

## THE EFFECTS OF UNRIPE BANANA FLOUR ON RESISTANT STARCH CONTENT AND QUALITY CHARACTERISTICS OF GLUTEN-FREE RICE COOKIES

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**Abstract:** Rice (*Oryza sativa* L.) is considered as a gluten-free food. However, using rice flour to prepare cookies has its limitations, such as poor texture and low sensory quality acceptance by consumers. This paper investigates the effects of substituting resistant starch (RS) content with banana flour on the quality of gluten-free rice cookies. Rice cookies were prepared by replacing wheat flour with unripe banana flour at 0% (control), 50%, 60%, 70%, 80% and 100%. The pasting properties of starch, physical properties of the cookies, texture profile, RS content and sensory evaluation were determined. The results indicated that as the degree of banana flour substitution increased, the peak, final and setback viscosities of blended flour also increased. Rice cookies containing unripe banana flour had lower diameters and spread ratio, but higher hardness than the control. RS content of gluten-free rice cookies ranged from 1.90% to 8.50% with the increase in banana flour content, while wheat cookies contained 2.85% of RS. For the sensory evaluation study, the gluten-free rice cookie with 70% unripe banana flour received the highest overall score and was comparable to wheat cookies. Partially replacing rice flour with unripe banana flour has the potential to produce gluten-free rice cookies with high RS content.

Keywords: gluten-free, rice cookie, wheat cookie, resistant starch, unripe banana flour.

### Introduction

Cookies are one of the most popular bakery products in the world, traditionally produced from soft wheat, which has low protein levels, with a weak gluten network and low hydration properties (Ma & Baik, 2018). The quality of cookies can be improved by increasing dietary fiber and adding malted wheat and fruit products (Yang *et al.*, 2020). However, gluten in bakery products made from wheat can cause inflammation in the small intestine and reduce the body's ability to absorb nutrients due to Celiac disease or gluten allergy. There is currently no medical treatment for this disease, and a strict gluten-free diet is recommended (Koehler *et al.*, 2014). Rice (*Oryza sativa* L.), as one of the most important global cereals, is considered to be a gluten-free food. However, using rice flour to prepare cookies has several limitations, such as poor texture, poor color, low

sensory quality acceptance by consumers and a high glycemic index.

Unripe banana (*Musa sapientum* L., Kluai Nam Wa) is a good source of dietary fiber, resistant starch, non-starch polysaccharides, including pectin, and other bioactive compounds (Faisant *et al.*, 1995; Moongngarm *et al.*, 2014; Segundo *et al.*, 2017). Resistant starch (RS) is a bioactive compound that has a high potential to improve dietary fiber and lower the glycemic index in food products. Resistant starch is a complex carbohydrate that resists digestion in the upper gastrointestinal tract. RS is fermented in the colon by microorganisms to produce short-chain fatty acids (SCFAs) that are beneficial for colonic health. Resistant starches are classified into five types as RS1 (physically inaccessible starches), RS2 (granular starches), RS3 (retrogradation starches), RS4 (chemical modified starches) and RS5 (amylose-lipid

complexes) (Champ, 2004; Brown *et al.*, 2006). Unripe banana flour is also a good source of total phenolic content with 746.67 mg catechol/100 g flour and 65.58% antioxidant activity (Haslinda *et al.*, 2009). Unripe banana flour contains resistant starch type 2 (RS2) (Brown *et al.*, 2006) at up to 48.88% (Moongngarm *et al.*, 2014). Unripe banana flour has been used in a variety of products to increase the RS content and improve digestibility. Agama-Acevedo *et al.* (2012) reported that the RS content increased from 2.3% to 8.4%, with 50% replacement of wheat flour with unripe banana flour in cookie products, while Ratnarari *et al.* (2018) reported a 14.6% RS content in wheat cookies substituted with unripe banana flour at 75%. The physicochemical properties of unripe banana flour are different from rice flour and may enhance the quality characteristics of gluten-free rice cookies. However, only a few studies have investigated the effects of replacing rice flour with unripe banana flour in gluten-free rice cookies. Therefore, here, the effects of unripe banana flour substitution in gluten-free rice cookies on pasting properties, physical properties and resistant starch content was investigated. A sensory evaluation was also conducted to compare composite rice and banana flour cookies with wheat flour cookies.

## Materials and Methods

Rice (*Oryza sativa* L.), indica cultivar Pathum Thani 1, was obtained from the Bureau of Rice Research and Development of Thailand. Wheat flour was purchased from a local supermarket. Bananas (*Musa sapientum* L., Kluai Nam Wa) were sourced from a farmer in Muang district, Prachinburi, Thailand. All chemical reagents were of analytical grade.

### Rice Flour and Banana Flour Preparation

Rice flour was produced by dry milling. The polished rice was dry-milled using an Alpine Pin Mill (160 Z, Augsburg, Germany). The rice flour was passed through a 100 mesh sieve, packed

in plastic bags and stored at 4 °C until required for use (Detchewa *et al.*, 2016). Unripe banana flour was produced from green bananas at Stage 2-3, according to the 7-color scale of the banana chart (Dadzie & Orchard, 1997), and prepared according to Moongngarm *et al.* (2014). Briefly, unripe banana slices were soaked in 0.5% citric acid solution for 15 minutes to avoid enzymatic browning and they were dried in an oven (TD024T, Bangkok, Thailand) at 50 °C until the moisture content was below 10%. The dried unripe banana slices were grounded using a grinder (HR 2115/01, Bangkok, Thailand) and passed through a 100 mesh sieve. The flour was stored in plastic bags and kept in cold storage at 4 °C until required for use.

### Preparation of Cookies

Formulations used to prepare the experimental cookies are presented in Table 1. The rice cookie formulations were replaced with banana flour at 0%, 50%, 60%, 70%, 80% and 100%. Replacement percentages were selected from a preliminary study. Ingredients were mixed using a mixer (KitchenAid, MI, USA) at a low speed for 20 minutes. Cookie dough was pressed using a cooking machine (Kuhn Rikon, AG, Switzerland) into star shapes. The cookies were then baked in a household oven at 150 °C for 20 minutes, allowed to cool for 30 min, and stored at room temperature in sealed plastic containers.

### Amylose Content Determination

The amylose contents in rice flour, unripe banana flour, wheat flour and composite flour were determined using the method by Juliano *et al.* (1981). The determination of the amylose content was carried out using the iodine-based colorimetric method. The absorbance was measured using a UV-visible spectrometer (Biochem Libra S32, UK) at 620 nm. The amylose content was determined with the standard amylose curve, using an analytical grade potato amylose starch (Sigma-Aldrich, UK).

Table 1: Formulations of wheat cookies and rice cookies added with unripe banana flour

Ingredient (gram)	Control	Rice:Unripe banana flour					
		100:0	50:50	40:60	30:70	20:80	0:100
Wheat flour	500	0	0	0	0	0	0
Rice flour	0	500	250	200	150	100	0
Unripe banana flour	0	0	250	300	350	400	500
Salted butter	240	240	240	240	240	240	240
Egg	110	110	110	110	110	110	110
Baking powder	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Butter flavor	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	150	150	150	150	150	150	150
Water	50	50	50	50	50	50	50

### ***Pasting Properties Determination***

The pasting properties of rice flour, unripe banana flour, wheat flour and composite flour were determined using a Rapid Visco Analyzer (Model 4S, Newport Scientific, Australia). The pasting profile was recorded as pasting temperature, peak viscosity, hot paste viscosity, breakdown, final viscosity, and setback, following Detchewa *et al.* (2012).

### ***Gluten Content Determination***

The gluten content was determined using a monoclonal R5-antibody-based sandwich enzyme-linked immunosorbent assay (ELISA), wheat/gluten (gliadin) ELISA Kit II (Morinaga Institute of Biological Inc., Japan) (Detchewa *et al.*, (2016).

### ***Resistant Starch Determination***

The resistant starch (RS) was determined according to the AACC method 32-40.01 using the Megazyme Resistant Starch Assay Kit (Megazyme International Ltd., Ireland) as described by Rungrusmee *et al.* (2020).

### ***Physical Characteristics of Cookies***

Cookie characteristics were determined 24 hours after baking. Ten cookies were measured for diameter (D) and thickness (T) using a caliper and the spread ratio (D/T) was calculated. The colors of the ten cookies were measured using a Hunter Lab (ColorFlex EZ, VA, USA) according to Yang *et al.* (2020). Measurements

were taken with the 10° standard observer and D65 standard illuminate, with results expressed following the CIELAB convention.

### ***Textural Characteristics of Cookies***

The textural properties of ten cookies were determined by a fracture test, using a texture analyzer (TA-XT plus; Stable Micro Systems, UK) and a three-point bend rig probe (HDP/3PB) according to the method described by Yang *et al.* (2020), with minor modifications. The experiment condition was a 20 mm probe travel distance, trigger force of 5 g and test speed of 2.0 mm/s. The hardness of the cookies was calculated as the maximum force (N) required to break the cookie.

### ***Sensory Evaluation***

Organoleptic testing of the cookies was performed by 30 untrained panelists using a 9-point hedonic scale (Chen *et al.*, 2020; Jan *et al.*, 2018; Stone & Sidel 2004; Curtis 2013). The scale was 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely. The panelists evaluated the cookie samples for appearance, color, texture, and overall acceptability.

### ***Statistical Analysis***

A completely randomized design (CRD) was applied for the data analysis of the amylose

content, gluten content, resistant starch content, and pasting properties. All experiments were performed in triplicates. The color and texture characteristics analyses were done in 8 repetitions. A randomized complete block design (RCBD) was used for sensory evaluation. Results were expressed as mean  $\pm$  standard deviation. The analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA) to determine the significant differences ( $p < 0.05$ ) among the mean observations.

## Results and Discussion

### *Amylose Content in Flour*

The quality of a cookie is highly dependent on the amylase content of the flour. The amylose contents of wheat, rice and unripe banana flour were 26.74%, 15.91% and 42.64%, respectively. The composite flour comprised a mixture of rice flour and unripe banana flour at different ratios, with amylose contents ranging from 15.91% to 42.64% (Figure 1). The amylose content of the composite flour increased with the increasing substitution levels of unripe banana flour since unripe banana flour had a higher amylose content than rice flour (42.64%). The amylose content affected the texture of the gluten-free rice cookies. Zhong *et al.* (2018) reported that adding high amylose maize

starch in cake mix affected the texture with increased hardness, cohesiveness, chewiness and resilience, and decreased springiness with increasing resistant starch. Moreover, Devi *et al.* (2019) found that wheat cookies made from high amylose wheat flour were of better quality than when using low amylose wheat flour.

### *Pasting Properties of Flour*

The pasting profiles of starches from different sources were significantly different (Table 2). Banana flour showed higher peak viscosity, hot paste viscosity, cold paste viscosity, and setback than rice flour and wheat flour, whereas wheat flour had the highest pasting temperature. Gluten protein in wheat flour acts as a water binding agent and binds with wheat starch, resulting in high pasting temperature (Ghiasi *et al.*, 1993). The effect of unripe banana flour on the pasting properties of rice flour is shown in Table 2. The addition of unripe banana flour increased peak viscosity, hot paste viscosity, final viscosity and setback viscosity. The setback viscosity indicates amylose re-association or retrogradation of starch after cooking, and has been correlated with the texture of various products (Huang *et al.*, 2018). The addition of unripe banana flour increased the setback from 1,619 to 1,885 cP. A high setback value of starch promoted good cookie quality (Devi *et al.*, 2019).

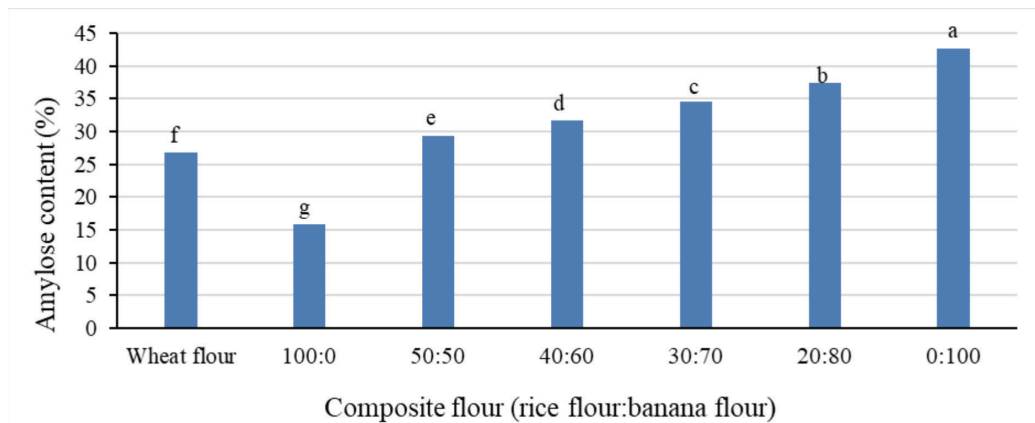


Figure 1: Amylose content in wheat, rice, banana and composite flour mixtures. Graphs with the different letters are significantly different ( $p < 0.05$ )

Table 2: Pasting properties of wheat, rice, unripe banana and composite flour mixtures

Sample	Pasting Properties						
	Pasting Temp (°C)	Peak Time (min)	Peak viscosity (cp)	Hot paste viscosity(cp)	Breakdown (cp)	Final Viscosity (cp)	Setback (cp)
Wheat flour	85.43±0.89 <sup>a</sup>	6.05±0.05 <sup>a</sup>	1911.67±18.58 <sup>d</sup>	1309.33±66.58 <sup>e</sup>	602.33±22.68 <sup>e</sup>	2474.67±85.82 <sup>e</sup>	1165.33±21.78 <sup>d</sup>
Rice flour: Unripe banana flour							
100:0 (0%)	81.38±0.66 <sup>b</sup>	5.57±0.05 <sup>b</sup>	3510.50±10.00 <sup>e</sup>	2041.50±21.92 <sup>d</sup>	1469.00±48.08 <sup>a</sup>	3660.50±9.20 <sup>d</sup>	1619.00±31.11 <sup>c</sup>
50:50(50%)	79.42±0.42 <sup>c</sup>	5.04±0.08 <sup>c</sup>	3821.66±28.74 <sup>bc</sup>	2347.33±64.36 <sup>c</sup>	1474.33±75.39 <sup>a</sup>	4090.00±10.58 <sup>c</sup>	1742.66±76.56 <sup>b</sup>
40:60(60%)	79.85±0.48 <sup>c</sup>	5.02±0.10 <sup>c</sup>	3847.33±34.02 <sup>bc</sup>	2631.66±65.65 <sup>b</sup>	1399.50±85.22 <sup>b</sup>	4392.67±62.03 <sup>b</sup>	1761.00±96.70 <sup>b</sup>
30:70(70%)	79.95±0.07 <sup>c</sup>	4.97±0.05 <sup>c</sup>	4008.50±34.76 <sup>b</sup>	2609.00±25.77 <sup>b</sup>	1215.66±28.99 <sup>b</sup>	4466.00±33.65 <sup>b</sup>	1857.00±67.88 <sup>a</sup>
20:80(80%)	79.55±0.07 <sup>c</sup>	4.97±0.05 <sup>c</sup>	4101.50±24.15 <sup>b</sup>	2789.00±22.33 <sup>b</sup>	1219.50±31.82 <sup>b</sup>	4653.00±57.98 <sup>b</sup>	1864.00±5.66 <sup>c</sup>
0:100(100%)	79.00±0.00 <sup>c</sup>	4.54±0.05 <sup>c</sup>	4249.00±26.27 <sup>a</sup>	3010.10±13.84 <sup>a</sup>	1239.50±12.43 <sup>b</sup>	4895.50±72.83 <sup>a</sup>	1885.00±41.01 <sup>a</sup>

Values are means ± standard deviation. Mean values for each characteristic followed by different letters within the same column are significantly different ( $p < 0.05$ )

### Gluten Content

The gluten content was determined using a monoclonal R5-antibody-based sandwich enzyme-linked immunosorbent assay (ELISA). Rice flour, unripe banana flour and composite flour cookies contained less than 1 ppm of gluten, but wheat cookies contained 153,510 ppm. Gluten-free rice cookies with unripe banana flour replacement at 0% to 100% contained gluten below 1 ppm. Thus, these cookies were gluten-free according to the US Food and Drug Administration (2019) guidelines that state gluten-free food as food containing less than 20 ppm of gluten.

### Physical Characteristics of Cookies

The moisture content and water activity ( $a_w$ ) play vital roles in cookie products and they have an effect on microbiology activity, texture, sensory evaluation and shelf life (Carter *et al.*, 2015). Moisture content, water activity and the color of cookies are shown in Table 3. The moisture content of gluten-free rice cookies was 2.13%. The substitution of unripe banana flour from 50% to 100% of rice flour resulted in an increase in the moisture content (2.92 to 3.36%) of cookies. Nevertheless, the moisture contents of gluten-free rice cookies with unripe banana flour and wheat cookies (3.34% moisture content) were not significantly different ( $p < 0.05$ ). Adding

unripe banana flour to rice flour from 0% to 100% w/w increased  $a_w$  of the cookies from 0.22% to 0.29%, whereas the  $a_w$  of wheat cookies was 0.28%. Alivola & Monterde (2018) reported that unripe banana flour had a high water absorption capacity (1.82 mL/g), while rice flour and wheat flour had a water absorption capacity of 1.56 and 1.31 mL/g, respectively. Thus, unripe banana flour could absorb and bind water more than rice and wheat flour, which resulted in rice cookies containing unripe banana flour having higher levels of  $a_w$  and moisture content compared with rice cookies without unripe banana flour.

According to Katz and Labuza, (1981), the moisture content of cookies should not be higher than 9% and  $a_w$  not higher than 0.35 to keep the quality of the product good. Carter *et al.* (2015) found that the crispness value of cookies changed as the water activity value changed. Cookies were softer at high moisture content and  $a_w$ , whereas cookies with low moisture content and low  $a_w$  were crispier. Our results showed that all cookies were crispy due to low moisture content. Moreover, the  $a_w$  values of all cookies were lower than 0.60, which may result in the prevention of microbial growth and food spoilage (Belitz & Grosch, 1999).

The color of gluten-free rice cookies with unripe banana flour was significantly different ( $p < 0.05$ ) compared with wheat cookies. The color parameters of gluten-free

rice cookies were  $L^*$  27.00,  $a^*$  7.80 and  $b^*$  20.96, with a lighter yellow color compared with wheat flour cookies ( $L^*$ 23.27,  $a^*$  7.39,  $b^*$  21.91). The replacement of rice flour with unripe banana flour decreased the lightness ( $L^*$ ) and  $a^*$  (redness) and  $b^*$  (yellowness) values. The unripe banana flour color is pale. Therefore, when rice flour was replaced with unripe banana flour, the lightness, redness, and yellowness were decreased. The results were similar to the study by Agama-Acevedo *et al.* (2009), which reported that the increase of banana flour in pastas decreased their lightness.

The dimensions, thickness and spread ratio are important quality parameters of cookies. The spread ratio evaluates the rising ability of cookies; a low spread ratio implies better rising ability (Olapade & Adeyemo, 2014). The diameter, thickness and spread ratios of wheat and gluten-free rice cookies are shown in Table 4. Gluten-free rice cookies without unripe banana flour had the highest diameter and lowest thickness, with a remarkable increase in spread ratio. The spread ratio of wheat cookies was higher than rice cookies, implying that wheat cookies had better rising ability. Gluten protein in wheat cookies increased

the viscosity of the dough (Nugraheni *et al.*, 2017). The addition of unripe banana flour in gluten-free rice cookies reduced the diameter as the substitution level increased from 60% to 100%, whereas the thickness increased significantly ( $p < 0.05$ ). This decrease in diameter and increase in thickness affected the spread ratio, which implied improved rising ability.

The results of the texture analysis are presented in Figure 2. Hardness is an important factor for sensory evaluation of cookies. Hardness was highest in wheat cookies with a value of 12.05 N, while gluten-free rice cookies (0% unripe banana flour) had the lowest hardness at 5.94 N. The hardness of cookies increased with the increasing amount of unripe banana flour. The texture of gluten-free rice cookies became firmer when unripe banana flour was increased from 50% to 100%. However, the hardness of gluten-free rice cookies with a replacement of 100% unripe banana flour was lower than that of wheat cookies. Wheat cookies showed the highest hardness due to the gluten protein in the wheat flour. Gluten-free rice cookies without unripe banana flour had the lowest hardness, but became harder when unripe banana flour was added. The addition of

Table 3: Moisture content, water activity and color of gluten-free cookies

Cookies	Moisture content	Water activity ( $a_w$ )	Color		
			$L^*$	$a^*$	$b^*$
Wheat flour	3.34±0.04 <sup>a</sup>	0.28±0.02 <sup>a</sup>	23.27±1.43 <sup>b</sup>	7.39±0.48 <sup>a</sup>	21.91±0.14 <sup>a</sup>
Composite flour (rice flour : unripe banana flour)					
100:0 (0%)	2.13±0.03 <sup>c</sup>	0.22±0.01 <sup>c</sup>	27.00±0.49 <sup>a</sup>	7.80±0.06 <sup>a</sup>	20.96±0.22 <sup>a</sup>
50:50 (50%)	2.92±0.04 <sup>b</sup>	0.26±0.01 <sup>b</sup>	23.49±1.35 <sup>b</sup>	6.33±0.28 <sup>bc</sup>	14.23±0.70 <sup>b</sup>
40:60 (60%)	3.07±0.04 <sup>b</sup>	0.26±0.01 <sup>b</sup>	21.32±1.32 <sup>b</sup>	6.52±0.18 <sup>b</sup>	13.86±0.45 <sup>b</sup>
30:70 (70%)	3.14±0.03 <sup>a</sup>	0.29±0.01 <sup>a</sup>	17.82±0.70 <sup>c</sup>	5.94±0.14 <sup>cd</sup>	12.35±0.50 <sup>b</sup>
20:80 (80%)	3.21±0.05 <sup>a</sup>	0.28±0.01 <sup>a</sup>	17.74±1.07 <sup>c</sup>	5.64±0.29 <sup>cd</sup>	12.18±0.57 <sup>bc</sup>
0:100(100%)	3.36±0.06 <sup>a</sup>	0.29±0.02 <sup>a</sup>	16.98±2.79 <sup>c</sup>	5.27±0.91 <sup>d</sup>	11.62±1.01 <sup>c</sup>

Values are means ± standard deviation. Mean values for each characteristic followed by different letters within the same column are significantly different ( $p < 0.05$ )

Table 4: Diameter, thickness and spread ratio of cookie samples

Cookies	Diameter (cm)	Thickness (cm)	Spread ratio (D/T)
Wheat cookies	3.24±0.05 <sup>c</sup>	0.66±0.28 <sup>bc</sup>	4.86±0.13 <sup>b</sup>
Composite flour (rice flour:unripe banana flour) (% unripe banana flour)			
100:0 (0%)	3.50±0.00 <sup>a</sup>	0.63±0.06 <sup>c</sup>	5.55±0.10 <sup>a</sup>
50:50 (50%)	3.47±0.05 <sup>a</sup>	0.73±0.03 <sup>ab</sup>	4.73±0.11 <sup>b</sup>
40:60 (60%)	3.40±0.00 <sup>b</sup>	0.73±0.06 <sup>ab</sup>	4.65±0.20 <sup>b</sup>
30:70 (70%)	3.33±0.05 <sup>c</sup>	0.75±0.05 <sup>ab</sup>	4.44±0.34 <sup>bc</sup>
20:80 (80%)	3.35±0.05 <sup>c</sup>	0.77±0.05 <sup>a</sup>	4.35±0.35 <sup>bc</sup>
0:100(100%)	3.30±0.05 <sup>c</sup>	0.80±0.00 <sup>a</sup>	4.13±0.00 <sup>c</sup>

Values are means ± standard deviation. Mean values for each characteristic followed by different letters within the same column are significantly different (p<0.05).

unripe banana flour in rice flour in Figure 1 and Table 2 showed higher amylose contents and setback viscosity, resulting in higher hardness in gluten-free rice cookies with unripe banana flour. The amylose content affected the hardness of the cookie samples, and this may be due to

cookies with high amylose content have higher retrogradation, therefore, the cookies with low amylose content showed softer texture. These results were supported by Yu *et al.* (2009), who reported that the hardness of cooked rice had a positive correlation with amylose contents.

Hardness(N)



Figure 2: The hardness of wheat cookies and gluten-free rice cookies. Graphs followed by different letters are significantly different (p<0.05)

### Resistant Starch Content

The resistant starch contents of gluten-free rice cookies replaced with unripe banana flour at 0%, 5 0%, 60%, 70%, 80% and 100% were 1.92%, 2.70%, 3.99%, 5.25%, 5.77% and 8.50%, respectively. The increase in resistant starch content is related to the amount of added unripe banana flour, whereas wheat cookies contained a RS content of 2.85% (Table 5). Gluten-free rice cookies with banana flour showed a higher resistant starch content than gluten-free rice flour (0% unripe banana flour). The highest amount of resistant starch content was found in gluten-free rice flour cookies replaced with 100% unripe banana flour (0% rice flour). Moongngarm *et al.* (2014) stated that unripe banana flour had a high RS content (48.88%), therefore, it was a good source for improving the RS content of food products. Moreover, since unripe banana flour has a high amylose content, baking at high temperature in the presence of free lipids formed amylose-lipid complexes. These results were observed in wheat cookies substituted with 50% unripe banana flour, containing 8.37% RS (Agama-Acevedo *et al.*, 2012). Ratnasari *et al.* (2018) reported that wheat cookies with 75% unripe banana flour replacement showed increased RS of 14.60%. Moreover, Tiboobun *et al.* (2011) reported

that rice noodles replaced with banana flour from 0% to 100% showed increased resistant starch from 7.49% to 13.15%. Digestible starch content in rice cookies decreased with the degree of substitution of unripe banana flour to rice flour, ranging between 71.08% and 73.13%. The total starch content of rice cookies increased with the increased substitution of unripe banana flour to rice flour. A similar result was found in rice noodles with the substitution of unripe banana flour (Tiboobun *et al.*, 2011).

### Surface Appearance

The surface appearance of the cookie samples is presented in Figure 3. Rice cookies had a lighter color than wheat flour and unripe banana flour cookies. The surface appearance of gluten-free rice cookie with unripe banana flour (Figure 3B to 3G) were more uneven and less uniform compared with those of wheat cookies (Figure 3A). These results were due to rice flour and banana flour having no gluten protein to form a strong molecular structure of dough, and, as a result, the cookie surface was uneven. Gluten-free rice cookies replaced with 70% unripe banana flour (Figure 3E) had a good appearance, while gluten-free rice cookies replaced with 80% and 100% unripe banana flour showed a rugged and rough surface, as indicated in Figure 3F and 3G.

Table 5: Resistant starch content in wheat cookies and composite flour cookies

Sample	Resistant starch (%)	Digestible starch (%)	Total starch (%)
Wheat cookies	2.85±0.70 <sup>d</sup>	65.95±0.25 <sup>d</sup>	68.8±0.73 <sup>c</sup>
Composite flour (rice flour:unripe banana flour)			
100:0 (0%)	1.92±0.05 <sup>c</sup>	73.13±0.21 <sup>a</sup>	75.05±0.14 <sup>d</sup>
50:50(50%)	2.70±0.10 <sup>d</sup>	72.55±0.45 <sup>ab</sup>	75.25±0.35 <sup>d</sup>
40:60 (60%)	3.99±0.19 <sup>c</sup>	71.91±0.97 <sup>bc</sup>	75.90±0.78 <sup>cd</sup>
30:70 (70%)	5.25±0.50 <sup>b</sup>	71.25±1.04 <sup>c</sup>	76.50±1.03 <sup>bc</sup>
20:80 (80%)	5.77±0.24 <sup>b</sup>	71.50±0.50 <sup>c</sup>	77.28±0.29 <sup>b</sup>
0:100 (100%)	8.50±0.14 <sup>a</sup>	71.08±0.50 <sup>c</sup>	79.58±0.16 <sup>a</sup>

Values are means ± standard deviation. Mean values for each characteristic followed by different letters within the same column are significantly different (p<0.05).



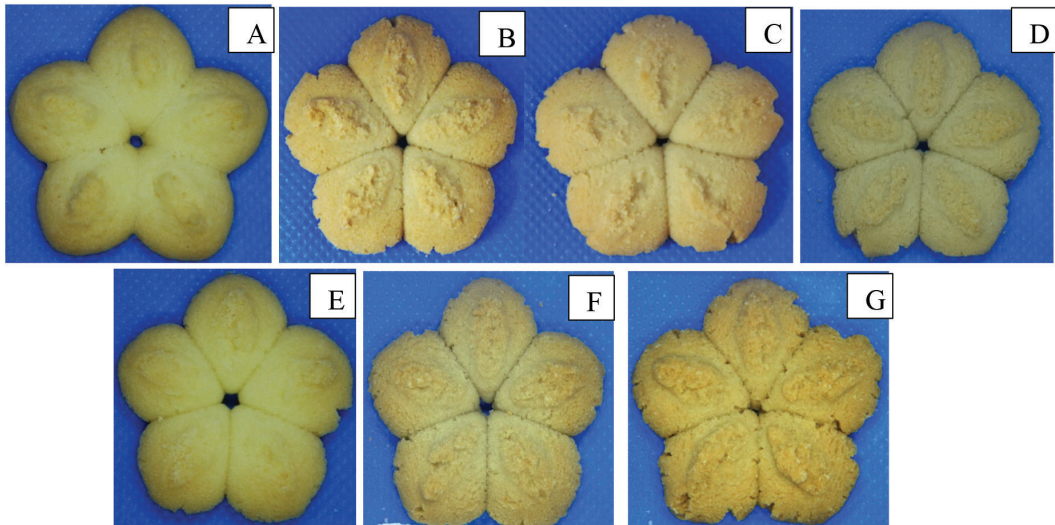


Figure 3: The appearance of wheat cookies (A), gluten-free rice cookies replaced with banana flour at 0% (B), 50% (C), 60% (D), 70% (E), 80% (F) and 100% (G)

### Sensory Evaluation

A nine-point hedonic scale was applied to evaluate sensory attributes of the cookies as shown in Table 6. The sensory evaluation assessed appearance, color, texture, flavor, and overall acceptability. The overall acceptability score of gluten-free cookies significantly increased when rice flour was replaced with unripe banana flour at 70%, comparable to the overall acceptability of wheat cookies ( $p < 0.05$ ). At higher levels of addition with unripe banana flour beyond 70%, the overall acceptability scores decreased because the texture of the cookies became hard and the products smelled like raw banana. The results indicated that the sensory attributes of gluten-free cookies prepared with rice flour to unripe banana flour ratio of 30:70 were comparable to that of wheat cookies. Gluten-free rice cookies with unripe banana flour at 80% and 100% had low scores for color, texture and overall acceptability. The addition of unripe banana flour at 80% and 100% caused a darkening (lower  $L^*$  value), less reddish (lower  $a^*$  value), and less yellowish (lower  $b^*$  value) color. This result concurred with Agama-Acevedo *et al.* (2009), who reported that an increased percentage of banana flour in pasta formulations decreased lightness.

The substitution of unripe banana flour in rice flour at up to 70% enhanced the overall acceptability of gluten-free cookies. This result agreed with Roman *et al.* (2019), who found that using banana starch to make gluten-free bread improved the odor and flavor of the product.

### Conclusions

Unripe banana flour had very high resistant starch content. The results confirmed the feasibility of using unripe banana flour to replace rice flour to produce gluten-free rice-based cookies with improved texture, color and high acceptability that is comparable to wheat flour cookies. The rice flour of gluten-free rice-based cookies can be replaced with unripe banana flour for up to 70%. This result could lead to the development of high resistant starch gluten-free rice cookies as a functional food product. However, further studies regarding the optimization of unripe banana flour and rice flour to maximize the RS content and sensory acceptability, as well as the glycemic index and nutrition quality are needed.

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Table 6: Sensory evaluation of wheat cookies and composite flour cookies

Cookies	Sensory attributes				
	Appearance	Color	Texture	Flavor	Overall acceptability
Wheat cookies	8.3±0.6 <sup>a</sup>	8.1±0.5 <sup>a</sup>	8.1±0.6 <sup>a</sup>	8.3±0.8 <sup>a</sup>	8.0±0.5 <sup>a</sup>
Composite flour (rice flour:unripe banana flour)					
100:0 (0%)	7.6±0.5 <sup>ab</sup>	7.4±0.6 <sup>a</sup>	6.4±0.3 <sup>b</sup>	6.0±1.5 <sup>bc</sup>	6.5±0.2 <sup>b</sup>
50:50 (50%)	7.2±0.3 <sup>b</sup>	7.1±0.2 <sup>ab</sup>	6.6±0.4 <sup>b</sup>	6.4±0.6 <sup>b</sup>	6.6±0.1 <sup>b</sup>
40:60 (60%)	7.2±0.4 <sup>b</sup>	7.1±0.3 <sup>ab</sup>	6.6±0.3 <sup>b</sup>	6.9±0.6 <sup>b</sup>	6.7±0.5 <sup>b</sup>
30:70 (70%)	7.6±0.4 <sup>ab</sup>	7.3±1.3 <sup>ab</sup>	7.3±0.3 <sup>a</sup>	7.2±0.3 <sup>a</sup>	7.8±0.5 <sup>a</sup>
20:80 (80%)	7.4±0.2 <sup>b</sup>	6.7±0.2 <sup>c</sup>	6.2±0.4 <sup>b</sup>	5.9±0.5 <sup>bc</sup>	6.1±0.2 <sup>c</sup>
0:100 (100%)	7.00±0.2 <sup>b</sup>	6.7±0.1 <sup>c</sup>	6.2±0.3 <sup>b</sup>	5.5±0.5 <sup>c</sup>	5.9±0.4 <sup>c</sup>

Values are means ± standard deviation. Mean values for each characteristic followed by different letters within the same column are significantly different ( $p < 0.05$ )

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