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Banana Peels as Potential Prebiotic and Functional Ingredient

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ABSTRACT

This study aims to determine the prebiotic potential of the banana peel on the growth of probiotic *Lactobacillus* spp. in vitro and to utilize the peel as a functional ingredient in preparing biscuits. Peels of dessert banana (*pisang berangan*) and plantain (*pisang nangka*) were oven-dried and homogenized, and the total sugar content was determined. Subsequently, different cultivation media were made by substituting the carbon source with Banana Peel Powder (BPP), Plantain Peel Powder (PPP), glucose, and inulin. These media were later fermented with probiotic *Lactobacillus* spp., extracted from a probiotic drink. The growth performance was accessed following 24 hours of incubation. BPP and PPP were incorporated into the preparation of biscuits as functional ingredients. A portion of wheat flour was substituted with 10%, 20%, and 30% of BPP and PPP, respectively, during the preparation of the biscuits. These biscuits were then analyzed for proximate composition, physical properties, and estimated Glycaemic Index (eGI). The supplementation of BPP and PPP in the media improved the probiotic bacteria's growth rate and generation time as the media had a significantly higher amount of *Lactobacillus* spp. compared to others. Both BPP- and PPP-supplemented media had significantly low pH, indicating intense metabolic activity of the bacteria utilizing the peels. Results also showed significant differences in the total dietary fiber and protein content of BBP- and PPP-incorporated biscuits. The addition of BPP and PPP did not significantly affect the physical properties of the biscuit, and such incorporation resulted in lower eGI when compared to the control. BPP and PPP possess potential prebiotic properties and can be utilized as functional ingredients. Further study is warranted to explore other prebiotic properties of banana peels and to investigate consumers' acceptance of banana peel-incorporated foods.

Keywords: banana peel, functional food, prebiotic

INTRODUCTION

Banana is a popular fruit widely cultivated and consumed in many tropical nations, including Malaysia, Thailand, and Indonesia (Zaini *et al.* 2022). To fulfil the increasing local demand for bananas, banana trees are widely cultivated in Malaysia (Fizar *et al.* 2022). High production of bananas is associated with the generation of banana by-products. Banana peel is usually discarded, thus generating massive amounts of household and industrial food waste (Ahmed *et al.* 2006).

Banana peel is an underpredicted functional food source as it contains soluble fibre oligosaccharide (Liang *et al.* 2022). Fructooligosaccharide was found in banana peels and had prebiotic properties (Azam *et al.* 2020). Besides soluble fibre, banana peel also contains insoluble fibre. Since banana peels have a remarkable amount of fibre; thus, they may exhibit a low Glycaemic Index (GI) value when the wheat flour is substituted with a specific ratio of banana peel powder (Chakraborty *et al.* 2021). Indeed, utilizing this agricultural waste as a functional food or value-added food item is critical for long-term sustainability, in line with the Sustainable Development Goal (SDG) No.12, which aims to ensure sustainable consumption and production patterns.

There is limited study on the prebiotic potential of banana peels. A few published studies have shown contradicted findings of banana peels (Hernández-Alcántara *et al.* 2016). Other studies have reported banana peels' antioxidant and antimicrobial properties, but there is still limited study of banana peels on the activity of *Lactobacillus* spp. growth. Besides,

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there is no study comparing the GI of banana peel-incorporated biscuits from several types of bananas. Only one study compared GI between wheat-based and unripe banana peel biscuits (Bakar *et al.* 2020). Therefore, this study aims to determine the prebiotic potential of the banana peel on the growth of probiotic *Lactobacillus* spp. in vitro and to utilize the peel as a functional ingredient in the preparation of biscuits.

METHODS

Design, location, and time

This was an in vitro experiment and it was carried out at the Nutrition Laboratory, Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, from July 2022 until February 2023.

Materials and tools

Banana peels were obtained from Kerepek Azadila, Puchong, Selangor, Malaysia, dried in an oven (Memmert GmbH, Germany) and ground (Waring Commercial, USA) to prepare banana peel powder. Anthrone reagent, concentrated H_2SO_4, 2.5 NHCl, sodium carbonate, centrifuge machine (Hettich, Germany), and water bath (Memmert GmbH, Germany) were used to determine the carbohydrate content of the banana peel powder. A probiotic drink was purchased from a nearby supermarket in Serdang, Selangor, and used as a source of probiotics, *Lactobacillus* spp. Both de Man Rogosa and Sharpe (MRS) agar and broth were prepared in a fume cupboard to culture and enumerate *Lactobacillus* spp. To prepare the batch culture medium, peptone, yeast extract, Tween 80, and L-cysteine hydrochloride were mixed with 1 L of distilled water and sterilised with autoclave (HVE-50, Hirayama, Japan). Petroleum ether, extraction thimble (Sigma-Aldrich, St. Louis, MO, USA), condenser, rotary evaporator (Büchi® R-200 Rotavapor System), were used in Soxhlet while concentrated H_2SO_4, HCl, and Kjeldahl's distillation unit (KT 200 Kjeltec™ Distillation Unit) were used in the Kjeldahl methods. For the total fiber determination, 78% ethanol, 95% ethanol, acetone, and megazyme total dietary fiber assay kit (NEOGEN, Michigan USA) were used. To estimate GI, 0.1 M potassium phosphate buffer solution (PBS) (pH 6.9), HCl, Potassium Hydroxide (KOH), pepsin, α-amylase, 3,5-Dinitrosalicylic acid (DNSA) reagent, and Visking Dialysis Tube, Cellulose (Size 2, inflat D14.3/26 mm) were used.

Procedure

Preparation of banana peel powder. The banana peel powder was prepared based on the method of Zahid *et al.* (2021) with some modifications. Dessert banana (*pisang berangan*) and plantain (*pisang nangka*) peels were washed with tap water and cut into small uniform pieces. They were dried in an oven at 50°C for 24 hours and ground into powder using a grinder. The dried Banana Peel Powder (BPP) and Plantain Peel Powder (PPP) were sieved to maintain a uniform particle size, packed in airtight bags, and stored in the chiller at 4°C until further use.

Determination of carbohydrate content in banana peel and banana peel-incorporated biscuits. The anthrone method by Ludwig and Goldberg (1956) was used to determine carbohydrate content. Samples were heated in a boiling water bath for 8 minutes after adding the anthrone reagent, and the absorbance was measured using a spectrophotometer at 620 nm. The carbohydrate content was extrapolated from a standard curve of glucose with varying concentrations (0.2–1 g/L).

Propagation of Lactobacillus spp.. Briefly, 0.01 mL of probiotic drink was aseptically spread onto MRS agar and incubated at 37°C aerobically for 24–48 hours. Then, one colony of *Lactobacillus* spp. was inoculated into MRS broth and incubated at 37°C aerobically for 48 hours. At 3-hour intervals, 1 mL was taken, where the Optical Density (OD) was measured, and log colony forming unit per milliliter (log CFU/mL) was calculated. A graph of log colony forming units (log CFU/mL) against optical density (OD600) of *Lactobacillus* spp. was plotted and these variables were positively correlated. Then the graph was used to extrapolate the amount of *Lactobacillus* spp.

Assessment of bacterial growth performance. The preparation of the batch culture medium and the fermentation of *Lactobacillus* spp. was based on the method in Azam *et al.* (2020) with some modifications. The amount of BPP and PPP with 2.39% w/v of carbohydrate content was extracted following the procedure by Pereira *et al.* (2018) to obtain the oligosaccharide. Peptone (1g/100 mL), yeast extract (0.5 g/100 mL), Tween 80 (0.1 mL/100 mL), and L-cysteine

hydrochloride (0.05 g/100 mL) were mixed in five vessels, respectively, with the extracted BPP and PPP solutions, 2.39% w/v of inulin, 2.39% w/v of glucose, and distilled water (control). All media were sterilized using an autoclave. The final volume in each vessel was 100 mL. One (1) colony of *Lactobacillus* spp. was inoculated separately into five different vessels containing batch culture media and incubated at 37°C for 24h. Samples from each vessel were taken (10 mL) at 15, 18, 21, and 24 hours of fermentation at 37°C for enumeration of *Lactobacillus* spp. and pH measurement.

Determination of mean growth rate constant (k) and mean generation time, (g) of Lactobacillus *spp.*. Formulas from Willey *et al.* (2018) were used to calculate the mean growth rate constant (k) and mean generation time (g) as follows:

$$k = (\log N_t - \log N_0)/(\log 2)t$$

$$g = 1/k$$

Where, $\log N_t$ = log CFU/mL at the end of fermentation

$\log N_0$ = log CFU/mL at the start of fermentation

t = final time of incubation

Preparation and proximate composition of BPP- and PPP-incorporated biscuits. One hundred (100) g of wheat flour, 56 g of sugar, 23.5 g of shortening, 1.1 g NaHCO3, 0.89 g NaCl, and 12 mL of milk were mixed to form biscuit dough (Arun *et al.* 2015). The Wheat Flour (WF) was substituted with 10% (BPP10 or PPP10), 20% (BPP20, or PPP20), and 30% (BPP30 or PPP30) of BPP and PPP, respectively. The doughs were then molded into walnut-sized balls approximately 4 cm in diameter, placed on a greased tray, and baked in the oven for 15 minutes at 180°C or until golden brown. The biscuits were cooled at room temperature and stored in a polyethylene bag before analysis. Evaluation of moisture (gravimetric method with oven drying – AOAC 2000), ash (carbonization and incineration in the furnace – AOAC 2000), fat (Soxhlet method – AOAC 1996), protein (Kjeldahl – AOAC 2000), carbohydrate (Anthrone method), and total dietary fiber (enzymatic-gravimetric method – AOAC 991.43) of the cookies were conducted according to AOAC methods (Helrich 1990).

Determination of physical properties of BPP- and PPP-incorporated biscuits. The diameter (D) and thickness (T) of the biscuits were measured using a ruler (Abu Bakar *et al.* 2018). The diameter of the biscuits was determined by arranging six biscuits edge to edge, and the overall diameter of the biscuits was measured. Then, the average diameter of the biscuits was recorded after six times of biscuit rearrangement. The procedure was repeated by stacking six biscuits on top of one another to determine the thickness of the biscuits. The averages of the diameter and thickness were reported in centimeters (cm) and used to calculate the spread ratio.

Estimation of glycaemic index. To predict the glycaemic response and estimate the Glycaemic iIndex (eGI) of the BPP- and PPP-incorporated biscuits, an in vitro starch hydrolysis was conducted according to Gibson *et al.* (2011). Briefly, samples were added into potassium phosphate buffer (pH 6.9) and kept at 37°C. Hydrochloric acid was used to adjust the pH of the samples to pH 2.5. Then, pepsin was added and placed in a water bath for 1 hour at 37°C. Potassium hydroxide was used to increase the samples' pH to 6.8. The mixtures were transferred into a dialysis tube after adding 2 mL α-amylase. Subsequently, the dialysis tube was placed in a buffer solution while the flask was set in a shaking water bath at 37°C. Forty (40) mL of buffer solution was extracted every 30-minute interval within 3 hours to determine the carbohydrate hydrolysis rate from the dialysis tube. 3,5-Dinitrosalicylic Acid (DNSA) reagent was used to estimate the amount of reduced sugar. The concentration-over-time curve (area under the curve) was determined, and the Hydrolysis Index (HI) was calculated by comparing the Area Under the Curve (AUC) of the samples and the AUC of maltose, which acts as the standard of reference food. Then, HI was calculated as HI=(Area under the curve, sample / Area under the curve, reference) x 100% and it was later used to determine eGI.

Data analysis

Statistical analysis was conducted by using IBM SPSS Statistics 26. Results were expressed as means±standard deviation. One-Way ANOVA analysis was used to compare the data, and Post Hoc Comparison (Tukey HSD) was used to determine significant differences between the variables. P-values of <0.05 were regarded to be significant.

RESULTS AND DISCUSSION

Carbohydrate content in BPP and PPP

BPP had 34.0% carbohydrates, while PPP contained higher carbohydrates, 61.9%. According to Tsado *et al.* (2021), dessert banana peel and plantain peel contain around 63.8% and 74.1% of carbohydrates, respectively, compared to this study's result. The varied nutritional content is due to the genetic makeup, environmental factors, and addition of nutrients to the soil (Durgadevi *et al.* 2019). The ripening state can also affect the composition of the peels (Khawas & Deka 2016). Plantain peel contains more total starch and soluble sugar (glucose, fructose, and sucrose) than dessert banana peel at any maturation stage (Emaga *et al.* 2007).

Growth performance of *Lactobacillus* spp. in BPP- and PPP-supplemented media

The bacteria count significantly differed in media with different carbon sources ($p<0.05$). Specifically, the growth of *Lactobacillus* spp. media supplemented with BPP and PPP was significantly higher than inulin, glucose, and without carbon source (Table 1). The finding of this study was in line with previous studies that showed the banana peel promotes the growth of probiotic bacteria (Syahpura *et al.* 2019; Zahid *et al.* 2021). Study showed that fructooligosaccharide is present in both dessert banana and plantain peel and possess prebiotic properties (Azam *et al.* 2020). Dessert banana peel contains more fructooligosaccharide than plantain peel (Syahpura *et al.* 2019). Thus, it can be postulated that the higher count of *Lactobacillus* spp. in a medium with BPP could be due to the presence of fructooligosaccharide.

The findings of Rossi *et al.* (2005) indicated that the potential of fructooligosaccharide in promoting the growth of *Lactobacillus* spp. was higher than inulin, supporting the findings of this study. The growth of *Lactobacillus* spp. is more rapid with the presence of fructooligosaccharide compared to inulin because short-chain fructooligosaccharide has a lower degree of polymerization (<10) compared to long-chain inulin (>20). Inulin is a fructan with a longer chain length and larger molecule, which requires more time for the bacteria to ferment (Parhi *et al.* 2021). Thus, bacteria take a shorter time to ferment fructooligosaccharide.

As shown in Table 1, there was a significant difference in the final pH of the media with a different carbon source ($p<0.05$). The pH of media supplemented with BPP and PPP was significantly lower than inulin, glucose, and no carbon source. The finding of this study was in line with previously reported study that showed the banana peel as a carbon source has a pH-lowering effect (Safdari *et al.* 2021). *Lactobacillus* spp. is lactic acid bacteria that can ferment carbohydrates to produce lactic acid (Wang *et al.* 2021). Thus, the activity of *Lactobacillus* spp. can be detected by measuring the pH of customized media.

The result showed that there was a significant difference between the mean growth rate constant (k) and mean generation time (g) of *Lactobacillus* spp. in media with a different carbon source ($p<0.05$) (Table 1). The mean growth rate

Table 1. Final bacteria count (log CFU/mL), final pH, and growth performance of *Lactobacillus* spp. employed with different carbon sources

Carbon source	Mean±SD			
	Log CFU/mL	Final pH	Growth rate constant, k (log CFU/mL h^{-1})	Generation time, g (h)
No carbon source	8.28 ± 0.01^{a}	4.65 ± 0.03^{a}	0.03 ± 0.00^{a}	33.83 ± 0.10^{a}
Glucose	$9.42\pm0.06^{b,f}$	$3.98\pm0.16^{b,f}$	$0.32\pm0.03^{b,f}$	$3.17\pm0.32^{b,f}$
Inulin	$9.49\pm0.15^{c,f}$	$3.87\pm0.05^{c,f}$	$0.32\pm0.02^{c,f}$	$3.09\pm0.15^{c,f}$
BPP	11.25 ± 0.04^{d}	3.43 ± 0.04^{d}	0.90 ± 0.07^{d}	1.11 ± 0.09^{d}
PPP	9.86 ± 0.07^{e}	3.78 ± 0.02^{e}	0.37 ± 0.02^{e}	2.68 ± 0.11^{e}

Results are expressed as mean±standard deviation of two independent experiments (n=2); Value with different superscript letters shows significant differences

BPP: Banana Peel Powder; PPP: Plantain Peel Powder

constant (k) and mean generation time (g) of *Lactobacillus* spp. in a medium supplemented with BPP as a carbon source was significantly higher than in a PPP-supplemented medium.

The generation time (g) of *Lactobacillus* spp. in a medium without a carbon source is significantly the longest. From the finding, it is estimated that around 33.83 hours to complete binary fission and produce the next generation, without any supplementation. Hence, the environment without a carbon source is the least preferred by *Lactobacillus* spp., as the bacteria can hardly undergo cell division. In contrast, *Lactobacillus* spp. only require around 1.11 hours to complete cell division in media with dessert banana peel. It shows that the environment is the most preferred by the *Lactobacillus* spp., where it divides actively, as found in the present study.

The generation time differs widely among different bacteria. Even within the bacteria of the same species, the generation time might also be different due to the environmental factor (Mason 1935). The finding of this study is slightly different from the previous study, where the generation time of *Lactobacillus* spp. is between 0.62 hours and 2.25 hours (Mason 1935; Ahmed *et al.* 2006). This indicated that the *Lactobacillus* spp. used in this study duplicate slower compared to other species.

Proximate composition of BPP- and PPP-incorporated biscuits

As shown in Table 2, the moisture content in BPP-incorporated biscuits was significantly lower as the level of BPP substitution increased ($p<0.05$). There was a significant difference and increasing trend in ash content between control and BPP-incorporated biscuits as the BPP level increased, while only PPP30 biscuits showed significance ($p<0.05$). Besides, the protein content and total dietary fiber (TDF) were significantly different between the control and BPP- and PPP-incorporated biscuits ($p<0.05$). Lower moisture content in BPP- and PPP-incorporated biscuits, indicates longer shelf life (Amarasinghe *et al.* 2021). Mahloko *et al.* (2019) also supported that microbial growth, mold, and insect manifestation could be resisted when the moisture content is less than 14%. It was expected that BPP- and PPP-incorporated biscuits to have low protein as their source from the wheat flour has been reduced. Nonetheless, these biscuits could be alternative sources of dietary fiber because they had higher TDF than wheat flour biscuits, as found in this study.

Diameter, thickness, and spread ratio of BPP- and PPP-incorporated biscuits

There was no significant difference in the diameter, thickness, and spread ratio of the biscuits ($p<0.05$) (Table 3). The results were contradicted by other studies since the diameter of the biscuits tend to decrease as the level of BPP increases (Arun *et al.* 2015; Abu Bakar *et al.* 2018; Mahloko *et al.* 2019). On the other hand, Mahloko *et al.* (2019) demonstrated that biscuits containing both BPP and PPP also did not show any significance from the control ($p<0.05$). As a result, the insignificant physical properties in both BPP- and PPP-incorporated biscuits

Table 2. Proximate composition of BPP- and PPP-incorporated biscuits

Parameter (%)	Control	BPP10	BPP20	BPP30	PPP10	PPP20	PPP30
Moisture	10.75±0.01^{a}	9.53±0.08^{ac}	9.13±0.30^{bc}	9.08±0.18^{bc}	10.62±0.29^{a}	10.60±0.25^{a}	10.16±0.18^{ac}
Ash	1.40±0.14^{a}	1.97±0.09^{ac}	2.72±0.26^{bc}	3.36±0.28^{b}	1.77±0.05^{ac}	2.15±0.03^{ac}	2.65±0.21^{bc}
Fat	15.09±0.12^{ac}	15.73±0.5^{1ac}	16.43±1.73^{ac}	19.29±1.74^{a}	13.25±0.47^{bc}	13.71±0.37^{ac}	14.59±0.21^{ac}
Protein	4.55±0.49^{a}	2.02±0.12^{b}	2.02±0.12^{b}	1.93±0.25^{b}	2.17± 0.09^{b}	2.45±0.00^{b}	2.51±0.08^{b}
TDF	1.76±0.11^{a}	2.98±0.03^{b}	3.00±0.02^{b}	3.25±0.03^{bd}	3.00±0.00^{bd}	3.15±0.12^{bd}	3.43±0.05^{cd}
TAC	67.26±0.9^{0a}	63.57±0.84^{a}	62.67±0.83^{a}	61.13±2.16^{a}	66.12±1.22^{a}	65.02±0.21^{a}	64.30±0.52^{a}

Results are expressed as mean±standard deviation of two independent experiments (n=2); Value with different superscript letters shows significant differences

BPP: Banana Peel Powder; PPP: Plantain Peel Powder; TDF: Total Dietary Fiber; TAC: Total Available Carbohydrate

Table 3. Physical properties of BPP- and PPP-incorporated biscuits

Parameter (%)	Control	BPP10	BPP20	BPP30	PPP10	PPP20	PPP30
Diameter (cm)	4.28±0.05[a]	4.31±0.14[a]	4.34±0.38[a]	4.42±0.23[a]	4.37±0.06[a]	4.42±0.14[a]	4.40±0.1[1a]
Thickness (cm)	8.21±0.60[a]	7.41±0.01[a]	7.30±0.50[a]	6.34±0.02[a]	8.15±0.69[a]	7.55±0.32[a]	7.66±0.23[a]
Spread ratio	5.22±0.33[a]	5.82±0.18[a]	5.96±0.91[a]	6.98±0.37[a]	5.38± 0.38[a]	5.86±0.06[a]	5.74±0.04[a]

Results are expressed as mean±standard deviation of two independent experiments (n=2); Value with different superscript letters shows significant differences
BPP: Banana Peel Powder; PPP: Plantain Peel Powder

demonstrated that the substitution of BPP and PPP did not cause major changes in the physical characteristics when compared to the standard one.

Hydrolysis Index (HI) and Estimated Glycaemic Index (eGI)

There was a significant difference in HI and eGI of control biscuits with both BPP- and PPP-incorporated biscuits ($p<0.05$) (Table 4). On the other hand, there was no significant difference in HI and eGI between BPP- and PPP-incorporated biscuits ($p>0.05$). The biscuits with the formulation of 100% wheat flour showed significantly greater values of HI and eGI than the other formulations. The incorporation of BPP and PPP in biscuits slows the starch hydrolysis rate due to the presence of fiber content. Indeed, BPP- and PPP-incorporated biscuits have much higher fiber content compared to wheat flour, thus resulting in lower HI and eGI. Furthermore, α-amylase has a lower capacity to digest the dietary fiber contained in the biscuits, thus, resulting in less digestion of starch (Chakraborty *et al.* 2021). High-fiber food allows the stomach to give signals to the liver to cease glucose production. Therefore, low hydrolysis rate and increment of glucose level can be seen over time. The lower hydrolysis rate determines a lower GI value (Bakar *et al.* 2020). Both BPP- and PPP-incorporated biscuits meet with the classification of medium GI food (56–69) compared to control biscuits that fell into the high GI food category (≥70) (Dona *et al.* 2010).

CONCLUSION

The cultivation of *Lactobacillus* spp. in customized batch culture media containing BPP and PPP as the sole carbon source resulted in intense bacterial metabolic activities as observed by high cell counts (log CFU/mL), decreased in pH values and acceptable results of mean growth rate constant (k) as well as mean generation time (g) besides obtaining satisfactory prebiotic properties. Besides, incorporating BPP and PPP in biscuits can increase the total dietary fiber content and decrease the protein content of biscuits. Furthermore, such incorporation slowed down starch digestion and possessed lower eGI. In contrast, the physical properties of both BPP- and PPP-incorporated biscuits were not significantly affected.

For the future study, the use of probiotic bacteria with different species and strains (*Bifidobacterium* spp. and *Eubacterium* spp.) and pathogenic bacteria (*E.coli*) are recommended to determine the prebiotic effect of the banana peel on different probiotics. Moreover, the banana peel fiber can be introduced for *Lactobacillus*

Table 4. Hydrolysis index and estimated glycaemic index of BPP- and PPP-incorporated biscuits

Parameter (%)	Control	BPP10	BPP20	BPP30	PPP10	PPP20	PPP30
HI	55.84±0.80[a]	34.94±2.93[b]	34.61±1.00[b]	34.78± 2.02[b]	34.73±3.19[b]	34.98±0.20[b]	34.98± 2.48[b]
eGI	70.36±0.44[a]	58.89±1.61[b]	58.7±0.55[b]	58.81±1.12[b]	58.78±1.75[b]	58.92±0.11[b]	58.91± 1.36[b]

Results are expressed as mean±standard deviation of two independent experiments (n=2); Value with different superscript letters shows significant differences
BPP: Banana Peel Powder; eGI: estimated Glycaemic Index; HI: Hydrolysis Index; PPP: Plantain Peel Powder

spp. fermentation since insoluble fiber might also promote the growth of probiotics. Since the finding presented here is based on in vitro study, further in vivo and human research can be pursued to validate the prebiotic properties of banana peel and its incorporated biscuits. In addition, sensory evaluation is recommended to assess consumer satisfaction and acceptance towards the appearance, odor, taste, color, hardness, and texture of the biscuit incorporated with banana peels.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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