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Research Paper

Diversity of Traditional Fermented Foods: Sucrose and Reducing Sugar Analysis of Various Fermented-Cassava

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Abstract

Cassava and its various fermented products are one of the local foods in Indonesia. The fermentation process can have different effects on food, both in taste and nutritional value. Changes in sucrose and reduced sugar levels during fermentation play a role in determining food effects in various health disorders. This study aims to describe the impact of cassava fermentation on different fermented products (tape, growol, gathot) in terms of sucrose and reducing sugar levels. This observational laboratory research was done through two stages, i.e., qualitative and quantitative analysis. The qualitative analysis examined carbohydrates' presence (monosaccharides, disaccharides, oligosaccharides, polysaccharides) through the Molisch test, Benedict's test, and the Iodine test. Quantitative analysis was carried out to determine sucrose and reducing sugar levels using three replicates with the Nelson-Somogyi test. The research was conducted in January-July 2019 at Universitas Respati Yogyakarta and Chem-mix Pratama Laboratory. The statistical analysis used was One Way Anova at a 5% significance level and continued with the Tukey test. Qualitative tests showed cassava, tape, growol, and gathot contained monosaccharides and polysaccharides. Benedict's test results showed that growol did not have reducing sugars. The quantitative test showed Growol's reducing sugar content was the lowest (0.32%) compared to cassava (0.95%), tape (14.87%), and gathot (11.12%). The highest sucrose content was in cassava (4.77%) followed by growol (2.36%), tape (1.47%), and gathot (0.78%). There were significant differences in sucrose and reducing sugars levels between cassava, tape, growol, and gathot ($p<0.001$). In conclusion, growol is a fermented food that can be used as a functional food.

Keywords *Cassava, Fermented Food, Reducing Sugar, Sucrose*

INTRODUCTION

Indonesia is a country with a diversity of foods, from food to drinks. This diversity of food makes Indonesia a tourist destination, especially if the food offers not only good taste but also health benefits. One of the foods the most easily found in Indonesia is cassava, which can be consumed as a staple food or as a snack processed using fermentation techniques in the form of tape, growol, gathot and others (Surono, 2016; Wijaya, 2019; Cempaka, 2021; Hutajulu et al., 2021).

Fermentation, a food processing technique that utilises the role of microbes and mould, is used in making tape, growol, and gathot (Rahayu, 2003; Surono, 2016; Cempaka, 2021). Various microbes involved in making tape, growol, and gathot such as *Saccharomyces cerevisiae, Lactobacillus plantarum, Lactobacillus fermentum, Actinobacteria, Pediococcus, Yeast* (Rahayu, 2003; Nuraida, 2015; Surono, 2016). The involvement of various microbes in the fermentation process is beneficial in preventing various diseases, one of which is diarrhoea caused by *Escherichia colli, Staphylococcus aureus*, and *Shigella dysentriae* (Nuraida, 2015).

Fermentation can cause chemical changes in complex organic compounds to become simpler organic compounds through enzymes produced by microbial growth and cause changes in the

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nutritional value of foodstuffs, one of which is a change in sucrose and reduced sugar levels. The fermentation that is too long can reduce the level of sucrose by breaking it down sucrose into monosaccharides (glucose and fructose). Furthermore, the more glucose is broken down into alcohol; the less reducing sugar will be in the fermentation process (Aryanta, 2000; Purnomo et al., 2013; Surono, 2016). Reducing sucrose and reducing sugar levels through this fermentation process can provide benefits, especially as an alternative food for various health problems such as obesity and diabetes mellitus, considering that excessive consumption of sugar can increase hunger and energy consumption (Aller et al., 2011; Sari et al., 2017). In addition, fermenting food prevents diabetes and obesity by increasing the capacity of antioxidants and anti-inflammatory compounds in food (Sivamaruthi et al., 2018; Cabello-Olmo et al., 2019; Jalili et al., 2023). A study showed that adding lactic acid bacteria from fermented cassava to make tempeh could improve tempeh's nutritional value and the composition of the microbiota in experimental animals with diabetes mellitus (Kusuma et al., 2021).

There are still limited studies related to changes in the characteristics of cassava carbohydrates during the fermentation process to tape, growol, and gathot, which are interesting to study, especially in terms of changes in sucrose and reducing sugar levels. This study wants to investigate the effects of cassava fermentation on sucrose and reducing sugar analysis.

LITERATURE REVIEW

This study aims to describe the effects of fermentation of cassava into various processed variants, namely tape, growol, and gathot in terms of testing the levels of sucrose and reducing sugar. Cassava is one of Indonesia's primary commodities; in fact Indonesia is the third-highest cassava-producing country after Nigeria and Thailand (Frediansyah, 2018). Cassava can be consumed simply through frying, steaming or boiling techniques or even processed into various products such as *kolak* and *bakwan*. Another processing technique that can optimize the nutritional value and health benefits of cassava is the fermentation technique.

Tape cassava is a fermented product from cassava which is made through the stages of washing, soaking, steaming, cooling at room temperature, sowing using yeast, packing using leaves, and fermenting for 2-3 days until the tape has a distinctive taste (Surono, 2016; Cempaka, 2021). Making growol starts with peeling, cutting cassava into small sizes, washing, soaking for about four days, filtering, pressing and drying, which ends with steaming (Surono, 2016). In comparison, gathot is made by peeling, washing, drying in the sun, and then fermentation in sacks for 1-2 weeks until a blackish brown colour is formed. The dried gathot is soaked for 2x24 hours and steamed for 30 minutes (Puspaningtyas et al., 2018).

During the fermentation process, there is a change in nutrients. The process of fermenting cassava into various products involves two stages. The first stage is the conversion of complex carbohydrates into simple carbohydrates carried out by microbes that produce the enzyme amylase. The second stage is the conversion of simple sugars into alcohol and acid compounds. This condition allows for changes in sucrose and reducing sugar levels in different fermentation techniques (Purnomo et al., 2013; Surono, 2016; Frediansyah, 2018).

Fermented foods are starting to be seen as functional food that can be used to treat certain diseases, such as obesity and diabetes, because of their valuable nutritional content, such as the presence of non-digestible food compounds such as resistant starch (Puspaningtyas, Sari and Kusuma, 2018). Fermentation also affects changes in the levels and types of food carbohydrates, such as levels of dietary fibre, reducing sugar and sucrose (Sari et al., 2017). The composition of different nutritional values can provide different health effects. It is interesting to study the differences in sucrose and to reduce sugar levels in cassava and various cassava-fermented products (tape, growol, and gathot). Most of the previous study was just concerned with

fermentation's effect on each product's proximate analysis and dietary fibre content analysis. The study of sucrose and reducing sugar analysis, especially the comparison between each cassava fermentation product, is limited. This study will compare sucrose and reducing sugar content among cassava fermentation products.

RESEARCH METHOD

Research Design

This type of research is observational laboratory research. The study was carried out in two stages: qualitative and quantitative. Qualitative analysis was carried out to examine the presence of carbohydrates (monosaccharides, disaccharides, oligosaccharides, and polysaccharides). Quantitative analysis was conducted to determine the levels of sucrose and reducing sugars in cassava and various fermented cassava products (tape, growol, and gathot) using three replicates for each sample.

The research was conducted from January to July 2019. Tape and growol production were carried out at the Dietetics and Culinary Laboratory, Faculty of Health Sciences, Universitas Respati Yogyakarta. While raw gathot is made by local farmer Ngudi Makmur in Gunung Kidul, Yogyakarta, then gathot is made at the Dietetics and Culinary Laboratory, Faculty of Health Sciences, Universitas Respati Yogyakarta. The Biomedical Laboratory, Faculty of Health Sciences, Universitas Respati Yogyakarta, conducted qualitative testing of carbohydrate types. Determination of sucrose and reducing sugar levels was carried out at the Pratama Chem-mix Laboratory, Banguntapan, Bantul.

Tools and Materials

The cassava used in this research is Klentengan cassava or commonly called Darul Hidayah cassava. Cassava is harvested at 8-11 months and obtained from the Ngudi Makmur Local Farmer in Gunung Kidul, Yogyakarta. The tools used included scales, baking sheets, basins, forks, steam pans, knives, thermometers, test tubes, mortar, pestle and stirrer.

Process of Making Tape, Growol, and Gathot

Tape cassava is made by steaming cassava for 20 minutes and then, cooling it, then sprinkling yeast with a cassava and yeast ratio of 1:0.5 and then packing it in banana leaves for two days.

The method of making growol is cassava that has been cleanly cut into ± 5 cm in size and then soaked in clean water with a ratio between cassava and water is 1:3 for four days. Then the cassava is washed seven times, filtered with a filter cloth, squeezed out of the water, last steamed for 20 minutes.

Gathot is made by drying cassava in the sun until all parts of the cassava are dry and then fermenting for two days in a clean sack to become gathotan. Gathotan is washed and then dried again for one day. The gathotan is then washed clean and then soaked in water for 12 hours at room temperature with a ratio of gathotan, and water is 1:4, then the gathotan is cut into 2x2x2 cm sizes first, then the gathotan is steamed for 30 minutes to become gathot.

Sucrose and Reducing Sugar Analysis

Qualitative analysis was carried out using Molisch's test, Benedict's test, and Iodine. The test was started by preparing an extraction solution. The extraction solution was prepared with 5 grams of each sample, namely cassava, tape, growol, and gathot pulverized using a pestle and mortar. The sample was dissolved in 20 ml of distilled water, filtered with filter paper and a funnel, and then the sediment-free solution was extracted as the extraction solution. Quantitative analysis of sucrose and reducing sugar levels were tested using the Nelson Somogyi method.

Statistical Analysis

The statistical analysis used was One Way Anova at a significance level of 5% and continued with the Tukey test to determine the difference in the average sucrose and reducing sugar levels in cassava, tape, growol, and gathot.

FINDINGS AND DISCUSSION

Characteristics of Tape, Growol, and Gathot

The cassava used in this study has specifications that are brownish-green stems, red stalks, finger-shaped leaves, brown outer skin, pink inner skin, and white tuber flesh. The cassava tape has the characteristics of a sweet taste, alcohol aroma, soft texture, and cream colour. While growol has the final characteristic of being white, it has a chewy texture, slightly sour taste, and distinctive aroma. Gathot's final characteristic is chewy textured, and blackish brown (Figure 1).

(a) (b) (c)

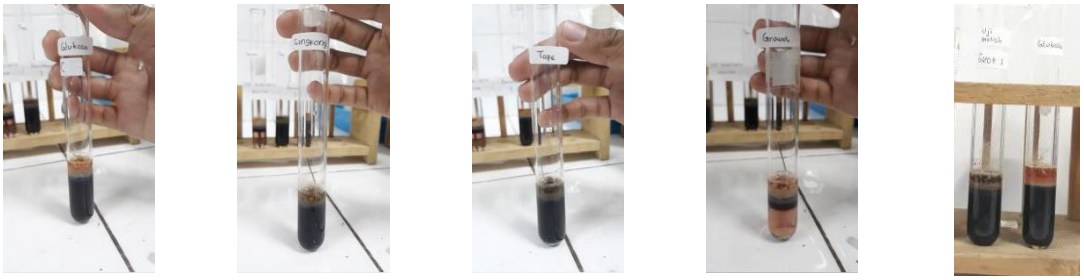
Figure 1. (a) Tape; (b) Growol; (c) Gathot

Tape is a food with a sweet and sour taste accompanied by the aroma of alcohol. Growol also has a taste sensation like tape with a more distinctive aroma. The presence of sour and sweet tastes and the formation of a distinctive aroma in tape and growol is caused by the fermentation process of cassava into tape or growol. The black colour in gathot is caused by spontaneous fermentation of the raw material (cassava) without soaking but in direct sunlight. The process of fermenting cassava into various products involves two stages: the conversion of starch into simple sugars, which is carried out by microbes that produce the enzyme amylase. The second stage is the change of sugar into alcohol and acid compounds. The sour taste produced in the making tape and growol is possibly due to the formation of acidic compounds in the second stage of fermentation (Purnomo, Lindayani and Hartayanie, 2013; Surono, 2016; Frediansyah, 2018).

Besides changing the taste and texture of food, the fermentation process can change the composition of the nutritional value of a food. The most striking change from the fermentation process is the change in the content of complex and simple carbohydrates (Surono, 2016; Sari et al., 2017; Puspaningtyas et al., 2018). Reducing sugar compounds can increase from 7.9% to 16% during the first four days of fermentation or can even decrease (Surono, 2016). A recent study showed decreased reducing sugars during fermentation (Indasah & Muhith, 2020). Carbohydrate testing can be done qualitatively and quantitatively. Qualitative testing was carried out using the Molisch, Benedict's, and Iodine tests, while quantitative testing was carried out using the Nelson Somogyi method.

Qualitative Analysis

The Molisch test is used to see the presence of monosaccharides in a food. The formation of a purple ring indicates a positive result in the test sample due to the reaction of alpha naphthol with 98% concentrated sulfuric acid (Schreck & Loffredo, 2017; Pooja et al., 2022). Figure 2 shows the presence of monosaccharides in cassava, tape, growol and gathot samples (indicated by the formation of purple rings).



(a) (b) (c) (d) (e)

Figure 2. (a) Standard Sample (Glucose); (b) Molisch Test Results on Cassava; (c) Molisch Test Results on Tape; (d) Molisch Test Results on Growol; (e) Molisch Test Results on Gathot

Benedict's test showed a brick-red colour change in cassava solution, cassava tape, and gathot. While there was no change in the brick red color in the growol solution (Figure 3). Benedict's test determines the presence of reducing sugars in food. Types of carbohydrates tested on reducing sugars include glucose and fructose. A positive result is indicated by the formation of a red or brick-red colour (Schreck & Loffredo, 2017; Pooja et al., 2022).

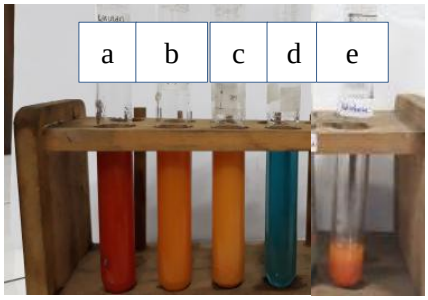


Figure 3. (a) Standard Sample (Glucose); (b) Benedict's Test Results on Cassava; (c) Benedict's Test Results on Tape; (d) Benedict's Test Results on Growol; (e) Benedict's Test Results on Gathot

The iodine test is used to check the presence of polysaccharides in food. The formation of black-blue colour indicates the presence of starch, while dark brown colour indicates the presence of glycogen (Schreck & Loffredo, 2017; Pooja et al., 2022). This test was only carried out on samples of cassava, tape, growol, and gathot (Figure 4). The addition of iodine solution to the sample causes a blue-black change in the cassava, growol, and gathot samples (indicating the presence of starch). While the addition of iodine solution to the tape sample showed a blackish-brown colour change (presence of glycogen).

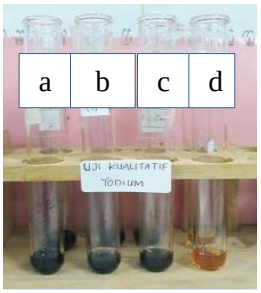


Figure 4. (a) Iodine Test Results on Cassava; (b) Iodine Test Results on Growol; (c) Iodine Test Results on Gathot; (d) Iodine Test Results on Tape

Quantitative Analysis

Table 1 illustrates the differences in sucrose and reducing sugar levels between cassava, tape, growol, and gathot. Sucrose levels from highest to lowest were found in cassava, growol, tape and gathot. Meanwhile, the highest to lowest reducing sugar levels were found in tape, gathot, cassava and growol.

Table 1. Test Results for Sucrose and Reducing Sugar Levels in Cassava and Various Cassava Fermented Products

Sample	Mean ± SD	
	Sucrose (%)	**Reducing Sugar (%)**
Cassava	4.77 ± 0.10 [a]	0.95 ± 0.01 [a]
Tape	1.47 ± 0.15 [b]	14.87 ± 0.06 [b]
Growol	2.36 ± 0.01 [c]	0.32 ± 0.01 [c]
Gathot	0.78 ± 0.10 [d]	11.12 ± 0.03 [d]

Note: Different letter notations (a,b,c,d) in the same column indicate a significant difference in the Tukey test ($p<0.001$)

The qualitative test results showed cassava, tape, growol, and gathot contained monosaccharides and polysaccharides. Meanwhile, Benedict's test results showed that growol did not have reducing sugars, unlike cassava, tape and gathot. The results of this qualitative test are consistent with the results of quantitative tests, which show that the results of growol's reducing sugar are the lowest (0.32%) compared to cassava (0.95%), tape (14.87%), and gathot (11.12%). In the quantitative test for disaccharides (sucrose), the highest sucrose content was found in cassava, followed by growol, then tape and gathot. In other words, there were significant differences in the sucrose and reducing sugars levels between cassava, tape, growol, and gathot ($p<0.001$).

Differences in the fermentation process may affect the nutritional content of each food ingredient. The tape fermentation process is carried out after the tape has been steamed first, the growol fermentation process is carried out by soaking the growol in water for some days and then steaming the growol. Meanwhile, the gathot fermentation process is carried out under the sun, fermented in a closed sack, and then steamed until cooked (Rahayu, 2003; Surono, 2016). This difference in the fermentation process will affect the differences in the environment for microbes. Of course, the main microbial differences in each fermentation process also cause differences in the nutritional value of food. The main microorganisms involved in making tape are Saccharomyces cerevisiae, Lactobacillus plantarum, Lactobacillus fermentum. While growol involves *Coryneform, Streptococcus, Bacillus, Actinobacteria, Lactobacillus, and Yeast.* Gathot also has different microorganisms, such as *Saccharomyces sp. TR7, Lactobacillus plantarum 250 Mut 7 FNCC* (Nuraida, 2015; Surono, 2016). Previous studies have shown that differences in microorganisms in the fermentation process lead to differences in crude protein and fiber levels in cassava products (Polyorach et al., 2018).

Sucrose levels in cassava are categorized as the highest when compared to processed cassava products because, in the fermentation process, microorganisms require sucrose and other simple sugars. Hence, the sucrose content in cassava is higher than gathot, growol, and tape. During

fermentation, sucrose will be hydrolyzed into glucose and fructose by the enzyme sucrase.

While the reduced sugar content of tape is the highest compared to cassava and other fermented products, this is possible because the long fermentation process allows microorganisms to continue hydrolyzing cassava starch so that more and more simple sugars are produced. The more simple sugars are produced, the more reducing sugars are produced. The fermentation environment can also affect the final content of reduced sugar. Tape and gathot are products that are fermented by packing using leaves and sacks, in contrast to growol, which is fermented by soaking in water.

Based on the results of sucrose and reduced sugars content, growol is a fermented product that is more recommended to be consumed by obese and/or diabetic people because it has a lower simple sugar content than tape or gathot. This is in line with previous studies, which show that growol has the potential effect to be used as a prebiotic food in the treatment and prevention of disease because the process of fermenting cassava into growol is able to reduce total sugar, reducing sugar and sucrose levels and is able to increase total dietary fibre, water-soluble dietary fibre, and water-insoluble dietary fibre (Puspaningtyas et al., 2019). Prebiotics are nutritional compounds that can provide health benefits, especially in maintaining the balance of the gastrointestinal microbiota, which will affect the prevention and treatment of obesity and diabetes (Cerdó et al., 2019; Megur et al., 2022; Santos et al., 2022).

A preclinical study proves that growol flour contributes to controlling diabetes. This can be seen from the decrease in fasting blood glucose levels of 99.12 mg/dL to 152.24 mg/dL (36.89% - 57.49%) compared to blood glucose levels before giving growol flour. In addition, there was an increase in insulin levels of 7.54% (32.03pg/mL) to 20.57% (86.79pg/mL) compared to insulin levels before giving growol flour. Growol can potentially be developed as a functional food for diabetes mellitus (Nofia et al., 2022).

CONCLUSIONS

The qualitative test showed that cassava and fermented cassava products contained monosaccharides and polysaccharides. Furthermore, based on the quantitative test, cassava is a food ingredient with the highest sucrose content, followed by growol, tape and gathot. Meanwhile, growol's reducing sugar is the lowest compared to other products. Judging from the levels of sucrose and reduced sugar, growol is a fermented food that is more recommended to be used as an alternative to functional food for obese and diabetic patients. However, further studies are needed regarding the growol potential in terms of dietary fiber, resistant starch, amylose, starch, amylopectin. The limitation of this study lies in qualitative testing, which is a subjective assessment that requires accuracy and expertise in assessing changes.

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All names involved in writing have a real contribution. Debora Helsius SB and Desty Ervira Puspaningtyas were involved in data collection, processing, and script writing. Inayah contributed to data processing and script writing. Puspita Mardika Sari was involved in data collection and script writing. Nanda Herdiyanti has contributed to data collection.

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