Exploring the potency of gathotan and gathot as diabetes functional food: resistant starch analysis

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Abstract. Indonesian traditional fermented food made of cassava, gathotan and gathot, was considered as diabetes functional food due to higher dietary fiber and lower carbohydrate content compared to cassava. Fermentation processes in gathotan and gathot involving lactic acid bacteria can potentially hydrolyze some natural starch into undigested resistant starch correlated with the value of glycemic index. Nonetheless, there has been no study on the gathotan and gathot potency as resistant starch sources. This study aimed to examine resistant starch content in cassava, gathotan, and gathot as diabetes functional food. Cassava was obtained from the traditional market in Yogyakarta. Gathotan was made through spontaneous fermentation while gathot was made by overnight soaking then steaming the gathotan. Resistant starch was analyzed by using enzymatic process through glucooxydase method. ANOVA test was used to analyze the resistant starch content difference in cassava, gathotan, and gathot at 5% significance level. The highest resistant starch content was found in cassava (24.44%), followed by gathotan (22.5%), and gathot (21.85%) (p=0.025). Fermentation process of cassava to gathotan and gathot decrease resistant starch content. Significant differences of resistant starch content were seen between cassava and gathot.

1. Introduction
Diabetes mellitus, a serious chronic disease, is the largest health problem in the world caused by insulin function decrease [1, 2]. The prevalence of diabetes mellitus increases year by year, especially for the past three decades and is growing rapidly in low- and middle-income countries [1, 3]. The worldwide prevalence of diabetes mellitus among adults has risen from 4.7% (1990) to 8.5% (2014) [2]. The global prevalence was expected to increase to 639 million by 2045 compared to the global prevalence in 2017 (451 million) [4]. Basic health research conducted by ministry of health showed that there was an increase in diabetes prevalence from 2007 (1.6%) to 2013 (2.1%) [5, 6]. Meanwhile, survey conducted by World Health Organization and International Diabetes Federation in 2016 and 2017 described the prevalence of diabetes mellitus in Indonesia was 6.6% and 6.7% respectively [7, 8].
Considering the increase of diabetes prevalence, deaths attributable to diabetes and high healthcare expenditure because of diabetes [4] a comprehensive strategy is needed to prevent and treat diabetes mellitus. Dietary management can be used to control blood sugar levels and other metabolic profile in diabetic patients [1]. Diet with high soluble and insoluble dietary fiber, high resistant starch and low glycaemic index can improve diabetes condition, especially controlling postprandial blood glucose levels and utilisation of blood glucose and fatty acid uptake to the muscle [9–12].

Indonesian traditional fermented food made of cassava, gathotan and gathot, was considered as diabetes functional food due to higher soluble and insoluble dietary fiber and lower carbohydrate content compared to cassava [13]. Recent intervention study showed that the most important characteristic of fiber associated with improved insulin sensitivity was the fermentability of the substrate. Fiber that is lost to caecum then potentially fermented by intestinal microflora to produce short chain fatty acid (SCFA), circulate free fatty acids (FFA) and regulate some physiological mediators and inflammatory markers so that it can influence health and decrease the risk of getting diabetes mellitus [14].

Not only does fermentation processes involving lactic acid bacteria in gathotan and gathot increase total dietary fiber and decrease carbohydrate content [13], it can potentially hydrolyze some natural starch into undigested resistant starch correlated with the value of glycemic index. Furthermore, the previous study described that cassava fermentation to gathotan and gathot can lower aflatoxin content and produced some antioxidant compounds [15, 16]. As a result, gathotan and gathot can be potentially used as diabetes functional food. To the best our knowledge, there has been no study on the potency of gathotan and gathot as resistant starch sources. The aim of this study is to examine resistant starch content in cassava, gathotan, and gathot as a preliminary study of diabetes functional food development.

2. Methods
This was an observational laboratory study analyzing resistant starch content in cassava flour, gathotan flour, and gathot flour. This study was conducted in Dietetic and Culinary Laboratory of Universitas Respati Yogyakarta on May until September 2017.

2.1. Materials
Pepsin from gastric porcine mucosa (Sigma Aldrich, Singapore), porcine pancreatic α-amylase (Sigma Aldrich, Singapore), Amyloglucosidase from Aspergillus niger (Sigma Aldrich, Singapore) and Glucose FS (Diasys, Germany) were purchased and used in this study.

2.2. Development of gathotan and gathot
Samples used in this study were cassava, gathotan, and gathot. Cassava obtained from local market in Yogyakarta was used to make fermented cassava; Gathotan and Gathot. In a brief, cassava was peeled, washed and dried for five days by sun drying. Dried cassava then was fermented for two weeks by laying dried cassava in a sack for spontaneous fermentation to occur. The cassava will turn into blackish color after two weeks of fermentation and will be called gathotan. Gathot was made by soaking gathotan for 2x24 hours then steaming for 30 minutes. The next step, cassava, gathotan, and gathot were made into flour by drying them in cabinet dryer, grinding with grinder, and sieving with 40 mesh size.

2.3. Resistant starch analysis
Resistant starch analysis was analyzed according to Goni et al. (1996). In brief, protein in 100 mg samples was hydrolyzed using 20 mg of pepsin at 40° C for 60 minutes at pH 2 prior to resistant starch analysis. The mixture then cooled at room temperature and the pH was adjusted back to 6.9 for hydrolysis of digestible starch using 40 mg of pancreatic α-amylase at 37° C for 16 hours. Hydrolysis was performed in magnetic stirrer and solution was continuously stirred during starch hydrolysis. The resulting solution was centrifuged for 15 minutes at 3000 rpm and pellet was collected. The pellets
were solubilized in 2 M KOH and digested further with amyloglucosidase at 60°C and pH 4.74 for 45 minutes to hydrolyze resistant starch. The mixture was centrifuged at 3000 rpm for 15 minutes and glucose was assayed in the supernatant using glucose FS kit according to manufacturer protocol [17, 18]. All analysis was performed in triplicates.

2.4. Statistical analysis
Data were presented as mean ± standard deviation. Shapiro wilk test was used to test normality of the data. Levene’s test was also performed for homogeneity of variance test. Because of both condition met, one way ANOVA continued by multiple comparisons Tukey HSD was performed to analysis the data. Significance level was set at 5%. All statistical analysis was performed in SPSS version 16.

3. Result and discussion
Analysis of resistant starch content showed that the highest resistant starch content was found in cassava (24.44%), followed by gathotan (22.50%), and gathot (21.85%) (Figure 1). From the statistical analysis result using one way ANOVA, there were significant differences in resistant starch content among the samples with p=0.025 (Table 1).

![Resistant starch content in cassava, gathotan, and gathot](image)

**Figure 1.** Resistant starch content in cassava, gathotan, and gathot.

| Products  | % Resistant starch content | p     |
|-----------|---------------------------|-------|
| Cassava   | 24.44±0.85<sup>a</sup>    | 0.025<sup>c</sup> |
| Gathotan  | 22.50±1.09<sup>ab</sup>   |       |
| Gathot    | 21.85±1.37<sup>b</sup>    |       |

<sup>a</sup>significant (p<0.05)

Different letter notations indicate differences between groups (a, b)

Gathotan is fungal fermented cassava product from Gunung Kidul, Yogyakarta, Indonesia. It is mainly colonized by *Botryodiplodia theobromae* Pat. Further fermentation of gathotan into gathot can change the texture and color of gathot. Gathotan has black color inside it as important characteristic of gathotan, and gathot has chewy texture [15, 16, 19].

Many processes in making cassava to gathotan and gathot potentially change the resistant starch levels. According to the previous research, resistant starch classified into some categories which are a non-digestible matrix (RS1), ungelatinized starch (RS2), retrograded starch (RS3), and chemically
modified starch (RS4). Processing method, storage condition and time had an effect on resistant starch formation. Sample with steam cooking process at atmospheric pressure had higher resistant starch than the samples steam cooked at higher pressure [20].

Figure 1 showed that fermentation of cassava into gathotan and fermentation gathotan to gathot can reduce resistant starch levels of the sample. Moreover, the resistant starch levels difference was looked between cassava and gathot. There was no resistant starch levels difference between cassava–gathotan and gathotan–gathot (Table 2).

This results is similar with the previous study evaluating effect of natural fermentation on resistant starch of legume based fermented foods. That study stated that fermentation significantly decreased the resistant starch content in all legume fermented products. The reduce levels of resistant starch in fermented food can be assumed as the effect of degradation of the complex starch into simple sugars and oligosaccharides by the enzymes produced by fungi or bacteria in fermented food and to a loss in the structural integrity of the starch granules [21]. Previous study stated that fermentation of cassava into gathotan may cause starch degradation [15, 22]. Texture changes, a chewy texture, of gathot may relate to the change in starch nature due to the enzymes or acid produced by fungi that can degrade starch fraction [19, 22].

Another study investigating the effect of soaking and autoclaving on resistant starch (RS3) content of tapioca pearls showed that autoclaving treatments increased RS3 content of tapioca pearls higher than soaking treatment. It may be due to decrease in intramolecular and intermolecular hydrogen bonds and or increase the crystallinity and amyllose to amylopectin ratio of tapioca pearl starch [23]. Analysis of amylose to amylopectin ratio in cassava, gathotan, and gathot may potentially be used to describe the resistant starch content difference among them. As observed before, as the amylase content increases, resistant starch also increases [24].

Although there are differences in the levels of resistant starch between cassava and gathot, cassava and processed cassava products are food sources that contain resistant starch which can be beneficial products to improve and maintain health [24]. Substitution of gathotan flour to gathotan noodle increased blood glucose level quicker but also it reduced blood glucose level to fasting blood glucose quicker than white bread as control meal due to the resistant starch [15]. Resistant starch is good for reducing blood glucose level and enhances glycemic control. Study conducted to seventeen individuals with type 2 diabetes mellitus for 12 weeks supplementation with 40 g resistant starch each day can lower postprandial glucose levels, lower fasting non-esterified fatty acid (NEFA) concentrations, increase muscle uptake of fatty acids and increase soleus intramyocellular lipid (S-IMCL), decrease TNF alpha [25]. Study carried out to normal and overweight women evinced that there was reduction in glycemic response by combining soluble fiber and resistant starch. Consumption of food containing moderate amount of soluble fiber and resistant starch can improve glucose metabolism [26]. The optimal intake of resistant starch to give positive effect for health is up to 45 gram/day [27].

Resistant starch, as a prebiotic and symbiotic, is non-digestible starch that can escape digestion from the small intestine and pass into the colon then is fermented by intestinal microflora to form short chain fatty acids (mainly acetate, propionate, butyrate) [28–32]. The increase of short chain fatty acid can give stimulus to enteroendocrine L-cells, adipose tissue, liver, and hypothalamus. The increase of peptide tyrosine tyrosine (PYY) and glucagon-like peptide 1 (GLP-1) is the response of enteroendocrine L-cells. This effect leads to a decrease body weight and fat mass. The hypothalamus gives response in the form of anorexigenic signaling increasing, appetite decreasing and food intake decreasing. Liver will compensate by decreasing glucose output and inflammation and also improving insulin sensitivity. Furthermore, adipose tissue decreases free fatty acid output and inflammation and increases adipocyte differentiation and insulin sensitivity [28, 33].

Besides the positive effect on appetite and nutrient intake changes, resistant starch can improve glucose metabolism, optimize energy metabolism, and balance the gut microbiota [34]. Microflora in human intestine ferments resistant starch and non-starch polysaccharides to short chain fatty acids. Then, colonic blood flow, fluid and electrolyte uptake will be stimulated by the short chain fatty acids.
Moreover, resistant starch acts as prebiotic which beneficially affect the host by enhancing the growth and activity of bacteria in the gastrointestinal tract selectively [29].

4. Conclusion and recommendation
Fermentation process of cassava to gathotan and gathot decrease resistant starch content. Significant differences of resistant starch content were seen between cassava and gathot. There was no resistant starch difference between cassava–gathotan and gathotan–gathot.

The next study can further examine the type of resistant starch formed from cassava fermentation process into gathotan and gathot and test the concentration of amylose and amylopectin considering the level of amylose to amylopectin ratio has an effect to resistant starch level. Moreover, the further research can analyze the effect of gathotan and gathot administration to metabolic mechanism and prebiotic profile of diabetes mellitus, such as to influence appetite and nutrient intake, improve glucose metabolism, improve lipid profile, optimize energy metabolism, balancing the gut microbiota, produce short chain fatty acids, stimulate the secretion of peptide tyrosine tyrosine (PYY) and glucagon-like peptide 1 (GLP-1), etc.

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