Evaluation of cookies quality enriched with resistant starch type 2 (RS2) and resistant starch type 3 (RS3) from banana (*Musa paradisiaca* formatypica)

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Abstract:

This research was aimed to evaluate cookies quality with the enrichment of resistant starch type 2 (RS2) and resistant starch type 3 (RS3) from unripe banana. Cookies were formulated with the addition of RS2 and RS3 with three levels of substitution: 10%, 20%, and 30% of the wheat flour basis. RS2 was obtained through water-alkaline extraction, while RS3 was obtained through starch modification by using autoclaving-cooling cycle method. Quality of cookies was evaluated on *in vitro* digestibility, hardness, color, and sensory acceptance. Both RS2 and RS3 was found to decrease the digestibility of cookies. However, effect of RS3 was more obvious compared to RS2 even at 10% of substitution level. In both type of RS, the higher substitution level resulted in the lower digestibility. Hardness of RS2 and RS3-enriched cookies at all substitution levels did not show any significant difference compared to control. Color measurement showed that both type of RS resulted in the significantly darker color of cookies, though the effect was more intense in RS3 substitution. The darker color of cookies was observed along with the increasing substitution level. Sensory acceptance test conducted for aroma, taste, hardness, and overall acceptance attributes showed that panelists rated all the RS2 and RS3-enriched cookies at all substitution levels equally with the control but lower for color attribute. The addition of RS3 at 20% level of substitution was suggested as it was found to be the most acceptable in all attributes tested, while having the significantly lower digestibility compared to control cookies.

Keywords: autoclaving-cooling, banana starch, cookies, *in vitro* digestibility, resistant starch

Introduction

Cookies are commonly consumed by people worldwide in a wide range of ages, starting from children until the elderly people. Cookies are easily served, having a long shelf-life, and generally come with convenient taste and texture which make it suitable to be consumed as snacks [1]. However, cookies contribute to a high intake of calories particularly due to its high carbohydrate content. The carbohydrate content may come from the use of wheat flour, sucrose, glucose, fructose syrup, hydrolyzed starch, or even corn syrup as its ingredient [2]. Cookies can contain up to 70% of sugar that can be easily digested resulting in the raise of blood glucose [3]. It may then create some health problems for certain people particularly those with the risk of diabetes or having an overweight problem.

Now that people are getting more health-conscious, a considerable interest has been given to the healthier food alternative yet with convenient sensory properties. Some attempts have been conducted to produce healthier cookies with low-intake of calories, such as by using a natural non-caloric sweetener [4], replacement of wheat flour with other flour such as pea and soybean flour [5], enrichment with fiber [6], and enrichment with resistant starch [7,8]. The use of resistant starch (RS) is however getting more attention as it performs similar functional properties with fiber in our digestive system but with minor influence on the sensory properties when applied to food product [9]. Traditional fiber was reported to affect texture, taste, and flavor of food enriched with the component, and thus becoming a shortcoming on its application [10]. On the other hand, RS offers some excellences over the fiber such as colorless, bland flavor, low water-holding capacity, and small particle size, which favorable for its application in varying food products including cookies [11]. Thus, RS with its low caloric content was hypothesized to give functional value of food by reducing the digestibility of product, while maintaining the sensory properties.

RS refers to the portion of starch and starch products that resist digestion as they pass through the gastrointestinal tract and may be fermented by microbiota in the large intestine, affecting some physiological functions such as reduction of glycemic response, hypocholesterolemic effect, and protective effect against colorectal cancer [12]. It should be noted that different sources of starch may affect the nutritional and functional properties of RS [8]. Among the available sources of starch, banana has been known as a potential source of resistant starch type II (RS2) which renowned for its health benefit [13] where its nutritional quality has been pointed out by several authors [14,15]. It also contains observable amount of starch particularly on its unripe state, which is up to 70-80% on a dry weight basis [13]. However, RS2 exhibits a shortage in terms of its stability upon heat treatment, while resistant starch type III (RS3) is found to be more heat-stable [9]. In this study, each RS2 and RS3 from banana source was tried to be incorporated in cookies formulation with three levels of substitution: 10%, 20%, and 30% of the wheat flour basis. RS3 was obtained through physical modification of autoclaving-cooling cycles treated to the banana starch. Effect of RS type and its concentration in the cookies formulation was evaluated on the following parameters: *in vitro* digestibility of cookies, texture, color, and sensory acceptance.

Materials and methods

Materials

The raw material used in this study was Indonesian local white *kepok* banana (*Musa paradisiaca* formatypica) of the plantain group *Musa* AAB (triploid cultivar), purchased from a local supplier in BSD, Tangerang, Banten. The selected bananas were green, hard, aged 90-120 days, and with no molds. The other materials used for production of cookies were eggs, wheat flour, margarine and sugar. The reagents used were sodium hydroxide technical grade, α-amylase enzyme (Sigma Aldrich, USA), maltose standard (Sigma-Aldrich, USA), phosphate buffer solution, acetic acid, ethanol 95%, iodine, potassium iodide, glucose standard (Merck, Darmstadt, Germany), amylose standard (Sigma-Aldrich, USA), starch (BDH Laboratory Supplies, England), acetate buffer, DNS solution and distilled water.

Extraction of Banana Starch [13, 16]

Banana was cut into thin slices and crushed and stirred in 0.1 N NaOH solution for 3 h, using blender machine. The mixture was then filtered with muslin cloth. The filtrate was collected, added with distilled water, and allowed for 2 h. The mixture was sieved with 120 µ siever and the supernatant was removed from the starch portion. The starch portion was stirred again in 0.1 N NaOH solution for 2 h, sieved with 120 µ, added with distilled water, and allowed overnight. The supernatant underwent further extraction with the addition of distilled and allowing it for 2 h to obtain another remaining starch portion. Supernatant was separated from the starch, and the starch was oven-dried at 40°C for 12 h. The dried starch was sieved using 100 µ siever and stored in a sealed container. The banana starch obtained in this extraction would be labeled later as RS2.

Preparation of RS3 through Autoclaving-cooling Cycles

Starch was added with distilled water to obtain 20% of starch concentration. The starch suspension was heated at 80°C for 5 min with constant stirring. The suspension was autoclaved at 121°C for 15 min and allowed at room temperature until it reached approximately 40°C. It was cooled at 4 °C and allowed for 24 h. The autoclaving-cooling was repeated for another two cycles. The suspension was dried in oven at 40°C, then ground into 100 µ particle size. The starch was sieved with and stored in a sealed container.

Formulation and Preparation of cookies

Cookies were formulated with the addition of resistant starch (RS2 and RS3) at 10% (F1), 20% (F2), and 30% (F3) substitution level of wheat flour basis. Control cookies were made with 100% wheat flour, without incorporation of RS. The detail formulation of cookies was presented in Table 1.

In vitro digestibility tests [17]

Sample (1 g) was suspended with 100 mL distilled water, stirred and heated until 90°C, then cooled down to room temperature. As much as 2 mL sample solution was put into test tubes quantitatively using micropipette, then 3 mL distilled water and 5 mL phosphate buffer pH 7 solution were added into the test tubes. Each sample was made in duplicate, one as the sample and the other was used as blank. Each tube was then covered and incubated at 37°C for 15 min. The sample was added with 5 mL α-amylase solution in 1 mg/mL phosphate buffer pH 7 solution and the blank solution was added with 5 mL phosphate buffer pH 7. Both tubes were incubated for 30 min and then both solution in test tubes were transferred to lidded test tubes containing 2 mL DNS. The solutions were then heated in boiled water for 12 min and cooled down. After that, as much as 8 mL distilled water was added into the solutions and stirred until homogenized using vortex. As much as 260 µL for each solution was pipetted into microplate and the absorbance was measured using microplate reader at wavenumber of 520 nm. The starch digestibility (in percentage) is calculated using this formula:

Table 1 Formulation of cookies

Ingredients	Control	F1	F2	F3
Wheat Flour (g)	125	112.5	100	87.5
Resistant Starch / RS (g)	0	12.5	25	37.5
Margarine (g)	75	75	75	75
White egg (g)	19	19	19	19
Egg Yolk	19	19	19	19
Sugar (g)	50	50	50	50
Baking powder (g)	2	2	2	2

$$\textit{In vitro}\text{ starch digestibility (\%)} = \frac{(A-a)}{(B-b)} \times 100$$

With,

A = maltose in sample (mg)

a = maltose in blank sample (mg)

B = maltose in starch (mg)

b = maltose in blank starch (mg)

Texture analysis of cookies

Texture of cookies was measured with Texture Analyzer (Stable Micro Systems Ltd, UK) using three point bending rig as a probe. The textural parameter measured in this test was hardness, as an important cooking quality of cookies. First, the individual sample of cookies was placed on the platform and the blade was attached to the crosshead of the instrument. The cutting knife would move downward until the cookie was broken. Hardness of the cookie was reported in gram unit. The texture analysis was performed five times for every sample.

Color analysis of cookies

Color of the cookies were quantitatively measured by using chromameter (Konica Minolta CR-40) and recorded in the L*a*b* color system. The L*a*b* color system consists of a luminance or lightness component (L*) and chromatic components: the (a*) component for green (-a) to red (+a) and the (b*) component from blue (-b) to yellow (+b). The colorimeter was calibrated using standard white plate. The measurement was repeated thrice for each sample.

Sensory analysis of cookies

Thirty-five untrained panelists were asked to evaluate the sensory attributes of the cookies with the hedonic test. Samples which consisted of control and resistant starch-enriched cookies (F1, F2 and F3) were randomly coded and given to the panelists simultaneously. Panelists were provided with drinking water to cleanse the palate between samples. Panelists were asked to rate each sample by giving a rating score, with the hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The sensory attributes being evaluated were color, aroma, taste, texture and overall acceptability.

Statistical Analysis

Statistical analysis was conducted using data analysis ToolPak in Microsoft Office Excel. The data for sensory hedonic test was analyzed by using Friedman test and continued with the Wilcoxon post-hoc test. Other statistical analysis in this study was carried out using One-way analysis of variance (ANOVA) with single factor and followed with Tukey HSD post-hoc test to evaluate significance of differences between the mean values of measured parameters. A p-value of ≤ 0.05 was considered as statistically significant.

Results and discussion

In vitro digestibility tests

Effect of resistant starch (RS2 and RS3) addition to the *in vitro* digestibility of cookies was presented in the Table 2. As hypothesized, the addition of both types of RS had led to the decrease of *in vitro* starch digestibility. The higher substitution level of RS resulted in the greater reduction of *in vitro* digestibility. RS3 was found to be more effective in reducing the starch digestibility, showing the lowest digestibility value at 30% level of substitution, which was significantly lower ($p<0.05$) compared to control. At the same level of substitution, RS2 showed higher digestibility than RS3. RS3 was formed due to the autoclaving-cooling treatment applied, through retrogradation mechanism. During retrogradation, the amylose molecules and amylopectin molecules realign between amylose-amylose and amylose-amylopectin which make the hydrogen bond stronger, forming double helix structure. The double helix structure will bind with another double helix structure to from crystallite which finally can increase the resistance of starch [18]. The higher effectivity of RS3 in reducing starch digestibility compared to RS2 was due to the better stability of RS3 [9]. It was also stated in [19], that RS3 exhibited substantially higher thermostability, compared to RS2.

Table 2. Result of *in vitro* digestibility test in formulated cookies compared to control

Type of RS	**Starch Digestibility (%)**			
	Control	**F1**	**F2**	**F3**
RS 2	56.68 ± 2.64^{a}	51.40 ± 1.53^{ab}	46.82 ± 3.40^{b}	42.93 ± 0.98^{b}
RS 3	56.68 ± 2.64^{a}	46.82 ± 1.87^{b}	40.22 ± 2.42^{bc}	32.22 ± 1.87^{c}

* Different superscript letter in the same row indicates significant difference

Texture analysis of cookies

Hardness is analyzed by measuring the force values needed to reach a certain deformation [7]. The hardness values of cookies enriched with RS2 and RS3 compared to control were presented in Figure 1. As seen in the chart, both RS2 and RS3 did not considerably affect hardness of cookies at all levels of substitution. However, generally RS resulted in the lower value for hardness compared to control, except for RS2 at 30% substitution level. The lowest hardness value was showed in cookies with RS3 enrichment at 20% substitution level. In similar research carried out in cookies enriched with different types of RS and fiber at 5%, 15%, and 25% substitution level, it was shown that generally fiber led to a harder texture of cookies [7]. The similar trend was also shown in other research conducted in tortillas product, where the addition of RS resulted in tortillas with less strength, that was easier to tear, and less dense compared to the flour-based tortilla [20]. However, a too low hardness value for cookies is also unexpected as it may be difficult in transportation and storage.

Color analysis of cookies

Color of cookies enriched with RS2 and RS3 were presented in Table 3 and 4, respectively. As seen in the Table 3, the L* value of cookies at the level of 10% was higher than control, meaning that it was less dark compared to control. However, as the substitution level was increasing, the L* value was getting lower. The a* value was found lower at the level of 10%, compared to control. While as the substitution level was increasing, the a* value was getting higher. The b* value was higher at 10% level compared to control and the value was getting lower as the substitution level was increasing. The increase in a* value and the decrease in L* and b* value could be translated into a more intense golden-brown color that could be seen visually along with the increase of RS substitution level.

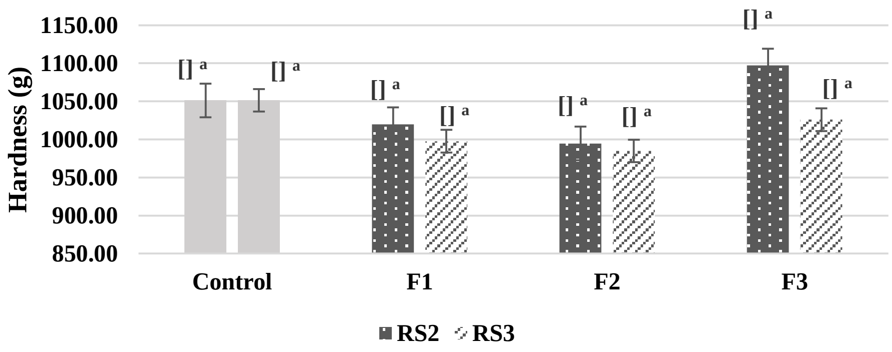

Figure 1. Hardness of RS-enriched cookies compared to control

In the substitution with RS3, the trend was similar with RS2, indicating the formation of a more intense golden-brown color in cookies enriched with resistant starch. Comparing to RS2 substitution, RS3 showed a more obvious effect of darkening in cookies. The significantly darker color in RS3 substitution, compared to RS2, was also observed in batter preparation with RS3 (Novelose 330 and C*Actistar) and RS2 (Hi-maize 260) addition [21]. The phenomenon happens in both types of RS substitution is due to the higher amount of reducing sugars that contribute to Maillard reactions and caramelization [22].

Sensory analysis

The addition of resistant starch in cookies formulation was expected to improve the functional value of cookies without impairing the sensory attributes. Thus, the sensory analysis is an important parameter for the cookies evaluation. Result of the hedonic sensory test for RS2 and RS3-enriched cookies was displayed in Table 5 and 6, respectively.

Table 3. Result of color measurement in RS2-enriched cookies

	Control	Formula		
		F1	F2	F3
L*	68.31 ± 0.12^{b}	70.56 ± 0.06^{a}	68.72 ± 0.20^{b}	62.64 ± 0.22^{c}
a*	7.68 ± 0.10^{b}	6.17 ± 0.05^{c}	7.70 ± 0.03^{b}	10.13 ± 0.10^{a}
b*	31.07 ± 0.06^{c}	32.06 ± 0.08^{a}	31.53 ± 0.06^{b}	28.72 ± 0.06^{d}

* Different superscript letter in the same row indicates significant difference

Table 4. Result of color measurement in RS3-enriched cookies

	Control	Formula		
		F1	F2	F3
L*	68.31 ± 0.12^{a}	67.26 ± 0.21^{b}	62.99 ± 0.24^{c}	60.54 ± 0.26^{d}
a*	7.68 ± 0.10^{c}	7.93 ± 0.12^{c}	9.07 ± 0.11^{b}	9.60 ± 0.26^{a}
b*	31.07 ± 0.06^{a}	30.73 ± 0.12^{b}	28.70 ± 0.10^{c}	26.49 ± 0.14^{d}

* Different superscript letter in the same row indicates significant difference

The addition of RS2 at all levels of substitution did not seem to have significant effect in almost all the sensory attributes tested, except in color attribute. In the color attribute, the RS2 addition did not lower the acceptance score significantly at 10% and 20% level of substitution. However, the score given at the level of 30% was found to decrease significantly. In texture attribute, the score for RS2-enriched cookies at the level of 20% was the highest among all samples tested, including control, however this highest score was not statistically significant. Correlating this result with the result of hardness analysis, it was assumed that the higher score given to the 20% RS2-substituted cookies might be due to softer texture of the cookies, compared to other samples. The overall acceptance also showed that 20% RS2-subsituted cookies was rated with the highest score compared to control and other samples. While for aroma and taste, all the samples were rated equally, showing that the addition of RS2 did not contribute an obvious effect on the two attributes.

Table 5. Result of Hedonic Sensory Test of Cookies Formulated with RS2

Attributes	Control	F1	F2	F3
Color	7.03 ± 1.18 ^{a}	6.60 ± 1.82^{ab}	7.37 ± 1.14^{a}	6.03 ± 1.67^{b}
Aroma	7.29 ± 1.02^{a}	6.54 ± 1.60^{a}	6.97 ± 1.07^{a}	7.14 ± 1.24^{a}
Taste	7.03 ± 1.38^{a}	6.91 ± 1.17^{a}	7.09 ± 1.04^{a}	7.17 ± 1.10^{a}
Texture	7.26 ± 1.40 ^{ab}	6.83 ± 1.25^{b}	7.83 ± 0.71^{a}	7.00 ± 1.21^{ab}
Overall Acceptance	7.31 ± 1.08 ^{ab}	6.77 ± 0.97^{b}	7.51 ± 0.89^{a}	7.14 ± 0.97^{ab}

* Different superscript letter in the same row indicates significant difference

Table 6. Result of Hedonic Sensory Test of Cookies Formulated with RS3

Attributes	Control	F1	F2	F3
Color	7.17 ± 1.20 ^{a}	7.34 ± 1.08^{a}	7.09 ± 1.20^{a}	5.77 ± 1.82^{b}
Aroma	6.89 ± 1.13 ^{a}	6.43 ± 1.40^{a}	6.63 ± 1.44^{a}	6.74 ± 1.77^{a}
Taste	7.00 ± 1.31 ^{a}	6.63 ± 1.21^{a}	6.77 ± 1.44^{a}	6.23 ± 2.00^{a}
Texture	6.83 ± 1.40 ^{ab}	6.71 ± 1.38^{ab}	7,40 ± 0.74^{a}	6.26 ± 2.13^{b}
Overall Acceptance	7.09 ± 1.34 ^{ab}	6.60 ± 1.42^{b}	7.40 ± 0.74^{ab}	6.86 ± 0.94^{ab}

* Different superscript letter in the same row indicates significant difference

The addition of RS3 was shown to exhibit a similar result in sensory evaluation, especially in the color attribute. Comparing to control, the cookies enriched with RS3 was observed with lower score in color attribute. A significant decrease of the score was found at 30% level of substitution. This was in line with the result of color measurement showing that cookies showed the significantly darkest color at that level of substitution. Similar to RS2, RS3-enriched cookies at 20% level was rated with highest score, compared to control and other samples, though it was not statistically significant. This might be related to the softer texture of this sample, which was also in a good accordance with the result of hardness analysis. In the aroma, taste, and overall acceptance, the scores given to the RS-enriched cookies were not significantly different from the control.

Conclusions

Incorporation of both types of RS in cookies formulation had been found effective in reducing the *in vitro* digestibility of cookies, suggesting the reduced caloric value of cookies. However, the effect was more obvious in RS3 addition. The increasing concentration of RS2 and RS3 applied in the formula had led to a lower digestibility value, but it was not significantly different between 20% and 30% level of substitution. Both types of RS in all substitution levels were not found to affect hardness of cookies significantly. In terms of color, RS2 and RS3 showed a darkening effect in cookies, with the higher intensity found in RS3. In the sensory acceptance, RS-enriched cookies were overall accepted equally with the control, except in color attribute at 30% substitution level, with the lower score for RS-enriched cookies. Finally, the use of RS3 at 20% substitution level was suggested as it was accepted better by the panelists while having the significantly lower *in vitro* digestibility value compared to control.

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