Effects of Fermentation Duration and Cooking Method on The Chemical Properties and Acceptability of Growol

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Effects of Fermentation Duration and Cooking Method on The Chemical Properties and Acceptability of *Growol*

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Abstract

Growol is a local staple food made from cassava that has been processed by spontaneous fermentation, which is achieved by soaking in water for 3-5 days. The fermented cassava is then cooked by steaming before consumption. *Growol* contains the bacteria *Lactobacillus* that can produce lactic acid. Currently, most *growol* producers soak the cassava for 2-3 days to reduce the sourness of *growol*. This causes a decrease in the pro-biotic food potential of *growol* as a small amount of the starch is hydrolyzed into sugar and acid, but with an increase in the amylose content. This increase in amylose produces retrograded starch that functions as resistant starch, although the texture of the *growol* remains hard. The purpose of this study was to modify the cooking method to produce a better preferred *growol* with a high lactic acid bacteria content and increased potential yield of retrograded starch. This research was done with a completely randomized design. The cassava used in this research was the local variety Martapura. Variations in fermentation duration were: 0, 24 and 48 hours, and cooking of the fermented cassava was varied: normal steaming for 15 minutes and two cycles of autoclave cooking at a temperature of 121°C. Moisture content, starch, acid, amylose and the total lactic acid bacteria of the cassava and fermented cassava were determined; *growol* samples were analyzed for their moisture content, acid and the acceptability by hedonic test method. The data were analyzed using SPSS-13 for Windows. The research showed that varying the duration of fermentation affected the fermented-cassava starch content and titratable acidity. The longer the fermentation duration, the lower the starch and amylose content of the fermented cassava, whereas the titratable acidity was increased. Based on the aroma, colour, texture and taste, the most acceptable *growol* was that cooked by steaming for 15 minutes with a fermentation process of 48 hours which resulted in a lactic acid bacteria content of $1 \times x10^5$ cfu/g.

Key words: fermented-cassava, cooking, growol, acceptability.

Introduction

Growol was a staple food in the Kulon Progo Regency, Yogyakarta, Indonesia a few decades ago. *Growol* is made from cassava using the following processing stages: peeling, washing, cutting, soaking/fermentation, washing, pressing, enumeration and steaming. Spontaneous fermentation of cassava in *growol* processing takes place over 3-5 days (Anonymous, 2015). Fermentation is part of the processing stage of *growol* that is carried out by soaking the cassava in water for several days. During cassava fermentation, lactic acid bacteria will grow and produce lactic acid. Putri *et al*. (2012) stated that the lactic acid bacteria types that predominantly grow in *growol* are *Lactobacillus plantarum* and *Lactobacillus rhamnosus*. Wariyah and Sri Luwihana (2015) determined that the total *Lactobacillus* in *growol* was 4.7×10^3 cfu/g. *Growol* has been proven to be effective in preventing diarrhea if consumed daily (Lestari, 2009, Prasetia and Kesetyaningsih, 2014).

Currently, the younger generation in Kulon Progo Regency, especially in Kalirejo village, do not like *growol* because of its sour taste, so to fulfill the desires of consumers *growol* producers have reduced the soaking time to two days. This reduction in soaking time hampers the optimal growth of lactic acid bacteria, thereby decreasing its potential as a pro-biotic. According to FAO/WHO (2002), pro-biotics are living organisms capable of providing beneficial effects on the health of their hosts, when consumed in sufficient quantities. On the other hand, a shorter fermentation time can increase the amylose content of the fermented cassava starch. According to Susilowati *et al.* (2008), the cassava harvested between the ages of 7 - 10 months contain of starch between 14.33% - 35.93% with an amylose content of between 12.37% and 18.91%. During fermentation there would be a breakdown of the starch molecules that could increase amylose (Putri *et al*., 2011). Ogbo and Okafor (2015) showed that in the cassava-based food processing, fermentation and cooking under certain conditions could improve retrograded starch that has the potential to metabolise starch resistance. In banana flour, fermentation to increase amylose can be achieved by soaking for twelve hours and heating at a temperature of 121ºC (Nurhayati, 2011).

The problem is that the *Lactobacillus* bacteria are not resistant to high temperatures. Erdiandini *et al.* (2015) stated that the viability of *Lactobacillus* bacteria decreases with the heating treatment. This condition could lower the sour taste of *growol*. Lactic acid bacteria are amylolytic bacteria. During cassava fermentation two processes occur. These are the enzymatic hydrolysis of carbohydrate substrate (starch) into sugar and conversion of sugar resulted lactic acid (Reddy *et al*., 2008), so decreasing the amount of bacteria would decrease the production of sugar and acid. The objective of this study was to evaluate the effect of varying the duration of fermentation and the cooking method of the fermented cassava on the chemical characteristics (amylose content, starch, titratable acidity) and the lactic acid bacteria resistance in *growol*.

Materials and Methods

Materials

The local cassava used as a raw material for *growol* production was Martapura variety and was purchased from the farmers' market in Sangon village, Kalirejo, Kokap, Kulon Progo, DIY, Indonesia. The cassava was harvested at the age of 12 months and was used no more than two days after harvesting. Chemicals for analysis of the starch content, titratable acidity and amylose, such as: KOH (Merck, 85%), indicator phenolphthalein $C_{20}O_{14}O_4$ (Merck, Darmstadt, 1%), ethanol (Merck, 100%); HCl (Merck, 37%) with pro-analysis qualification and MRS media for total lactic acid bacteria (LAB) analysis came from Oxoid Ltd.

Equipments

The equipment used in this research was: a set of cassava fermentation equipment, autoclave (Pressure sterilizer model 1925X Wisconsin Aluminium Foundry C. Inc. 838 South 16th St. Manitowoc, WI 54220), steamer pan (Bima stainless steel), UV Vis Spectrophotometer (Shimadzu UV mini 1240) for analysis of amylose and starch, balance (OHAUS Pioneer PA214), oven (Memmert DIN 40050 IP 20), vortex (Maxi Mix II TY 37600), sensory testing equipment and glassware for chemical analysis from Pyrex Iwaki (Iwaki glass under LIC).

Research procedure

The fresh cassava was analysed for its water content using the static gravimetric method and for its starch content with the Direct Acid Hydrolysis method (AOAC, 1990), the amylose content was analysed by the colorimetric method (Williams *et al*., 1970), titratable acidity (Apriyantono *et al*., 1989). Processing of *growol* refers to Sutanti *et al.* (2013) and Wariyah and Luwihana (2015). The cassava was peeled and cut into 5 cm lengths, furthermore soaked in water at a cassava/water ratio of 1/3 (w/v) and with variations of soaking/fermentation duration of cassava of: 0 hour (control), 24 hours and 48 hours.

The fermented cassava was analyzed for moisture content, starch, total lactic acid bacteria (LAB) (Fardiaz, 1993 in Hidayat *et al*, 2013) and amylose content. Furthermore, it was cooked using various cooking methods: steaming for 15 minutes (such as is done to make normal *growol*) and cooking using two cycle autoclaves at 121°C for 15 minutes (Ashwar *et al.,* 2016). The resulting *growol* samples were analyzed for titratable acidity, moisture content, total (LAB) and acceptability of the *growo,l* which was determined by Hedonic Test (Krammer and Twigg, 1966).

Design experiment

This research used Completely Randomized Design with the factors of fermentation duration and cooking method. The difference between treatments was determined by F test then any significant difference between samples was determined by Duncan's Multiples Range Test (DMRT) that was analyzed by SPSS 13.0.

Results and Discussion

Chemical characteristics of fermented cassava

Table 1. showed the chemical characteristics of fermented cassava. Cassava used for making *growol* has a water content of 60.00% ± 0.49% (wb), starch 49.03% ± 1.68% (db) or about 19.60% ± 0.43% (wb), and amylose 46.95% ± 0.23% (db) or 16.72% ± 0.50% (wb). Susilowati (2008) recorded a cassava starch content of between 14.33% and 35.93% with amylose between 12.37% and 18.91%, but the amount of starch and amylose content was also dependent on the cassava varieties. The major components of cassava are starch and water, which can function as a source of carbohydrates in *growol* as a staple food.

Table 1. The characteristics of cassava and fermented cassava

Cooking method	Fermentation duration (days)	Moisture (% wb)	Titratable acidity (%wb)	Starch (%db)	Amylose (%db)
Fresh cassava	-	60.00±0.49	0.49±0.01	49.03±1.68	46.95±0.23
Steaming	24	58.47±3.95**	0.38±0.01[a*]	75.60±1.41[b*]	48.46±0.70c*
	48	57.49±5.19	0.52±0.08[c]	64.15±0.42[a]	36.12±0.21a
Autoclave two cycles	24	57.77±4.46	0.38±0.02[a]	75.57±6.52[b]	47.83±0.47b
	48	54.59±2.29	0.48±0.06[bc]	65.27±4.60[a]	35.83±1.12a

*Means in a column with similar superscript, not significantly different at α =0.05.
**not significantly different.

After fermentation, the moisture content of the fermented cassavas was 54.59% ± 2.29% - 58.47% ± 3.95% (wb) and not significantly different, the starch was about 64.15% ± 0.42% -75.60% ± 1.41% (db), amylose 35.83% ± 1.12% - 48.46% ± 0.70% (db). The water content of the fermented cassava was lower than the fresh cassava, because before cooking the fermented cassava it was pressed and crushed to reduce water until it reached certain softness. In addition, during washing, the fermented cassava was separated from the fiber component, which resulted in a relatively higher starch content. The length of the fermentation had an effect on the starch content of the fermented cassava. The longer the fermentation the lower the starch content. According to Reddy *et al.* (2008), during fermentation, the starch was hydrolyzed into sugar and then lactic acid. This was shown by increasing the titratable acidity in fermentation for 48 hours. The amylose content of the fermented cassava increased slightly. Putri *et al*. (2011) stated that cassava fermentation for 48 hours at temperature of 30°C resulted in a stable amylose content of the fermented cassava but was still dependent on the type of *Lactobacillus* during fermentation.

Growol characteristics

Growol is fermented cassava (Table 1) that has been treated with two different methods of heating, i.e. normal steaming for 15 minutes and cooking using two cycle autoclaves at 121°C for 15 minutes. The resulting analyses of moisture content and titratable acidity of the *growol* are shown in Table 2, and the total lactic acid bacteria is shown in Table 3.

Table 2. Moisture content and titratable acidity of *growol*

Cooking method	Fermentation duration (days))	Air (% wb)**	Keasaman tertitrasi (%wb)*
Steaming	0	59.70±0.50	0.21±0.03[ab]
	24	60.03±4.17	0.20±0.03[a]
	48	58.01±5.19	0.26±0.06[c]
Autoclave two cycles	0	60.02±1.80	0.23±0.03[ab]
	24	57.52±4.87	0.20±0.03[a]
	48	54.63±0.36	0.22±0.04[ab]

*Means in a column with similar superscript are not significantly different at α =0.05.
** not significantly different.

The results of the analysis showed that the moisture content of *growol* was not significantly different, whereas the titratable acidity was significantly different. *Growol* soaked for 48 hours and cooked by steaming for 15 minutes had the highest titratable acidity. Before steaming, *growol* with fermentation duration of 48 hours had the highest titrated acidity, so was the most acidic *growol*. However, all treatments showed that there were decreases in acidity after cooking. This was probably due to part of the lactic acid evaporating during the heating process. According to Komesu *et al.* (2017), lactic acid stability was affected by the temperature and heating time. The higher the temperature and the longer the heating time, the faster the lactic acid degraded. Steaming treatment for 15 minutes was a mild heating condition compared to cooking using autoclaves two cycles at 121°C, therefore the acidity of *growol* tended to be higher.

Besides acidity, a characteristic of *growol* is the *Lactobacillus* bacteria content. The results indicated that the total lactic acid bacteria (LAB) on the fermented cassava increased with an increase in fermentation duration; the lengthier the time of fermentation, the more the total LAB, both in samples prepared for steaming and for cooking in the autoclave. Putri *et al*. (2012) found that the amount of bacteria after cassava fermentation of 48 hours was about $7.5\times x10^{10}$ cfu/g. However, after cooking no LAB was present in the *growol*, except in *growol* which had been treated with soaking/fermentation for 48 hours and was cooked by steaming. Wariyah and Luwihana (2015) found that in *growol* treated with fermentation for 3 days and then steamed, the total LAB was 4.7×10^3 cfu/g. Cooking conditions greatly affected the total LAB. The LAB could grow well at 37°C. Therefore, the higher the temperatures of LAB and the longer they were sustained, the more the resistance fell.

Table 3. Total Lactic acid bacteria (LAB) for fermented cassava and *growol*

Cooking method	Fermentation duration (days)	Total LAB (cfu/g)	
		Fermented cassava	*Growol*
Fresh cassava	0	5.00×10^5	-
Steaming	24	3.27×10^7	-
	48	4.80×10^8	1.0×10^5
Autoclave two cycles	24	4.90×10^7	-
	48	8.10×10^7	-

*Means those in a column with similar superscript are not significantly different at α =0.05.

Acceptability of growol

The acceptability of *growol* was determined based on preferences for the aroma, colour, texture, taste and overall preferences as shown in Table 4. The acceptability of *growol* showed a significant difference. The aroma, texture, taste and overall preferences of *growol* made from cassava fermented (24 and 48 hours) and cooked by steaming, and a sample made without fermentation (0 hours) which was cooked by using autoclave were not significantly different and were judged to be favourable.

Table 4. Acceptability of *growol**

Cooking method	Fermentation duration (days)	Aroma	Warna	Tekstur	Rasa	Kesukaan keseluruhan
Steaming	0	2.35[a]	2.90[b]	2.70[a]	2.85[a]	2.75[a]
	24	2.65[a]	2.40[ab]	3.55[abc]	3.15[a]	3.10[a]
	48	2.45[a]	2.50[ab]	3.00[ab]	2.45[a]	2.60[a]
Autoclave two cycles	0	2.35[a]	2.00[a]	3.00[ab]	2.85[a]	2.75[a]
	24	5.35[b]	3.70[c]	4.20[c]	5.30[b]	5.05[b]
	48	5.35[b]	3.00[b]	3.85[bc]	5.25[b]	5.15[b]

*Means those in a column with similar superscript are not significantly different at α =0.05.

Whereas two samples from fermented cassava with fermentation times of 24 and 48 hours and cooked by using autoclave were not different and were disliked. The Factors that determined the acceptance of *growol* to the panelists were: smell and taste being rather sour, white colour and chewy texture. In *growol* with 24 hours and 48 hours fermentation treatment and cooking with autoclave, the aroma, colour, texture, taste and overall preferences in categories were less acceptable. This was due to a decrease in the aroma, a brownish color, a bland taste and a very soft texture.

A long heating time and a high temperature (autoclave two cycles and temperature 121°C), caused aromatic substance loss. The colour became brown due to intensive Maillard browning. According to Fennema (1985), Maillard's reaction could be due to a reaction between the amino-protein group and the reduction in sugar. According to Wariyah and Luwihana (2016), a reduction in the sugar of fermented cassava became greater with an increase in fermentation time, Hence the browning effect. The texture of the *growol* was also not favoured because it was too soft. This was due to a higher heating temperature and longer fermentation, which resulted in more hydrolyzed starch and more simple sugars, so the *growol* texture was softer.

Conclusion

The duration of cassava fermentation had a significant effect on the chemical characteristics of fermented cassava. The longer the fermentation, the starch content was decreased, and the titratable acidity was increased, but the amylose remained relatively stable. The duration of fermentation and the method of cooking affected the bacterial resistance and *growol* preferences. Cooking the fermented cassava by steaming resulted in a preferred *growol*, while cooking with autoclave produced *growol* which was not well liked *Growol* made with 48 hours of fermentation and cooked by steaming the fermented cassava still contains LAB, while other treatments did not retain LAB. Based on the aroma, colour, texture and flavor of *growol* and its LAB resistance, cassava fermented for 48 hours and cooked by steaming resulted in an acceptable *growol* with a total LAB 1.0×10^5 cfu/g.

Acknowledgement

This research was financially supported by the Directorate of Research and Community Service, Ministry of Research, Technology and Higher Education of the Republic of Indonesia via the Applied Research Grant Program in 2018 with the Letter of Assignment No. 001/HB-LIT/II/2018, 15 February 2018.

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