The Effects of Treatments on Batu Banana Flour and Percentage of Wheat Substitution on The Resistant Starch, In Vitro Starch Digestibility Content and Palatability of Cookies Made with Banana (Musa ba

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The Effects of Treatments on Batu Banana Flour and Percentage of Wheat Substitution on The Resistant Starch, In Vitro Starch Digestibility Content and Palatability of Cookies Made with Banana (*Musa balbisiana* Colla) Flour

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Abstract. Diabetes mellitus (DM) is the most common endocrine disease worldwide. Resistant starch is polysaccharide that is recommended for DM patient diets. One of the staple crops containing resistant starch is banana. It is the fourth most important staple crop in the world and critical for food security, best suited plant in warm, frost-free, and coastal climates area. Among banana varieties, Batu bananas (*Musa balbisiana* Colla) had the highest content of resistant starch (~39%), but its use as a food ingredient is limited. Inclusion of Batu banana flour into cookies manufacturing would both increase the economic value of Batu bananas and provide alternative snacks for DM patients. Here we sought to examine whether cookies made with modified Batu banana flour would be a suitable snack for DM patients. This study used a completely randomized design with two factors: substitution of Batu banana flour (25%, 50%,75%) for wheat-based flour and Batu banana flour treatment methods (no treatment, autoclaving-cooling, autoclaving-cooling-spontaneous fermentation). The resistant starch and *in vitro* starch digestibility levels were analyzed using two-way ANOVA and Tukey test, whereas the acceptance level was analyzed by Friedman and Wilcoxon tests. The content of resistant starch and *in vitro* starch digestibility of the different treatments ranged from 3.10 to 15.79% and 16.03 to 52.59%, respectively. Both factors differed significantly ($p<0.05$) with respect to Batu banana flour substitution, but not to processing method ($p>0.05$). Meanwhile, palatability in terms of color, aroma, texture, and flavor differed significantly among the different treatments and starch contents ($p<0.05$). Together these results show that Batu banana flour could be a promising ingredient for the production of snacks suitable for consumption by DM patients.
Keywords: Batu banana, cookies, resistant starch, *in vitro* starch digestibility

1. Introduction
Diabetes mellitus (DM) is the most common endocrine disease worldwide and represents a growing public health problem [1]. According to the International Diabetes Federation, 382 million adults

worldwide (8.3 %) have DM, and 80% live in countries with low and middle income. If this trend continues, by 2035 592 million people, or nearly 10% of the adult population, will suffer from diabetes [2].

Type 2 diabetes mellitus is most common in adults. Type 2 diabetes mellitus patients can regulate and monitor their carbohydrate intake to control blood glucose levels. Moreover, type 2 diabetes patients are encouraged to consume complex carbohydrates or polysaccharides as these sugars require longer digestion times that in turn slow the glycemic response [3]. One type of polysaccharide recommended for type 2 diabetes patients is resistant starch, which undergoes unhydrolysis to D-glucose in the intestine within two hours of consumption before fermentation in the colon [4]. Resistant starch is present in rice, peas, potatoes, and bananas [5].

The banana is a tropical/subtropical plant best suited to warm, frost-free, coastal climates [6]. The production, consumption and trade of banana is in high volumes in the world. Banana is the fourth most important staple crop in the world and is critical for food security in many tropical countries. This makes banana to be the prime leading fruit crop in terms of volume and value in the world market [7-8].
Among banana varieties, Batu bananas (*Musa balbisiana* Colla) had the highest content of resistant starch (39.35%) [5][9]. Moreover, during processing the resistant starch content can be increased with autoclaving-cooling cycles and spontaneous fermentation. A previous study showed that spontaneous fermentation of sliced bananas combined with one cycle of autoclaving-coolingincreased the resistant starch content of banana flour more to more than 17% of dry weight [10].

Despite its potential benefits, the use of Batu bananas as a food or food ingredient is limited. Banana flour has benefits for inclusion in semi-finished products because it can be saved longer, is easy to incorporate, and can be cooked faster [11]. Cookies are a commonly consumed snack in Indonesia, as evidenced by a 2013 survey conducted by Basic Health Research (RISKESDAS) found that 13.4 % of Indonesians consume cookies more than once a day [12-13].
The objective of this study is to study resistant starch and *in vitro* starch digestibility contents as well as the palatability of cookies made with modified Batu banana flour.

2. Materials and methods

2.1. Materials
Cookies were made from flour produced from unripe Batu bananas obtained from the Pemalang district in Indonesia, wheat flour, stevia sugar, egg yolks, regular tub margarine, vanilla powder, single-acting baking powder, cocoa powder (windmolen), and table salt.

2.2. Production of cookies with banana flour
For production of banana flour cookies, 25 g egg yolks and 70 g margarine were mixed for 2-3 minutes before the other ingredients (3 g stevia sugar, 0.5 g vanilla powder, 0.3 g table salt, 0.2 g baking powder, 5 gr cocoa powder, wheat flour and batu banana flour formulation) were incorporated to produce a homogenous batter. The dough was applied to baking sheets in uniform amounts using a syringe before baking at 150 °C for 15-20 minutes.

2.3. Preparation of modified banana flour using spontaneous fermentation and autoclaving-cooling cycles
Methods used to increase resistant starch content in this study were autoclaving-cooling (AC) and autoclaving-cooling spontaneous fermentation (AF). Untreated banana flour (WT) was also examined. For all treatments, dried Batu bananas (microwave drying, 450 watt, 25 min) were pulverized and passed through a #60 mesh sieve (0.23 mm). Untreated Batu bananas were simply dried (450 watt, 25 min). Spontaneous fermentation was carried out at room temperature for 24 hours in distilled water. One cycle of autoclaving-cooling involved autoclaving at 121 °C for 15 minutes followed by cooling at 4 °C for 24 hours.

2.4. Chemical analysis
Resistant starch content and *in vitro* starch digestibility was determined according to methods described by Kim *et al*. (2003) and Anderson *et al*. (2002), respectively [14-15]. The water, ash, fat, protein, and carbohydrate content was determined by AOAC 1995 [16], AOAC 2006 [17], soxhlet method AOAC

1995, bradford method [18], and by difference, respectively. The materials used for chemical analysis were 0.05 M phosphate buffer (NaH_2PO_4 & $NaHPO_4$) pH 6, Endo α-amylase (termamyl), NaOH, protease enzymes (Pronase E), HCl, amyloglucosidase and α-amylase enzymes (Cargill), ethanol, Whatman filter paper 42, Na-phosphate buffer (0.02 M and 0.1 M pH 7), 3,5-dinitrosalisilat (DNS), Na-K tartaric acid, and distilled water.

2.5 Palatability analysis

Product palatability was assessed using a hedonic test with a four-point scale wherein, 1 = dislike very much, 2 = dislike, 3 = like, and 4 = like very much. Thirty panelists recruited by the Nutrition Science Department, Diponegoro University tested cookie palatability.

2.5. Statistical Analysis

Resistant starch content and *in vitro* starch digestibility data were analyzed using a two-way ANOVA followed by a Tukey test. Palatability data were analyzed using a Friedman test followed by a Wilcoxon test.

3. Results and discussion

Banana flour produced using the AF method had a lower ash content relative to the AC and WT methods. This decreased ash content likely resulted from the soaking and fermentation steps [10].

Table 1. Chemical composition of Batu banana flour

Composition	Autoclaving cooling + fermentation (AF)	Autoclaving cooling (AC)	Without treatment (WT)
Water (%)	5.19 ± 0.02*	4.93 ± 0,02	6.87 ± 0,01
Ash (%)	2.59 ± 0,01	5.46 ± 0,04	5.29 ± 0,03
Fat (%)	12.18 ± 0,03	10.00 ± 0,14	7.47 ± 0,03
Protein (%)	6.70 ± 0,14	6.93 ± 0,03	6.93 ± 0,04
Carbohydrate (%)	73.34 ± 0,21	72.67 ± 0,24	73.43 ± 0,11
Resistant starch (%)	36.74 ± 1,26	31.54 ± 1,90	25.94 ± 0,85

*Mean ± SD of two trials is shown

For all processing methods, the fat and protein content of the banana flour tended to be higher in this study compared to values obtained in an earlier [10]. This difference could be attributed to the different varieties of bananas that were used or differences in processing, particularly the use of microwave for drying. A microwave was used here for drying the fruit because microwaving shortened the drying time and yielded a final product that had better flavor and nutritional value compared to flours made using conventional methods [19]. Furthermore, less protein was lost with a shortened drying time [20].

3.1. Resistant starch content and in vitro starch digestibility of cookies made with Batu banana flour

There were significant differences in the mean content of resistant starch as a function of the percentage banana flour substituted ($p=0.000$), whereas the starch contents of flours produced using different methods were similar ($p=0.284$) (Table 2). Moreover, the interaction of Batu banana flour substitution and method also had no difference in resistant starch content ($p=0.987$). An advanced Tukey test showed significant differences in starch content for each amount of banana flour substituted ($p<0.05$).

The results for method of flour preparation were consistent with those of Nurhayati et al., who found that the amylose content in agung semeru banana flour made using three different methods (autoclaving-cooling spontaneous fermentation, autoclaving-cooling and no treatment) had some degree of significant difference, although the difference was not large. Thus, it is possible that banana flour treated by different methods and used for cookie production had similar amylose contents, which positively correlate with resistant starch content [21-22].

Meanwhile, higher amounts of resistant starch were seen with higher amounts of substituted banana flour, which was expected given that refined wheat-based flour has a low content of resistant starch (0.65% w/w) [5][23].

Table 2. Resistant starch content (%) of Batu banana flour cookies.

Batu Banana Flour Substitution	Resistant Starch Content			P
	Flour Processing Method			
	Autoclaving cooling + fermentation (AF)	Autoclaving cooling (AC)	Without Treatment (WT)	
25%	4.52 ± 1.16*	3.59 ± 0.51	3.10 ± 0.49	
50%	12.07 ± 1.59	10.57 ± 1.59	10.14 ± 0.67	0.000
75%	15.79 ± 2.81	15.48 ± 0.40	14.60 ± 2.46	
P		0.987		

*Mean ± SD of two trials is shown

A study by Musita [5] showed that the resistant starch content of Batu banana flour cookies was less than that of untreated Batu banana fruit. This result could be due to the release of amylose from granules during baking that could increase starch solubility, which is associated with decreased resistant starch content [24]. Another study showed that heat treatments such as baking could decrease the Insoluble Dietary Fiber (IDF) content of which resistant starch is a component of cereal and legumes [25].

Moreover, the addition of ingredients containing fats and proteins such as eggs and margarine can reduce resistant starch content, as was seen for potato starch treated with autoclaving and cooling followed by the addition of albumin before rehydration at 20 °C [26]. Furthermore, heating of starches to 100 °C in the presence of fats induced the formation of amylose-lipid complexes, which can reduce formation of resistant starch during recrystallization due to lipid binding to amylose [26].

Statistical analysis showed that there were differences in the mean in vitro starch digestibility among the different banana flour substitutions (p=0,000), whereas the methods did not have significant changes in starch content (p=0.193). The interaction of banana flour substitution and preparation method with in vitro starch digestibility also showed no significant differences (p=0.973). Among cookies prepared with different amounts of banana flour substituted for wheat flour, an advanced Tukey test showed differences between the concentrations ($p<0.05$) (Table 2).

Table 3. Analysis of in vitro starch digestibility (%) of cookies prepared with banana flour

Batu banana flour substitution	In vitro starch Digestibility			P
	Flour processing method			
	Autoclaving cooling + fermentation (AF)	Autoclaving cooling (AC)	Without treatment (WT)	
25%	50.18 ± 2.78*	51.92 ± 1.84	52.59 ± 1.73	
50%	33.28 ± 1.26	34.74 ± 2.45	35.92 ± 1.81	0.000
75%	16.03 ± 1.53	16.31 ± 1.29	17.31 ± 1.12	
P		0.193		

*Mean ± SD of two trials is shown

Starch digestibility is the ease with which a starch type is hydrolyzed into simpler units by disgestive enzymes [27]. The in vitro starch digestibility in cookies produced with Batu banana flour was similar among the various flour processing methods, but the amount of banana flour substituted during cookie production did have a significant effect on the in vitro starch digestibility (Table 3).

A low value for in vitro starch digestibility can be produced by autoclaving-cooling cycles, wherein starch molecules rearrange between amylose-amylose and amylose-amylopectin to yield a stronger bond between starch molecules that is more resistant to digestion [26]. Lower in vitro starch digestibility was associated with a higher content of resistant starch and vice versa (Tables 2 and 3).

In vitro starch digestibility can also be affected by interactions between starches and proteins that reduce the susceptibility of starch to enzymatic hydrolysis due to embedding of starch granules in protein matrices in the corneous endosperm of plants. Starch gelatinization arising during processing can form starch and protein complexes that reduce both starch digestibility and protein content [28].

3.2. Palatability of Batu banana flour cookies

Study participants noted significant differences in color ($p = 0.001$), aroma ($p = 0.000$), texture ($p = 0.003$), and flavor ($p = 0.000$) of cookies produced with varying amounts of Batu banana flour (Fig. 1; Wilcoxon test).

Based on a hedonic test of color, cookies with made with 25% Batu banana flour produced using the AC method had the highest hedonic scores, with a mean 3.00 (like), whereas cookies made with a substitution of 75% Batu banana flour made with the AC method had the lowest score (2.30, dislike), likely because these cookies had the darkest color. Flours produced by AC, AF, and WT methods had increasingly lighter color, suggesting that the brown color of the AC method arose from the Maillard reaction or other non-enzymatic browning reactions that occur when foods are cooked at high temperatures and sugar-reducing agents react with compounds bearing a NH_2 group (e.g., proteins, amino acids, peptides, and ammonium) [29-30].

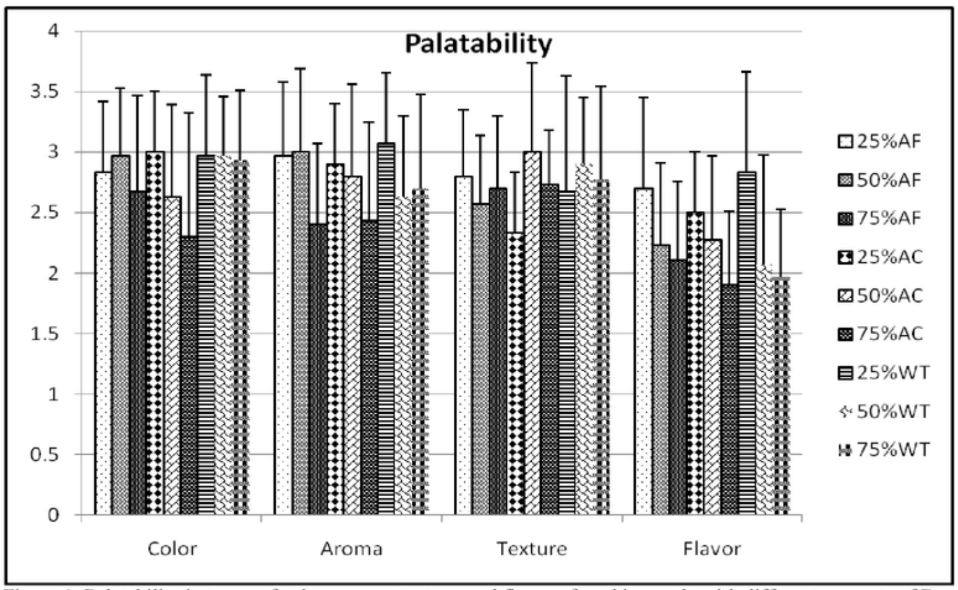

Figure 1. Palatability in terms of color, aroma, texture, and flavor of cookies made with different amounts of Batu banana flour processed using different methods. Flour processing methods are indicated by AF: autoclaving-cooling followed by spontaneous fermentation; AC: autoclaving-cooling only; WT: no treatment. Data represent mean ± SD.

Cookies produced with Batu banana flour processed by AF had lighter color than the AC method, likely because bananas that underwent spontaneous fermentation that involved soaking in distilled water for 24 hours can have delayed enzymatic browning reactions [30].

Meanwhile, scores for aroma were highest for cookies produced with 25% WT banana flour (mean=3.07, like), whereas cookies with 75% AF banana flour had the lowest preference scores (mean = 2.40, dislike). In terms of aroma, cookies made with banana flour with no treatment had higher scores than cookies with 75% banana flour processed either by the AC or AF method, which again may be due to Maillard or heat-induced reactions that result in loss of aromatic compounds [31], which, along with individual perception, form the basis for aroma [32].

Cookies made with substitution of 50% AC Batu banana flour were judged to have the best texture (mean =3.00, like), whereas cookies with substitution of 25% AC banana flour had the lowest scores (mean = 2.33, dislike) and were noted to have a less crispy texture. Indeed, cookies that had higher levels of banana flour had greater crispiness that may be due to gelatinization processes that occur during baking that break intramolecular hydrogen bonds in proteins and carbohydrates [33].

The presence of free hydroxyls will absorb water that swells starch granules. The more hydroxyl groups contributed by starch molecules in banana flour, the greater its capacity to to absorb water, such that when drying occurs, the water carrying capacity is reduced and the crispy texture increases [33]. For flavor, cookies with 25% WT Batu banana flour substitution had the best socres (mean = 2.83, like) and those with 75% substitution of AC banana flour had the lowest scores (mean = 1.9, dislike).

3.3. Consumption of resistant starch affects blood glucose levels

Some research suggest that the compsumtion of foods containing resistant starch has a positive effect on glycemic control. Research on mice, pigs, and humans indicate that resistant starch can lower fasting blood glucose concentrations, increase insulin secretion, and improve insulin sensitivity [27]. A study using mice with type 2 diabetes mellitus showed an increased pancreatic beta cell mass and insulin sensitivity after the mice consumed feed with 30% resistant starch content for 10 weeks. A study in humans showed improvements in glucose tolerance, blood glucose, insulin, and colonic fermentation condition in healthy or type 2 diabetes mellitus subjects after consuming food containing resistant starch [27].

Some evidence showed that the mechanism by which blood glucose levels are regulated might be associated with resistant starch fermentation in the colon and SCFA (Short Chain Fatty Acid) production. SCFAs are organic fatty acids with 1 to 6 carbon atoms with a straight and branched chain that are produced in the distal intestine by bacterial fermentation of fibrous food and allow resistant starches to escape the gastrointestinal tract and enter the colon. The most common forms of SCFA are formic, acetic, propionic, butyric, valeric, isovaleric, and caproic acid, but in the colon 90-95% of SCFA is acetic, propionic, and butyric acids [34].

One form of SCFA is butyrate that can induce L-cells to secrete an incretin as intestinal peptide YY (PYY, also known as peptide tyrosine tyrosine or glucagon-like-peptide-1/GLP-1 hormone) [27]. Incretin hormone secretion from endocrine cells of the small intestine occurs in response to consumption of carbohydrates, proteins, and fats. The incretin hormone has many functions, including appetite suppresion. The main form of incretin hormone is GLP-1, which controls post prandial glucose by increasing insulin secretion, stimulating insulin gene expression, inhibiting glucagon synthesis, and delaying gastric emptying. However, the mechanism by which butyric SFCA increases the secretion of GLP-1 is unclear [34, 35].

Several studies of patients with controlled type 2 diabetes mellitus showed increasing levels of GLP-1 after cosumption of 40 g resistant starch daily for 2 weeks. Thus, long-term supplementation with resistant starch may have the potential to increase blood glucose tolerance in patients with type 2 diabetes mellitus [36].

4. Conclusion

The levels of resistant starch and in vitro starch digestibility significantly differed according to amount of Batu banana flour included during production of banana flour cookies, but the flour processing method had no effect. All palatability parameters, including color, aroma, texture, and flavor, showed differences among the amount of banana flour incorporated. The optimal palatability was seen for cookies produced with substitution of 25% Batu banana flour produced using the AF method. These results show that Batu banana flour could be a useful ingredient to produce desirable snacks having high levels of resistant starch and in vitro digestibility that would allow type 2 diabetes patients to control their blood glucose levels.

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