



Total Phenolic Content, Antioxidant Activity and Glycemic Values of Non-Meat Burger Patties

Dzul Fadly^{1*}, Sulvi Purwayantie¹, Andi Imam Arundhana^{2,3}

¹ Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, 78124, Indonesia

² Nutrition Department, School of Public Health, Hasanuddin University, Makassar, Indonesia

³ Postgraduate Student, Central Clinical School, the University of Sydney, NSW, Australia

Abstract

Food choices with high antioxidant and low glycemic values may benefit the body's health. It will be thoughtful to know the antioxidant activity and glycemic values of the food that consumed. Such in the case of non-meat products, including patty burger, which intended for meat patty burger substitution. This study aims to analyze the values of total phenolic content, antioxidant activity, and glycemic values of the non-meat burger patty. The total phenolic content was determined by Folin-ciocalteu method. Antioxidant activity was determined by DPPH method. The glycemic values were determined by an incremental area under the curve (iAUC) method. The values of total phenolic content and antioxidant activity of product was in line. More phenolic content results in a higher antioxidant activity. The patty has a lower glycemic response compared to a reference food. It has a high glycemic index, but low glycemic load. In conclusion, Non-meat burger patty has phenolic substances result in antioxidant activity, while its consumption with the right serving size may contribute a low glycemic effect and protect blood glucose stability.

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1. Introduction

During the last decades, the need for functional food has been increased. Lifestyle changes likely tend to be “back to nature” driven people to increase the utilization of natural food to obtain several benefits such as antioxidants. Antioxidants have been known as essential substances related to non-communicable diseases (NCDs), such as cancer. Antioxidants may prevent the oxidation process, the initial step of degenerative disease development. One of the most well-known biochemical substances that have high antioxidant activity is a Phenols. This compound acts as an antioxidant, providing H⁺ to neutralize free radicals. (1) found that phenolic compounds may prevent deterioration through quenching of radical reaction responsible for lipid oxidation.

In addition to antioxidant compounds, the glycemic value of food is one of the essential aspects of utilizing food to maintaining a healthy body. The human body will respond to carbohydrate intake in the form of changes in blood glucose levels, which is represented as a glycemic response (GR), Glycemic Index (GI), and Glycemic Load (GL). In

* Correspondence : Dzul Fadly  dzul.fadly@faperta.untan.ac.id

normal conditions, the stomach will digest carbohydrate, which is then absorbed in the form of monosaccharides, thus affecting blood glucose level (2,3).

Therefore, food selection with low glycemic values may be beneficial for maintaining post-prandial blood glucose stability. The higher glycemic values the greater risk of degenerative diseases, including diabetes mellitus (2). This study was aimed to determine the total value of phenolic content, antioxidant activity, glycemic response, glycemic index, and glycemic load of non-meat burger patties products.

2. Materials and Methods

The study protocol has been approved by the Ethics Division of Faculty of Medicine, Tanjungpura University (No: 3964/UN22.9/DL/2019). The participants were also required to sign a consent form prior to interview process.

2.1. Materials

The *non-meat burger patties*, the main material of the study, was developed in the previous study (4). The materials used in antioxidant activity measurement were free radical DPPH, and materials used in total phenolic content measurement was reagent Folin-Ciocalteu. The materials used in glycemic values measurement were alcohol swab, mineral water, lancets, *EasyTouch* glucometer, glucose anhydrous as the reference food, non-meat burger patties as a test food.

2.2. Procedures

This study was experimental laboratory-based study conducted in Food Technology Laboratory, Tanjungpura University in 2019.

2.2.1. Total Phenolic Content Analysis

The total phenolic content determination was executed by Folin-Ciocalteu colorimetric method (5). This method begins with the preparation of a blank solution and gallic acid as a standard solution. A blank solution was prepared by means of 2 mL 96% ethanol into a 10 mL test tube. Gallic acid solution was prepared by making a stock solution of 800 ppm concentration in 100 mL. Then, 10 mg of gallic acid was dissolved in 50 mL 96% ethanol in an extract bottle of 60 mL. Then dilution was carried out with concentrations of 0, 50, 100, 150, 200, and 250 ppm at a volume of 2 mL.

Sample preparation was initially prepared by homogenization. A total of 0.5 samples were extracted with 5 mL methanol, stored at room temperature for 2 hours in the dark. The sample was then centrifuged. The supernatant extract was then used for analysis. 25 μ L extract was oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. Leave for 60 minutes at room temperature. Then, the measurement of λ absorption at 760 nm. The total value of phenol was interpreted in milligrams equivalent to gallic acid per gram of extract (mg GAE / g extract). The determination of the GAE mg / g value was based on calculations from the simple linear regression equation of the gallic acid standard curve.

Total Phenolic calculation:

$$mg\ GAE/g = \frac{(x \times V\ total)/1000}{G}$$

Explanation:

x = Sample concentration (mg/L)

V total = Total volume of test solution (mL)

G = extracts weighed (g)

1000 = conversion factor to total solution volume (mL)

2.2.2. Antioxidant Activity

Antioxidant activity was determined by the DPPH method (6). The sample was diluted into methanol (1 mg/mL) with a concentration of 100 ppm. In a total volume of 1 mL, a test solution consisting of 500 μ L of sample and 500 μ L DPPH (125 μ M in ethanol) was added to the test solution. The test sample solution was dissolved and then allowed to stand at room temperature and dark for 30 minutes. Then, the absorbance was measured at λ 517 nm using a spectrophotometer.

$$\text{Inhibition (\%)} = (A - B) / B \times 100$$

Explanation:

A = absorption of control (0 mM)

B = absorption of sample (mM)

2.2.3. Glycemic Values

The glycemic values were including the glycemic response (GR), glycemic index (GI), and glycemic load (GL). The number of subjects used in determining the glucose response was 11 people. The subject must meet the inclusion and exclusion criteria. Inclusion criteria in this study were subjects aged 18-30 years; they have a normal body mass index (BMI), which is 18.50 - 22.90 kg / m² (7), and healthy. The exclusion criteria applied included having a history of diabetes (from parents, grandparents, and grandmothers); having a digestive problem; being on medication; drinking alcohol, and smoking. Both of these criteria were used to minimize the value of bias in measuring glycemic values.

The glycemic values measurement was carried out based on (8) with modifications (9). The reference food used in measuring the glycemic values in this study is 25 g of pure anhydrous glucose. The intervened test food was non-meat burger patties. The test food must be consumed equivalent to 25 g of available carbohydrate.

Subjects who meet the criteria were required to fast 10-12 hours before the intervention begins, except drinking mineral water. In the first week, the subject was given a reference food that is 25 g of pure glucose that had been dissolved in 250 ml of water and to be finished within a 5-10-minute consumption time limit. For two hours after consuming the reference food, blood samples (1-2 μ L) were taken with finger-prick capillary blood samples in a row at 0 minutes (before consumption), 30, 60, 90, and 120 using the EasyTouch® glucometer device. One week later, the same thing was done on test foods.

The GR obtained from each measurement time point was then made in the form of a curve with blood glucose as the Y-axis and time as the X-axis. The value of subject blood glucose after consuming the reference food and test food was known as the GR value at each measurement time. Measurement of the GR in this study was carried out on a reference food in the form of 25 g of pure glucose and a test food that is 160 g of non-meat patty formulation of F2 containing carbohydrates equivalent to 25 g of glucose.

The GI was then calculated by comparing the area under the glucose value curve between the test food and the reference food (pure glucose). The calculation method used was the incremental area under the curve (iAUC) method. This method is one of the best ways to calculate the GI (10). Furthermore, The GL was calculated by multiplying of glycemic index value by the amount of available carbohydrate in 1 serving size. The serving size of non-meat burger patty was 50 g with total carbohydrate was 14.66%.

Glycemic Index calculation:

$$GI = \frac{AUC\ TF}{AUC\ RF} \times 100\ %$$

Explanation:

AUC TF = The area under the curve of blood glucose of the test food

AUC RF = The area under the curve of blood glucose of the reference food

Glycemic Load calculation:

$$GL = \frac{A \times B}{100}$$

Explanation:

A = GI value of the test food

B = available carbohydrate in 1 serving size of the test food

2.3. Data Analysis

The results of the determination of total phenolic content and antioxidant activity were analyzed by one way ANOVA followed by Duncan's multiple range test using SPSS for windows. The result of blood glucose response after the intervention of reference and test food were recapitulated and processed by Microsoft Excel 2013. The GI then determined and was categorized as low (0 - 55), medium (56 - 69), and high (≥ 70). The GL was determined and was classified as low (0-10), medium (11-19), and high (≥ 20) (11).

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activity

Analysis of total phenolic content was performed on three formulations of the non-meat burger patty product (Table 1). There was no significant in phenol value between F1, F2, and F3 formula (0.04 ± 0.02 ; 0.10 ± 0.05 ; 0.09 ± 0.01 , respectively).

Table 1. Total phenolic contain and antioxidant activity.

Parameters	Non-meat patty burgers (means \pm SD)			P
	F1	F2	F3	
Total Phenolic Content (mg GAE/g)	0.04 ± 0.02^a	0.10 ± 0.05^a	0.09 ± 0.01^a	0.219
Antioxidant Activity (% inhibition on 500 ppm)	51.78 ± 1.88^a	68.43 ± 5.57^b	45.17 ± 5.76^a	0.003

Numbers followed with difference superscript in the same row considered statistically different ($p < 0.05$).

F1: kidney beans 30%: maize 30%: oyster mushroom 40%; F2: kidney beans 30%: maize 40%: oyster mushroom 30%; F3: kidney beans 40%: maize 30%: oyster mushroom 30%.

The antioxidant activity of non-meat burger patty products at a concentration of 500 ppm was $51.78 \pm 1.88\%$ in F1, 68.43 ± 5.57 in F2, and 45.17 ± 5.76 in F3 (Table 1.0). The percentage of inhibition of F1 was significantly different from F2, but not from F3 ($p < 0.05$). The percentage of inhibition of F2 was significantly different from both F1 and F3. Furthermore, the percentage of inhibition of F3 was not significantly different from F1, but from F2.

The total phenol content of non-meat patty burger was not significantly different between those three formulations. F2 has the highest phenol value. The higher phenol in a substance showed an increase in antioxidant activity. Some studies proved that polyphenols were one of the essential keys to inhibit the oxidative damage and were important contributors in determining the antioxidant capacity derived from plants (12,13). In addition, research conducted by (14) on local types of maize in Italy showed a positive correlation between total phenolic content and antioxidant activity measured by the ORAC method. In a study conducted by (15) on two types of oyster mushrooms showed that phenol compounds had an essential role in determining the antioxidant activity in mushrooms compared to its amino acid content. In oyster mushrooms, the more phenol would have better antioxidant activity. (16) and (17) found that antioxidant activity was determined primarily by the content of phenol values, which were the main indicators of antioxidant activity in legume. In legumes, 40 -71% of antioxidant activity was determined by phenol compounds and 20 – 39 % was by flavonol compounds

In non-meat patty burger formulation, the highest phenol and antioxidant activity were on a formula with more maize concentration. It seems that Increasing the amount of maize may affect antioxidant activity more than oyster mushrooms or red beans.

The inhibition percentage represents the antioxidant activity on the DPPH solution. The percentage of inhibition was resulted from the number of free radicals that was neutralized by antioxidants contained in 500 ppm sample. This inhibition can be seen from the change in purple color in the DPPH solution to be fade (18). DPPH (1,1-Diphenyl-2-Picrylhydrazil) is a substance that has proton free radicals that will capture other free radicals. The effect of antioxidant inhibition on DPPH is through the ability of antioxidants to donate H^+ (19). In non-meat patty burger, DPPH free radicals were neutralized by antioxidants through capturing hydroxyl ions (ortho-dihydroxyl and 3-Hydroxyl) derived from phenols (20).

3.2. Glycemic Values

Prediction of glycemic values was measured in 11 subjects (Table 2.). The subject was 18-22 years old. In addition, the subjects have a bodyweight between 45-63 kg with an average value of 54 kg, Height between 1.47 - 1.68 m with an average value of 1.59 m, and a BMI value of 19.20 - 22.64 with an average value of 21.36 kg / m². The subjects had met the inclusion and exclusion criteria. The subjects were declared healthy, had normal nutritional status according to BMI classification of WHO Asia Pacific (7), and were eligible as subjects in this study.

Before the intervention, subjects were conditioned to fast 0-12 hours. As much as 25 g of pure glucose was used as a reference food. The test food consumed was non-meat burger patties formula F2, which has the best characteristics based on (4). The product must be consumed equivalent to 25 g of available carbohydrate ($14.66 \pm 0.38\%$), which is 160 g. The results of the glycemic value measurement are presented in Table 3.

Table 2. Nutritional status of subjects

Subjects	Bodyweights (Kg)	Height (M)	Body Mass Index (Kg/M ²)
1	50	1.50	22.22
2	45	1.47	20.82
3	58	1.63	21.83
4	60	1.65	22.04
5	57	1.63	21.45
6	56	1.68	19.84
7	51	1.63	19.20
8	53	1.53	22.64
9	50	1.52	21.64
10	51	1.57	20.69
11	63	1.67	22.59
Means ± SD	54.00 ± 5.27	1.59 ± 0.07	21.36 ± 1.12

Table 3. Glycemic Response (GR), Glycemic Index (GI) and Glycemic Load (GL)

Intervened Food	GR (mg/dL) Minute -					GI	GL
	0	30	60	90	120		
Reference food (Glucose)	93	148	160	119	79	-	-
Test Food (Non-meat patty burger)	93	153	112	91	80	88.2	6.8

Reference food = 25 g glucose. GR and GI value obtained from average blood glucose values after consumed 160 g test food (consist of 25 g available carbohydrate). GL value of 1 serving size = 50 g test food.

The GR of the food was plotted on the graph shown in Figure 1. Before consuming both foods, the mean blood glucose value of the subject was 93 mg/dL (normal range of fasting blood glucose). After 30 minutes, the increase of blood glucose after consuming reference food was higher than test food (148 mg/dL and 153 mg/dL, respectively). Blood glucose levels after 60 minutes for test food were decreasing (112 mg/dL), but reference food was still increasing (160 mg/dL). After 90 minutes, there was a decrease in both, that was 91 mg/dL (test food) and 119 mg/dL (reference food). Furthermore, after 180 minutes, both eventually returned to the normal blood glucose value (80 mg/dL).

Glycemic values of foods are quite crucial as regards hyperglycemia management. GR is a state of blood glucose after eating. The GR may differ from each other, and it is measured in two hours after eating. The GR of non-meat patty burger is lower compared to the reference food. Those responses show that the test food has a GI value is lower than 100. The reference food (pure glucose) has a 100% digestibility and is biologically absorbable (10). Based on the blood glucose response, the GI of non-meat burger patty is 88 and categorized as high GI (21,22). The high GI value of non-meat burger patty influenced by 1) The use of sago starch as a binding agent; 2) Food preparation (soaking, grinding, boiling, and steaming) cause the fibrous layer of grains which slows the work of the digestive enzymes has been lost; 3) High level of carbohydrate maturity due to food processing; 4) Small granules due to the milling and blending process.

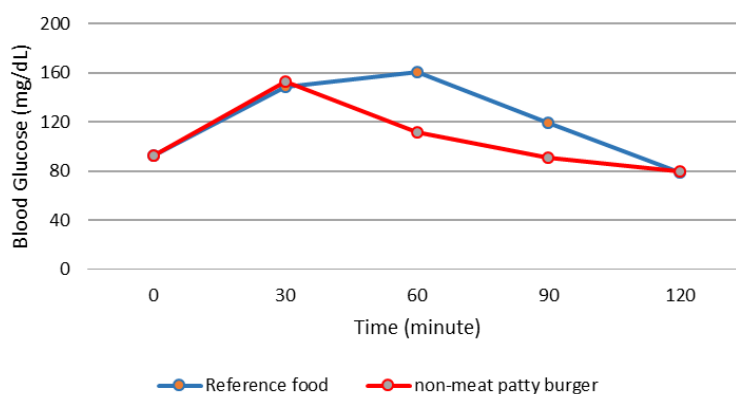


Figure 1. Glycemic Response

GL is the rate of glucose response after consuming foods containing carbohydrates in one serving size. In this case, one serving size suggested for a non-meat patty burger is 50 g. In other words, GL is an indicator of blood glucose response and insulin response induced by one serving size of food. Based on the results, it is known that the GL value of the non-meat burger patty in one serving size (50 g) is about 6.8 and categorized as low GL (23,24).

Low carbohydrate diets are recommended for people with hyperglycemia and diabetes. The quality of carbohydrates should also be considered thoroughly to maintain postprandial blood glucose. Carbohydrates with a high GI may result in increasing the blood glucose, followed by the increase of insulin concentration in the blood. Then, low GI food may be an excellent source to supply carbohydrates needed without causing an adverse effect (25). Studies have found that some food may have different categorize of GI and GL values. A low GL food with the suggested serving size is considered as a healthy food despite the restriction to the serving size in consumption is still advised (26).

4. Conclusions

It is shown inline values between total phenolic content and antioxidant activity of non-meat burger patty. The glycemic response of non-meat patty burger is lower than the reference food. The non-meat burger patty has a high glycemic index, but if consumed in the right serving size will result in a low blood glucose.

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Author Contributions

The study involved, Dzul Fadly and Sulvi Purwayantie conceived and designed the experiments; Dzul Fadly performed the experiments; Andi Imam Arundhana analyzed the data; Sulvi Purwayantie contributed the analysis materials, and Andi Imam Arundhana and Dzul Fadly wrote the paper.

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