt, which was assessed using the free radical 2,2-Diphenyl-1-Picrilhydrazyl (DPPH). The result showed that all arrowroot tuber yogurts have antioxidant activity, with the ability to inhibit DPPH by 59.30-86.62%. The arrowroot tuber yogurt had a pH level of 4.29-4.81, total lactic acid of 0.87-0.95%, and total lactic acid bacteria of 7.5×10^7-7.6×10^9 CFU/mL, all of which complied with the characteristics of the probiotic drink standard set by the Indonesian National Standard 2981:2009. These findings suggest that arrowroot tuber yogurt has the potential to be developed into a healthy drink.

Keywords: Antioxidant, arrowroot, fermentation, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*

INTRODUCTION

Fermentation has been a well-known food processing method for a long time. Historically, milk was the primary ingredient used in fermentation, altering its original taste to sour and producing a distinctive smell. Today, people in several countries ferment milk and other food materials, including vegetables. This process utilizes Lactic Acid Bacteria (LAB), such as *Lactobacillus bulgaricus*, commonly used in milk fermentation to produce yogurt. Yogurt contains mixed milk nutrients and metabolites produced from the fermentation process, including proteins, vitamins, and minerals. It also contains many organic acids, such as glucuronic, propionic, folic, and lactic acid. Essentially, yogurt provides more nutrients than milk and has high nutritional values (Aryana and Olson 2017). As a result, it has become a widely recommended healthy drink to improve health, particularly related to digestive health. Yogurt is also commonly included in many healthy diet programs due to its antioxidant, anti-hypertensive, anti-diabetic, and anti-hypercholesterolemia properties (Melini et al. 2019).

Many free radicals in the body are known to be one of the main causes of disease. This is because the free radical can interfere with the passage of biochemical processes in the cell. Naturally, free radicals are produced from cellular biochemical reactions and have many functions, including tissue homeostasis, signal regulation, and cell death control. The cells also have a defensive system to prevent any damage caused by these free radicals, including the presence of enzymes such as catalase and superoxide dismutase. However, excessively high concentrations of free radicals may prevent proper regulation of metabolism (Halliwell 2020; Harris and DeNicola 2020). This can be prevented by adhering to a healthy diet that includes yogurt and other fermented foods that are scientifically and empirically proven to have antioxidant activity. Fermented vegetables and fruits such as apples, pears, and carrots show increased antioxidant activity during fermentation and increased metabolite levels with antioxidant activity, such as flavonoids and phenols (Yang et al. 2018).

In recent years, there has been a trend to add fruit and vegetable concentrates to yogurt to increase health benefits. These additions also increase fiber content, as vegetables are a good source of fiber. Arrowroot (*Maranta arundinacea* L.) is a fiber-rich tuber containing phenolic compounds such as phenols, lignins, anthraquinones, flavonoids, tannins, and phenylpropanoids. (Putra and Estiasih 2016). Arrowroot is primarily used as a source of natural fiber to form biomaterials due to its biodegradability. It also has useful applications in biomedical materials and has been found to have favorable pharmacological effects, such as antioxidant activity (Tarique et al. 2021). In Indonesia, arrowroot is underutilized, especially in the health sector, and there are few studies on the bioactivity of this plant. Arrowroot tubers have antioxidant activity by lowering levels of MDA (Malondialdehyde), SGOT (Serum Glutamic Oxaloacetic Transaminase), and SGPT (Serum Glutamic Pyruvic Transaminase) in rats (Ramadhani et al. 2017). Previous studies have shown that arrowroot tuber yogurt fermented by *Lactobacillus fermentum* and *Lacticaseibacillus casei* and incubated for 12 hours could lower total cholesterol levels in rats. Furthermore, fermentation of this tuber with *L. fermentum* and *Lactiplantibacillus plantarum* reduced total cholesterol levels. The cholesterol-lowering effect of yogurt with *L. fermentum* is reported to be higher than that of simvastatin activity (Noviardi et al. 2020; Yuningtyas et al. 2021). These health benefits suggest that arrowroot has the potential for further development.

Arrowroot yogurt is not yet widely known, and its product variety is limited due to its production's limited types of fermented microbes. Normal yogurt cultures, such as *Lactobacillus delbrueckii* sub-sp. *bulgaricus* and *Streptococcus thermophilus* are not bile-resistant or acid tolerant and, therefore, cannot survive in the intestinal tract but may help lessen lactose intolerance symptoms. Various strains of LAB are considered probiotics, and two of the most documented strains, *L. casei*, and *L. plantarum*, can colonize the intestine, are bile resistant, and survive passage through the human gastrointestinal tract without induction of systemic immune or inflammatory responses (Abdelazez et al. 2018). Meanwhile, *L. plantarum* is rarely used in yogurt production (Corrieu and Béal 2016). This study introduced a new approach using arrowroot tubers as a substrate in fermentation with a combination of *L. casei* and *L. plantarum*. The aim of this study is to produce arrowroot tuber yogurt using *L. plantarum*, *L. casei*, and a combination of both. Each yogurt product was characterized by pH, total lactic acid, and total lactic acid bacteria, and their antioxidant activity was also measured. This study may serve as the basis of information for developing arrowroot tuber yogurt as a healthy drink.

MATERIALS AND METHODS

The arrowroot (*Maranta arundinacea* L.) tubers (8 months planting) used in this study were obtained from the Cell and Plant Tissue Culture Laboratory, Research Center of Biology, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia. Cultures of *L. casei* and *L. plantarum* were obtained from Indonesia Culture Collection Laboratory (InaCC), National Research and Innovation Agency, Cibinong, West Java, Indonesia.

Production of arrowroot tubers yogurt

The arrowroot tuber yogurt was produced according to a method from a previous study, which began by boiling the arrowroot tuber at 80°C for 20 min (Yuningtyas et al. 2021). Therefore, 100 g of arrowroot tubers was added to 300 mL of aquadest and blended to obtain a smooth suspension. Next, liquid skimmed milk was added to the suspension in a 2:1 ratio (arrowroot tubers: liquid skimmed milk). The mixture was pasteurized at 61-63°C for 30 min. Furthermore, the suspension medium of arrowroot tubers was inoculated by lactic acid bacteria starter culture with the formulation A (*L. plantarum*: *L. casei* (1: 1 %v/v)), B (*L. plantarum* 2% v/v), and C (*L. casei* 2 % v/v). Each bacterial media and starter mixture was incubated at 37°C for 12 hours.

Determination of pH value

The pH value of arrowroot tuber yogurt was measured at room temperature using a pre-calibrated pH meter (Laqua, Japan) with pH values ranging from 0-14.

Determination of total lactic acid

The total lactic acid content in arrowroot tuber yogurt was measured by titration (Yuningtyas et al. 2021). A total of 10 ml of yogurt solution was titrated with 0.1 N NaOH (Merck), using phenolphthalein as the pH indicator (Merck).

Determination of total lactic acid bacteria

The Total Plate Count (TPC) method determined the total lactic acid bacteria in arrowroot tuber yogurt (Yuningtyas et al. 2021). First, the yogurt solution was serially diluted, and then 1 ml of the solution was pipetted into the De Man Rogosa Sharpe (MRS) agar (Sigma-Aldrich). Furthermore, it was incubated at 37°C for 48 h, and the growing colonies were counted using colony counters.

Antioxidant activity test

The antioxidant activity was determined using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) (Sigma) (Alfarabi et al. 2022). Before the test, the yogurt solution was centrifuged (Kubota 5910, Japan) at 4,000 rpm. The supernatant obtained was tested with 0.1 mM DPPH. After being incubated at room temperature for 30 min, the absorbance was measured at 517 nm (Shimadzu Series UV mini-1240, Japan). Ascorbic acid (Sigma) was used as a positive control in this test, while DPPH solution was the negative control. The antioxidation power of the sample was calculated as the percentage of DPPH reduction using the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance negative control} - \text{Absorbance Sample}}{\text{Absorbance negative control}} \times 100\%$$

RESULTS AND DISCUSSION

Characteristics of arrowroot tuber yogurt

Arrowroot (*Maranta arundinacea*) is a locally available rhizomatous herbaceous plant with high levels of fructooligosaccharides, which may possess prebiotic properties useful in manufacturing bio-yogurt. The results showed that after fermentation, the pH of the arrowroot tuber suspension decreased from 7.0 to 4.29, 4.65, and 4.81 for formulations C, A, and B, respectively. The total lactic acid produced from the fermentation process was 0.87%, 0.89%, and 0.95% for formulations B, A, and C, respectively. Meanwhile, the total lactic acid bacteria in the fermentation product was 7.5×10^7, 1.8×10^8, and 7.6×10^9 CFU/mL for formulations B, C, and A, respectively. The characteristics of the arrowroot tuber yogurt met the probiotic drink standards set by the Indonesian National Standards Agency (SNI) (Table 1). This standard should have a pH value ranging from 4.0 to 5.0, total lactic acid of 0.5-2.0%, and minimum total acid bacteria lactate of 10^6 to 10^7 CFU/mL (National Standardization Agency of Indonesia 2009).

This study used arrowroot tuber suspension containing various carbohydrates as a growth medium for LAB. The decreased pH value observed after fermentation can be attributed to the production of lactic acid by LAB, which results from the metabolism of carbohydrates. These metabolites are then secreted from the cells, lowering the suspension's pH (Gänzle 2015). The fermentation process is anaerobic, and LAB grows optimally at a low pH of 4.0-6.0. Therefore, the production of lactic acid is a natural process conducted by the bacteria to maintain the optimum pH conditions of their growth environment (Feng et al. 2018). A low pH value due to the presence of lactic acid produced by LAB can prevent the growth of pathogenic bacteria in food. The LAB is used in some food industries to produce fermented products with antibacterial properties as a biopreservative to extend the shelf life and preserve the nutritional properties of food products (Özogul and Hamed 2018).

The sugars contained in arrowroot tuber flour are raffinose and oligofructose. These sugars are indigestible by humans and have the potential as prebiotics. The previous study showed that indigestible oligosaccharides are prebiotics that can increase normal microflora in the gastrointestinal tract. This leads to oligosaccharide fermentation by the microflora, which increases Short-Chain Fatty Acids (SCFAs), decreases intraluminal pH, and inhibits an increase in intestinal permeability. Consequently, it prevents pathogenic bacteria from passing through the intestinal epithelial barrier (Fidianingsih et al. 2022). Most probiotic bacteria grow slowly in milk, and the acid production level is usually too slow to support an adequate fermentation process in yogurt. However, standard yogurt culture consisting of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* work together and cause accelerated and efficient lactic acid production during fermentation. In this study, there was no deceleration of bacterial growth. The arrowroot tuber yogurt has a total LAB of 7.5×10^7-7.6×10^9 CFU/mL, higher than the standard value of at least 10^6 to 10^7 CFU/mL (National Standardization Agency of Indonesia 2009). This result confirmed the previous study that yogurt produced with arrowroot tuber has a high population of *Lactobacillus* than the control (yogurt without arrowroot tuber) (Abesinghe et al. 2012).

The results showed that the lowest pH value was observed in formulation C, where *L. casei* was used as LAB. This can be attributed to the highest total lactic acid content found in formulation C compared to other formulations. The highest total LAB was found in formulation A, a fermentation process using a mixture of *L. plantarum* and *L. casei*. The results indicate that fermented products that used a combination of LAB did not necessarily produce the highest total lactic acid and the lowest pH value. Abdelazez et al. (2018) found that *L. plantarum* did not acidify milk during 24 h and 72 h of fermentation at 37°C but grew well and remained at 108 CFU/mL level during 21 days of cold storage. Hill et al. (2018) also found that the pH decrease during fermentation was faster in the milk inoculated with *L. casei* starter. Batch fermentation studies with no pH control suggest that lactose slightly inhibits the cell growth of *L. plantarum* in the exponential growth phase, with no effects in the stationary and death phases (Lee et al. 2017). Therefore, further studies on the interaction between LAB in arrowroot tuber yogurt should be carried out to explain the mechanism of this interaction, such as metabolic processes that occur during fermentation and in the growth of LAB.

Antioxidant activity

The fermented products of arrowroot tuber could exhibit antioxidant activity, as demonstrated by its ability to inhibit DPPH as a free radical in the antioxidant assay. All samples showed 59-86% free radical inhibition, indicating that fermented products have the potential as a source of natural antioxidants (Figure 1). Antioxidants are molecules that can neutralize free radicals by either donating or using electrons together, thereby pairing the valence electrons in free radicals (Wang et al. 2021). As a stable free radical at room temperature, the DPPH reaction into a stable molecule can occur by two mechanisms, donation or electron sharing from antioxidant molecules. The antioxidant mechanism of arrowroot tuber yogurt in the DPPH reaction was unclear. Furthermore, this yogurt must be analyzed using different antioxidant methods (different reactions) than the DPPH to enhance the understanding of the antioxidant reaction concept from arrowroot tuber yogurt. The Ferric-Reducing Antioxidant Power (FRAP) method can be used for further testing to determine the electron transfer reactions (Cerretani and Bendini 2010). Another applicable method is the Thiobarbituric Acid Reactive Substances (TBARS) method, which measures lipid oxidation from oxidation products (Ghani et al. 2017).

Table 1. pH Value, total lactic acid, and total lactic acid bacteria from fermented arrowroot (*Maranta arundinacea*) tubers

Analysis	Formulation			SNI 2981:2009*
	A	B	C	
pH	4.65 ± 0.02	4.81 ± 0.03	4.29 ± 0.03	4.0-5.0
Total lactic acid (%)	0.89 ± 0.25	0.87 ± 0.08	0.95 ± 0.17	0.5-2.0
Total lactic acid bacteria (CFU/mL)	7.6×10^9	7.5×10^7	1.8×10^8	10^6-10^7 (minimum)

Note: A. Fermented *L. plantarum*: *L. casei* (1:1 %v/v), B. Fermented *L. plantarum* (2% v/v), C: Fermented *L. casei* (2%v/v), *: Indonesian national standard (SNI) reference for probiotic drinks

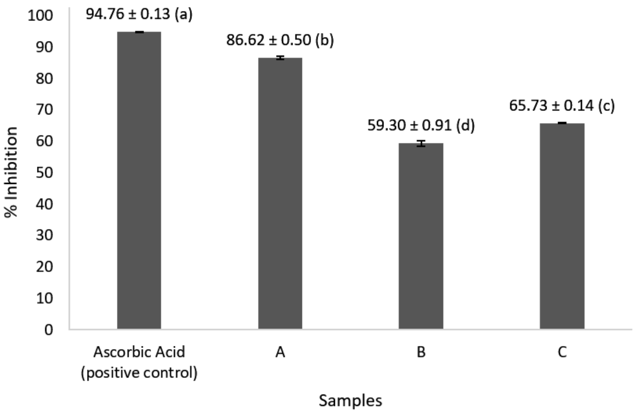

Figure 1. Antioxidant activity of fermented arrowroot tuber product. Different scores on the top of the bar chart indicate the inhibition percentage of each sample. A: fermented with *L. plantarum*: *L. casei* (1:1 %v/v), B: fermented with *L. plantarum* (2% v/v), C: fermented with *L. casei* (2% v/v)

Fresh arrowroot tubers contain many secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds (Shintu et al. 2015; Ieamkheng et al. 2022). Phenolic compounds in arrowroot tubers can be detected in tubers and leaves, with the total phenol content in tubers up to 3.8g/100g (Kusbandari and Susanti 2017). These molecules have also been shown to have antioxidant activity. Antioxidant properties of yogurt also result from the presence of casein, whey proteins, peptides, amino acids, uric acid, vitamins (A, C, D, and E), β-carotene, enzymic systems (SOD, catalase, and glutathione peroxidase), lactic acid, Conjugated Linoleic Acid (CLA), and coenzyme Q10 (Fardet and Rock 2018). Some of these molecules are extracellular metabolites of LAB produced during fermentation. Therefore, LAB is a group of bacteria with antioxidant activity tolerant to oxidative stress conditions. These bacteria can capture free radicals, chelate metal ions, produce antioxidant enzymes, and modulate intestine flora (Feng and Wang 2020). Many LAB strains can chelate metal ions, such as Fe^{2+}, which can be a free radical in metabolic processes, such as *L. casei*, which chelates Fe^{2+} and Cu^{2+} (Lee et al. 2005). Suzuki et al. (2013) identified two compounds, L-3-(4-Hydroxyphenyl) Lactic Acid (HPLA) and L-Indole-3-Lactic Acid (ILA), from the cultures of *L. plantarum* and *L. casei* with radical scavenging activity. However, the spectroscopic analyses showed that these compounds were strain-specific and not the main antioxidants. Bioactive peptides in yogurt, such as Valine-Proline-Proline (VPP) and Isoleucine-Proline-Proline (IPP), were produced from milk protein fermentation by *L. casei*. These peptides have been reported to have radical scavenging activity (Rutella et al. 2016).

The ability to produce antioxidant enzymes and lactic acid from LAB is crucial in modulating the intestine flora. The intestinal epithelium produces a lot of Reactive Oxygen Species (ROS) as a defense against the highly proliferating pathogenic cells. This leads to decreased levels of ROS and pathogenic cells in the intestines, although this mechanism is not completely clear (Jones and Neish 2017). Therefore, we can assume that the arrowroot tuber yogurt has two sources of antioxidants than the original yogurt or the arrowroot tuber alone. The antioxidant activity of arrowroot tuber yogurt in this study showed high free radical inhibition than other arrowroot tuber-based products. A previous study reported that the free radical inhibition of arrowroot flour was 1.62%, fried arrowroot tuber was 0.65%, and arrowroot tuber steam was 1.44% (Yuniastuti et al. 2017). Wu and Liao (2017) also found that arrowroot flour-based biomembrane had an antioxidant activity of about 15-23.6%.

In conclusion, this study showed that arrowroot tuber yogurts have antioxidant activity, with the highest activity observed in formulation A was a 1:1% v/v mixture of *L. plantarum* and *L. casei*. This formulation had the highest number of LAB but not the highest lactic acid content, indicating the production of other metabolites with antioxidant activity. Furthermore, there was no correlation between the pH value and the total amount of lactic acid on the highest antioxidant activity. Therefore, the arrowroot tuber yogurts in this study's Indonesian National Standard reference (SNI) for probiotic drinks indicate their potential as a prebiotic drink. However, further studies on metabolite profiling and organoleptic tests must be conducted.

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