The Level of Flavonoid and the Antioxidant Activity of the Growol Flour

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Abstract: Growol a fermented product made from cassava, has the potential as a functional food. The food is origin from the Kulon Progo area, Yogyakarta. Growol flour contains high dietary fiber and can be consumed as alternative food for people with Diabetes Mellitus (DM). This study aims to determine the concentration of flavonoid and the antioxidant activity of growol flour. The flavonoid levels were determined by using the $FeCl_3$ method followed by a spectrophotometry assay. The antioxidant activity of growol was measured by using the *2,2-Diphenyl Picrylhydrazyl* (DPPH) method. The assays showed that the average level of growol's flavonoid was 6.8004 mg/100g while the average antioxidant activity showed that inhibition percentage was 37.232%. Since growol flour contains flavonoids and have antioxidant activity, the food has the potential to be consumed as an optional diet therapy for people with diabetes.

1 INTRODUCTION

Cassava is originating from the Americas. The plant also called wood potato or *kasepe* and Manihot Esculenta Crantz in Latin. Cassava contains flavonoids, alkaloids, saponins, phenolics, and glycosides (Hasanah et al., 2020; Yi et al., 2011). It is known as a source of staple food after rice and corn and is one of the plants that contain cyanogenic. Despite its well-known "cyanogenic" content that indicated its toxicity, processing cassava by fermentation method is a way to reduce these toxic components as is true for other plant cyanogenic glycosides (Pramudita et al., 2017; Wu et al., 2012). Growol is one of the popular fermented foods made from cassava and considered safe.

Growol is a product of fermented cassava from Kulon Progo, Yogyakarta, that has the potential as a functional food. The growol fermentation goes through an immersion stage for 3 days which can reduce the level of cyanogenic toxicity so that it is at a safe level. In addition, during this process, fermentation by probiotic lactic acid bacteria (LAB) occurs. LAB is gram-positive and beneficial for human health including stimulating the immune system, balancing intestinal flora, reducing the risk of tumors, improving blood lipids, premature aging, and its function in antioxidant activity (Ayeni et al., 2011; Kuda et al., 2010; Lee et al., 2010). Fermentation of foods can also increase antioxidant activity by increasing the release of flavonoids from plant foods, making it a useful method to increase the supply of natural antioxidants (Hur et al., 2014).

Flavonoids are bioactive compounds that can act as antioxidants. Antioxidant compounds can inhibit the entry of free radicals into the human body by giving electrons to free radical molecules so that these molecules become stable (Hasim et al., 2016). To our knowledge, the flavonoid levels and the antioxidant activity in growol has not been well studied. In previous studies, only research related to the analysis of prebiotics contained in growol flour was carried out (Puspaningtyas et al., 2019). Thus, this study aims to determine the flavonoid levels and the antioxidant activity of growol flour.

2 METHODS

This research was conducted at the Central Laboratory of Food and Nutrition Studies (PSPG) in

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June 2021. The type of research in this study was experimental laboratory in vitro to determine the levels of flavonoids and the antioxidant activity in growol flour.

2.1 Instrument and Material

The instrument used in this research are knife, container, steam pot, cabinet dryer, grinder, 60 mesh sieve, test tube, scale, UV-Vis spectrophotometer. The main ingredients used in the production of growol flour are cassava, ethanol 96% and methanol.

2.2 Growol Production

The process of making growol is carried out with modifications and refers to previous research (Puspaningtyas et al., 2019).

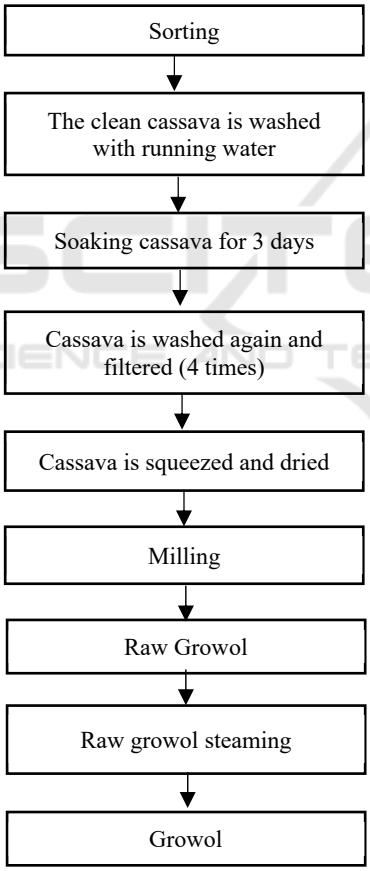

Figure 1: The process of making growol

2.3 Growol Flour Production

Growol flour manufacture refers to previous research (Puspaningtyas et al., 2019).

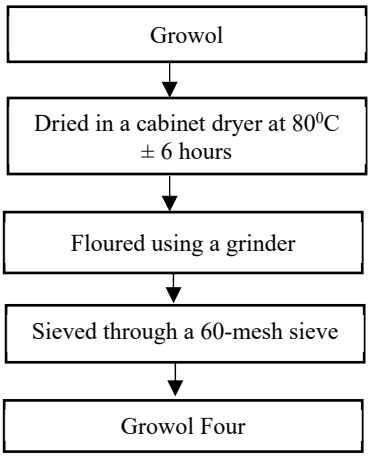

Figure 2: The process of growol flout poduction

2.4 Measuring Flavonoid Levels

About of 1-2 grams of growol flour was weighed and put in a test tube, and then added with 10 ml of 96% ethanol. Next, 1 ml of mother liquor and add 5 ml of FeCl3 solution (forming a red color) was added. It was then diluted to 10 ml using 96% ethanol. UV-Vis spectrophotometer was used for tera with a wavelength of 520 nm.

2.5 Measuring Antioxidant Activity

2.5.1 Antioxidant Activity/Radical Scavenging Activity

Antioxidant Activity/ Radical Scavenging Activity (RSA) is conducted refer to previous research (Yen & Cheng, 1995). About 1-2 g of growol flour was weighed and dissolved using methanol at a certain concentration. Then, 1 ml of mother liquor was taken and put in a test tube. Next, 1 ml of 1,1, 2,2–Diphenyl Picryl Hydrazyl (DPPH) solution, 200 micromolars, was added. Then, it was incubated in a dark room for 30 minutes. It was diluted to 5ml using methanol. A blank was made by adding 1 ml of DPPH solution into 4 ml of ethanol. The tera was at a 515 nm wavelength.

$$\% \text{ Inhibition} = \frac{A\ blangko - A\ sample}{A\ blangko} \text{x} 100\% \quad (1)$$

3 RESULTS AND DISCUSSION

Growol is a fermented food typical of Kulon Progo made from cassava through a process of soaking,

washing, grinding, and steaming to produce mature growol (Puspaningtyas et al., 2019).

The levels of total flavonoid in growol flour were determined by using UV-Vis spectrophotometer with calorimetric method. The use of a spectrophotometer in determining flavonoids is due to the presence of a conjugated aromatic system in flavonoids which can show strong absorption bands in the ultraviolet and visible spectrum regions. The maximum wavelength used is 520 nm.

FeCl3 is used for testing the levels of flavonoid in growol flour, followed by UV-Vis spectrophotometer. Flavonoids are one of the natural compounds that can act as antioxidants and hypolipidemics (Nwose et al., 2017). They can also act as antidiabetic including improvements in glycolysis pathways, mitochondrial function, improving insulin sensitivity, reducing oxidative stress and gluconeogenesis (Vinayagam and Xu, 2015). Nwose et al., (2017) state that flavonoid in cassava has anti-inflammatory, hypolipidemic, and management effects on metabolic syndrome disease. The levels of flavonoid are presented Table 1.

Table 1: Flavonoid Levels in Growol Flour.

Sample	Results (mg/100g)	Average (mg/100g)
Growol Flour	6.7317	6.8004
	6.8691	

The flavonoids in growol flour is the result of the fermentation process in growol. During the fermentation, there was an increase in flavonoids due to the presence of sugar-breaking enzymes and degraded phenolic complex compounds because of lactic acid bacteria activity. These enzymes support the formation of flavonoid compounds due to the addition of phenol groups (Dwiputri, 2018).

These studies have shown that regular flavonoid consumption of 5-100 mg/kg by oral or intraperitoneal injection can provide an antihyperglycemic effects (Ghorbani Ahmad, 2017). In experimental animal studies with type 2 diabetes mellitus, regular flavonoid consumption of 5-100 mg/kg can decreased fasting blood glucose and non-fasting blood glucose (A. Hunyadi et al., 2012; C.Y. Hsu et al., 2014; R. Jadhav, 2012). The mechanism of regular flavonoid consumption on the antihyperglycemic effect is the reduced absorption of glucose from the small intestine by inhibiting glucosidase and α-amylase involved in carbohydrate digestion (O.M. Ahmed et al., 2010; R. Jadhav, 2012; S. Jo, E. Ka et al., 2009; Y.Q. Li et al., 2009). Our study showed that the levels of total flavonoid in growol flour is 6.8004 mg/100 g. The application of growol flour with a concentration of 6.8004 mg/100g can also able to provide antihyperglycemic treatment.

Antioxidant activity is influenced by active compounds contained in foods such as flavonoids (Dewi, S. R. et al., 2018). The antioxidant activity of growol flour is measured using the 2,2-Diphenyl Picryl Hydrazyl (DPPH) method. DPPH is a method of detecting antioxidant activity in a simple, fast, and inexpensive way, and requires only a small number of samples. This method is a stable and free radical at room temperature and is very soluble in methanol and is very easily oxidized by temperature and air. In addition, DPPH is in powder form and is purple black in color (Molyneux, 2004). The results of antioxidant activity test presented in Table 2

The antioxidant activity in fermented food can inhibit the entry of free radicals into the body and improve the levels of glucose and lipid in the blood (Bajaj & Khan, 2012; Ceriello & Testa, 2009; Li et al., 2012). The result of antioxidant activity test showed that inhibition percentage found in growol flour is 37.2317% due to the presence of lactic acid bacteria formed during immersion and the fermentation process (Li et al., 2012; Nugraheni, 2011; Putri et al., 2012). The antioxidant activity is due to the presence of flavonoids in growol flour.

Table 2: Antioxidant Activity in Growol Flour.

Sample	Results (%)	Average (%)
Growol Flour	37.1244	37.2317
	37.3390	

4 CONCLUSIONS

The levels of flavonoid and the antioxidant activity with percentage inhibition in growol flour were 6.8004 mg/100g and 37.2317% influenced by LAB during the fermentation process in making the growol. The levels of flavonoids and the antioxidant activity in growol flour can be an alternative prevention or diet therapy for people with diabetes mellitus.

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