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RESEARCH ARTICLE

Standardization of *Growol* processing and the effect of different processing processes on the potential of *Growol* as functional food

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ABSTRACT:

One of the Indonesian traditional food, *growol* is a fermented cassava product of the home industries. It is believed that *growol* can cure diseases in the digestive tract. This research aims to analyze the standardization of *growol* processing and the effects of various processing processes, i.e. steaming, baking, frying, and cooling, on *growol* potential as traditional food. Four variations of fermentation treatments were used to investigate the standardization of *growol* processing, namely the fermentation process. Based on the organoleptic test encompassing appearance, texture, taste, and overall preference and the LAB level test, the best *growol* was that with the fermentation process "not changing the water in three days" (TG3). As such, we figured out the standardization of *growol* processing comprising preparing the basic ingredients, performing the fermentation "not changing the soaking water in three days", washing five times (b/v) draining, crushing, steaming, and molding. The glycemic index of baked, steamed, cooled, without-processing, and fried *growol* was 97, 94, 93, 91, and 89, respectively. With a GI > 70, without processing, steamed, baked, fried, and cooled *growol* could not be consumed as functional pro-diabetic food. The LAB test indicated that the LAB level of without-processing, baked, fried, steamed, and cooled *growol* was 1.32×10^7 cfu/g, 6.7×10^3 cfu/g, 1.53×10^3 cfu/g, 1.27×10^3 cfu/g, and 2.7×10^2 cfu/g, respectively. We could then infer that without processing *growol* and steamed, baked, fried, or cooled *growol* had potential as a functional prebiotic food.

KEYWORDS: Standardized Processing Process, Fermented Cassava, Prodiabetic, Probiotic.

INTRODUCTION:

One of the Indonesian traditional food, *growol* is a fermented cassava product of the home industries. As usual, people consume it as snacks or as a substitute for rice. Also, it is believed that *growol* can cure diseases in the digestive tract. In traditional markets, *Growol* comes with several variants due to different processing processes used by producers. *Growol* is traditionally fermented by immersing cassava into the water. When being immersed, cassava will also be fermented by Lactic Acid Bacteria (LAB), especially *Lactobacillus casei* subsp. *rhamnosus* TGR 2.

Some Lactobacillus can break down carbohydrates into lactic acid; they are *Lactobacillus*, *Lactococcus*, and *Streptococcus*². Lactic acid bacteria (LAB) are a group of gram-positive bacteria, do not produce spores, are round or rods that produce lactic acid as the main metabolic end product during carbohydrate fermentation. Generally, catalase^{3,4} is negative but sometimes pseudo catalase is detected in cultures grown at low sugar concentrations⁵. Lactic acid bacteria^{6,7,8,9,10,11} are named according to their ability to produce lactic acid as the major (and sometimes the sole) product of sugar fermentation.

The bacteria are renowned for their ability to survive in acidic conditions in the digestive tract, be resistant to bile salt concentrations, and have the potential for antimicrobial activities¹²⁻¹³. Those characters make *growol* potential as functional food from which we can

get probiotics and prebiotics¹⁴. Several studies have shown that probiotic products have positive effects on human health, including reducing the lactose intolerance reaction, affecting the balance of the intestinal microflora, and increasing the body's immunity. Research on LAB activity in processed lactic acid fermentation products in the form of *growol* in suppressing disease *in vitro* has been conducted by several researchers¹⁵. *Growol* made of cassava is cassava which has a GI lower than that in rice. Accordingly, *growol* can be regarded as a staple food substitute for rice which can be used in the prevention and therapy of diabetes mellitus or prodiabetic¹⁶. Unfortunately, the standardization of *growol* processing and its potential as functional prodiabetic and probiotic food is not adequately researched. As such, this research aims to examine what the standardization of *growol* processing is and what the effects of different processing processes, namely steaming, baking, frying, and cooling, on *growol* characteristics, which are the appearance, texture, taste, degree of preference, glycemic index (GI), and level of Lactic Acid Bacteria (LAB). Lactic acid bacteria (LAB) have been used for a long time as beneficial bacteria in food processing¹⁸.

RESEARCH METHODS:

Materials:

The material used in this research was cassava (*Manihot esculenta*) whose characteristics were having red inner skin and white flesh and including the non-bitter cassava species bought at Yogyakarta. Pro-analysis chemicals (Merk), e.g., HCL, MR-BCG indicators, H₃BO₃, H₂SO₄, catalyst in the form of a mixture of Na₂SO₄ and HgO, petroleum ether, and others were used for proximate analysis.

MRSA (*deMann Rogosa Sharpe Agar*) media were used for the analysis of lactic acid bacteria and pure glucose or was used as standard food for the Glycemic Index test.

Standardization of *Growol* Processing:

The process was initiated by preparing basic materials. We selected and a peeled fresh whole cassava which had an intact physical condition. The peeled cassava was chopped into ±5cm sizes, yielding a uniform material size. The materials were then washed up twice using running water. Cassava which had been purified from contaminants was then fermented by immersing it in the well water at a ratio of 1:3 (b/v). There were four variations of fermentation treatment, i.e., G3 (change the immersing water, three days), TG3 (not changing the immersing water, three days), G4 (changing the immersing water, four days), and TG4 (not changing the immersing water, four days). We then washed the material five times (the ratio of 1:1 (b/v)) and draining and crushed it. The making process of raw *growol* was ended by steaming and molding processes to produce

ready-to-eat *growol*.

Experimental Design to Determine the Best *Growol*:

This experiment was to investigate the effects of four variations of fermentation treatments on *growol* characteristics, i.e., organoleptic characters and the level of LAB. We used a completely randomized design or 2-factor RAL¹⁹. The first factor was the fermentation/immersion method and the second one was the fermentation time. The fermentation method consisted of two treatments, namely by changing and not changing the immersing water. The fermentation time also comprised two treatments, which were 3- and 4-day fermentation. The results of interaction and organoleptic tests were tested using ANOVA statistics and the DMRT real difference test¹⁹. The tests were carried out using a computer program SPSS 14.0 for Windows Evaluation version at a 95% confidence level. We also performed a LAB level test using MRSA (*deMann Rogosa Sharpe Agar*) and TPC media. Based on the statistic analysis, we determined the fermentation process which generated the best *growol*. The best *growol* produced was then tested for proximate composition as the basis for GI testing. The proximate testing on the best *growol* included water content, protein, fat, ash, according to AOAC²⁰, and carbohydrates (by difference).

Best *Growol* Processing with Different Processing:

The best *growol* was treated with four processing variations, which were steaming, baking, frying, and cooling. As a control, steamed *growol* without any additional processing was used. We steamed *growol* for 15 minutes, baked it at 180°C for 15 minutes, deep frying it in boiling oil for 15 minutes, and cooling it down by storing it at a refrigerator temperature for 24 hours.

Organoleptic Test, GI Test, and LAB Test for the Best *Growol* with Various Processing:

Organoleptic testing encompassing appearance, texture, taste, and overall preference, statistical analysis using ANOVA, and DMRT real 18 difference test¹⁹ was run using a computer 19 program SPSS 14.0 for Windows evaluation version at a 95% confidence level of 95%. Glycemic index testing was conducted on ten volunteers, i.e., students in Panti Rapih Health Institute Yogyakarta, Indonesia. The volunteers were instructed to do fasting for approximately ten hours before consuming food tested for its GI. In one testing, the volunteer consumed one type of food whose GI was measured. The blood sugar 26level measurement began at 0 minutes (before food consumption) and 27 then at 30, 60, 90, and 120 minutes after the volunteers consumed food 28 whose GI had been measured. GI was quantified using the Trapezoid method²¹. Meanwhile, the LAB level testing used MRSA (*deMann Rogosa Sharpe Agar*) and TPC media.

RESULT AND DISCUSSION:

Cassava (*Manihot esculenta Crantz*) is a major staple food in tropical countries. Cassava belongs to the family *Euphorbiaceae* and is a perennial woody shrub producing enlarged tuberous roots. The roots are the main storage organs and in some areas, it is cultivated as the perennial or annual plant with the storage roots being harvested during the first or second year²². Cassava contains anti-nutritional factors and toxins, it must be properly prepared before consumption, and improper preparation of cassava can leave enough residual cyanide to cause acute cyanide intoxication and goiters and may even cause a toxic or partial paralysis²³. Cassava contains high phosphor and calcium, and these substances contribute to significant ash content.

Indonesia is a country that is rich in local food, one of which is fermented foods from cassava such as *growol*. *Growol* is a traditional food from Kulonprogo, Yogyakarta, Indonesia, which is made from cassava through a spontaneous fermentation stage by immersing it in *growol*²⁴. *Growol* can be used as an effective. Diet solution in disease therapy, both in infectious diseases such as diarrhea and metabolic disorders such as diabetes, dyslipidemia, and coronary heart disease. This is in line with several studies which state that fermented foods can be developed into functional foods with functions, primary, secondary, and tertiary which can be developed in the prevention of diseases, both infectious and non-communicable diseases²⁵. Several studies related to disease prevention and management have been conducted before. There is a significant relationship between *growol* consumption and the incidence of diarrhea. The more often the *growol* consumption rate, the less frequent diarrhea¹⁶. In addition, *growol* administration can significantly be used in the prevention of diarrhea caused by Enteropathogenic *Escherichia coli*. *Growol* is also a functional food that involves the fermentation of probiotic bacteria. An increase in the amount of food fiber in the fermentation of cassava into *growol* can improve the mass of feces so that the consistency of the stool becomes denser. In addition, the presence of food fiber can increase the sensation of being full longer²⁶.

Standardization of *Growol* Processing:

1. Effects of Different Fermentation Treatments on the Organoleptic Characteristics of *Growol*:

The effects of different fermentation treatments on the organoleptic characteristics of *growol* are indicated in Table 1. In Table 1, it is obvious that all samples were not significantly different in terms of appearance and taste. Samples given a 3-day fermentation treatment

(TG3 and G3) had a more chewy texture and were more preferable than the samples with 4-day fermentation treatments (TG4 and G4).

Table 1. Results of organoleptic test on *growol* with different fermentation treatments

<i>Growol</i> Sample	Appearance*)	Texture*)	Taste*)	Overall Preference*)
G3	2.5750a	2.7500 ^b	2.5750a	2.5500 ^b
TG3	2.5000a	2.8250 ^b	2.5000a	2.5250 ^b
G4	2.5750a	1.9500a	2.5750a	2.2000a ^b
TG4	2.5750a	1.9750a	2.5750a	1.9000a

*) The same notation in one column indicated no significant difference

2. Effects of Different Fermentation Treatments on the Lactic Acid Bacteria (LAB) Levels of *Growol*:

The effects of the fermentation method and time on the LAB levels of *growol* are listed in Table 2.

Table 2. LAB levels of *growol* with different fermentation treatments

<i>Growol</i> Sample	LAB Level (cfu/g) ^{*)} at 0 Hours	LAB Level (cfu/g) ^{*)} at 24 Hours
Control ^{**)}	-	1.46 × 10 ⁵ a
G3	9.0 × 10 ¹ a	1.26 × 10 ⁷ b
TG3	4.0 × 10 ¹ a	1.32 × 10 ⁷ b
G4	2.0 × 10 ¹ a	2.04 × 10 ⁶ a
TG4	2.0 × 10 ¹ a	2.68 × 10 ⁶ a

*) The same notation in one column indicated no significant difference

**) *Growol* bought in a traditional market

In Table 2, all samples contained LAB probiotics higher than that in the control *growol* bought at a traditional market. The highest LAB level was in TG3 and G3 samples.

As presented in Table 1 and Table 2, *growol* with 3-day fermentation treatments (G3 and TG3) had between organoleptic characteristics, higher preferable level, and higher LAB level if compared to that fermented for four days (G4 and TG4). Despite their LAB levels which were not significantly different, *growol* with a 3-day fermentation treatment “not changing the water” (TG3) had more preminent making processes than that with a 3-day fermentation treatment “changing the water” (G3). The first one was more efficient and economic as producers did not need to change the water in three days.

3. Comparison between the Best *Growol* (TG3) and *Growol* from Traditional Markets:

Table 3 and Table 4 show the comparison in nutritional composition and organoleptic characteristics between the best *growol* (TG3) and *growol* from traditional markets.

Table 3. Comparison of the result of the organoleptic test and nutritional composition between the best growol (TG3) and growol from traditional markets

Growol Sample	Appearance*)	Texture*)	Taste*)	Overall Appearance*)	Composition Nutrition (%)
Best growol(TG3)	3.0333 bc	2.4000 bcd	2.0333 ab	2.5000 c	Protein: 0.92 Fat: 0 Water: 66.95 Ash: 0.16 KH (by Diff): 32.0
Growol from markets	2.3000 ab	2.0000 ab	2.4000 b	2.000 ab	Protein: 0.856±0.06 Fat: 1.23±0.10 Water: 56.74±0.06 Ash: 1.03±0.06 KH (by Diff): 32.44

*) The same notation in one column indicated no significant difference

Table 3 indicates that concerning nutritional composition, we did not see any significant difference between TG3 growol and growol from the traditional market, however, Table 4 shows that the first growol came with better organoleptic characteristics than the latter. Therefore, we could safely argue that growol produced using standardized processing had better organoleptic characteristics. Also, it was more preferred and had higher LAB levels than the other bought at traditional markets.

Effects of Different Processing on Growol Potentials as Functional Food:

Processing the best growol with steaming, baking, frying, and cooling yielded the following data.

1. Results of Organoleptic Test on Growol with Different Processing

Table 4. Results of organoleptic test on growol with Different Processing

Growol Sample	Appearance *)	Texture*)	Taste *)	Overall Appearance *)
Without-processing Growol	3.0333 bc	2.4000 bcd	2.0333 ab	2.5000 c
Steamed growol	2.30000 ab	2.8000 d	2.1000 ab	2.4667 bc
Cooled growol	2.6667 b	2.5667 cd	2.4667 b	2.6333 c
Baked growol	2.6000 b	2.2333 bc	2.1333 ab	2.3333 abc
Fried growol	1.9333 a	1.7000 a	1.8667 a	1.9333 a

*) The same notation in one column indicated no significant difference

Table 4 shows that without processing growol and growol with steaming, cooling, or baking processes had appealing appearances, chewy textures, and taste which were similar to that of fried growol. However, based on the overall preference test, fried growol was the least preferred.

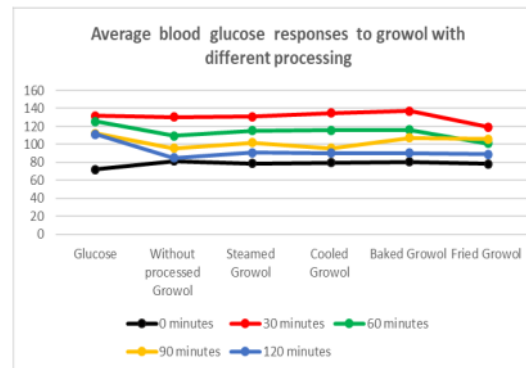
2. Blood Glucose Responses to Growol with Different Processing:

Tables and curves showing the blood glucose responses to growol with different processing and the

glucose standard food are presented below.

Table 5. Average blood glucose responses to growol with different processing

	0 Minute s	30 Minutes	60 Minute s	90 Minute s	120 Minute s
Glucose	72.3	131.9	125.4	112.3	111.1
Without-processing growol	81.5	130.4	109.5	95.6	85.2
Steamed growol	78.8	130.7	115.3	101.8	90.8
Cooled growol	79.5	134.7	115.6	95.7	90.6
Baked growol	80.6	137.3	116.1	107.5	90.2
Fried growol	78.3	119.2	100.9	106	89



Picture. Average blood glucose responses to growol with different processing

Blood glucose responses after the consumption of without-processing growol and growol with steaming, baking, frying, and cooling processes were lower than after the consumption of glucose standard food. In other words, the starch in cassava as the essential material of growol was not completely hydrolyzed to glucose during fermentation. The more the starch hydrolyzed to glucose, the more increased blood glucose responses.

3. Glycemic Index (GI) of *Growol* with Different Processing:

Glycemic index (GI) is the ranking of foods based on postprandial glucose response compared with a reference food. High glycemic index foods produce high concentrations of blood glucose and increase insulin demand and could plausibly contribute to the development of type 2 diabetes. GI is usually applied in the context of the quantity of the food and the amount of carbohydrate in the food that is consumed. A related measure, the glycemic load (GL), factors this by multiplying²⁷.

The GI of the food in question by the carbohydrate content of the consumed serving. Low GI diets are essential to address blood glucose control as consumption of foods with a high GI is hypothesized to contribute to insulin resistance, which is associated with an increased risk of diabetes mellitus, obesity, cardiovascular disease, and some cancers²⁷.

Based on the curve of blood glucose responses, we could determine the Glycemic Index (GI) of *growol* with different processing, as shown in Table 6

Table 6. Glycemic index of *growol* with different processing

Sample	Article Error	Glycemic Index	Amount of LAB (CFU/g sample)
<i>rowol</i> with a baking process		78	1.32×10^7
<i>Growol</i> with a steaming process		77.41	6.7×10^3
<i>Growol</i> with a cooling process		73.49	1.53×10^3
<i>Growol</i> without processing		78.94	1.27×10^3
<i>Growol</i> with a frying process		75.43	2.7×10^2

Glycemic index is rated on 1 to 100. Foods that raise the blood glucose quickly after a meal are known as high glycemic index meals and they are assigned a value of 70 and above while foods that release glucose slowly into the bloodstream are known as low glycemic index foods and their values are 55 and below. There are so many controversies surrounding the contribution of starchy foods such as cassava to the incidence of diabetes mellitus²⁷.

Health problems associated with high blood glucose such as obesity, metabolic syndrome, diabetes mellitus are due to high glycemic index foods²⁸. Therefore, clinical trials have shown that low glycemic diets improve glycemic control in diabetes, increase insulin sensitivity; reduce food intake and body weight²⁹. This supports the contribution made by that certain carbohydrates are digested rapidly and releasing their glucose into the bloodstream.

In this research produced IG of baked, steamed, cooled, without-processing, and fried *growol* was 97, 94, 93, 91, and 89, respectively. These tubers can be traditionally

processed into various forms, one of which is fried granules (*Garri*) from fermented²⁷, grated cassava. In a similar study carried out by on the glycemic indices of different cassava products, the glycemic index for *growol* was 92.

Based on the test of *lactic acid bacteria* level, all processing affected the growth of LAB without damaging all LAB in *growol*. This attested that lactic acid bacteria in *growol* could retain their growth at neither low nor high temperature. This supported the theory that *Lactobacillus casei* LAB would grow optimally at 30°C, whereas *Bifidobacterium bifidum* would grow optimally at 37°C and could grow at a minimum temperature of 37°C and a maximum temperature of 48°C. *Lactobacillus casei* strain *Shirota* (*Yakult* bacteria) grew at 15-41°C (optimum at 37°C) and bacterial activities would be slowed down at a temperature less than 15°C. This finding indicated that room temperature could give LAB growth conditions to *growol*. As such, storing *growol* at room temperature would likely provide an optimum growth condition for the LAB of *growol*, optimizing the LAB or probiotic levels in *growol* and supporting its potentials as a functional probiotic food. A type of lactic acid bacteria that only has some enzymes to break down amylose and starch, and certain lactic acid bacteria which is only able to break down protein into simpler substances^(2, 30).

CONCLUSIONS:

The standardization of *growol* processing consisted of the following stages, namely preparing basic materials, conducting the fermentation by not changing the immersing water for three days, washing five times (b/v), draining, crushing, steaming, and molding. *Growol* made with the standardized processing had an appealing appearance, chewy texture, strong taste, and LAB levels of 1.32×10^7 cfu/g. Without- processing *growol* and *growol* with steaming, cooling, and baking processes had the appealing appearance, chewy texture, and taste which was not much different from and more preferred than fried *growol*. Additionally, the GI of baked, steamed, cooled, without-processing, 35 and fried *growol* was 97, 94, 93, 91, and 89, respectively. Furthermore, LAB testing indicated that the LAB level of without-processing, baked, fried, steamed, and cooled *growol* was 1.32×10^7 cfu/g, 6.7×10^3 cfu/g, 1.53×10^3 cfu/g, 1.27×10^3 cfu/g, and 2.7×10^2 cfu/g, respectively. *Growol* was not potential as functional prodiabetic food but potential as a functional probiotic food.

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