

## Gluten-free noodle made from gathotan flour: antioxidant activity and effect of consumption on blood glucose level

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### Abstract

This work evaluated gathotan noodle on its antioxidant activity and effect of consumption on blood glucose concentration. Gathotan is fermented cassava product originated from Central and East Java, Indonesia, colonised mainly by dematiaceous fungus *Botryodiplodia theobromae* Pat. Gathotan flour was made into gluten-free noodle using pregelatinised gathotan flour consisting of 1:7 ratio of flour and water. Pregelatinised flour was mixed with dry gathotan flour of 5:6 ratio. Antioxidant activity of gathotan flour and noodle was expressed as total phenolic compounds, scavenging ability, and vitamin E equivalents. Seven healthy non-diabetic participants with body mass index ranging from 18-22 were employed in the test to measure blood glucose concentration after gathotan noodle consumption. A commercial kit was used in the determination of blood glucose concentration. White bread was used as control, and for developing a baseline. Results showed that both gathotan flour and noodle had antioxidant activity, but it reduced in gathotan noodle. Gathotan flour had scavenging ability of 90.33%, 18.92 mg/100 g equivalent to vitamin E, and 419.43 mg/100 g total phenolic compounds. Whilst, gathotan noodle had 84.26 % inhibition of DPPH, 7.9 mg/100 g equivalents to vitamin E, and 14.13 mg/100 g total phenolic compounds. Consumption of gathotan noodle increased blood glucose level quicker compared to that of white bread consumption. However, gathotan noodle consumption also reduced blood glucose level back to fasting level quicker than control meal. Gathotan noodle showed a possible antidiabetic effect, since at the end of two hours testing period, it reduced blood glucose level to be lower than fasting level.

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### Introduction

Cassava is an important root tuber for feeding populations in developing countries in Asia, Africa, and South America, comprises 97% of tubers consumed (Scott *et al.*, 2000). It is a good candidate in food security program due to its ability to grow well in marginal land. The production is projected to rise reaching about US\$ 12-13 billions in year 2020, with 1-2 % annual increase (Scott *et al.*, 2000), despite its cyanogenic glycoside which can reduce palatability or even make it inedible. An upper motor neuro disease called konzo was reported to be associated with prolonged consumption of bitter cassava having high cyanogenic glycosides content (Adamolekun, 2010). There are several methods to decrease cyanoglycosides in cassava as summarised by Nambisan (2011), those highly effective were crushing combined with sundrying, and combination of grating-fermentation-dewatering-drying. Soaking for 3-4 days was also able to reduce cyanoglycosides to safe level to prevent konzo disease (Banea *et al.*, 2013). Some traditional cassava products are well-known locally and considered as safe, including

gathot.

Gathot is a traditional fermented cassava product originated from Central and East Java, Indonesia. Important characteristics of gathot is very chewy texture and black in colour. Purwandari (2000) described several ways of making gathotan (uncooked gathot). In general, gathotan was prepared traditionally by peeling cassava tubers and then letting them to stand in an open space for several weeks by maintaining humidity, thus allowing different types of fungi grow outside and inside the tubers. Main characteristics of gathotan is black colour of inside part of tuber, and chewy texture of gathot (cooked gathotan). To make gathot, gathotan piece was soaked and steamed. Black colour of gathot or gathotan was due to growth of a dematiaceous fungus, *Botryodiplodia theobromae* Pat., the only fungus always isolated from inside gathotan piece (Purwandari, 2000). *B. theobromae* is a plant pathogen, highly prevalent in tropics, infecting tubers or fruit including sweet potato (Kihurani *et al.*, 2008), mango (Sivakumar *et al.*, 2011), cashew (Muniz *et al.*, 2012), cocoa bean (Fagbohun *et al.*, 2011), guava (Pawar, 2012), or Indian rosewood (Kausar *et*

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al., 2009). However, dematiaceous fungi contained melanin, a black pigment, which can give antioxidant effect (Govindappa *et al.*, 2011). Therefore, there is a possibility of antioxidant activity in gathotan or product(s) made from gathotan.

Some works have reported on the safety of fungal contaminated of fungal fermented cassava. Although gathotan was reported to have high level of aflatoxin B1 in 1972 (Purwandari 2000), analysis of gathotan from 15 production areas in Java using high pressure liquid chromatography and enzyme-linked immunosorbent assay did not show any trace of the toxin (Purwandari, 2000). Moreover, toxigenic strain of *Aspergillus flavus* was not found in any sample, although *A. flavus* colonies were always isolated from surface of gathotan chunk (Purwandari, 2000). More recent work (Adjovi *et al.*, 2014) also showed that *A. flavus* colonies were isolated from all cassava samples collected in Africa, but there was no aflatoxin B1 nor toxigenic strain of *A. flavus* in the samples. Thus, in term of aflatoxin content, gathotan may be considered as free from the toxin.

Chewy characteristic of gathot makes it possible to make gathotan noodle with acceptable sensory properties. Thus, we have studied the making of gathotan noodle and its textural and sensory evaluation, and concluded that noodle made with pregelatinisation gathotan flour having ratio of water and flour 1:7, and ratio of pregelatinisation flour and dry gathotan flour 5:6, had best textural and sensory properties.

As fungal fermentation of cassava during gathotan making may cause starch degradation, it is probable that starch digestibility was altered by the process. Therefore, it is important to know blood glucose profile after consumption of gathotan noodle. This work deals with assessing blood glucose level upon gathotan noodle consumption, and antioxidant activity in gathotan noodle.

## Materials and Methods

### Preparation of gathotan flour

Gathotan was made following a method described by Purwandari (2000) with some modifications. Cassava tubers bought from local market in Kamal, Madura, Indonesia, were peeled, washed, and half dried in the sun. The tubers were then put in a closed container, added with gathotan powder from previous batch, 5% of weight of cassava tuber. After 3 days incubation at room temperature, tubers were then sun-dried until fully dry. Some moisture control was done by spraying water into the tubers, until patchy black area was developed inside tuber. Dry gathotan piece

was ground to powder, and passed through 60 mesh sieve. Gathotan flour was kept in a tight container and stored in a fridge until used.

### Noodle making

Noodle was made by mixing dry gathotan flour with pregelatinised gathotan flour. Pregelatinised gathotan flour consisted of 1 part of flour and 7 parts of distilled water, and then cooked on a water bath, and stirred gently until all part was gelatinised. Noodle dough contained a mixture of 5 parts of pregelatinised flour and 6 parts of dry gathotan flour. The mixture was kneaded until smooth dough was formed. Dough was then let to stand for 30 minutes in a tight container at room temperature. Then it was formed into smooth sheet using a roller. The sheet was steamed until all parts was gelatinised. The sheet was then cut into strips, and dried in a cabinet dryer. Dry gathotan noodle was kept in a plastic bag, stored in a dry place until used.

### Measurement of antioxidant activities

Antioxidant activities were determined using previous method by Shin *et al.* (2007) with some modifications. As much as 10 g gathotan noodle was crushed into smaller chunks, and then mixed with 100 mL methanol before putting in a Waring blender (type 8010S/8010G, Waring Laboratory Science, Winsted, CT, USA) and homogenized for about 10 min until smooth suspension was formed. Suspension was filtered using #1 Whatman paper, and filtrate was evaporated in a rotary evaporator (RV 8, IKA®-Werke, GmbH and Co. KG, Germany) at 45°C, for 30 min. Dried sample was then diluted with distilled water before analysis using colorimetric method. Folin-Ciocalteu reagent for determination of total phenolic content (10%, 0.5 mL) was added into the mixture (0.1 mL), and allowed to stand for 8 min at room temperature. Na<sub>2</sub>CO<sub>3</sub> (4.5 mL, 2%) was then mixed with the mixture, and kept in the dark room for 60 min at room temperature. Absorbance was measured in a spectrophotometer (SP-3000, Plus Optima, Japan) at wave length of 765 nm, and result was expressed as galic acid equivalents/100 g sample.

DPPH scavenging activity was determined using previous method (Arabshi-Delouee and Urooj, 2007), with some modifications. Dried sample was diluted in methanol to make a suspension containing 20-100 µg dried extract. Then, 5 mL of the suspension was mixed with 1 mL DPPH (1 mmol/L in methanol), mixed vigorously using a vortex (Vortex 3, IKA, Krackeler Sci. Ltd. Albany, NY), and left in the dark at room temperature for 30 min, then measured the

absorbance with a spectrophotometer at 517 nm (SP-3000, Plus Optima, Japan). DPPH scavenging was calculated relative to control according to the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Result was also expressed as vitamin E equivalents/100 g sample, using  $\alpha$ -D tocopherol.

#### Measurement of blood glucose level

Participants from University students were selected based on their body mass index (BMI) and diabetes history in their family, and smoking or alcohol drinking habit, after they agreed to be involved in the test. The age of seven selected participants was between 18-21 years old, with body mass index between 17-22, had no history of diabetes within family, and do not smoke nor drink alcohol. They were invited to a brief session explaining the procedure. The following day, they were asked to fast overnight (10-12 hours), allowed only for drinking water. After fasting, a drop of blood from finger was taken by giving a prick, and blood glucose concentration was measured using a glucometer (Gluco DR™/AGM-2100 Biosensor, equipped with GlucoDr™ AGS Biosensor Strip). Participants were then asked to consume 95 gram white bread equals to 50 g of flour, in 10 minutes. Then, every 15 minutes blood sample was taken and blood glucose level was determined with glucometer. Test was carried out up to 120 minutes. Every set of test was repeated three times, with at least five days interval between each test. White bread used as control. Gathotan noodle as much as 50 gram was cooked in boiling water until fully cooked, then mixed with fried seasoning containing garlic, white pepper, salt, and candle nut, and served. Participant were allowed to drink up to 250 mL water during the two hours test. Area under curve (AUC) was measured by trapezoid rule. Relative glycemic response was determined by dividing AUC of gathotan noodle over AUC of white bread, times 100.

#### Data processing

All parameters for antioxidant activity and blood glucose concentration were repeated three times, and then run in a statistical package SPSS® version 16 (SPSS, Inc.), for analysis of variance. A statistical difference among means was determined using Tukey method at 5% confidence level.

## Result and Discussion

### Antioxidant in gathotan noodle

Scavenging ability of gathotan flour was 90.33%, and reduced slightly in gathotan noodle (84.26%) (Table 1), whereas equivalent to vitamin E of gathotan noodle was only about half of the concentration in gathotan flour. Concentration of phenolic compounds in gathotan reduced substantially from 419.43 mg/100 g in gathotan flour to 14.13 mg/100 g in gathotan noodle.

*Botryodiplodia theobromae* Pat. was reported to have radical scavenging activity as shown by reduction of lipid peroxidase in cancer-induced rat, due to taxol production by the fungus (Pandi *et al.*, 2010). Antioxidant activity may also come from hydroxycoumarine scopoletin produced when cassava tubers undergo postharvest physiological deterioration which does not involve microbial activity (Bayaoumi *et al.*, 2010). Cooking of noodle caused antioxidant loss by more than half (Choy *et al.*, 2013). Reduction of total phenolic acid was also shown in semolina pasta. Total phenolic acid in semolina flour was 155  $\mu\text{g/g}$ , while that in uncooked pasta was 124  $\mu\text{g/g}$  (Fares *et al.*, 2010), showing about 20% reduction during the process of making pasta. In our work, although gathotan flour contained relatively high concentration of phenolic compounds (419 $\pm$ 19.50 mg/100 g), it diminished considerably in dried noodle (14.13 $\pm$ 0.03 mg/100 g). Free phenolic compounds easily loss from material during mixing with water (Fares *et al.*, 2010), heating (Fares *et al.*, 2010) including steaming (Qin *et al.*, 2013), and in contact with oxygen (Fares *et al.*, 2010). Drying and steaming also reduced DPP scavenging activity (Qin *et al.*, 2013). However, other report showed increase in bound phenolic acid upon cooking (Fares *et al.*, 2010), which was thought to relate to release of phenolic acids from cell wall (Fares *et al.*, 2010). We experienced difficulty in extraction of phenolic compounds from noodle matrix, which seemed to contribute to low concentration being detected in gathotan noodle.

*Botryodiplodia theobromae* Pat. is a dematiaceous fungus that most likely produces melanin, a black pigment. Although we did not find any publication on relation between melanin and antioxidant activity, polysaccharide of black fungus showed increasing effect of myocardium antioxidant enzymes activities (Jiangwei *et al.*, 2011). Some black fungi containing melanin such as *Alternaria alternata* produced phenolic compounds (Govindappa *et al.*, 2011).



Table 1. Antioxidant in gathotan flour and gathotan noodle

Parameter of antioxidant activities	Gathotan flour	Gathotan noodle
Scavenging ability (% inhibition)	90.33±0.00	84.26±0.00
Equivalent to vitamin E (mg/100 g)	18.92±0.06	7.90±0.05
Concentration of phenolic compounds (mg/100 g)	419.43±19.50	14.13±0.03

Table 2. Blood glucose concentration of volunteers upon white bread or gathotan noodle intake

Time (minutes)	Blood glucose concentration (mg/dL)	
	White bread	Gathotan noodle
0	94.00±10.01 <sup>ab</sup>	94.14±8.05 <sup>ab</sup>
15	102.00±10.01 <sup>b</sup>	144.79±8.05 <sup>c</sup>
30	123.93±10.01 <sup>bc</sup>	144.07±8.05 <sup>c</sup>
45	137.00±10.01 <sup>c</sup>	127.64±8.05 <sup>c</sup>
60	134.21±10.01 <sup>c</sup>	107.93±8.05 <sup>bc</sup>
75	123.93±10.01 <sup>bc</sup>	93.21±8.05 <sup>ab</sup>
90	111.86±10.01 <sup>bc</sup>	100.93±8.05 <sup>b</sup>
105	103.21±10.01 <sup>bc</sup>	91.29±8.05 <sup>ab</sup>
120	101.36±10.01 <sup>b</sup>	77.00 <sup>a</sup> ±8.05 <sup>a</sup>

Same superscripts within a column indicate no significant difference ( $P > 0.05$ )

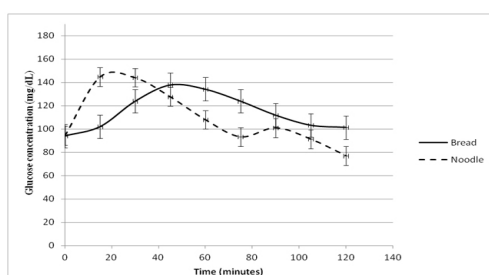


Figure 1. Blood glucose concentration of volunteers after bread or gathotan noodle consumption

### Blood glucose concentration

Blood glucose concentration increased rapidly and significantly ( $P < 0.05$ ) after 15 minutes of consumption of gathotan noodle, to reach peak of  $144.79 \pm 8.05$  mg/dL (Figure 1, Table 2). Whilst, white bread consumption resulted in highest glucose concentration ( $137.00 \pm 10.01$  mg/dL) later at 45 minutes after consumption. A quick reduction in blood glucose concentration was shown by volunteers consuming gathotan noodle, where blood glucose concentration was back to fasting level at 75 minutes after consumption ( $93.21 \pm 8.05$  mg/dL). Meanwhile, blood glucose concentration of those consuming white bread reduced gradually to approach fasting level at 120 minutes after consumption ( $101.36 \pm 10.01$  mg/dL). We noted a slight increase of blood glucose level among gathotan noodle volunteers at 100 minutes after consumption ( $100.93 \pm 8.05$  mg/dL), then there was a steady reduction towards the end of testing period. Calculation of AUC resulted in relatively same area for both white bread and gathotan noodle, leading to 95.95 glycemic index of gathotan noodle.

This likely indicates that gathotan noodle contained dextrin as result of enzyme degradation by *B. theobromae* during fermentation. *B. theobromae* was reported to have  $\beta$ -glucosidase which metabolised starch into glucose and dextrin (Umezurike, 1971; Umezurike, 1975). Although gathotan had lower glycemic index than base line, it is still categorized as high. Similar to our result, some types of dextrin did not alter glycemic index of baseline of glucose

response (Wolf *et al.*, 2001). Other types of dextrin even showed higher area under curve as compared to their native starch (Deng *et al.*, 2001). Potato dextrin or corn dextrin had glycemic index, area under curve (AUC), and certainly also blood glucose level higher than their corresponding native starch (Deng *et al.*, 2001). Soluble dietary fiber in muffin gave even larger AUC and higher blood glucose concentration than in muffin without it (Willis *et al.*, 2011). Bread containing annealed yam flour also showed minimal change in glucose response from baseline (Mojiono *et al.*, 2013). Therefore, rapid change of glucose level after gathotan consumption may relate to glucose content as product of  $\beta$ -glucosidase digestion of starch, and also by the presence of type of dextrin that was rapidly digested. Rapidly digestible starch was capable of fast increasing glucose response, and subsequently quickly reducing it (Lee *et al.*, 2012). A fast reduction of blood glucose level was also shown after gathotan noodle consumption.

Gathotan noodle could be considered to show rather prominent feature on blood glucose concentration, where it caused quick reduction of blood glucose concentration to return to fasting level at 75 minutes. The reducing effect was even faster than what was shown by anti-diabetic herbs decoction (Ejike *et al.*, 2013). As the glucose reduction did not occur during the first 30 minutes, it was probable that gathotan noodle modulate insulin sensitization and secretion rather than modulation of digestion (Ejike *et al.*, 2013). A striking sharp reducing tendency towards the end of testing period need to be carefully evaluated to make sure that it will not lead to hypoglycemia when blood glucose concentration reaches  $< 72$  mg/dL (Bolli *et al.*, 2009). The effect of gathotan noodle consumption for people with diabetes may also need to be studied, with possibility of reduction of fasting glucose level (Qureshi *et al.*, 2002).

### Conclusion

Gathotan noodle had antioxidant activity as expressed by scavenging ability and equivalent to vitamin E, and showed fast increase followed by quick decrease in blood glucose response upon consumption.

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