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RESEARCH ARTICLE

The Effect of Cavendish Banana (*Musa acuminata*) Puree Concentration on the Microbiological, Physicochemical, and Sensory Properties of Rice Bran Yogurt

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ABSTRACT

Cavendish banana is a widely known fruit for its high saccharide content and many volatile aroma compounds (esters and alcohols). Integrating Cavendish banana into rice bran yogurt [RBY] is expected to enhance its sensory properties. However, its contents of starch, simple sugars, and organic acids may affect LAB's growth, which impacts the physicochemical and sensory properties of RBY. The aim of this study was to ascertain the microbiological, physicochemical, and sensory properties of RBY integrated with Cavendish banana puree [CBP] at various concentrations. Experimental yogurts were made from full cream UHT milk supplemented with 1% rice bran and different levels of CBP (0%, 5%, 10%, 15%, 20%, and 25% (w/v)) and then inoculated with a yogurt starter. Results showed that the integration of CBP has significantly ($P < 0.05$) affected the LAB counts, pH, and sensory preferences of RBY (appearance and mouthfeel). However, it has no significant ($P > 0.05$) effect on the titratable acidity (as %lactic acid), syneresis, and taste preference of RBY. Increment levels of CBP integration reduced the appearance and mouthfeel preferences, while taste preference tended to increase up to 15% level of integration. CBP exerted a praising effect on the microbiological, physicochemical, and sensory properties of RBY.

KEYWORDS: *Yogurt, Rice bran, Cavendish banana puree, Fermentation*

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Introduction

Yogurt is functional food obtained from the fermentation of milk by lactic acid bacteria (LAB), *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Yogurt is the most commonly used vehicle for delivering probiotics into the body, that is generally regarded as safe (Nyanzi et al., 2021). At present, innovations have been made to increase the health benefits of yogurt, including by adding dietary fiber. One of the fiber-rich ingredients that is rarely used is rice bran.

Rice bran is a by-product of the rice milling process, which is rich in dietary fiber, i.e., cellulose, hemicellulose, lignin, arabinoxylan, β-glucan, gum, and pectin (Hoogenkamp et al., 2017; Zhuang et al., 2019; Chandel et al., 2020). A study of rice bran yogurt by Demirici et al. (2017) has shown that the addition of rice bran was able to increase the counts of *S. thermophilus*, *L. bulgaricus*, and *L. casei* in yogurt. Hasani et al. (2016) reported that yogurt enriched with rice bran increased the viability of *L. acidophilus*, but unfortunately this yogurt was not well accepted as its flavor was not pleasant. This weakness could be overcome by adding fruit, such as Cavendish banana.

Cavendish banana (*Musa acuminata*, AAA group, Cavendish subgroup) is one of the commercially cultivated varieties of banana in Indonesia. It has a sweet taste and according to Wills et al. (1984) total sugars present in ripe Cavendish banana was about 17.2 g/100 g. Bananas have a large number of volatile compounds that play a significant role in providing flavor perception. Thompson et al. (2019) stated that more than 250 volatile compounds have been identified in bananas. According to Gonçalves et al. (2018) major volatile compounds that contribute to banana aroma are volatile esters. Aurore et al. (2011) found that Cavendish was the variety richest in esters, which probably explains its more aromatic note.

Predominant sugars in bananas namely sucrose, glucose, and fructose could also serve as a substrate for bacterial fermentation (Srisuvor et al., 2013). In addition, bananas also contain inulin and FOS known as prebiotics (Fidina et al., 2018). Wills et al. (1984) reported that dietary fiber present in ripe Cavendish banana was about 2.5 g/100 g. Therefore, the addition of Cavendish banana is expected to have a positive effect on LAB and improve the flavor of rice bran yogurt.

This study aimed to ascertain the microbiological, physicochemical, and sensory properties of rice bran yogurt (RBY) integrated with Cavendish banana puree (CBP) at various concentrations.

Materials and Method

Materials

Cavendish bananas (*Musa acuminata*, AAA group, Cavendish subgroup), full cream UHT milk, rice bran powder, skim milk powder, granulated sugar, and gelatine were purchased from local supermarkets in Surabaya. Commercial freeze-dried yogurt starter cultures was used (Yogourmet, Lyo-San Inc., Lachute, Québec, Canada) containing *S. thermophilus*, *L. bulgaricus*, and *L. acidophilus*.

Instrumentation

Colony counter (ColonyStar 8502-2835, Funke Gerber, Germany), thermostatic water bath (XMTD-204, Nuohai, China), laminar air flow cabinet (AV-100, Telstar, Spain), incubator (BD 115, WTC Binder, Germany), and pH meter (SI Analytics Lab 855, Xylem Brand, Germany).

Preparation of Cavendish Banana Puree (CBP)

Ripe Cavendish bananas were washed, then blanched using steam at 90 ± 5˚C for 5 minutes, and subsequently cooled in ice water at 5˚C for 10 minutes. After that, Cavendish bananas were peeled and the seeds were removed, then the pulp was pureed. CBP was then stored in a freezer at -18˚C and will be thawed before usage.

Production of Rice Bran Yogurt (RBY)

The production of RBY was performed in four (4) replications. Each replication was prepared in the same ways, 2% (w/v) skim milk powder and 5% (w/v) granulated sugar were added to the full cream UHT milk, which was stirred and heated to 90 ± 2˚C for 5 minutes. When the milk temperature cooled down to 80˚C, 1% (w/v) rice bran powder and 0.5% (w/v) gelatine were added. The milk mixture was then divided into six (6) containers according to the formulation and when the temperature reached 60˚C, each mixture was incorporated with 0%, 5%, 10%, 15%, 20%, and 25% (w/v) CBP. The mixes were inoculated with 0.5% (w/v) starter when the temperature reached 43˚C. Subsequently, the inoculated samples were poured into sterile plastic cups and fermented at 43 ± 2˚C for 5 hours. RBY was then stored in a refrigerator at 4 ± 1˚C. All samples were subjected to microbiological, physicochemical, and sensory analyses after 19 hours of storage and it is assumed as day 0. Analysis for syneresis was also performed on day 7.

Microbiological Analysis

The LAB counts were performed by using the pour plate technique. One gram of each RBY sample was weighed aseptically and diluted with 99 mL of 0.1% sterile peptone water (dilution of 10^{-2}). Furthermore, 0.5 mL from 10^{-2} was pipetted into 4.5 mL of 0.1% sterile peptone water to obtain 10^{-3} and it was repeated until 10^{-9}. 1 mL of the sample from the 10^{-5}-10^{-9} dilutions was put into a sterile petri dish (duplo), then MRS agar (cooled to 50˚C) was added, and subsequently homogenized. The plates were then incubated at 37˚C for 48 hours.

Physicochemical Analysis

pH value of the yogurt samples was measured by using a pH meter. The electrode is immersed into 20 mL sample until stable readings were obtained (Fidina et al., 2018). Titratable acidity (as % lactic acid) was determined via acid-base titration method. Ten grams of yogurt samples were diluted to 100 mL with distilled water. 10 mL of the diluted sample was titrated with 0.1 N NaOH and phenolphthalein indicator until a stable pink solution was formed. The titratable acidity was calculated using Eq. 1. (Widagdha and Nisa, 2015).

$$Titratable\ Acidity\ (\%) = \frac{V_{NaOH} \times N_{NaOH} \times Eqv.Wt._{Lactic\ Acid} \times Dilution\ Factor}{W_{Sample} \times 1000} \times 100\% \qquad (1)$$

Syneresis measurement was carried out using the siphon method. Spontaneous whey separated from the undisturbed yogurt samples was taken by using a pipette. The syneresis was expressed as the percent weight of whey over the initial weight of the yogurt samples as seen in Eq. 2. (Saleh et al., 2020).

$$Syneresis\ (\%) = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100\% \qquad (2)$$

pH and titratable acidity analyses were performed in triplicate, while syneresis analysis was performed in duplicate.

Sensory Evaluation

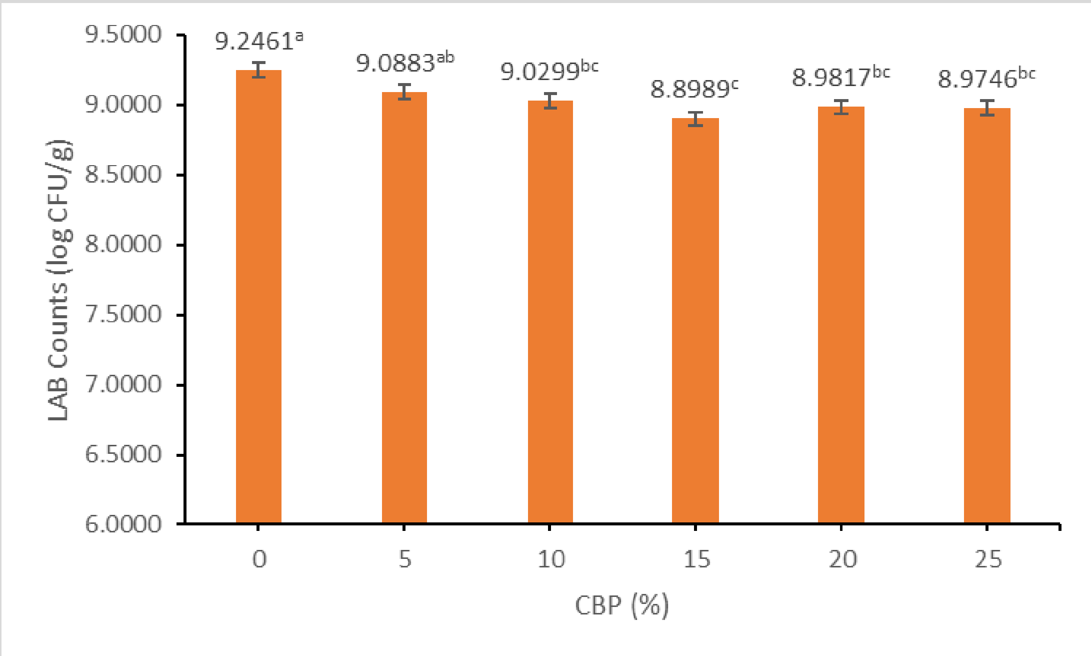
The evaluation of appearance, taste, and mouthfeel for all the yogurt samples was performed by 40 untrained panelists using the Hedonic Scale Scoring method. Yogurt samples (about 25 ml of each treatment) were provided in a plastic cup and encoded with three (3) different numbers. Panelists were then asked to fill out the questionnaire using a seven-point hedonic scale, i.e. 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like very much.

Statistical Analysis

The data were analyzed statistically using IBM SPSS Statistics 19. Data from four replications were analyzed by Analysis of Variance (ANOVA) at 5%, in a randomized block design (RBD). When ANOVA revealed a significant effect ($P < 0.05$), means were compared by Duncan's Multiple Range Test (DMRT) at 5% significance level. P-value less than 0.05 ($P < 0.05$) was accepted as statistically significant.

Results and Discussion

Microbiological Properties



Means with different superscripts were significantly different in ANOVA and subsequently grouped by Duncan's test ($P < 0.05$).

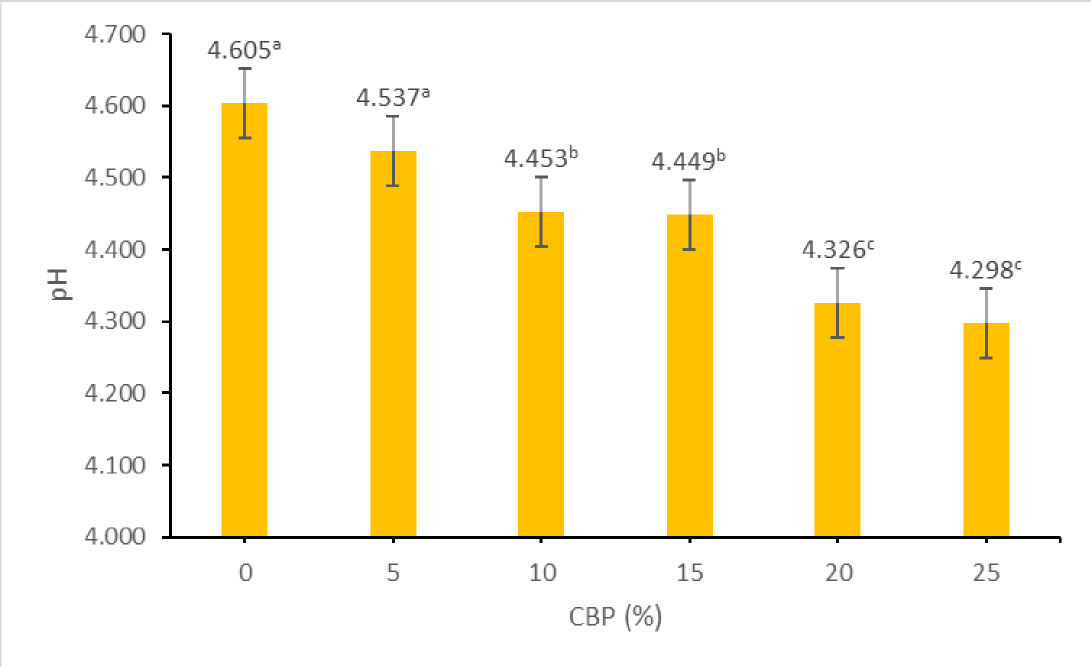
Figure 1. The average of LAB counts on day 0.

Figure 1. demonstrated that there were significant ($P < 0.05$) differences in terms of LAB counts between the RBY with and without CBP. When CBP concentrations increase, LAB counts tend to decrease. The highest LAB counts (9.2461 log CFU/g) showed at RBY without CBP. This occurrence may be caused by the decrease in milk mixture amount as the CBP concentrations increase. According to Sobowale et al. (2011), *S. thermophilus* and some strains of *L. bulgaricus* utilize lactose as the main carbon source compared to other simple sugars. On the other hand, milk protein also serves as a nitrogen source for LAB to synthesize cell walls and nucleic acids (Özer, 2015; Niyonzima et al., 2020). Therefore, the reduction of milk mixture causes the availability of lactose and protein to also decrease so that the activity and growth of LAB are inhibited.

Wills et al. (1984) pointed out that starch content in ripe Cavendish was about 1.9 g/100 g. During the puree-making process, Cavendish bananas were blanched with steam, cooled in ice water, crushed, frozen, then thawed before being used. These processes might cause the starch to retrograde and form type 3 resistant starch (RS3). Yogurt containing RS3 was characterized by a very compact structure and lack of pores while observed using SEM (scanning electron microscope) (He et al., 2019). Therefore, yogurt became more viscous and it is suspected to inhibit the diffusion of nutrients into LAB cells. He et al. (2019) stated that yogurt with RS3 might change the water activity and affect bacterial metabolism as RS3 could not act as a carbon source for bacteria.

Figure 1. showed a slight increase in LAB counts at 20% CBP and 25% CBP. This is likely due to simple sugars derived from CBP. Glucose, fructose, and sucrose content in ripe Cavendish were about 3.6 g, 2.4 g, and 11.2 g respectively per 100 g (Wills et al., 1984). These simple sugars can also act as a carbon source for LAB metabolism, especially by *L. acidophilus*. The results conducted by Srinivas et al. (1990) showed the order of sugar utilization by *L. acidophilus*, namely glucose ≥ fructose > sucrose ≥ lactose > galactose.

Physicochemical Properties



Means with different superscripts were significantly different in ANOVA and subsequently grouped by Duncan's test (P < 0.05).
Figure 2. The average of pH on day 0.

The pH of RBY decreases significantly (P < 0.05) as the increment concentrations of CBP integration (Figure 2.). This is mainly due to the accumulation of lactic acid as described by Corrieu and Béal (2016) that glucose derived from lactose will be utilized by *S. thermophilus*, *L. bugaricus*, and *L. acidophilus* through the Embden–Meyerhof–Parnas (EMP) pathway producing lactic acid. The glucose, fructose, and sucrose derived from CBP were also suitable substrates for LAB metabolism. According to Srinivas et al. (1990), *L. acidophilus* is able to utilize glucose, fructose, and sucrose faster than lactose. Sobowale et al. (2011) also found that *S. thermophilus* and *L. bugaricus* were also able to utilize sucrose and fructose well, although not as fast as lactose and glucose.

The decrease of pH in RBY could also be influenced by organic acids naturally found in bananas. Malic acid and citric acid are the two main organic acids found in bananas (Bhuiyan, 2020). According to Wills et al. (1984), malic acid and citric acid content in ripe Cavendish were about 0.25 g/100 g and 0.14 g/100 g respectively. Malic acid and citric acid are polyprotic acids that can release more than one H^+ when dissociated, while lactic acid is a monoprotic acid that can only release one H^+ when dissociated. As the concentrations of CBP increase, there were also more H^+ that could be dissociated from these acids, so the pH measurement will show a lower value.

There were no significant (P > 0.05) differences in titratable acidity of RBY integrated with various CBP concentrations (Figure 3.). However, the titratable acidity tends to decrease as the CBP concentrations increase. This occurrence is probably influenced by the viscosity level of RBY as explained by He et al. (2019). A large amount of RS3 can cause changes in the water activity and it will affect the diffusion of nutrients into LAB cells.

The titratable acidity values in RBY have a similar trend with LAB counts (Figure 1.). It showed a close relationship between LAB counts and its metabolic products. The decrease in LAB counts will cause the production of lactic acid to decrease and vice versa. The increase in titratable acidity at 20% CBP and 25% CBP could be due to the glucose, fructose, and sucrose content derived from CBP that is also utilized by LAB for metabolism.

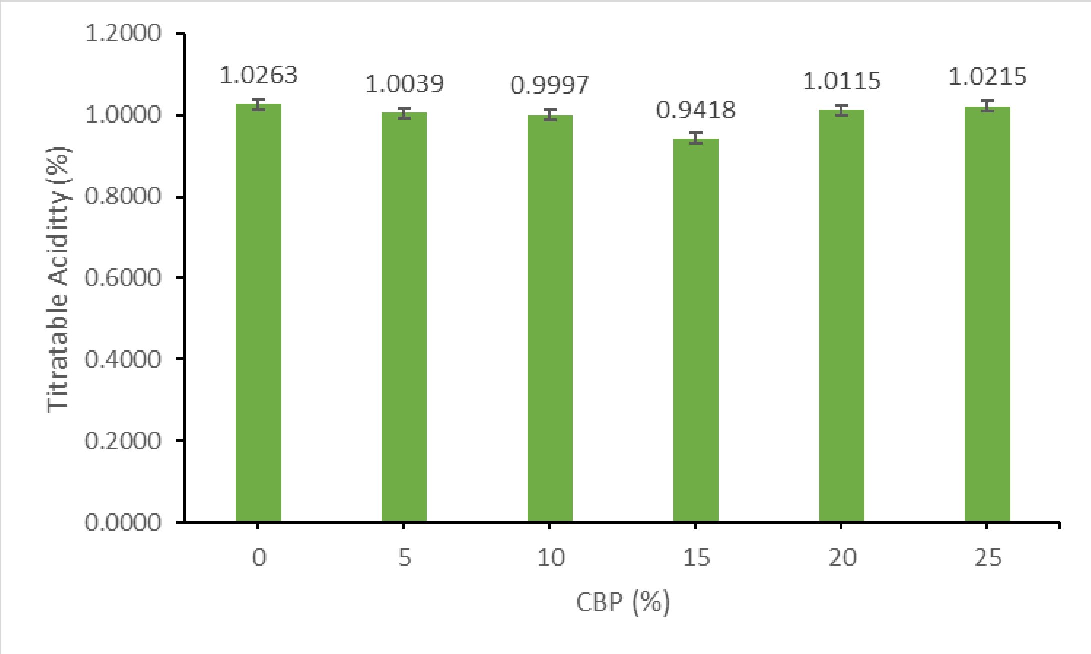


Figure 3. The average of titratable acidity on day 0.

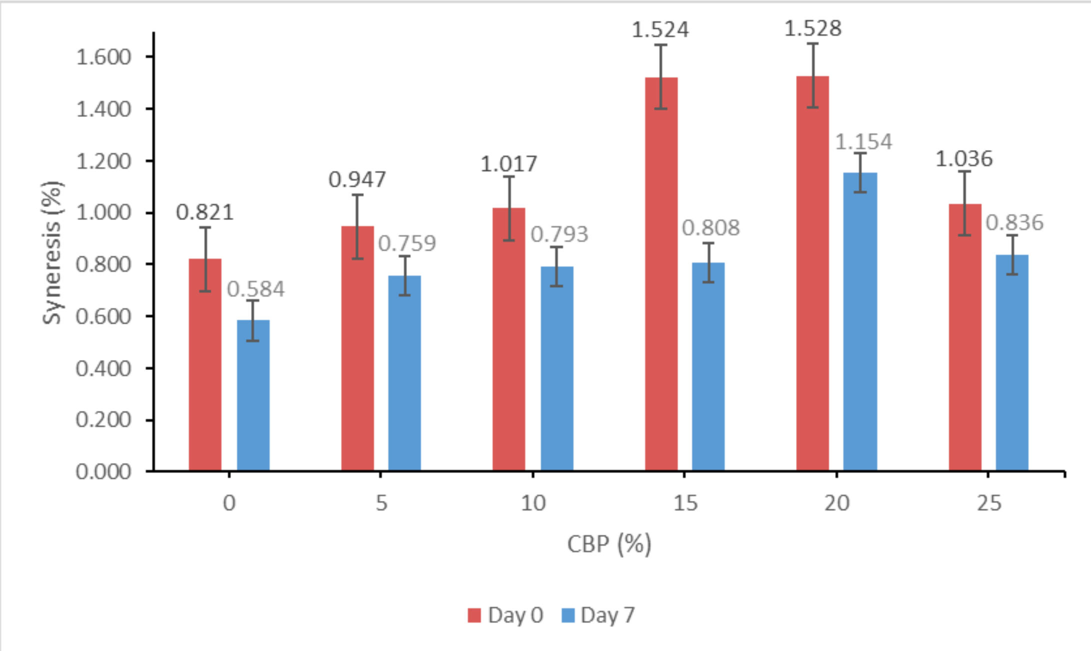


Figure 4. The average of syneresis on day 0 and day 7.

Syneresis is one of the main drawbacks that will affect the attractiveness of yogurt. Figure 4. showed that the syneresis of RBY (with and without CBP) was not significantly (P > 0.05) different both on day 0 and day 7. However, there is an upward trend of syneresis along with the increase in CBP concentrations. This may be occurred due to the transfer of water from CBP into the yogurt system. Research by Yap et al. (2017) found that the water content of banana puree from ripe Cavendish was 84.0 g/100 g. Hence, it is presumed that the higher concentrations of CBP would make more water move into the yogurt system. The occurrence of water transfer will cause will cause the gel matrix of the yogurt to be broken and cannot hold the extra water, thus causing syneresis to increase (Vareltzis et al., 2016).

There was a decrease in syneresis both on day 0 and day 7 at 25% CBP. It can be due to the accumulation of fiber, starch, and sugar from CBP integration. Bakirci and Kavaz (2008) stated that fiber from bananas has high water holding capacity (WHC), resulting in yogurt with a firmer texture.

Sensory Properties

Table 1. Mean scores of appearance, taste, and mouthfeel.

Treatment	Sensory Characteristic		
	Appearance	Taste	Mouthfeel
CBP 0%	5.80 ± 1.36[a]	4.80 ± 1.87	6.05 ± 1.45[a]
CBP 5%	5.75 ± 1.24[a]	5.20 ± 1.62	5.58 ± 1.20[ab]
CBP 10%	5.08 ± 1.19[b]	5.15 ± 1.55	5.20 ± 1.26[b]
CBP 15%	4.25 ± 1.37[c]	5.25 ± 1.28	4.40 ± 1.57[c]
CBP 20%	3.98 ± 1.51[cd]	4.65 ± 1.63	3.55 ± 1.48[d]
CBP 25%	3.50 ± 1.47[d]	4.83 ± 1.82	3.33 ± 1.65[d]

Means with different superscripts within a column were significantly different in ANOVA and subsequently grouped by Duncan's test ($P < 0.05$).

The mean scores for the sensory evaluation of RBY were presented in Table 1. CBP incorporation significantly ($P < 0.05$) affected the appearance and mouthfeel preference of RBY, meanwhile the taste was not significantly ($P > 0.05$) affected by CBP incorporation. The preference score of RBY appearance declined as the CBP concentrations increased. It happens because RBY has more sediment as the CBP incorporation levels increase. In terms of color, the sediment has brown color and it is thought to be mainly from CBP which has undergone an enzymatic browning process during the puree-making process. Yogurt generally has a light color, so the dark-colored yogurt tends to be disliked by the panelists. Based on the panelists' score for the appearance, it was found that yogurt with a lot of sediment and dark color was disliked by the panelists, but RBY with the addition of 5% CBP was still acceptable.

The taste score of RBY incorporated with CBP was higher than the control (RBY without CBP) up to 15% level of incorporation. It could be due to the high sugar content and volatile compounds derived from ripe bananas. Aurore et al. (2011) claimed that free volatile compounds identified in bananas were complex mixtures of several classes of components: carbonyls, esters, alcohols, and acids. Sucrose is the predominant sugar that mainly contributes to the Cavendish banana sweet taste. According to Yap et al. (2017), the sucrose content of banana puree from ripe Cavendish was 4.13 g/100 g. Therefore, it also provides a role in forming the final taste of RBY. However, there was a decrease in taste scores at 20% CBP and 25% CBP due to the sour taste from the accumulation of organic acids naturally derived from bananas. It is also presumed that sucrose, glucose, and fructose from bananas were being utilized by LAB producing lactic acid. RBY with 15% CBP was the most preferred by the panelists because it has a balanced taste between sour and sweet.

The preference score of mouthfeels declined as the CBP concentrations increased. The origin of this phenomenon is referring to the presence of sediment which makes the RBY does not have a smooth texture. It can also be due to the presence of fiber derived from CBP and rice bran. These findings are in agreement with Hasani et al. (2016) who stated that all-fiber fortified products will have a gritty texture. Insoluble fiber may also play a major role in influencing the mouthfeel of RBY. Bananas contain insoluble fibers such as cellulose, lignin, and hemicellulose, at 0.28%, 0.03%, and 0.96% respectively (Kayisu et al., 1981). Based on the mouthfeel preference score, it is known that the panelists do not like yogurt with a coarse texture. However, RBY with 5% CBP was still acceptable to the panelists and was not significantly different from the control.

Conclusion

The integration of CBP to partially replace the milk mixtures resulted in RBY with lower LAB counts, pH, titratable acidity, and higher syneresis. Since CBP integration resulted in sediment, therefore the appearance and mouthfeel acceptances were lower than the control. However, taste preference was higher than the control, up to 15% level of integration. If we consider the microbiological, physicochemical, and sensory properties of RBY, CBP can be added to yogurts at level of 5%. This study demonstrated that RS3 formed during the puree-making process displays prebiotic potential and warranted further research. Future study is also needed to investigate total solids, viscosity, and water holding capacity since Cavendish bananas contain starch and pectin which can bind and trap water. LAB's viability during storage can also be investigated to determine the shelf life of yogurts.

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Conflict of Interest

The authors declare no conflict of interest.

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