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Characteristics of Bread with The Substitution of Fermented Purple Yam Flour (*Dioscorea alata*)

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ABSTRACT

Fermented purple yam flour is known to have high swelling power, which is capable of improving its ability as a substitute for wheat flour in bread production. Therefore, this study aimed to determine the effect of the substitution of wheat flour with fermented purple yam flour (FF), at 0%, 10%, 15%, 20%, 25%, and 30% concentrations on the physical, chemical, and sensory characteristics of bread. The result showed that FF concentration has no significant effect on the moisture content, specific loaf volume, hedonic aroma, and taste but significantly affected porosity, anthocyanin content, antioxidant activity, hedonic color, texture, and overall acceptance of the bread. Furthermore, the best treatment obtained was at the 25% substitution level, which produced bread with 29.30% moisture content, 27.18% porosity, 3.06 cm^3/g specific loaf volume, and 5.30 mg/g anthocyanin. It also produced 61.03% antioxidant activity, color 3.88, texture 4.00, taste 3.92, and overall acceptance 4.00 (Likes). The maximum substitution rate of fermented purple yam flour was 25%.

Keywords: Antioxidant; bread; fermented purple yam flour; porosity; specific loaf volume

INTRODUCTION

Purple yam (*Dioscorea alata*) is a prospective local food plant that produces tuber used as a food source. It grows easily and does not require any particular treatment to produce good tubers. During a single harvest period, one plant can yield up to 10 kg of tuber, with the optimal harvesting time being 8 to 9 months when the tubers are intended for food consumption. The older the tuber, the higher its bioactive components content but the lower its starch content. This yam tuber, which is rich in carbohydrates and bioactive components, can be developed into functional food raw materials, such as bread. It can be processed into flour using several methods to retain its beautiful color and bioactive components. Some of these methods include the use of citric and ascorbic acid, types of blanching, or a combination of those practices when preparing the flour (Akubor, 2013; Imanningsih, Muchtadi, Wresdiyati, & Komari, 2013; Lavlinesia, Ulyarti, Yulia, Francisca, & Purnawati, 2019).

The substitution of wheat with gluten-free flour produces a bread with a low level of expansion, as indicated by a low specific loaf volume (SLV), which is unacceptable to the consumers (Amandikwa, Iwe, Uzomah, & Olawuni, 2015). This study faced a challenge, which include the production of quality bread from gluten-free ingredients, such as purple yam flour. The gluten-free bread had a firmer texture, less appealing color, and drier mouthfeel than gluten-containing bread. According to Ohimain (2014), the structural protein in wheat flour helps the formation of elastic dough, resulting in the production of soft bread with good expansion. Previous studies have shown that the role of gluten in wheat cannot fully be replaced by other ingredients in bread

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production. However, the formulation of fermented flour and other ingredients may help meet the criteria for good gluten free-bread (Marti et al., 2017; Monthe et al., 2019; Shrivastava & Chakraborty, 2018). The starch of the fermented flour is known to have high swelling power, which helps the development of the free-gluten dough (Monthe et al., 2019).

(Aini, Wijonarko, & Sustriawan, 2016) used tape to produce fermented flour. Tape is a fermented product made out of cooked glutinous rice or cooked cassava tuber. Furthermore, tape yeast contains microbes, such as *Saccharomyces cerevisiae*, which are useful in hydrolyzing starch into sugar.

This study aimed to determine the substitution effect of wheat flour with fermented purple yam flour on the physical, chemical, and sensory characteristics of bread. It was also conducted to determine the optimum substitution level, which produces bread with the best physical, chemical, and sensory properties.

METHODS

Materials

The materials used in this study included purple yam tubers (*Dioscorea alata*) harvested in Jambi, tape yeast (NKL brand), high protein wheat flour of premium *Cakra Kembar* brand, table salt, granulated sugar, instant bread yeast, margarine, and powdered milk. Meanwhile, the materials used for analysis were methanol (Supelco, pro anallysis), DPPH (Sigma Aldrich), HCl (Merck, 37%), and nitrogen gas.

The equipment used was bread makers (Magbread, Ardin, made in Indonesia), H-C-6 centrifuges, scanner (HP type deskjet Ink Advantage 2135, USA), and Thermo Scientific Genesys 20 spectrophotometers.

Research Design

This study adopted a completely randomized design with six treatment levels of fermented purple yam flour (FF) concentration and three replications to obtain 18 experimental units. The treatment used is the concentration of fermented purple yam flour as a substitute for wheat flour, namely P0= 100% wheat flour (control), P1= wheat flour 90%:10% FF, P2= wheat flour 85%:15% FF, P3 = wheat flour 80%:20% FF, P4 = wheat flour 75%:25% FF, and P5 = wheat flour 70%:30% FF.

Fermented Purple Yam Flour (Aini et al., 2016; Ulyarti, Yulia, Nazarudin, Armando, & Erawaty, 2021)

Purple yam tubers were washed, peeled, and sliced into 5 mm thickness, then soaked in water (ratio of purple yam and water = 1:2), and 2% tape yeast (20 g/L) was added. After fermentation for 40 hours at a temperature of 30°C, the sliced tubers were drained and rinsed until the pH of the washing water was neutral. The oven drying process lasted for 24 hours at a temperature of 33 °C or until the chips were easily broken. Subsequently, the purple yam chips were ground and sieved using a standard 60 mesh sieve. Finally, the flour is packed in plastic clips and stored at room temperature.

Bread Processing (Ulyarti & Nazarudin, 2022)

The necessary materials are prepared in advance, after which the ingredients are placed in the bread maker with an initial volume of 90 mL water. The ingredients included 150 g flour (the amount of wheat flour and modified purple yam flour depends on the treatment), 15 g sugar, 2.25 g instant yeast, 2.7 g salt, 12 g butter, and 4.5 g full cream milk powder. The bread maker is then turned on by selecting a minimal weight and medium crust color level, and the dough was fermented twice for 35 and 60 minutes. In between the fermentation, the dough was kneaded for 15 minutes and the baking process was carried out at 180°C for 25 minutes. The baked bread is removed from the pan, allowed to cool to room temperature, and ready for analysis.

Moisture Content

Moisture content is determined using the oven method (AOAC, 1995).

Porosity (Kurniawan, Waluyo, & Sebayang, 2011)

The inside of the bread was cut into 4x4 cm size and scanned and the porosity was measured on the scan result using ImageJ software. Each sample photo was cut to 3x3 cm, loaded into ImageJ, and set to 8 bits. The process involved in measuring the percentage area includes a selection of analyzing the menu, then setting measurements and checking the measured checklist. By selecting the image menu and adjusting the threshold, the image undergoes changes and the pores become more visible. Additionally, the image was analyzed for pore by selecting the menu and conducting measurements. Finally, porosity is expressed in percent area, and it is calculated by dividing it by the total pore area.

Specific loaf volume (Marti et al., 2017)

Specific loaf volume (SLV) is the volume ratio of a bread to its weight and calculated using Equation 1 and 2. The volume of the mold was measured by inserting the beads of 1cm in diameter until a flat surface is achieved. Afterward, the volume of the beads is calculated using a measuring cup and recorded as V1. The volume of the bread was then measured by filling the mold containing the bread with beads and was recorded as V2.

$$\text{Bread volume} = \text{V1} - \text{V2} \quad (1)$$

$$\text{Specific loaf volume (SLV)} = \frac{\text{Bread volume}}{\text{Bread weight}} \quad (2)$$

Anthocyanin content (Ulyarti, Nazarudin, & Lisani, 2019)

A total of 1 g mashed bread sample was dissolved in 10 mL of acidic methanol, containing 95% methanol and 1 N HCl 85:15, in a centrifugation tube. Nitrogen gas was added and shaken for 30 minutes, then centrifuged at 3000 rpm for 10 minutes. The supernatant was measured with a UV-Vis spectrophotometer at wavelengths of 535 nm and 700 nm. The Equation 3 is used to determine anthocyanin content.

Anthocyanins (mg cyanidin 3-glucoside equivalent/g sample) =

$$\frac{(A535-A700)}{e} \text{ x (total volume of methanol extract) x BM x (1/wt)} \quad (3)$$

Antioxidant Activity (Molyneux, 2004)

The test was conducted by dissolving 1 g of the bread sample in 10 mL methanol, and vortexed for 1 minute. The antioxidant activity was determined by adding 0.2 mL of the solution to the vial and 3.8 mL of 0.05 μM DPPH solution. Furthermore, the mixture was homogenized and left in the dark for 30 minutes and absorption was then measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The positive control used was ascorbic acid, administered under the same treatment as the sample. Finally, the Equation 4 is used to calculate the antioxidant activity.

$$Inhibition\ (\%) = \frac{Sample\ absorbance - Control\ absorbance}{Control\ absorbance}\ x\ 100\% \quad (4)$$

Sensory Test

Sensory testing was conducted to determine the level of preference or feasibility of the product, to ensure accessibility by panelists or consumers. The method used was the hedonic approach (preference test) with 25 untreated panelists, which covered color, aroma, taste, texture, and overall acceptance. During this process, the panelists were asked to provide an assessment based on their preference level using 5 levels, namely "like very much", "like", "quite like", "dislike", and "dislike very much", with scores of 5, 4, 3, 2, and 1, respectively.

Data Analysis

The data obtained were analyzed using ANOVA, with 1% and 5% levels of significance. In a case where there is a significant effect of the treatment ($p<0.05$), the data were further analyzed using Duncan new Multiple Range Test (DnMRT).

RESULTS AND DISCUSSION

Bread Physical Characteristics

Moisture content is a chemical characteristic that is very influential on food products because it affects the appearance, texture, and taste of food, as well as determines the freshness and durability of the food material. Although there is no statistically significant difference, Table 1 shows that the moisture content of the bread tends to increase in direct proportion to the concentrations of fermented purple yam flour. The content of purple yam starch is higher than the wheat flour counterpart. According to Winarti (Winarti, Sunarti, & Richana, 2011), the starch content of purple yam flour is 82.12%. The range in the moisture content of loaves in this study was between 26.04% to 29.90%. This

Table 1. Physical properties of bread with different levels of FF concentration

FF concentration (%)	Moisture content (%)	Porosity (%)	Specific loaf volume (cm^3/g)
0	26.04	35.27±4.4[b]	3.37±0.1
10	26.91	34.26±4.4[ab]	3.34±0.3
15	27.25	31.23±4.3[ab]	3.20±0.1
20	28.79	29.00±6.0[ab]	3.10±0.1
25	29.30	27.18±1.7[ab]	3.06±0.3
30	29.90	26.60±3.9[a]	2.90±0.2

Note: Numbers followed by the same letter in a column are not significantly different according to DnMRT ($p > 0.05$). Data for moisture content and specific loaf volume were not tested using DnMRT as the ANOVA test showed no differences in mean values data meets the criteria in SNI 01-3840-1995 regarding the Quality Standard of Bread, which states that the maximum moisture content is 40% w/w.

The porosity mean value of bread showed a decreasing trend with an increasing concentration of fermented purple yam flour. This indicated that bread was significantly affected by the concentration ($p<0.05$), as shown in Figure 1 and Table 1. The porosity is directly proportional to the expansion volume, which is influenced by the gluten formed from wheat flour and the protein-starch-sugar-fat matrix of the constituents (Xin et al., 2022). FF at a concentration of up to 30% produced an elastic dough structure that can help the development process. A similar result was obtained in fermented brown rice flour used in the steamed bread (Ilowefah et al., 2014). Fermentation changes the characteristics of the flour, specifically the crude fiber content, which decreases from 13.6% to 5.34%. In addition, there was an increase in water absorption, which is consistent with the report of (Ulyarti, Surhaini, Wahyuni, & Nazarudin, 2022). The decrease in fiber content and the increase in water absorption may help form a dough that is more resistant to gas, thereby enhancing better expansion.

The specific expansion volume is a ratio of the volume and weight of a bread product. A high rate of bread expansion provides an attractive appearance but will be less attractive when these parameters decrease. The volume expansion of loaves is one of the important factors in consumer acceptance. The bread with a considerable expansion volume suggests that the dough has a good capacity to bind CO_2 gas during fermentation (Justicia, Liviawaty, & Hamdani, 2012).

The ANOVA result showed that the substitution of wheat flour with fermented purple yam flour had no significant effect on the specific loaf volume, as indicated by $p>0.05$. This result confirms the ability of fermented flour to help in the development of a less-gluten dough (Monthe et al., 2019). The specific loaf volume of bread in this study was in the range of 2.9 cm3/g to 3.37 cm3/g, which was consistent with those reported previously for bread made with soy flour substitution (Jideani & Onwubali, 2009) ranging from 2.54 to 4.03 cm^3/g.

Anthocyanin Content

Anthocyanins are bioactive ingredients that occur naturally in purple yam flour and are unstable. The stability of this content is affected by pH, temperature, light, concentration, the presence of metal ions, oxygen, sugar content, enzymes, and the effect of sulfur dioxide (Cavalcanti, Santos, & Meireles, 2011). In Table 2, the variance analysis showed that the fermented purple yam flour concentration had a significant effect on bread anthocyanin contents, as indicated by $p < 0.05$. In addition, the anthocyanin contents in the purple yam flour ranged from 122 to 136 mg/g. A previous study showed that the baking process may reduce anthocyanin content by up to 28% (Cordeiro et al., 2021). In this study, a higher reduction in the anthocyanin content in the bread was observed. The factors responsible for the different heat stability may include the source and type of anthocyanin (Cordeiro et al., 2021).

Antioxidant Activity

Similar to anthocyanin content, the concentration of FF had a significant effect on the antioxidant activity

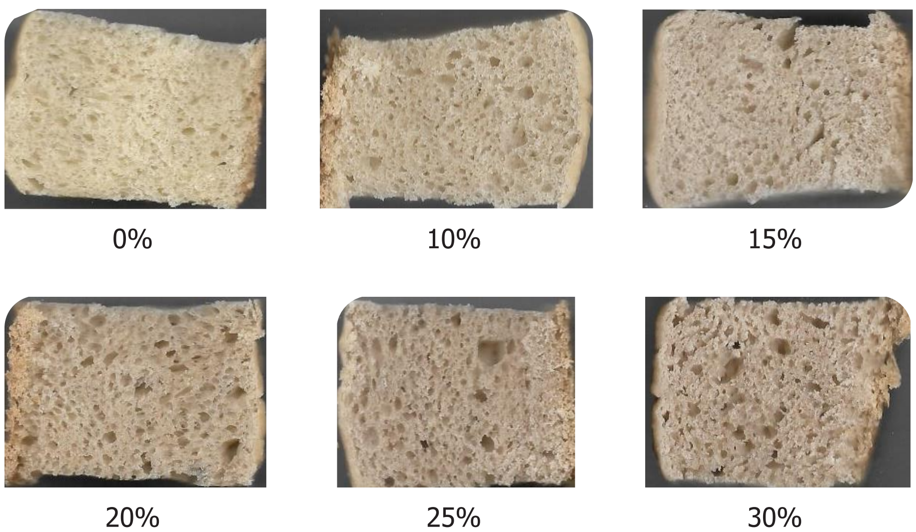

Figure 1. Image of the sliced bread with different levels of FF concentration

Table 2. Average values of anthocyanin contents and antioxidant activity of bread with various FF concentrations

FF concentration (%)	Anthocyanin contents (mg cyanidin 3-glucoside equivalent/g sample)	Antioxidant activity (%)
0	0[a]	16.50±2.5 [a]
10	3.86±0.08 [b]	53.98±0.2 [b]
15	4.03±1.0 [b]	55.94±0.2 [b]
20	4.52±1.7 [b]	60.32±0.2 [c]
25	5.30±2.5 [b]	61.03±3.5 [c]
30	5.55±2.2 [b]	62.43±1.1 [c]

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

Table 3. Sensory characteristics of the bread with different levels of FF concentration

Sensory Characteristics	FF concentration					
	0%	10%	15%	20%	25%	30%
Color	4.08±0.6[c]	3.60±0.8[abc]	3.48±0.7[ab]	3.56±0.7[abc]	3.88±0.8[bc]	3.16±0.9[a]
Aroma	4.00±0.4	3.52±0.5	3.56±0.5	3.68±0.8	3.68±0.1	3.52±0.5
Taste	3.84±0.4	3.72±0.6	3.60±0.6	3.56±0.7	3.92±0.4	3.36±0.8
Texture	3.88±0.6[b]	3.48±0.6[ab]	3.08±0.6[a]	3.24±0.8[a]	4.00±0.7[b]	3.44±0.9[ab]
Overall Acceptance	4.08±0.4[c]	3.80±0.6[bc]	3.36±0.7[a]	3.48±0.8[ab]	4.00±0.7[c]	3.40±0.7[ab]

Note: 5 (like very much), 4 (like), 3 (quite like), 2 (dislike), 1 (dislike very much)
Numbers followed by the same letter in a row are not significantly different according to DnMRT ($p > 0.05$). Data for aroma and taste were not tested using DnMRT as the ANOVA test showed no differences in mean values

of bread, as indicated by $p < 0.05$ (Table 2). Since anthocyanin function as an antioxidant, an increase in the total anthocyanin contents was positively correlated with a rise in the antioxidant activity of fermented purple yam bread. Furthermore, the level of FF substitution increases in direct proportion to the content of antioxidant compounds. According to Ulyarti et al. (2021), purple yam flour fermented by *Lactobacillus plantarum* for 48 hours has an antioxidant activity of 77.65%. A previous study observed lower antioxidant activities of all bread as an impact of heat instability during baking (Cordeiro et al., 2021). The antioxidant activity of the bread may also come from other not come compounds produced from the Maillard reaction, such as melanoidin, which is formed during baking (Shen, Tebben, Chen, & Li, 2018).

Sensory Properties

Color as the first trait seen by consumers is a part of the product appearance and is an important sensory assessment parameter. The addition of FF to the bread reduces the preference for bread color, as shown in Table 3. Furthermore, the purple color changed during baking as an impact of the heating instability of anthocyanin. Table 3 showed that the concentration of FF has no significant effect on the aroma and taste of the bread, as indicated by $p>0.05$. This is understandable because the taste and aroma of fermented purple yam flour are neutral. Texture, a parameter that greatly impacts consumer overall acceptance was significantly affected by the FF concentration.

CONCLUSION

In conclusion, the substitution of wheat flour with fermented purple yam flour significantly affected porosity, anthocyanin contents, antioxidant activity, color, texture, and overall acceptance of the bread. However, this substation had no significant effect on moisture content, specific loaf volume, aroma, and taste of the bread. Wheat could be substituted with FF to a maximum of 25% without changing its primary characteristics (specific loaf volume) and produced bread with the highest hedonic value.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest regarding the publication of this study.

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