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Proximate and antioxidant analysis of steamed cake made from flour of green bean sprout (*Vigna radiata*) and ambon banana peel (*Musa paradisiaca* var. sapientum (L.) Kunt.) as main ingredients

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Abstract: This research aims to determine: (1) antioxidant levels in banana peel flour and mung bean sprouts; (2) protein, water, fat, carbohydrate and ash content of steamed cake made from ambon banana peel flour and green bean sprout flour. This type of research is experimental. The DPPH method was used to analyze antioxidants, Kjeldahl for protein analysis and Soxhlet for fat analysis. The research findings revealed that the antioxidant activity was weak (165.58 ppm) in banana peel flour and very weak (360.30 ppm) in green bean sprout flour. The highest protein content of 7.01% was found in sample T_0. The highest fat content of 0.77% was found in sample T_0. The highest carbohydrate of 53.44% was found in sample T_0. The highest water content of 43.95% was found in sample T_2. The highest ash content of 1.15% was found in the T_3 sample.

Keywords: Antioxidants, steamed cakes, green beans, ambon bananas, proximate, flour

Abstrak: Riset ini bertujuan mengetahui: (1) kadar antioksidan pada tepung kulit pisang dan kecambah kacang hijau; (2) kadar protein, air, lemak, karbohidrat dan abu dari bolu kukus yang terbuat dari tepung kulit pisang ambon dan kecambah kacang hijau. Jenis penelitian ini adalah eksperimental. Metode DPPH digunakan untuk menganalisis antioksidan, Kjeldahl untuk analisis protein dan Soxhlet untuk analisis lemak. Temuan penelitian mengunkap bahwa aktivitas antioksidan lemah (165,58 ppm) pada tepung kulit pisangdan sangat lemah (360,30 ppm) pada tepung kecambah kacang hijau. Kandungan protein tertinggi sebesar 7,01% terdapat pada sampel T_0. Kandungan lemak tertinggi sebesar 0,77% terdapat pada sampel T_0. Karbohidrat tertinggi sebesar 53,44% terdapat pada sampel T_0. Kandungan air tertinggi sebesar 43,95% terdapat pada sampel T_2. Kandungan abu tertinggi sebesar 1,15% terdapat pada sampel T_3.

Kata kunci: Antioksidan, bolu kukus, kacang hijau, pisang ambon, proksimat, tepung

INTRODUCTION

The steamed cake is a type of cake that is enjoyed by all ages, both children and adults (Sari et al., 2019). This cake is usually made from wheat flour. Wheat flour causes dough to rise due to the gluten content. The use of excessive flour can be harmful to health. The high glycemic content increases blood sugar levels (Singh et al., 2020; Yanti et al., 2019). Efforts to improve the quality of steamed cake can be seen from the quality of flour as the main ingredient, including antioxidant content. Antioxidants function to prevent oxidation or release oxidized compounds, by contributing hydrogen atoms or electrons (Sinaga, 2016;

Ulmillah et al., 2022; Yadav et al., 2016). Natural materials expected to be antioxidant sources are green bean sprouts and ambon banana peels.

Green bean sprouts contain vitamins C, E, and A, riboflavin, K, niacin, thiamin, pantothenic acid, vitamin B6, folate, choline, and β-carotene. Essential amino acids found in green bean sprouts include tryptophan, methionine, threonine, leucine, isoleucine, phenylalanine, lysine, and valine. The mineral content includes calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), copper (Cu), sodium (Na), selenium (Se), potassium (K), manganese (Mn) and zinc (Zn) (Fanzurna & Taufik, 2020).

The banana peel has high nutritional value, particularly vitamins, and minerals, so it can be used as a flour ingredient. The content of ambon banana peel is fiber and antioxidant. Antioxidant compounds in ambon banana peel are catechins, gallocatechin, and epicatechins and are classified as flavonoids. Based on previous research, it is known that ambon banana peel has a relatively high antioxidant activity compared to the fruit flesh. The antioxidant activity of ambon banana peel reaches 92.25% at a concentration of 125.00 mg/ml while in the fruit flesh only 70.00% at a concentration of 50.00 mg/ml (Cahyani et al., 2019).

The combination of using green bean sprout flour and banana peel flour as the main ingredients of steamed cake can increase the antioxidant content. In addition, both types of flour can function as an alternative to wheat flour, which is commonly used in making steamed cake. Wheat flour is not suitable as a basic ingredient in cake and consumed by people with diabetes mellitus because the glycemic content can trigger an increase in blood sugar levels (Yanti et al., 2019). Therefore, in addition to knowing the antioxidant content, it is also important to know the quality of the nutritional content of the steamed cake. Proximate analysis is one method that can be used to determine protein, fat, moisture, ash, and carbohydrate levels. By conducting this analysis, it is hoped that the nutritional content in both types of flour is good and safe for people with diabetes mellitus.

The purpose of this study is to determine the antioxidant level test on flour of green bean sprouts and flour of banana peel, determine the protein level, fat level, water level, ash level and carbohydrate level in steamed cake from flour of green bean sprouts and flour of ambon banana peel.

METHOD

This research was conducted in July at the Laboratory of Biology II, Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA), University of Lampung. The type of research is experimental. This research uses a Completely Randomized Design (CRD) with four different treatment variations (Table 1).

The Making of Green Bean Sprout Flour

Five hundred grams of green beans are soaked in 500 ml water for 12 hours. Then they are drained and rinsed again with clean water, and then spread out in a container with

holes lined with cotton or cloth and covered with a damp cloth. The green beans are sprayed with water using a hand sprayer every 6 hours. The sprouts that are produced are drained first and then sun-dried for 5 days, and then dried in an oven at a temperature of ±40°C until a certain degree of dryness is achieved. The dried sprouts are then peeled, ground and sieved into flour (Dirga, 2018).

Table 1. The formulation for making steamed cake

Material	Quantity Concentration			
	100% (T_0)	**80% (T_1)**	**60% (T_2)**	**40% (T_3)**
Wheat Flour (g)	250	200	150	100
Green Bean Sprout Flour (g)	0	25	50	75
Ambon Banana Skin Flour (g)	0	25	50	75
Powdered Milk (mL)	150	150	150	150
SP (tsp)	1	1	1	1
Chicken Egg (pieces)	2	2	2	2
Granulated Sugar (g)	220	220	220	220

Note:
T_0 = 100% (wheat flour (Control))
T_1 = 80 % (200g wheat flour : 25g green bean sprout flour : 25g banana peel flour)
T_2 = 60% (150g wheat flour : 50g green bean sprout flour : 50g banana peel flour)
T_3 = 40% (100g wheat flour : 75g green bean sprout flour : 75g banana peel flour)

The Making of Banana Peel Flour

Wash the ambon bananas. After washing the fruit, steam it and remove the banana peel. Before drying, the banana peels are sun-dried for a week to reduce the moisture content and prevent sticking to the grinder. Then, drying is done for 1.5 hours at a temperature of ±40°C. After drying, it is ground using a grinder and sieved into flour (Cahyani et al., 2019).

Making Steamed Cake

The procedure for making steamed cake is to heat the steamer with medium heat. Add 220 g of granulated sugar, 2 eggs, and 1 tsp of baking powder into the mixer, then mix at high speed until it becomes white and thick. Reduce the mixer speed, add wheat flour, sprouted green bean flour, and ambon banana peel flour according to the concentration, then add 150 ml of milk powder. After that, mix at high speed until the dough is evenly mixed. Pour the dough into a steamed cake mold that has been lined with paper cups until it is full. Steam at 100°C for 20 minutes.

Extracting Flour

330 g of sprouted green bean flour is macerated with 96% ethanol solvent. Then, the crude is divided into three sample parts, 110 g each. Next, the crude is dissolved in ethanol in the Erlenmayer tube. Then, the mixture is stirred until it is evenly mixed and

closed tightly and wrapped in black plastic, then left for 72 hours. Every 24 hours, stirring is done at a speed of 50 rpm for 15 minutes. After 72 hours, the mixture is filtered. Then, it is immersed again for 24 hours, filtered again and then concentrated with rotary vacuum evaporator at 50°C. And evaporated using waterbath until a thick extract is obtained (Syafarina et al., 2017). Then, the same steps are taken to make the extraction of ambon banana peel flour.

Antioxidant Test

2 mL of test flour solution of kecambah with concentrations (25, 50, 75, 100 and 125 g/L) were added with 1 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution and 0.8 mL f tris HCL buffer, and vortexed for 2 minutes. Then, the absorbance was measured during e last 5 minutes out of 30 minutes incubation using a spectrophotometer with a wavelength f 517 nm (Wulan et al., 2019). The antioxidant level was determined using the following quation:

Inhibition (%)=(Abs. Control-Abs. Sample)/(Abs. Control) x 100%

The parameter used is IC_{50}. IC_{50} is the concentration needed to reduce DPPH by 50%, and it is calculated using linear regression. The % inhibition is the y-axis, and the concentration of the sample is the x-axis. The smaller the IC_{50}, the stronger the antioxidant level (Wulan et al., 2019). Using the equation y = a + bx, the IC50 value is computed: (Rahman et al., 2016).

y = a + bx
50 = a + bx
(x) $IC_{50} = \frac{50-a}{b}$

Protein Content Test

Destruction Stage

Weigh 2 g of steamed cake and grind it. The fine sample is put into a Kjeldahl flask, and a boiling stone is added. Then add 5 g K_2SO_4, 200 mg $CuSO_4$ and 30 ml of concentrated H_2SO_4 to the flask. All the materials in the flask are heated in an acid cabinet, first with a small fire, and after the smoke disappears, the fire is heated. The heating is ended until a clear green liquid is obtained. Then cooled, and then add aquades to a total volume of 150 ml. Then add 50% NaOH slowly until the liquid is basic (Purnama et al., 2019).

Distillation Stage

Immediately attach the Kjeldahl flask to the distillation apparatus. Then perform the distillation quickly until the ammonia is completely evaporated. The distillate is collected in an Erlenmeyer flask that has been added with 50 ml of 0.1 N HCl and 3 drops of phenolphthalein indicator 1%. Make sure the glass pipe of the distillator enters into the 0.1

N HCl solution. The distillate is ended with a drop of distillate that does not react to base (Purnama et al., 2019).

Titration Stage

The distillate obtained is titrated with a standard NaOH (0.1 N). The end of titration is marked by a change in color of the solution to light red (Munthe et al., 2016).

Blank Treatment

The blank treatment is done in the same way as above, but in the blank only aquades is used instead of steamed cake (Munthe et al., 2016).

Calculation

Determining protein content is calculated using the following formula: (Sumantri, 2018)

$$\frac{\text{v NaOH blank-VNaOHsample}}{\text{berat sample}} \text{x N NaOH x 14,008 x 100\% x fk}$$

% protein = % N x convertion factor.

The Test for Lipid Content Using the Soxhlet Method

Weigh 1 g of steamed cake, put it into a paper thimble that has been lined with cotton. Then stuff the paper thimble containing the sample with cotton, dry it in an oven at a temperature of ± 80°C for 1 hour. After that, put it into a Soxhlet apparatus that has been connected to a flask containing dried boiling stones and known weight. Extract with hexane solvent for 6 hours. Then the solvent is distilled. Dry the lipid extract in an oven at a temperature of 105°C. Then cool it in a desiccator and weigh (Angelia, 2016).

Lipid content (%)= $\frac{W - W1}{W2}$ x 100%

W = Sample weight (g)

W1 = Lipid weight before extraction (g)

W2 = Lipid weight after extraction (g)

The Test for Water Content

Fifteen minutes are spent in an oven heated to 105 °C drying porcelain cups. After 15 minutes of drying in a desiccator, the sample was weighed. Five grams of steamed cake sample is placed in a porcelain cup and then dried in an oven at ± 105^{0}C for six hours. Next, it was weighed after 30 minutes in a desiccator (Sakul et al., 2019). Water content calculation:

Water content = = $\frac{\text{Initial sample weight - Final sample weight}}{\text{Initial sample weight}}$ x 100%

The Ash Content Test

A clean porcelain dish is heated in a furnace at a temperature of ± 400°C for 1 hour, then cooled in a desiccator for 1 hour and weighed. 5 g of the cake sample is placed in a porcelain dish and then placed in a furnace at a temperature of ±550°C for 1 hour. After that, it is cooled in a desiccator and weighed (Angelia, 2016).

Calculation of Ash Content:

$$\text{Ash Content (\%)} = = \frac{\text{Ash Weight}}{\text{Sample Weight}} \text{ x } 100\%$$

Test for Carbohydrate Content

The carbohydrate content is calculated by determining the difference between the amounts of fat, protein, water, and ash content (Sakul et al., 2019). The formula is as follows: % Carbohydrates = 100% - % (Protein + fat + ash + water)

RESULTS AND DISCUSSION

Antioxidant in Flour

This study used the DPPH technique with UV-Vis spectrophotometry at a wavelength of 517 nm for the antioxidant test. The antioxidant test results are shown in Table 2.

Table 2. Results of antioxidant testing

The sample	Concentration (ppm)	Average Absorbance	% Inhibition	Linear Equation	IC_{50} Value
Ethanol extracts of green bean sprout flour	25	0,254	53,82	y = -0,0091x +53,286 R^2 = 0,0207	360,30 ppm
	50	0,255	53,64		
	75	0,282	48,55		
	100	0,247	55		
	125	0,264	52		
Ethanol extracts of ambon banana peel flour	25	0,242	56	y = -0,0255x +54,219 R^2 = 0,0221	165,58 ppm
	50	0,298	45,82		
	75	0,213	61,27		
	100	0,300	45,45		
	125	0,258	53		

Table 2 shows that the green bean sprout flour sample obtained and IC_{50} value of 360.30 ppm and the ambon banana skin flour obtained an IC_{50} value of 165.58 ppm. The calculation results of the IC_{50} value of green bean sprout flour are categorized as very weak because it obtained a value of 360.30 ppm. The ambon banana skin flour obtained a value of 165.58 and is categorized as a weak antioxidant. The higher the% inhibition value of a material, the stronger the inhibitory ability of the material against free radicals but inversely proportional to the IC_{50} value.

The weakness of antioxidants in flour may be influenced by the drying and sun-drying time of green bean sprouts which is too long. In ambon banana skin, it is influenced by the

thickness during the process of taking the inner skin and heating during the boiling process. During boiling, tissue integration occurs in food materials causing direct substrate contact, leading to the extraction of antioxidant activity and oxidation reactions (Cahyani et al., 2019).

Proximate Analysis

Proximate analysis of steamed cake includes protein, fat, moisture content, ash content, and carbohydrates. The results of the analysis can be seen in Table 3.

Table 3. The results of the proximate analysis of steamed cake

Parameter	Test Results				SNI Requirements
	T_O	T_1	T_2	T_3	
Protein (%)	7,01	6,40	6,26	6,98	Minimum 9%
Water Content (%)	38,27	42,96	43,95	41,10	Maximum 5%
Fat (%)	0,77	0,27	0,21	0,45	Minimum 9,5 %
Carbohydrates (%)	53,44	49,52	48,59	50,32	Minimum 70%
Ash Content (%)	0,51	0,85	0,99	1,15	Maximum l 1,5%

Table 3 presents proximate analysis data. The table shows the test results of various parameters of the product against the SNI (Indonesian National Standard) requirements. Each column represents the results of each formula (T_O, T_1, T_2, T_3). It can be seen that the protein content is below the minimum requirement of 9%, the fat content is below the minimum of 9.5%, the water content is above the maximum of 5%, the ash content is above the maximum of 1.5%, and the carbohydrate content is below the minimum of 70%.

The protein content in formula T_0 is 7.01%, while T_1 is 6.40%. In T_2, the protein content is 6.26%, and in T_3 is 6.98%. The lowest protein content is in formula T_3. The protein content in green bean sprout flour (per 100g) is 28.50%. Germination for 48 hours is able to increase the protein content in flour. Germination for 48 hours has the most optimal germination time, giving the production of phenolic compounds and tocopherols (Dirga, 2018). In banana skin flour itself, it causes a decrease in protein content. This is because banana skin flour has a low protein content, banana skin flour has a protein content of 4.40% (Lestari et al., 2018).

Fat content in this study uses the Soxhlet method. The lowest fat content is in formula T_2 at 0.21%. The fat content is in the control formula at 0.77%. In formulas T_1 and T_3, fat content is obtained at 0.27% and 0.45%. The fat content in green bean sprout flour is 11.33%. This decrease in fat content is caused by the germination process, fat is a food reserve used as an energy source. Ambon banana skin flour has a fat content of 1.38% lower than green bean sprout flour (Rachim et al., 2020).

The highest moisture content is in formula T_3 and the lowest is in the control formula T_0. Ambon bananas contain high moisture, so ambon banana skin also has high moisture content. In steamed cakes with ambon banana skin, it has a rough texture. Coarse fibers have strong water absorption capabilities because they have large polymer sizes and hydroxyl

groups that can bind large amounts of water. The water absorbed by coarse fibers is a type of bound water, but the water evaporated from the fermentation process is free water (Paramita et al., 2020).

The ash content in this study does not exceed 4% of the total weight of food. Ash content indicates the mineral content in a food. The ash content of a material is influenced by technical factors during planting, such as the intensity of fertilization, soil type and climate. Fertile soil contains many minerals, which increases the mineral content of ambon banana skin. The greater the ash content of a material, the greater the mineral content in a food (Proverawati et al., 2019).

The highest carbohydrate content is in formula T_0 as a control. The carbohydrate content in ambon banana skin flour is 25.09%. The main function of carbohydrates itself is as one of the main sources of body energy. While in ambon banana skin flour has a carbohydrate content of 46.41%. The carbohydrate content of legumes is usually between 24%-68%. The decrease in carbohydrate content is possibly caused by carbohydrates used as an energy source during germination (Rachim et al., 2020).

CONCLUSION

This research concludes the antioxidant activity was weak (165.58 ppm) in ambon banana peel flour and very weak (360.30 ppm) in green bean sprout flour. The highest protein content of 7.01% was found in sample T_0. The highest fat content of 0.77% was found in sample T_0. The highest carbohydrate of 53.44% was found in sample T_0. The highest water content of 43.95% was found in sample T_2. The highest ash content of 1.15% was found in the T_3 sample.

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