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Cover Page Footnote

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Functional Properties of Purple Water Yam Flour Modified by *Lactobacillus plantarum*

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Abstract

Purple water yam flour modified by *Lactobacillus plantarum* fermentation may be used as an ingredient in flour-based functional food. The purpose of this research is to determine the effect of fermentation time on the functional properties of purple water yam flour modified using *L. plantarum*. This research used a randomized block design with six treatments: without fermentation (control) and 12, 24, 36, 48, and 60 h of fermentation. Results showed that lactic acid bacteria initially grow well with increasing viability over 48 h of fermentation. Growth and viability rates began to decrease as the organisms entered the declining or dying phase. Statistical analysis showed that fermentation time affects the solubility at 75 °C, chromatic color values L* and b*, and antioxidant activity of flour. Fermentation for 36 h produced the best modified purple water yam flour with an antioxidant activity of 69.82%, bulk density of 0.817 g/mL, water absorption capacity of 3.31 g/g, oil absorption capacity of 1.20 g/g, solubility of 0.45%, L* of 80.33, a* of 16.33, and b* of −5.33. The results indicate that purple water yam flour modified by *L. plantarum* fermentation for 36 h could be developed as a functional food ingredient.

Keywords: fermentation, Lactobacillus plantarum, purple water yam flour, functional properties

Introduction

Water yam or *Dioscorea alata* grows easily in many types of soil and is hardly attacked by plant diseases and pests. The tuber of water yam is known to possess functional properties that provide health benefits, such as antioxidant activity. Water yam tubers found in Indonesia may be yellow, orange, white, purple, and dark purple [1]. Among these tubers, purple water yam contains the most beneficial components, such as dietary fibers and minerals [2] and secondary metabolites [3]. Secondary metabolites are components produced during plant metabolism using primary metabolites as their precursors and may be classified into three major groups: terpenoid, phenolics, and nitrogen-containing compounds; alkaloids make up the third largest secondary metabolite group [4]. Secondary metabolites found in various food materials, such as purple water yam tubers, may act as antioxidants. Thus, the tubers are often processed into functional foods with increasing popularity.

Purple water yam tubers have high water contents and may be easily damaged. Proper processing into flour, for example, is one method to improve the durability of the tubers. However, direct processing from tuber to flour could reduce the beneficial components of the former, such as its anthocyanins [5] or antioxidant compounds. In addition, purple water yam flour has lower water (WAC) and oil (OAC) absorption capacities than wheat flour, which means flour from this crop could fail as a wheat flour substitute in wheat-based products.

Lactic acid fermentation is widely used to improve the functional properties of flour from different sources, such as cassava, wheat, sorghum, and corn [6-9]. Some lactic acid bacteria produce enzymes that allow them to have amylolytic, proteolytic, lignolytic, pectinolytic, and/or cellulolytic properties [10-12]. The amylolytic action of the bacteria causes starch hydrolysis, which leads to increased water solubility in the resulting flour [7]. The proteolytic action of the bacteria also increases the protein digestibility of starch in fermented flour [11]. The lignolytic, pectinolytic, and cellulolytic properties of lactic acid bacteria help degrade cell walls to release secondary metabolites bound to cell wall components [12]. *Lactobacillus plantarum* is one of

hundreds of species of *Lactobacillus* considered as commercial probiotics [13]. It is widely used in fermentation because its optimum temperature is in the range of room temperature. Fermentation has also been reported to be successful in increasing the WAC and OAC of fermented flour [6,7]. These potential benefits motivated the authors to carry out research using *L. plantarum* to modify purple water yam flour and improve its functional properties, especially its WAC and OAC, and retain beneficial components such as antioxidants.

Materials and Methods

Materials. The experiment was carried out at the Faculties of Agricultural Technology and Husbandry, University of Jambi. Purple water yam tuber was harvested from a local farm in Jambi City. *L. plantarum* was kindly provided by the laboratory at the Faculty of Husbandry, University of Jambi. De Man, Rogosa, and Sharpe (MRS) broth and agar (Merck) were used to grow *L. plantarum*. 2,2-Diphenyl-1-picrylhydrazyl (DPPH; Sigma–Aldrich) was used to analyze antioxidant activity.

This research used a randomized block design with six levels of treatment (fermentation time: 0, 12, 24, 36, 48, and 60 h) and three replicates per treatment. Flour without fermentation served as the control.

Preparation of the fermentation medium. One segment of of *L. plantarum* colonised agar was transferred into test tubes containing 10 mL of MRS broth using inoculating loop and incubated at 37 °C for 48 h. Exactly 5 mL of this culture was inoculated in 500 mL of MRS broth and incubated for another 8 h. This culture was designated as the work culture and used to produce the fermentation medium. The fermentation medium was obtained by mixing 12 mL of work culture with adequate drinking water to make up a 600 mL solution.

Flour processing [5]. Purple water yam flour without fermentation (control) was processed following the method of Ulyarti *et al*. [5]. The tuber was cleaned, peeled, washed, and cut into small slices of approximately 0.5 mm. The slices were steam-blanched at 86 °C for 34 s, cooled to room temperature, and then dried in the oven at 50 °C for 24 h. The dried chips were ground and sifted through a 60-mesh sieve. The flour was stored in a plastic bag at room temperature until further analysis.

The fermented flours were processed using procedures similar to those employed for the control flour with some modification. After blanching treatment, the slices were cooled and soaked in *L. plantarum* medium according to the assigned treatment (i.e., 12, 24, 36, 48, or 60 h). Five hundred grams of yam slices were soaked in 600 mL of medium containing 2% (v/v) *L. plantarum*. Fermentation was carried out in closed plastic container at 30 °C. After the assigned time had elapsed, the fermented slices were washed with distilled water and drained. This washing step was repeated until neutral drained water (pH 7) was obtained. The slices were then dried, ground, sifted, and stored as described above.

Total plate count. Total plate counts were used to assess the total amount of lactic acid bacteria growing in the water yam fermentation medium. Here 1 mL of fermentation medium was aseptically mixed with 9 mL of sterile 0.85% NaCl solution; the resulting solution was considered a dilution of 10^{-1}. The fermentation medium was further diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. From each dilution, 1 mL of solution was plated in Petri dishes containing 10 mL of MRS agar. The agar and medium samples were mixed by moving the Petri on the table in a number 8 pattern. When the agar and medium had set, the Petri dishes were incubated upside down at 37 °C for 48 h. Total lactic acid bacteria was calculated using Eq. (1).

$$\text{Total lactic acid bacteria per mL} = \Sigma \text{ colony} \times \frac{1}{\text{dillution factor}} \qquad (1)$$

Functional properties of flour. Besides antioxidant activity, six other functional properties of flour were determined in this experiment: flour bulk density [14], WAC [15], OAC [15], swelling power (SP) [16], solubility [16], and color [17].

Antioxidant activity using DPPH radical scavenging activity. Sample preparation was carried out following the method described by Baba et al. [18]. One gram of flour sample was dissolved in 10 mL of distilled water and stirred for 2 h. The suspension was centrifuged at 3500 rpm for 10 mins, and the supernatant was collected for antioxidant assay.

DPPH radical scavenging activity was measured using the method described by Sharma et al. [19] with some modification. Exactly 0.2 mL of the supernatant from the sample preparation was placed in a vial. Then, 3.8 mL of 0.05 µM DPPH was added to the vial, and the latter was shaken slowly and kept in the dark for 30 mins. The absorbance of the sample solution was measured using a UV-VIS spectrophotometer at 517 nm. The control solution was obtained by using methanol as the sample supernatant. The absorbance data were used to calculate DPPH radical scavenging activity as shown in Eq. (2). Ascorbic acid was used as a comparison or positive control.

$$\text{Antioxidant activity (\%)} = \frac{\text{A control - A. sample}}{\text{A control}} \times 100 \qquad (2)$$

Data analysis. The data were analyzed by analysis of variance (ANOVA) and Duncan's new multiple range test (DnMRT).

Result and Discussion

Total lactic acid bacteria in the fermentation medium. The total amount of lactic acid bacteria grown during fermentation is shown in Figure 1. This curve shows initial growth during the first 24 h of fermentation. Bacterial growth reached the stationary phase after 24–48 h of fermentation. After 48 h of fermentation, the total amount of lactic acid bacteria decreased, thereby indicating that 48 h is the optimum fermentation time to produce fermented flour. The absence of a lag phase during initial fermentation indicates that lactic acid bacteria easily adapt to and readily grow in the fermentation medium. During fermentation, the bacteria use carbohydrates in the water yam tuber as a carbon source for growth. These carbohydrates are then converted into lactic acid, which, in turn, decreases the acidity of the fermentation medium. The pH of the fermentation medium noticeably decreased after 12 h of fermentation. However, increases in fermentation time did not remarkably change the pH of the fermentation medium, as shown in Table 1.

Bulk density. Bulk density refers to the weight of a certain volume of flour. A higher bulk density means the same volume of flour is heavier. Powder products usually have bulk densities in the range of 0.30–0.80 g/mL. Fermentation using *L. plantarum* did not affect the bulk density of water yam flour (Table 1). This result is different from a previous report by Efendi [20], who found that fermentation time decreases the bulk density of fermented cassava flour. Fermentation softens the cell tissues of cassava, thereby allowing water to evaporate during drying and producing empty cavities in the cassava granules. These empty cavities endow fermented cassava flour with high porosity. In contrast to those of cassava, the cell tissues of water yam do not soften during fermentation.

Water and oil absorption capacities. WAC refers to the ability of flour to absorb and retain water in the food system [6]. WAC depends on the presence of hydrophilic components, such as proteins with hydrophilic substituents, or amylose [20]. However, other researchers have proposed that the presence of amylose decreases water retention because of the close association between starch molecules in the flour granules and the availability of water in the water binding sites of starch [21]. Another study reported that fermentation increases

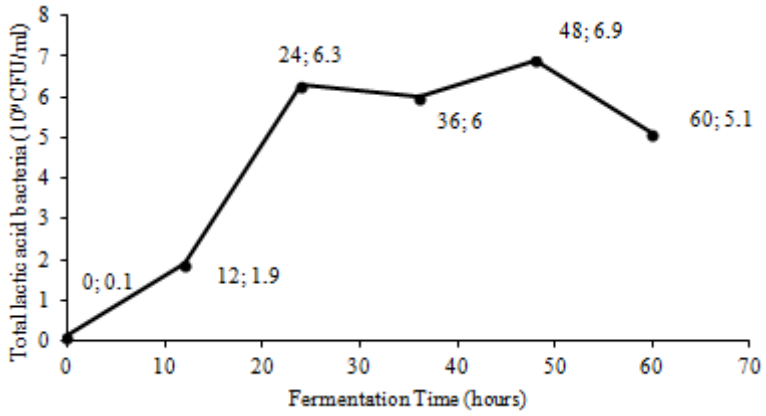

Figure 1. Growth Curve of Lactic Acid Bacteria during Purple Water Yam Tuber Fermentation

Table 1. pH, Bulk Density, and Water and Oil Absorption Capacities of Water Yam Flour at Different Fermentation Times

Fermentation (hour)	pH	Bulk Density (g/mL)	WAC (g/g)	OAC (g/g)
0	6.23 b	0.832 a	3.04 a	1.18 a
12	4.36 a	0.831 a	2.99 a	1.17 a
24	4.33 a	0.820 a	3.13 a	1.19 a
36	4.29 a	0.817 a	3.31 a	1.20 a
48	4.20 a	0.817 a	3.28 a	1.19 a
60	4.26 a	0.802 a	3.34 a	1.21 a

Note: Numbers followed by the same letter in a column are not significantly different according to DnMRT ($p > 0.05$).
WAC = water absorption capacity, OAC = oil absorption capacity.

WAC [7], which could explain why the ability to absorb water is mainly affected by the attributes of starch, the main component of flour. The results of the current research showed that the WAC of water yam flour is not affected by fermentation time. No significant change in the WAC of water yam flour was detected when the fermentation time was increased (Table 1). Lactic acid fermentation of water yam for up to 60 h may not provide sufficient acid hydrolysis to enhance the ability of starch to absorb water. This finding is in agreement with the results of Aini et al., who reported that fermentation time does not affect the WAC of modified corn flour [6]. However, when the WAC of wheat flour (1.5 g/g) [22] was compared with that of purple water yam flour, the latter was much higher even without fermentation.

Oil absorption capacity (OAC) reflects the ability of flour to absorb oil. The porous nature of flour could increase its OAC. In this study, no difference in the porosity of the water yam flours was observed, as indicated by their fairly similar bulk densities. This finding may explain why the OAC of the water yam flours did not change over 60 h of lactic acid fermentation (Table 1). Similar to WAC, the OAC of purple water yam flour at all levels of treatment was consistently higher than that of wheat flour, which is 0.88 mL/g or 0.81 g/g when the wheat flour density of 0.924 g/mL is used for conversion [22]

Swelling power and solubility. Swelling power (SP) is a main property of starch in flour. A higher SP means the starch is able to absorb more water and hold it in its granules, causing them to swell. SP is related to the ability of a starch granule to maintain its integrity before it breaks down because of the excess water it contains. As seen in Table 2, all of the flour samples demonstrated higher SP with increasing temperature. No statistical difference in the SP of flours obtained at different fermentation times was observed. This result is different from the results of fermented *Canavalia ensiformis* flour, which showed an increase in SP with increasing fermentation time [7]. Lactic acid fermentation for up to 60 h did not change the molecular arrangement of the starch granules. Water yam starch is known to have strong intermolecular and intramolecular bonds that are more difficult to break compared with those of other starches, such as cassava starch [23].

Similar to SP, solubility is also a predominant feature of starch. When starch granules absorb water during heating, some of them solubilize into the water. As seen in Table 2, solubility increased as the heating temperature increased and no significant difference in solubility, except for that measured at 75 °C, was observed over different fermentation times. This result reveals that lactic acid fermentation for up to 60 h does not change the molecular bonds, soluble material contents, or solubility of the flour.

Table 2. Swelling Power (SP) and Solubility (S) of Water Yam Flours at Different Fermentation Times

Temperature		Fermentation Time (hour)					
		0	12	24	36	48	60
65 °C	SP (g/g)	8.76 a	8.73 a	8.58 a	8.93 a	8.94 a	8.42 a
	S (%)	1.72 a	0.83 a	0.67 a	0.75 a	0.77 a	1.82 a
70 °C	SP (g/g)	10.85 a	10.96 a	11.03 a	10.28 a	9.78 a	9.74 a
	S (%)	1.04 a	0.32 a	0.34 a	0.68 a	0.52 a	0.97 a
75 °C	SP (g/g)	11.43 a	12.56 a	12.74 a	11.98 a	11.97 a	11.89 a
	S (%)	**0.31 a**	**0.30 a**	**0.52 a**	**0.45 a**	**1.08 b**	**1.13 b**
80 °C	SP (g/g)	13.58 a	13.86 a	14.84 a	12.96 a	13.10 a	13.74 a
	S (%)	0.96 a	0.58 a	0.41 a	0.28 a	0.58 a	1.21 a
85 °C	SP (g/g)	13.71 a	15.05 a	14.61 a	14.03 a	13.98 a	14.72 a
	S (%)	1.15 a	0.55 a	0.35 a	0.60 a	0.53 a	1.30 a
90 °C	SP (g/g)	14.69 a	14.75 a	16.00 a	14.73 a	14.80 a	15.23 a
	S (%)	0.82 a	0.61 a	049 a	0.50 a	0.57 a	1.11 a
95 °C	SP (g/g)	15.23 a	15.24 a	15.68 a	15.21 a	14.89 a	15.54 a
	S (%)	1.41 a	0.81 a	0.77 a	0.75 a	0.82 a	1.63 a

Note: Numbers followed by the same letter in the same row are not significantly different according to DnMRT ($p > 0.05$).

Table 3. Color Values and Antioxidant Activity of Water Yam Flour at Different Fermentation Times

Fermentation time (hour)	Color parameter			Antioxidant activity (%)
	L*	a*	b*	
0	74.67 a	17.67 a	−13.00 a	46.47 a
12	80.00 ab	13.33 a	−5.67 b	45.57 a
24	82.33 b	17.33 a	−667 b	52.53 ab
36	80.33 ab	16.33 a	−5.33 b	69.82 bc
48	76.33 a	15.33 a	−5.67 b	77.66 c
60	84.00 b	15.33 a	−3.67 b	88.24 c

Note: Numbers followed by the same letter in the same column are not significantly different according to DnMRT ($p > 0.05$).

Color properties. Color plays a significant role in consumers' acceptance of a product, including flour. L* describes lightness and ranges in value from 100 (white) to 0 (black), a* describes redness (range, 0–80) and greenness (range, 0–80), and b* describes yellowness (range, 0–70) and blueness (range, −70–0) [24]. The average and DnMR test results are presented in Table 3. As seen in this table, fermentation time significantly affected ($p < 0.05$) L* and b*. The lightness of fermented flour increased as the fermentation time increased. The longer the fermentation time, the larger the amount of the pigment component dissolved in the fermentation medium, which results in a lighter flour color. The browning reaction, which usually interferes with the production of yam flour, is also inhibited by the acid produced during fermentation, leading to a lighter flour color. The fermented water yam flour obtained in the present study (Table 3) is lighter than the fermented yam flour observed in another study, which had an L* of 79.24 [25].

Antioxidant activity. Fermentation is known to positively affect antioxidant activity [8,12,26]. In this study, lactic acid fermentation increased the antioxidant activity of purple water yam flour. The longer the fermentation time, the higher the antioxidant activity of the resulting flour (range, 45.57%–88.24%; Table 3). However, fermentation of the tubers for 36, 48, and 60 h resulted in antioxidant activities that were not significantly different ($p < 5\%$), thereby confirming that 36 h of fermentation is sufficient to produce maximum antioxidant activity in purple water yam flour. Purple water yam flour fermented for 48 and 60 h had higher antioxidant activity than ascorbic acid, which had value of 71.17%. Given the values obtained and the classification of ascorbic acid as a strong antioxidant [27], the antioxidant activity of flour fermented for 48 and 60 h could be classified as strong. The fermented purple water yam flour produced in the present study showed much higher antioxidant activity than wheat–barley blend flour [19]. The increase in antioxidant activity of the fermented flour may be related to the release of bound phenolic compounds after fermentation. Phenolics are bound to the structural components of cell walls, such as cellulose, hemicellulose, lignin, and pectin [12]. Fermentation releases phenolic compounds from these structures and increases the antioxidant activity of the resulting flour.

Conclusion

L. plantarum reaches maximum growth after 48 h of fermentation in purple water yam tubers. Fermentation time affected the solubility at 75 ℃, L*, b*, and antioxidant activity of the resulting flour. The best fermentation time to produce modified purple water yam flour was 36 hours. The resulting flour had a bulk density of 0.817 g/mL, WAC of 3.31 g/g, OAC of 1.20 g/g, solubility of 0.45%, L* of 80.33, a* of 16.33, b* of −5.33, and antioxidant activity of 69.82%. The WAC and OAC of modified purple water yam flour were much higher than those of wheat flour. These results indicate that water purple yam flour could be used as a functional food ingredient.

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