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# The effect of different concentration of banana ambon puree on the physicochemical, microbiological, and organoleptic properties of rice bran yogurt

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**Abstract.** The addition of Ambon Banana Puree (ABP) has potential to improve the functional and organoleptic properties of rice bran yogurt. Hence, this study was aim to determine the effect of different concentrations of ABP on physicochemical, microbiological, and organoleptic properties of rice bran yogurt. The latter was prepared with ABP at concentrations of 0%, 5%, 10%, 15%, 20%, and 25% (w/v) and each reproduced 4 (four) times. Different concentrations of ABP showed significant effect ($P < 0.05$) on syneresis day 7, pH value, total LAB, and organoleptic of mouthfeel and appearance. Results highlighted that different ABP concentrations had no significant effect ($P > 0.05$) on syneresis day 0, total lactic acid of rice bran yogurt, and organoleptic property of taste. Based on these findings, rice bran yogurt with different concentrations of ABP had syneresis on day 0 was 0.089-0.500%, syneresis day 7 was 0.130-0.522 %, pH 4.349-4.593, and total lactic acid 0.9733%-1.0513%. Furthermore, organoleptic testing presenting the appearance preferences ranged from 2.15 to 6.07 (dislike-like), taste 4.10-4.78 (neutral-sufficient), and mouthfeel 3.65- 5.53 (imperfect to like). The result of total LAB was 8.7335-9.1543 log cfu/g. ABP has a favourable effect on the physicochemical, microbiological, and organoleptic properties of rice bran yogurt.

**Keywords**: yogurt, rice bran, banana ambon

## 1. Introduction

The consumption of yogurt in Indonesia continuous increasing from 2012 to 2016. This is evidenced through the enhancement of yogurt imported products at 6.12% annually [15]. The increasing of yogurt consumption indicates that this product is feasible to be developed as a functional food trend. Yogurt is a product from milk coagulation due to the activity of

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microorganism, that made with or without the addition of skim milk, caseinate, and whey concentrate [34]. Yogurt is made from sinbiotic activity of LAB (Lactic Acid Bacteria), such as *Streptococcus thermophillus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium spp*., *Lactobacillus reuteri*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, dan *Lactobacillus johnsonii* [22]. These bacteria cause enzymatic reaction during yogurt fermentation [3]. The minimum number of probiotic bacteria in food product is $10^7$ CFU/g [16]. According to [14], the benefits of probiotic are improving symptoms of lactose intolerant, improving health, increasing the body's immune response, and reducing the risk of colon cancer. Based on [35], colon cancer causes 1.8 million cases of death annually. Colon cancer is caused by low consumption of fibrous foods. One of the foods that are high in fiber is rice bran.

Rice bran is the outer layer of rice that is wasted during milling process. Rice bran contains dietary fiber, such as cellulose, hemicellulose, pectin, arabinosilan, lignin, and β-glucan [18]. In previous research [29] and [32] research, adding rice bran to yogurt can increase total LAB, increase nutritional value, decrease syneresis value, and increase WHC (Water Holding Capacity) in yogurt. However, increased concentration of rice bran causes off flavor in yogurt [29]. Rice bran contains the dominant phenolic compound in the form of 4-vinylphenol which causes unpleasant aroma after cooking [11]. The unpleasant smell of rice bran is also caused by lipid oxidation after the milling process [23]. The addition of 1% rice bran produced total LAB value which was not significantly different ($P > 0.05$) with yogurt without the addition of bran [32]. The functional properties of yogurt rice bran can be enhanced by the addition of banana puree. Based on the research [19], adding kepok banana puree can increase physicochemical and microbiological properties in accordance with yogurt standards. One type of banana that can be used is ambon banana.

Ambon banana (*Musa paradisiaca L*) is a type of banana that is widely consumed in Indonesia. Ambon banana in ripening state contain of 6.49 g of fructose, 5.68 g of glucose, and 17.65 g of sucrose per 100 g [28]. Based on [24], LAB will grow quickly in rich media. Monosaccharide will be more easier to use in LAB metabolic process, so it is more faster to produce lactic acid and other metabolite.

Therefore, the aim of this study was to investigate the effect of different concentrations of ABP on physicochemical, microbiological, and organoleptic properties of rice bran yogurt.

## 2. Materials and Methods

### *2.1. Materials*

UHT milk "Ultramilk", skim milk powder, sugar, rice bran powder "dr. Liem", commercial *freeze-dried yogurt starter* "Yogourmet" that consists of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, gelatin powder, and ambon banana (*Musa paradisiacal L*) at early stage of ripening.

### *2.2. Preparation of ABP*

Ambon banana at early stage of ripening were washed with clean water and drained. Ambon banana were blanched using steam blanching method (90°C±5°C for 5 minutes) and soaked in cold water (5°C±5°C for 10 minutes). After that, the peel and seeds were removed. The ambon banana were then blended in 15-30 seconds, kept in plastic, and stored in freezing temperature -18°C±2°C.

### *2.3. Preparation of rice bran yogurt with different concentration of ABP*

UHT milk was mixed with 5% sugar and 2% skim milk powder then pasteurized in 90°C±2, hold temperature for 5 minutes. During pasteurization, stir the milk continuously to avoid cream layer formation. 1% rice bran and 0.5% gelatin powder were added at temperature 80°C±5°C, stirred and allowed it to cool to 60°C±5°C. The mixture was then divided in six parts as follows: 0%, 5%, 10%, 15%, 20%, and 25% ABP. Freeze dried yogurt starter (0.5%) were inoculated and incubated in 43°C±2°C for 5 hours then stored in 4°C±1°C for 19±1 hours.

### *2.4. Determination of syneresis*

Determination of syneresis based on previous research [7], with modification. Syneresis of yogurt stored at low temperature [4°C±1°C for 19±1 hours] and was determined at day-0 and day-7 with two repetition. Syneresis was indicated by separated whey on yogurt surface. To derive the syneresis, take the whey on yogurt with paper which can then be calculated using (final weight – cup weight)/sample weight x 100%.

### *2.5. Determination of pH and titratable acidity*

The pH of yogurt was measured with "SI Analytics Lab-855" instrument. Titratable acidity of yogurt determined based on previous research [30], approximately 10 g sample diluted with aquades in volumetrick flask, then 10 mL filtrate and *phenolphthalein* indicator titrated with NaOH 0.1N. Total lactic acid was calculated using this following equation:

$$\text{Total lactic acid} = \frac{Volume\ NaOH\ (ml) x\ N\ x\ BM\ lactic\ acid}{Volume\ sampel\ (ml) x\ 1000}\ x\ 100\%$$

### *2.6. Determination of total lactic acid bacteria with total plate count method*

Total plate count method refers to previous study [7]. Total plate count analysis was determined after 5 hours fermentation and stored in refrigerator at 4°C±1°C for 19±1 hours. Colony counts were converted to log CFU/g.

### *2.7. Assessment of sensory evaluation of rice bran yogurt with different concentration of ABP*

The preference test of the panelists using the Hedonic Scale Scoring method for appearance, taste and mouthfeel. Appearance assessed namely color, viscosity (curd formation) and the presence of sedimentation (deposition). The scale used is a sensory scale with value conversion as follows 1 = very dislike; 2 = dislike; 3 = imperfect; 4 = neutral; 5 = sufficient; 6 = like; 7 = really like. The organoleptic test performed by 40 untrained panelists. The test will be carried out on a RAK basis as much as 25 mL of sample per concentration. Each of the panelists will tested each sample and asked to fill in a questionnaire that has been provided.

### *2.8. Statistical Analysis*

The data were analyzed by "IBM SPSS Statistics 19". Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) with $\alpha = 5\%$ were used to differentiate between the mean values.

# 3. Results and Discussion

## *3.1. Physico-chemical Properties*

Syneresis is the spontaneous release of the liquid phase (whey) from the gel phase (curd) due to contraction of the gel phase [10]. Based on ANOVA at α = 5%, different concentrations of ABP had no significant effect on syneresis of day-0, but had a significant effect on day-7 in rice bran yogurt. As demonstrated in **Fig. 1.** the syneresis on day-0 and day-7 tended to decrease from 0% to 10% concentration and increased at 15% to 25% concentration.

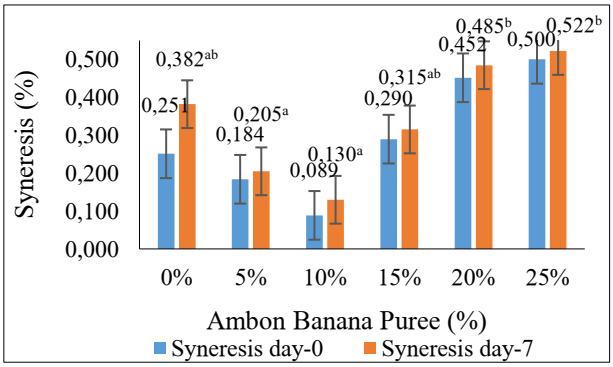


**Fig. 1.** The syneresis value of rice bran yogurt at different concentrations of ABP. Mean values at different superscripts capital letter (a-c) which are significantly different (P<0.05) based on DMRT.

The decrease in syneresis from 0% to 10% concentration was due to the fiber and starch components of bananas. The fiber content in ABP is 8.82 ± 0.25 g per 100 g [1]. The starch content in ABP is 66.90±4.88 g per 100 g [1], where the proportions of amylose were 22.89±0.37% and amylopectin were 77.11±0.59% [4]. These results were consistent with research conducted by [10], regarding the addition of banana puree to yogurt where the higher the addition of ABP, the resulting decreased syneresis.

The increase in syneresis from a concentration of 15% to 25% is due to a decrease in pH [2]. A decrease in pH that exceeds the pI of casein causes the casein compound to become unstable and causes casein micelles tend to repel so that the trapped water will be released and syneresis occurs. According to research [8], at a lower pH there is an excessive increase in the bond between caseins. This increase in casein binding causes shrinkage or contraction of the protein gel which stimulates the formation of curd along with the separation of whey [8].

On the day-7 syneresis has a higher value than day 0 syneresis. According to research [33], syneresis increases because there is a decrease in pH during storage. According to research [6] experiment on banana yogurt, higher syneresis was produced on longer storage, because the longer the gel formed the high tendency to release water.

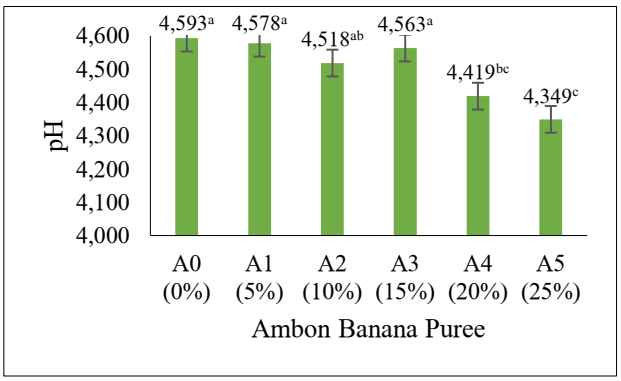


**Fig. 2.** The pH value of rice bran yogurt at different concentrations of ABP. Mean values at different superscripts capital letter (a-c) which are significantly different (P<0.05) based on DMRT.

Based on statistical analysis, different concentrations of ABP had significant effect on pH in rice bran yogurt. Rice bran yogurt with 0%, 5%, 10%, and 15% ABP had no significant difference (P>0.05) due to buffering agent effect. Buffering agent neutralize lactic acid from LAB fermentation [26]. The capacity of the buffering agent depends on the distribution of minerals and proteins between the liquid and solid phases of the dairy product [9].
The different concentrations of ABP affect the mineral distribution in rice bran yogurt. Based on [25], ambon banana contains 20 mg of calcium, 30 mg of phosphorus, 0.2 mg of iron, 10 mg of sodium, 0.20 mg of copper, and 0.2 mg of zinc. **Fig. 2.** shows that increased concentration of ABP caused significant decrease (P<0.05) of pH in rice bran yogurt. It is because the measured pH value is $H^+$ ions derived from lactic acid produced by LAB during fermentation and organic acids from ABP.

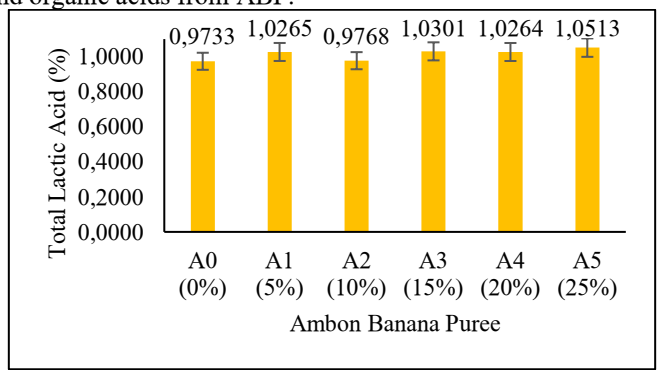


**Fig. 3.** The total lactic acid value of rice bran yogurt at different concentrations of ABP. Mean values at different superscripts capital letter (a-c) which are significantly different (P<0.05) based on DMRT

The results of total lactic acid are shown in **Fig. 3.** The different concentrations of ABP had no significant effect (P>0.05) in rice bran yogurt. RS 3 in ABP inhibits the nutrients diffusion of LAB metabolism.

### *3.2. Total Lactic Acid Bacteria*

Based on ANOVA at α=5%, different concentrations of ABP had a significant effect on total LAB in rice bran yogurt. The results of the DMRT showed that total LAB in 0% ABP rice bran yogurt was not significantly different from 5%. However, 0% ABP rice bran yogurt showed significant difference from 10%, 15%, 20%, and 25%. LAB cells have adjusted to their environmental conditions. According to previous research [21], LAB growth is

influenced by the medium in which it lives, such as pH, nutrients content, and temperature. The nutritional content in the media will affect the growth and survival of LAB [12].

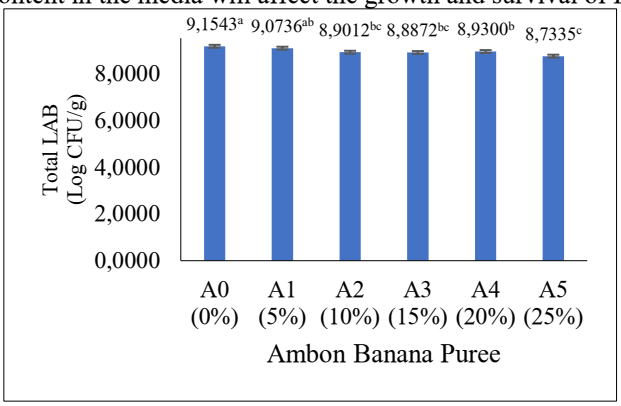


**Fig. 4.** The total LAB of rice bran yogurt at different concentrations of ABP. Mean values of different superscripts capital letter (a-c) which are significantly different (P<0.05) based on DMRT.

**Fig. 4.** demonstrates an increase of ABP concentration that causes a decrease in the total value of LAB. The decrease in total LAB may be attributed by several reason. The increase concentration of ABP will decrease the availability of UHT milk in yogurt rice bran. *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* are starter cultures that are classified as dairy lactic acid bacteria [12]. LABs such as *L. bulgaricus* and *S. thermophilus* will use a secondary transfer called LacS to transfer lactose into cells. Furthermore, the lactose that accumulates in the cells will be hydrolyzed by cytoplasmic β-galactosidase into glucose and galactose. Glucose will be phosphorylated to glucose-6-phosphate and enter Embden Mayerhorf Parnas system [24].

The other factor of decreasing total LAB is starch content in banana ambon puree. Banana ambon in ripening phase contains of 66.90 g total starch consisting of 45.87 g resistant starch and 21.03 g non-resistant starch [1]. According to research [31], there are 1-20% conversion of starch into sugar during banana ripening process.

Furthermore, steam blanching and freezing at -18°C in ABP process causes the formation of RS (resistant starch) type 3. RS type 3 in banana that have undergone steaming, cooling, and freezing is higher than unripe banana phase [36]. The research of [13] reported that the addition of RS type 3 produced yogurt with continuous and compact network structure. It causes by the formation of casein micellar aggregates with RS type 3.

## 3.3. Sensory Evaluation

### *3.3.1. Appearance*

Based on ANOVA at α = 5%, different concentrations of ABP had a significant effect on appearance preference in rice bran yogurt. **Fig. 5** highlighted the preference for appearance which was decreased at higher concentrations. Appearances assessed in this experiment include color and the presence of solids. The higher the concentration of ABP, the duller the color will be.

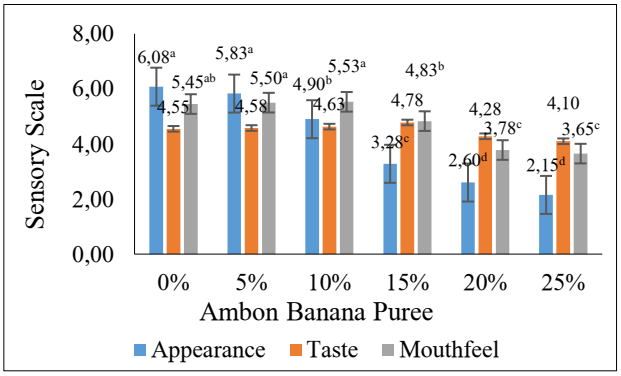


**Fig. 5.** The organoleptic value of rice bran yogurt at different concentrations of ABP. Mean values of different superscripts capital letter (a-c) which are significantly different (P<0.05) based on Duncan Multiple Range test.

This brown color is due to non-enzymatic browning which attributed to the presence of the PPO (polyphenol oxidase) enzyme in bananas. The presence of a dull color can also be caused by the oxidation of rice bran. The rice bran used in this experiment is full-fat rice bran that has not undergone fat removal, so the presence of unsaturated fatty acids in the rice bran facilitates oxidation and gives a dull color.

Furthermore, at higher concentrations, there were more solids produced. The presence of solids resulted as bananas were not fully gelatinized. The gelatinization temperature of Ambon banana is 76±0.06ºC [20]. In the experiment, ABP was added at a temperature of 60 ºC, this caused the starch from the bananas to not fully gelatinize and give an unfavorable appearance.

The ratio between the amorphous part and the crystalline part also affects the gelatinization. The higher the amorphous part, the easier the starch will undergo the gelatinization process because the amorphous part can absorb more water so that the starch granules will swell and form a gel. Ambon banana starch has a larger crystalline component, so the amylopectin component is more and it is more difficult to form a gel. According to research [4], the proportion of amylose was 22.89±0.37% and amylopectin 77.11±0.59% in Ambon banana.

### *3.3.2. Taste preference*

Based on ANOVA at α = 5%, different concentrations of ABP had no significant effect on taste preference in rice bran yogurt. **Fig. 5** depicted that there was an increase from a concentration of 0% to 15% and a decrease from a concentration of 20% to 25% in organoleptic testing of taste.

In organoleptic testing of taste, preference increased at higher concentrations of ABP and the resulting concentration of 15% was the most preferred. This is because at this concentration the taste is not too sour and the unpleasant aroma and taste of the rice bran are masked by the aroma and taste of the ABP. At higher concentrations (20% and 25%) the preference value decreases, this indicates that the panelists tend to dislike yogurt with a sour taste that is too sharp. The decrease in taste value can be caused by the proteolytic activity of bacteria and the production of higher acidity [2]. Meanwhile, at low concentrations (0%, 5%, 10%), the panelists were still able to detect the unpleasant aroma and taste of rice bran so that it was less desirable, but not statistically significant. In the previous research [5], regarding rice bran substitution in the manufacture of yogurt explains that higher rice bran substitution results in lower taste acceptance, this is because the aroma and taste of rice bran are usually

less favored by consumers. The unfavorable aroma and taste of rice bran is caused by the presence of free fatty acids which are oxidized by the lipoxygenase enzyme to form peroxides, ketones, and aldehydes that cause a rancid odor [17].

### *3.3.3. Mouthfeel*

Mouthfeel is the rheological property of food during the first step in the mouth. Based on ANOVA at $\alpha = 5\%$, different concentrations of ABP had a significant effect on mouthfeel preference in rice bran yogurt. As demonstrated in **Fig. 5,** there is an increase from a concentration of 0% to 10% and a decrease from a concentration of 15% to 25% in organoleptic testing of mouthfeel. Concentration of 10% produces the highest preference value. At higher concentrations there are more solids in yogurt. The solids in yogurt come from ABP and rice bran. ABP has 3.53±0.00 g per 100 g dry weight of soluble dietary fiber and 2.94±0.03 g per 100 g dry weight of insoluble dietary fiber [28]. The experiments of [27] produced a total dietary fiber of rice bran accounted to 23.34 g per 100 g consisting of 21.17 g per 10 g of insoluble dietary fiber and 2.17 g per 100 g of soluble dietary fiber.

## 4. Conclusion

The different concentration of ABP gave a significant effect on the syneresis on day-7, pH, total lactic acid bacteria, appearance and mouthfeel preferences of rice bran yogurt. On the other hand, different concentration of ABP had no significant effect on total lactic acid and the syneresis on day-0 of rice bran yogurt.

Total lactic acid bacteria had increased by the addition of ABP up to 10% based on statistical analysis. Overall, total lactic acid bacteria ranged from 8,7335 - 9,1543 log CFU/g. Furthermore, all of yogurt samples had pH ranged from 4.349 to 4.593 and total lactic acid ranged from 0.9733% to 1.0513%. Based on the sensory evaluation, the average of consumer preferences ranged from 2.15 to 6.08 (dislike to like), taste preference ranged from 4.10 to 4.78 (neutral to sufficient), and mouthfeel preferences ranged from between 3.65 to 5.53 (imperfect to like).

The addition of 5% ABP was the recommended concentration for rice bran yogurt. As it has total lactic acid bacteria and pH with no significant different when compared to 0% ABP. For future study, we suggest to investigate the viability of lactic acid bacteria based on the high carbohydrate value of ABP.

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