

Effect Sourdough from Snake Fruit (*Salacca zalacca*) on Microbiota and Texture of Rice Flour Bread

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ABSTRACT

The use of fruit in the manufacture of sourdough provides nutrients and substrates that can improve the microbiota in sourdough and affect the characteristics of sourdough bread. The study aimed to determine the differences in the characteristics of the snake fruit sourdough microbiota on the effect of the texture of rice flour white bread. The snake fruit is peeled and cut to be fermented for several days, then the water from the snake fruit fermentation is mixed with flour to be fermented into the sourdough. Furthermore, it is applied to make plain bread with rice flour as a leavening agent. The results showed that the snake fruit starter had a LAB of 8.77 Log CFU/g and yeast 7.72 Log CFU/g with a pH of 3.62. Snake fruit sourdough (SDS) has a shorter fermentation time of 3 days to reach ripe sourdough and a pH of 3.25. The amount of Lactic acid bacteria (LAB) and yeast snake fruit sourdough (SDS) was higher than control sourdough (SDF) as follows BAL SDS 9.19 log cfu/g and Yeast SDS 9.42 log cfu/g while LAB SDF 9.03 Log CFU/g and Yeast SDF 9.07 Log CFU/g. The specific volume of SDS is similar to that of commercial SDF and baker's yeast. SDS has a small pore size of 16.29 mm² so the cells found are higher, namely 6.31 cells/cm². The use of snake fruit sourdough produced bread with higher hardness, gumminess, and chewiness, but the springiness, cohesivity, and resilience were similar to those of control sourdough bread and commercial yeast bread. In this study, the use of SDS was faster in fermentation time on day 3 SDS had reached its optimal level compared to SDF.

Keywords: lactic acid bacteria (LAB); rice flour bread; snake fruit Sourdough (SDS); snake fruit starter; control sourdough (SDF)

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INTRODUCTION

Bread is a bakery product consumed by many people, one of which is white bread products (Lockyer & Spiro, 2020). In the process of making bread, the basic ingredient used is gluten, which builds a strong protein network so that bread with a chewy texture, elastic dough, and a tasty crumb volume is obtained (Diowksz & Sadowska, 2021). However, 1% of the world's population is incompatible with any gluten-based product (Pellegrini & Agostoni, 2015). In addition, consumption

of gluten can also cause Celiac Disease through the interaction of peptide gliadin with the small intestine mucosa, which triggers an innate immune response in the form of the release of IL-8 and IL-15 from epithelial cells and dendritic cells of the lamina propria which induces damage to the intestinal mucosa and causes intestinal inflammation (Kahaly et al., 2018; Serena et al., 2015). Based on this, gluten-free foods can help normalize intestinal function for people with Celiac Disease by reducing cytokine-triggering bacteria that cause intestinal inflammation

(Hansen et al., 2018). The solution to this problem is to make gluten-free flour-based food products, one of which is rice flour.

Rice flour is made from finely ground rice, and the majority of it does not contain gluten, so it is good for celiac disease (Twinomuhwezi et al., 2020). Rice flour, as gluten-free flour in bread making, has a particle size similar to wheat flour, is white in color, has a fresh taste, and has the same ability to absorb water as wheat flour and produces a final product with high digestibility and is hypoallergenic (Park et al., 2014). Research from Diowksz et al., (2020) stated that gluten-free flour has spoilage properties, low quality, an easily damaged appearance, and an unpleasant taste in the mouth. In addition, research by Fratelli et al., (2021) stated that bread made from rice flour produces a lower volume and higher hardness compared to bread made from wheat flour which contains gluten. Therefore, this research uses a leavening agent from natural yeast, sourdough.

Sourdough is a spontaneously fermented mixture of flour and water which has a sharp sour aroma as an ingredient in the development of bread from cereal flour and is classified as a traditional product that has a high nutraceutical factor due to the positive interaction between yeast and LAB and biochemical reactions (Sakandar et al., 2019). During flour fermentation, lactic acid bacteria (LAB) in sourdough produce many metabolites, such as organic acids, enzymes, and exopolysaccharides, which have been shown to positively affect bread quality (Campo et al., 2016). Based on research by Wolter et al., (2014), the Acidification of gluten-free flour can increase the porosity of bread crumbs, reduce dough hardness, reduce starch hydrolysis, increase sensory and be able to reduce the glycemic index. The use of sourdough not only affects the physical characteristics but also affects the aroma of the bread produce.

Sourdough, also called natural yeast, has a conducive habitat for microbes that can support the growth of more than 50 species of lactic acid bacteria (LAB) and more than

20 species of yeast Lau et al., (2021). Variations in bacterial and yeast species in sourdough starters can affect taste, aroma, storage time, and even the nutritional quality of bread (Reese et al., 2020). Based on research by Gordún et al., (2015) showed that the addition of natural ingredients such as apple juice, yogurt, and wine to the initial starters of sourdough can increase microbial diversity and can trigger the emergence of different aromas and different textures on bread because this research uses natural ingredients in the form of fruits to make snake fruit sourdough.

Snake fruit is a tropical fruit from the Arecaceae (Palm-palma) tribe and is an annual plant that is not affected by the season. Snake fruit has a high carbohydrate content, so it can be used as a habitat for sourdough microbes to grow. The microbial community in sourdough will ferment the carbohydrates in the flour and produce carbon dioxide gas which makes the bread dough rise before baking Landis et al., (2021). However, the use of snake fruit in this study has limited sources of information because snake fruit has yet to be extensively studied as a starter for microbiota characteristics in sourdough. So this research can be referred to as renewal research. Thus, the purpose of this study was to analyze the number of snake fruit sourdough microbiota and determine the effect of snake fruit sourdough on the texture characteristics of rice flour white bread.

MATERIALS AND METHODS

Material and equipment

The ingredient used in this study was snake fruit (Pondoh variety) obtained from Turi Village, Sleman Yogyakarta. The snake fruit used was ripe, with a harvesting age of around 6 months. Its characteristics include the tip of the fruit's skin (the pointed part of the fruit) feeling soft when pressed, a shiny appearance, and an easy detachment from the stem when picked. Meanwhile, the analytical materials used were NaOH (Merck, Germany), oxalic acid

(Merck, Germany), phenolphthalein indicators (Merck, Germany), peptone (Oxoid, England), peptone water (Merck, Germany), Yeast extract (Merck, Germany), MRS broth (Merck, Germany), and distilled water (Java Santoso). The equipment used for this research in bread making includes a mixer and for analysis uses the Texture Analysis Machine (TA1 Series), Image j, and Cannon 550D products.

Snake fruit starter making

The snake fruit water fermentation was based on the method Yu *et al.*, (2018). The Snake fruit was cleaned and then cut was weighed as much as 100g, 20g of sugar, and 250g of mineral water placed in a glass jar, and fermented in an incubator at 30°C for duration 0, 12, 36, 48, 60, 72 hours

Sourdough making

The method for preparing natural yeast follows the approach described by Yu *et al.*, (2018). The selected snake fruit starter was filtered, and the liquid obtained (30g) was mixed with 30g of wheat flour and fermented. The control sourdough (SDF) was prepared by mixing wheat flour with water only in 1:1 ratio (30g of wheat to 30 of water). Both sourdough is fermented for 24 hours to activate the starter and build the bubbles. After 24 hours, both sourdoughs were fed by adding 60g of wheat flour and 60g of water. Fermentation was then continued, with observations made every 6 hours.

Rice bread making

The raw materials for making white bread include rice flour (Deli, Indonesia), tapioca flour (Rose Brand), potato flour (point), sugar, salt, margarine, mineral water, Hydroxy Propyl Methyl Cellulose, Psyllium husk, and sourdough according to (Yu *et al.*, 2018). All the ingredients are mixed using mixer for ± 10 minutes, the dough is then put into the tin and fermented at 30 °C for 240 minutes, and the dough is baked at 185-200 °C for 40 minutes.

Determination of pH and titratable acidity (TTA)

Analysis of pH and titrated acid levels in snake fruit starters and sourdough was carried out based on research (Bartkiene *et al.*, 2014). TTA was measured by dissolving 10 g of snake starter or sourdough in 100 mL of distilled water (Yu *et al.*, 2018). Then, 25 mL of the sample solution was put into the Erlenmeyer, and three drops of phenolphthalein were given. NaOH 0.1 N is used in the titration until the sample solution turns pink.

Enumeration of Lactic Acid Bacteria (LAB) and Yeast

Analysis for total LAB used the method (Bartkiene *et al.*, 2014) and for yeast used the method of (Ripari *et al.*, 2016). The samples tested were snake fruit starter, snake fruit sourdough (SDS) and control sourdough (SDF). 10 g sample was homogenized in 90 ml of peptone water with 0.1% (w/v) peptone and 0.8% (w/v) NaCl. 10g sample was homogenized in 90 ml of peptone water with 0.1% (w/v) peptone and 0.8% (w/v) NaCl. The sample was diluted with 8-9 fold dilution for LAB and 4-5 fold dilution for yeast. The total LAB (Lactic Acid Bacteria) was calculated using MRS agar media and yeast using YPD (Yeast Peptone Dextrose) agar media with a pH of 4.3. After incubation at 37°C based on Minervini *et al.*, (2012), the number of colonies (CFU/g) was estimated.

Specific volume analysis of bread

White bread is calculated based on the Chinma *et al.*, (2016) method using the rapeseed displacement method according to AACC (2000). The specific volume calculation is obtained from the volume divided by the weight of the white bread (ml/g).

Microstructural analysis of bread

Microstructural analysis was carried out by cutting the bread and then taking pictures with digital camera canon type 550D. Data on microstructure can be

obtained using Image software v.1.49. The data obtained are cell density (cells/cm²), and mean cell area (mm²) (Zhou et al., 2022).

Texture analysis of bread

Texture profile analysis (TPA) was carried out based on the method of research by Yu et al., (2018) using a texture analyzer according to directions from the AACC, (1999). Bread cut with a thickness of 20 mm is compressed to 50% of the initial bread height at a speed of 1.5 mm/s. The data obtained includes hardness, gumminess, chewiness, springiness, cohesiveness, and resilience.

Statistical analysis

Data were analyzed using SPSS 25.0 statistics. Significance was determined using a one-way analysis of variance (ANOVA) and if there

was a difference, continued Duncan's test was.

RESULTS AND DISCUSSION

pH and titratable acidity (TTA) of snake fruit starter

The selection of starter is presented in Figure 1a, the pH and Titratable acidity (TTA) in Figure 1b, and the number of Lactic Acid Bacteria (LAB) and total yeast in Figure 1c, respectively.

Figure 1a. shows that the highest peak of bubbles is found in the starter fermented for 48 hours. This allegedly shows that the number of yeast capable of producing CO₂ is highest in these conditions, so the starter is used to make sourdough. Evaluation of the starter's pH and TTA (Figure 1b) showed that the bubble formation results aligned with the pH, TTA, LAB, and yeast growth values. The pH will decrease as fermentation progresses, in contrast to TTA.

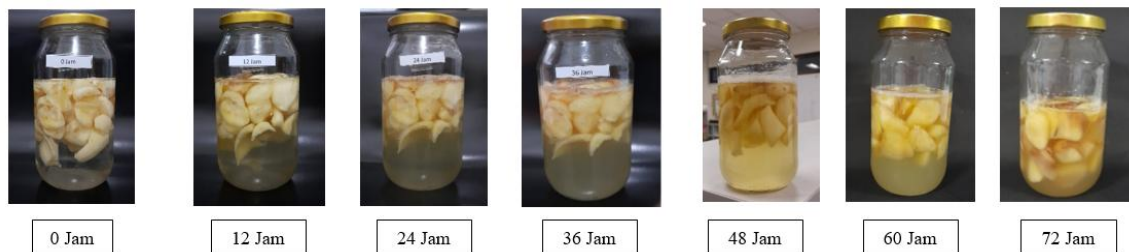


Figure 1a. Snake fruit fermentation starter for 72 hours at 30 °C

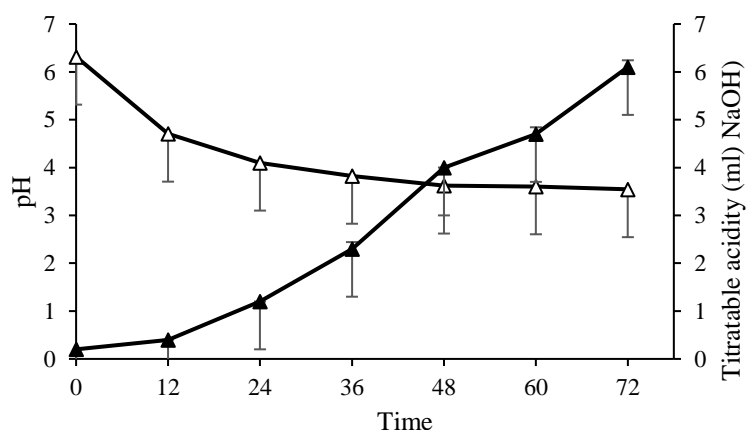


Figure 1b. pH and titratable acidity (TTA) during fermentation on snake fruit fermentation starter

Note: ▲ = Titratable acidity (TTA) Δ= pH; during fermentation

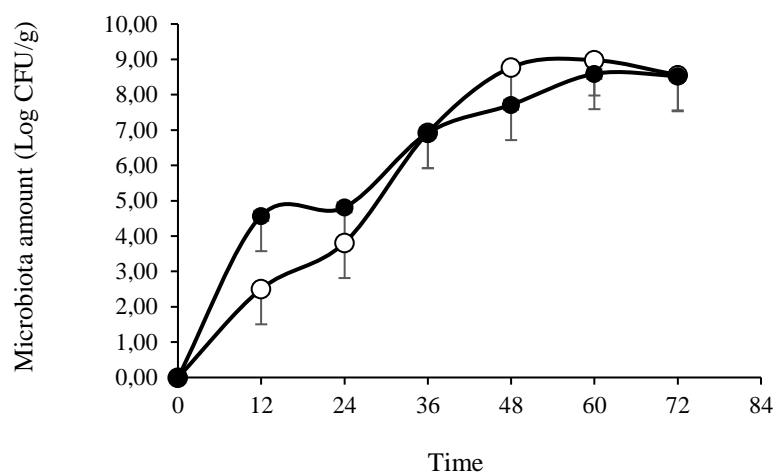


Figure 1c. Growth rate of lactic acid bacteria (LAB) and yeast on the snake fruit starter
 Note: ○= Lactic acid bacteria (LAB) ●= Yeast

During the 48-hour fermentation, the pH value decreased to 3.62 at the TTA value of 4 ml NaOH, while the LAB and yeast (Figure 1c) values increased to 8.77 log CFU/g (LAB) and 7.72 log CFU/g (yeast). The decrease in pH during fermentation is due to LAB and acetic acid. This research is in line with the findings Yu et al., (2018) that fermentation can increase TTA in pear and orange starters. The increase in the acidity of fruit starter during fermentation is caused by the high content of organic acids produced by lactic acid bacteria and yeast, as mentioned by (Omedi et al., 2021) said that increasing the sugar content during fermentation or proofing on added fruit is the main ingredient that causes high acid conditions or decreased pH during fermentation.

The sourdough production

The sourdough production is presented in Figures 2a and 2b, pH and titratable acidity (TTA) of sourdough in Figure 3a, and the LAB and yeast in Figure 3b. Snake fruit starter reached acidic conditions and high microbiota growth at 48 hours, snake fruit starter was added to wheat flour to make sourdough or natural yeast. During the of sourdough making, it produces acidic conditions, which means a decrease in pH and an increase in TTA, both in snake

fruit sourdough (SDS) and sourdough control (SDF). The increasing number of microbiota in the dough causes acidic conditions in sourdough. However, both showed differences in the acid conditions and the number of microbiota. The maturity of the starter culture at the 48 hours can be seen from the appearance after feeding, which volume increases 3-4 hours after feeding. There is an increase in the concentration of the starter culture, a sweet and sour aroma, and small bubbles that are evenly distributed (Figure 2a and 2b).

Figures 2a and 2b show that snake fruit sourdough can develop into mature sourdough, which is indicated by the increase in sourdough doubling. It is faster than sourdough control, which is made with flour and water. The maturity level of sourdough is indicated by an increase in volume up to 2-3 times from the original, a decrease in pH, as well as an increase in the TTA value from the previous day and becoming constant on the following day. Sourdough maturity from SDS is achieved on day 3, while control (SDF) is achieved on day 7, meaning that the bread will be obtained from SDS on day 3 and SDF on day 7. This phenomenon was similar to Minervini et al., (2012) whose added pears and grapes ripen two days faster than the controls.

Based on Figure 3a, the initial pH value of the snake fruit sourdough (SDS) was lower than that of the control sourdough (SDF). This is because the liquid in SDS had already undergone fermentation during the snake fruit liquid starter-making process, allowing bacteria and yeast to grow. These microorganisms produce acids during fermentation, which results in a lower pH. In contrast, the control sourdough (SDF) was made by simply mixing wheat flour and water, meaning that fermentation only began after these ingredients were combined.

The pH of mature sourdough was 3.8-4, achieved on the third day by snake fruit sourdough, while sourdough control achieved that pH on day 5. So, the maturity level of snake fruit sourdough (SDS) is

obtained faster, namely three days, while control sourdough (SDF) takes longer, namely five days. It can be seen in Figures 2a and 2b that the volume of the snake fruit sourdough on the 3 days increased twice compared to the control sourdough

Figure 3b showed that snake fruit sourdough (SDS) had more number of microbes, LAB 9.19 Log CFU/g and Yeast 9.42 Log CFU/g, respectively, while water-flour sourdough (SDF) contained LAB 9.03 Log CFU/g and Yeast 9.07 Log CFU/g. The more yeast and LAB of SDS in snake fruit sourdough than controls because of increased sugar content during fermentation or proofing on sourdough can increase yeast activity and gas production Omedi et al., (2021).

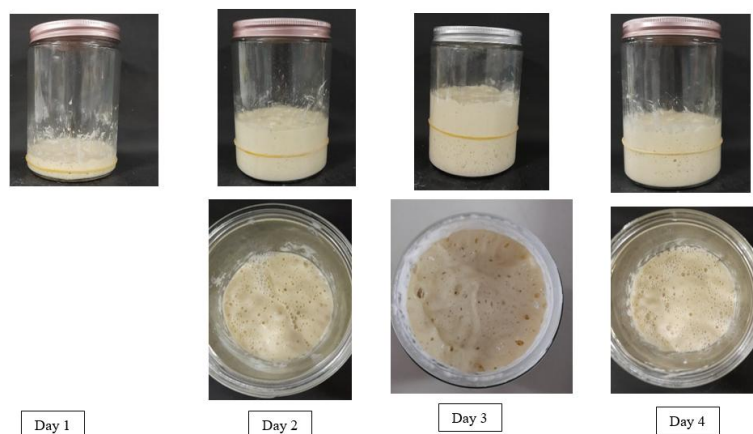


Figure 2a. Snake fruit sourdough fermentation at 30°C

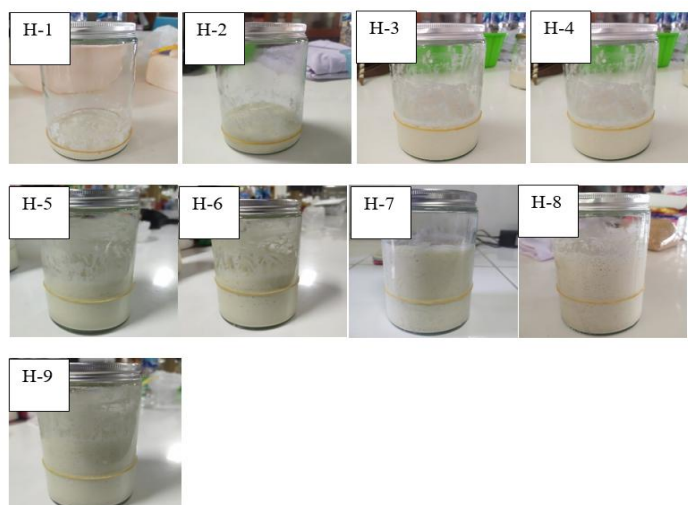


Figure 2b. Sourdough control(SDF) Fermentation at 30 °C

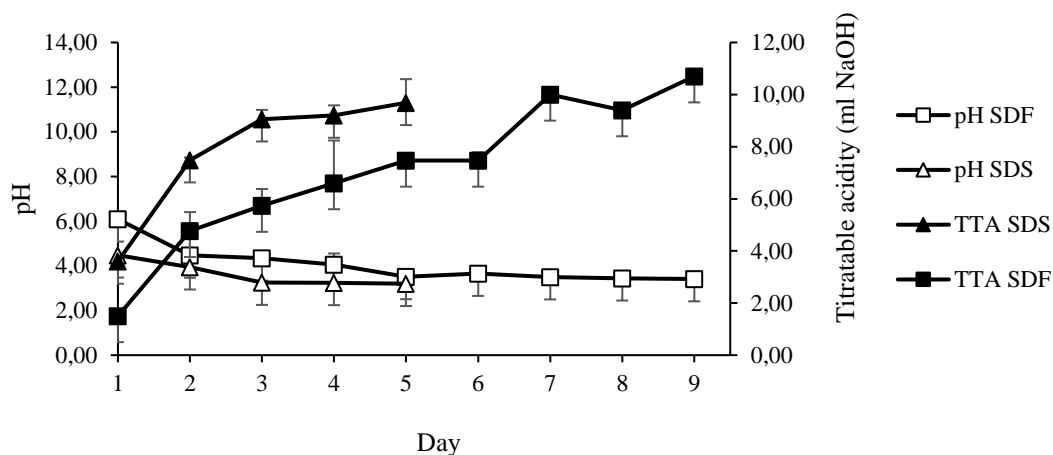


Figure 3a. pH and titratable acidity (TTA) of sourdough (SDS)
 Note: Δ= PH snake fruit sourdough (SDS) ▲= TTA snake fruit sourdough (SDS);
 □= pH control sourdough (SDF) ■= TTA control sourdough (SDF)

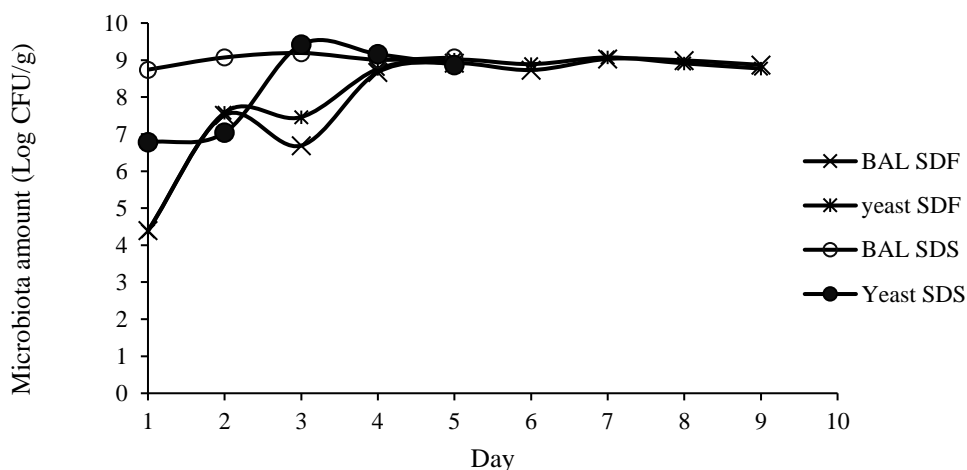


Figure 3b. The growth rate of lactic acid bacteria (LAB) and yeast of sourdough (SDS)
 Note: ○= LAB snake fruit sourdough (SDS) ●= Yeast snake fruit sourdough (SDS)
 ×= BAL control sourdough (SDF) *=Yeast control sourdough (SDF)

The characteristic of rice flour bread

The result of rice flour bread products using SDS, SDF and commercial yeast (CY), showed different characteristics. These differences in texture characteristic can be seen in Table 1, while microstructure characteristics are presented in Figure 4 and Table 2.

There was a difference in the texture of SDS rice flour bread and control bread of SDF, where the bread of SDS has higher levels of hardness, gumminess, chewiness, springiness, cohesiveness, and resilience

compared to the bread of SDF. These results are in line with Omedi et al., (2021), where the *hardness* level of wheat bread with pitaya sourdough is very high. This is due to the presence of fruit substrate, where the production of organic acid metabolites from lactic acid bacteria can increase the *hardness* of breadcrumbs. The results indicate that using a snake fruit starter produces a firmer crumb texture. This analysis is intended to characterize the bread's texture, while preference for softness or firmness depends on consumer preference.

Table 1. The texture of rice flour bread against the type of yeast.

Types of starter	Hardness	Gumminess	Chewiness	Springiness	Cohesiviness	Resilience
SDS	27.515 ± 1.83 ^b	17.771± 2.63 ^b	16.127 ± 2.60 ^b	0.905 ± 0.126 ^a	0.645 ± 0.056 ^a	0.565 ± 0.065 ^a
SDF	21.576 ± 0.63 ^a	12.784± 1.15 ^a	11.429 ± 0.96 ^a	0.894 ± 0.006 ^a	0.592 ± 0.036 ^a	0.510 ± 0.038 ^a
CY	20.075 ± 1.38 ^a	12.082± 1.16 ^a	10.815 ± 1.22 ^a	0.894 ± 0.123 ^a	0.601 ± 0.017 ^a	0.519 ± 0.011 ^a

Note: Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's test. Snake fruit sourdough (SDS), Control sourdough (SDF), Commercial yeast (CY).

Table 2. Characteristics of rice flour bread against yeast types

Types of starter	Parameter		
	Cell Density (cells/cm ²)	Mean Cell Area (mm ²)	Volume Spesifik (ml/g)
SDS	6.31 ± 1.15 c	16.29 ± 3.16 a	1.92±0.03 ^a
SDF	2.23 ± 0.27 a	47.22 ± 5.38 b	1.91±0.04 ^a
CY	4.36 ± 0.59 b	23.58 ± 3.20 a	1.89±0.02 ^a

Note: Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's test. Snake fruit sourdough (SDS), Control sourdough (SDF), Commercial yeast (CY).

**Figure 4.** Microstructure of rice flour bread by *Image J*

Note: A. Snake fruit sourdough (SDS); B. Control sourdough (SDF); C. Commercial yeast (CY).

The research on the impact of sourdough on rice flour bread microstructure has revealed significant effects on bread volume, cell density, and mean cell area. These findings, detailed in Table 2, provide valuable insights into the bread characteristics. Notably, the specific volume of the three types of bread showed no significant difference, aligning with previous research (Yu *et al.*, 2018), where the volume of bread using pear sourdough is higher than the volume of bread using pear orange sourdough. This is caused by several factors, namely 1) the activity of yeast metabolites increases due to the presence of heterofermentative conditions of lactic acid bacteria so that they produce more carbon dioxide gas during fermentation, 2)

suitable acidic conditions increase the gas retention capacity in the bread structure, 3) the accumulation of water-soluble pentosan which causes changes in water distribution which can increase the volume of bread.

The results show significant differences in cell density and mean cell area between the three types of rice bread. Cell density on sourdough snake fruit rice flour (SDS) bread was higher than sourdough control rice flour (SDF) and commercial yeast (CY) white bread. However, the low mean cell area is caused by the high acid conditions in the snake fruit sourdough dough. When applied in bread making, the acidic conditions certainly increase when the proofing is carried out for 4 hours, along with the increase in yeast.

According to (Trappey *et al.*, 2015) the high cell density on white bread will tend to result in fewer cells. The cell density is influenced by the fiber content, which promotes and regulates the expansion of gas within specific particle sizes. Fiber can also cause gas retention restrictions. This statement is proven by the mean cell area of snake fruit sourdough, which is lower than control sourdough rice flour white bread and commercial yeast.

CONCLUSION

The starter of snake fruit fermentation can shorten the fermentation of sourdough. The number of microbiota in snake fruit sourdough was 9.19 log CFU/g and 9.42 log CFU/g for lactic acid bacteria and yeast, respectively. The amount of microbiota found on the 3rd day of sourdough was the optimal number. The effect of snake fruit sourdough on the texture characteristics of rice bread creates higher hardness, gumminess, chewiness, springiness, cohesiveness, and resilience than other leavened agents

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