

HASIL CEK_Development of Bangle Rhizome and Purple Sweet Potato Flour Biscuit and Its in vivo Antioxidant Activity in High-Fat Diet-Induced Rats

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Research Article

Development of Bangle Rhizome and Purple Sweet Potato Flour Biscuit and Its *in vivo* Antioxidant Activity in High-Fat Diet-Induced Rats

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ABSTRACT

Bangle rhizome and purple sweet potato could be used as functional food to overcome health problems such as hyperlipidemia. The anti-hyperlipidemic, nutritional properties of the above-said root vegetables could be formulated into a more community-preferred food in the form of biscuits. For this reason, this study aims to formulate biscuits from the mixed flour of bangle rhizome and purple sweet potato. Next, the prepared product's antioxidant activity and lipid-lowering properties are tested *in vivo* in high-fat diet-induced Wistar rats. In this study, bangle rhizome and purple sweet potato were turned into flour and formulated into three types of biscuits comprising different ratios of bangle rhizome and purple sweet potato flour (5:39 % w/w (F1), 3:41 % w/w (F2), and 2:42 % w/w (F3)). The study found that the baked products showed good organoleptic and physical properties, yielding golden- to brown-colored biscuits with a distinctive aroma and vaguely bitter after-taste, with F3 showing the highest hardness (8.94 0.18). The proximate analysis test showed that the biscuits achieved three of the six SNI 01-2973-2011 quality requirements. The best formula (F3) exhibited acceptable *in vivo* antioxidant catalase (5.12 0.16 U/mL) and glutathione peroxidase activity (64.44 2.11 U/mg) in high-fat diet Wistar rats tested for 28 days. The F3 formula was deemed the best, yielding biscuits with low moisture content and good crispiness. The formulated biscuits increased catalase's antioxidant activity (285.47%) and glutathione peroxidase (265.08%) more than the negative control. Hence, the study demonstrated that bangle rhizome and purple sweet potato-containing biscuits were potentially useful functional foods for improving antioxidant activity in high-fat diet-induced Wistar rats.

Keywords: Antioxidant, Bangle rhizome, Biscuit, Hyperlipidemia, Purple sweet potato

Introduction

Metabolic disorders can contribute to cardiovascular damage, a risk exacerbated by obesity, characterized by low chronic inflammation with increased oxidative stress [1]. Reduced physical activity and an increased high-energy diet are two main contributors to obesity [2]. Therefore, the best option for mitigating obesity in individuals is by reducing food portions and consuming low-energy-dense foods [3]. Consuming functional foods

containing active compounds could also be an option for weight management and overcoming metabolic disorders [4].

Biscuits are one of the popular foods among consumers and, if innovatively formulated, could be helpful as functional foods to address food-related disorders [5]. However, biscuits are energy-dense food because wheat flour, sucrose, and fat are the main ingredients [6]. For a healthier option, the wheat-based flour in biscuits could be substi-

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tuted with bangle rhizome- and purple sweet potato flour. Hence, consumers could reap the health benefits of these root vegetables to deal with food-related ailments.

According to the literature, bangle rhizome (*Zingiber cassumunar* Roxb.) and purple sweet potato (*Ipomoea batatas* L.) are plants commonly used as food and medicine. Bangle rhizome contains various active compounds such as phenylbutanoid, cyclohexene derivatives, vanillin, terpenoids, sitosterol, and curcuminoids [7]. The active components in bangle rhizomes are said to increase antioxidant activity and minimize the side effects of a high-fat diet [8]. Conversely, the anthocyanin-rich purple sweet potato can significantly reduce aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) levels against CCl₄ injury. Anthocyanins are powerful antioxidants capable of restoring metabolic activity in our bodies by normalizing superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels [9].

For this reason, this study aimed to determine the best ratio of the bangle rhizome- and purple sweet potato flours to make organoleptically acceptable biscuits. The biscuits were then assessed for their organoleptic and physical properties, followed by testing *in-vivo* for antioxidant activity in high-fat diet-induced Wistar rats.

Material and Methods

Preparation of bangle rhizome and purple sweet potato flour

Bangle rhizome and purple sweet potato were washed under running water to remove soil and dirt before peeling. The flour was then sun-dried for 3-4 days, milled, and then sieved through a 100 mesh sieve.

Preparation of bangle rhizome and purple sweet potato biscuit

To prepare the biscuits, the ratio of the bangle rhizome- and purple sweet potato flour were investigated for 5:39%, w/w (F1), 3:41%, w/w (F2), and 2:42%, w/w (F3), to yield three types of biscuit formula. Other ingredients we held constant and made up the total weight of biscuit dough: egg yolks (15%), low-fat margarine (15%), low-fat milk (4%), refined sugar (21.5%), and baking powder (0.5%). Firstly, the biscuit was prepared by beating the margarine and egg yolks until the color of the mixture turned pale. Then, milk

powder was added and the mixture was beaten for a further 1-5 mins. Next, the bangle rhizome- and purple sweet potato flour were added and homogenized using a mixer for 5-10 mins. The dough was placed into a mould lined with a margarine-greased baking sheet and baked at 150°C for 35 mins. The biscuits were left to cool (30 min) and stored in an airtight container before analysis.

Organoleptic observation of biscuit

The formulated organoleptic properties of the prepared biscuit were based on the Standar Nasional Indonesia (SNI 2973-2011). The tests relied on human senses to describe the powder form and the normal smell, taste, and color according to the raw materials used. In this study, feedback from a total of 3 randomly selected consumers was used in gauging the organoleptic properties of the formulated biscuits.

Physical analysis of biscuit

A five-piece stack of biscuits was weighed, then the average weight was calculated [10]. The thickness of the stacked biscuits was also measured using a digital caliper, and the values were averaged in units (mm) [11]. Meanwhile, the hardness of each biscuit type was measured using a penetrometer.

Proximate analysis of flour and biscuit

The flour and biscuit samples were then subjected to proximate analysis for the following parameters: moisture, ash, protein, fat, and crude fiber, determined according to the AOAC procedure [12]. Meanwhile, the carbohydrate contents were calculated by the difference in the values.

Total Plate Count (TPC) analysis of biscuit

The biscuits were milled until smooth and then dissolved in Butterfield's phosphate buffer (BPB) to prepare a dilution series of 10⁻¹ to 10⁻⁴. A 1 mL aliquot was transferred from each dilution into a sterile petri dish and duplicated. Then, molten Plate Count Agar (PCA) agar medium (12-15 mL, 45°C) was poured into each petri dish and then incubated at 37°C for 48 h. Then, only petri dish containing 25 to 250 colonies were use for the colone enumeration (Eq. 1).

$$\text{TPC (colonies/g)} = n \times F \dots \dots \dots 1$$

Where n is the average colony of two Petri dishes from one dilution, expressed in colonies per gram (colonies/g), while F is the dilution factor of the selected colony mean.

In Vivo Antioxidant Activity Test Animals

For brevity, this research which used 24 male Wistar rats, was approved by the Ahmad Dahlan University Research Ethics Committee (No. 012105028). Only male Wistar rats were used in this study to reduce hormonal influences. Before the treatment, all experimental animals were acclimatized for 7 days.

Experimental design

Twenty-four male Wistar rats were divided into four groups; the first group was given the standard feed, and the negative control group was given high-fat diet (HFD) feed. Meanwhile, the positive control group was given HFD and Nutrive benecol 9 mL/kg BW a day. The treatment group was given HFD and the F3 biscuit at 1,944 g per day. The HFD was a mixture of standard feed 300 g, egg yolks 20 g, butter 100 g, and meat fat 10 g, before pelleting and dried. The feed treatment on the rats was done for 28 days. The animals were sacrificed and dissected on the 29th day, and the livers were harvested for subsequent analysis.

Liver homogenate preparation

A 2.5 g rat liver was weighed and transferred into phosphate buffer saline (PBS) solution (10 mL) containing KCl solution (23 g/L) and finely chopped in cold conditions. The slurry was centrifuged at 4000 rpm for 10 min at 4°C until a clear supernatant (homogenate) was obtained. This homogenate was analyzed for the antioxidant activity of glutathione peroxidase and catalase enzymes [13].

Measurement of glutathione peroxidase (GPx) activity

A 200 µL aliquot of the clear liver supernatant was transferred into 200 µL of phosphate buffer (0.1 M, pH 7.0) containing EDTA (0.1 mM), 200 µL of reduced glutathione (GSH) (10 mM) and 200 µL of glutathione reductase (2.4 units). The mixture was incubated for 10 min at 37°C before NADPH (200 µL, 1.5 mM) was added and incubated for a further 3 min. Next, 200 µL H₂O₂ (1.5 mM) was added to the mixture and vortexed. The absorbance was read with a

spectrophotometer (Shimadzu UV-1280) at 340 nm after 1-2 min incubation. The Glutathione peroxidase (GPx) was calculated as follows (Eq. 2):

$$GPx \text{ (M unit)} = \frac{Abs \times Vt \times 2 \times 1000 \times 1/mg \text{ protein}}{6,22 \times Vs}$$

..... 2

Remarks:

Abs = change in absorbance

Vt = total volume (ml)

6.22 = 2 moles of GSH, which is equivalent to 1 mole of NADPH

1000 = change from 1 to milli units

Vs = Sample volume

Measurement of catalase (CAT) activity

The catalase activity was measured using the BioVision Catalase Activity Colorimetric / Fluorometric Assay Kit (Catalog #K773-100). The measurements were done according to the manufacturer's instructions.

Results and Discussion

Organoleptic observation of biscuit

Of the three biscuit formulas created in this study, all baked products yielded brown-colored biscuits with a distinctive aroma and a slightly bitter aftertaste. The outcome seen here was in line with the SNI 2973-2011 requirement, which states that the color of biscuits must be brown or golden yellow, with a normal or distinctive aroma and taste. Noteworthy, all three biscuits (F1 to F3) were crunchy, but the F3 had a slightly harder texture when broken. This was likely due to the higher purple sweet potato flour composition in F3 (rhizome flour: purple sweet potato flour, 2:42%, w/w). The higher amylose content correlated well with the increased hardness in the F3 biscuits. Literature has shown that a higher amylose content in dough reduces water absorption, consequently affecting the final texture of baked goods, i.e., biscuits [14].

Physical analysis of biscuit

The study found that the triplicated samples of the F1 to F3 biscuits showed relatively similar diameter, thickness, and hardness (Table 1). However, the F3 composition (rhizome flour: purple

Table 1. Physical analysis of bangle rhizome- and purple sweet potato flour biscuits

Biscuit	Thickness (mm)	Weight (gram)	Hardness (N)
F1	6.60 ± 0.01	11.60 ± 0.20	8.67 ± 0.37
F2	6.20 ± 0.00	11.55 ± 0.25	8.56 ± 0.40
F3	5.90 ± 0.01	11.68 ± 0.34	8.94 ± 0.18

Table 2. Proximate analysis of bangle rhizome and purple sweet potato flour biscuits

No	Parameter (%)	Result Analysis			SNI (2973:2011)
		F1	F2	F3	
1.	Moisture Content (%)	7.48 ± 0.07 [#]	7.04 ± 0.02 [#]	6.52 ± 0.00 [#]	<5%
2.	Ash Content	2.75 ± 0.04 [#]	3.14 ± 0.03 [#]	3.00 ± 0.06 [#]	< 1.6%
3.	Fat content	18.82 ± 0.13 [*]	18.22 ± 0.03 [*]	18.30 ± 0.14 [*]	Min 9.5 %
4.	Protein content	5.84 ± 0.09 [*]	6.00 ± 0.22 [*]	6.00 ± 0.16 [*]	(5-9)%
5.	Carbohydrate Content	65.09 ± 0.07 [*]	65.55 ± 0.20 [*]	66.16 ± 0.26 [*]	<70%
6.	Crude Fiber Content	1.70 ± 0.48 [#]	1.58 ± 0.28 [#]	1.37 ± 0.05 [#]	Max. 0.5%

Notes: (*) meets the quality requirements, and (#) does not meet the quality requirements of SNI 01-2973-2011

sweet potato flour, 2:42%, w/w) exhibited the highest fracture strength (hardness). Hence, the biscuits' hardness was flour composition-dependent, aside from the consequence of ingredients that interacted during baking [15].

Proximate analysis of biscuit

Table 2 enlists the proximate analysis results for biscuits F1 to F3. As can be seen, several parameters did not meet the requirements of SNI 2973-2011. The affected parameters were water content (6.52 0.00 to 7.48 0.07 %), ash content (2.75 0.04 to 3.14 0.03 %), and total fiber content (1.37 0.05 to 1.70 0.48 %). Moisture content is one of the important parameters in biscuits, as low moisture content increases shelf-life and potentially reduces microbial contamination [16].

Of the three formulas, the moisture content was outside the permissible range stated in the SNI 01-2973-2011 (<5%), with F3 exhibiting the lowest water content (6.525%). The lower water content in F3 might be related to the inherently lower water content for the purple sweet potato flour (8.81 ± 0.14) (Table 3). Conversely, the higher the moisture content of the bangle rhizome flour (9.22%), the also increases water- and moisture contents of the resulting biscuits F1 and F2 (7.48 and 7.04%, respectively) (Table 2). The higher water content in these two biscuit groups is also influenced by the flour's water-binding capacity used in the formulation (F1 and F2) [17].

In addition, the fiber content in the biscuits also did not meet the SNI 2973-2011 standard (maximum 0.5%). It was evident that the bangle rhizome- and purple sweet potato flours could in-

crease the biscuits' fiber contents. This is nutritious as foods with high crude fiber content have lower calories, sugar, and fat. These traits are useful in reducing obesity and heart disease [18].

Total Plate Count (TPC) analysis of biscuit

According to the literature, the Total Plate Count (TPC) is one of the parameters that determines food quality. That said, the number of microbial colonies contained in biscuits must not exceed the maximum allowable limit at 1×10^4 colonies/g, based on SNI 2973-2011. Results revealed that the microbial (TPC) in the F1 to F3 formulas were 3.0×10^2 colonies/g (F1); 2.6×10^3 colonies/g (F2); 2.3×10^3 colonies/g (F3), all of which were within the specified SNI quality.

Antioxidant activity of biscuit

In this study, we chose F3 as the best formulation for the subsequent antioxidant activity test, and the results are depicted in Figure 1. The negative control group showed a significantly decreased antioxidant activity (catalase and glutathione peroxidase ($p < 0.05$) compared to the normal group. Meanwhile, the positive control group showed an increase in antioxidant activity compared to the negative control group ($p < 0.05$). The outcome here assented to our earlier study that showed that HFD, which elevates cholesterol and triglyceride levels, was inversely proportional to reduced antioxidant activity [19]. A high-fat diet could lead to hypercholesterolemia, thrombus accumulation, and free radicals. Subsequently, atherosclerosis from the damaging action of free radicals was due to increased lipid peroxidation, which elevated oxidative stress [20].

Table 3. Proximate analysis of bangle rhizome and purple sweet potato flour

No	Parameters (%)	Result Analysis	
		Bangle Rhizome Flour	Purple sweet potato Flour
1.	Moisture Content	9.22±0.35	8.81±0.14
2.	Ash Content	6.42±0.06	2.61±0.03
3.	Fat content	8.84±0.13	0.53±0.13
4.	Protein content	5.58±0.63	3.76±0.01
5.	Carbohydrate Content	69.92±0.35	84.92±0.22

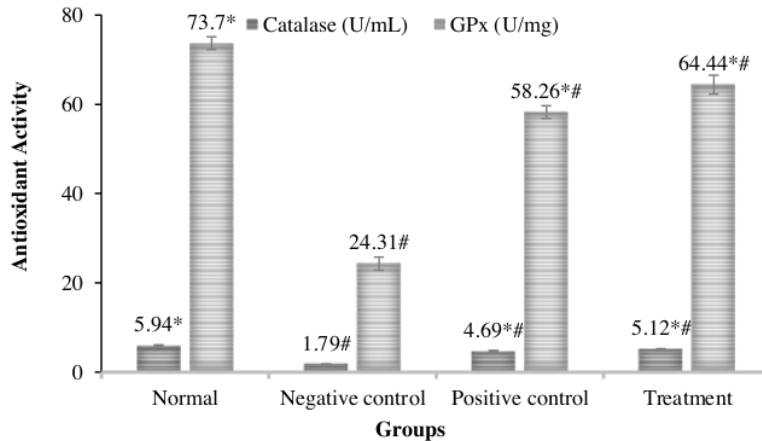


Figure 1. Catalase and glutathione peroxidase (GPx) activity of bangle rhizome- and purple sweet potato flour biscuits. Notes: * significantly different than the negative control group ($p < 0.05$), # significantly different than the normal group ($p < 0.05$).

As can be seen, the substitution of wheat flour with bangle rhizome- and purple sweet potato flours resulted in a significant increase in the antioxidant activity of GPx and catalase ($p < 0.05$) than to the negative control group. Catalase, SOD, GPx, and other antioxidant enzymes are important defense systems against oxidative stress. This is because the GPx and catalase catalyze the decomposition of H_2O_2 into oxygen and water molecules, which prevents H_2O_2 from damaging the cells [21].

Bangle rhizome flour containing oils showed antioxidant activity that inhibited 2,2-diphenyl-1-picrylhydrazyl radicals [22]. The phenolic compounds in bangle rhizomes are natural antioxidant agents that could reduce the activity of free radical species [23]. Plus, anthocyanins in the purple sweet potato flour guard against inflammation triggered by oxidative stress, in addition to reducing various other oxidative stress markers. This mechanism reduces enzymes that promote the proliferative process and protect against reduced nitric oxide levels [24]. In addition, polyphenols can

increase GSH levels [25]. Similarly, GSH is another antioxidant that provides reducing equivalents for glutathione peroxidase (GPx) in catalyzing the reduction of H_2O_2 and lipid hydroperoxides [26].

Conclusion

In this study, the biscuits created from a mixture of bangle rhizome- and purple sweet potato flour were found effective in boosting the activity of antioxidant enzymes GPx and catalase in rats induced by HFD.

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