Journal of Applied Agricultural Science and Technology
7(3): 225-235 (2023)
E-ISSN: 2621-2528
ISSN: 2621-4709

MICROBIOTA PROPERTIES AND TEXTURE OF RICE FLOUR BREAD WITH PINEAPPLE STARTER

Icca Ratna Danila, Rini Yanti*, Dian Anggraini Suroto

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author
Email: riniyanti@ugm.ac.id

***Abstract**. Changing the ingredients also can change the properties of the bread. In this study, bread was made using rice flour and sourdough. Sourdough with Lactic Acid Bacteria (LAB) and yeast can also be added with pineapple starter to help microbiota growth. The study aimed to determine the amount of microbiota produced from sourdough with pineapple addition and its effect on the texture of rice flour bread. Pineapples with water and sugar were fermented to make pineapple starter. Pineapple starter is then used in sourdough making and fermented around 3-6 days. The mature sourdough was used in bread making. The addition of pineapple, in the form of a starter, made the pineapple sourdough (PS) have lower pH and higher titratable acidity (TA), total Lactic Acid Bacteria (LAB), and total yeast than sourdough without the addition of pineapple starter or wheat sourdough (WS). The pH and TA of PS at the peak were 3.25 and 2.67 mL of NaOH. The total LAB of PS at the peak was 9.27 Log CFU/g, and the total yeast was 9.30 Log CFU/g. PS reached its peak on the third day, while wheat sourdough (WS) reached it on the sixth day. The pineapple sourdough bread (PSB) had the lowest specific volume, and the highest texture properties compared to control bread (CB) and wheat sourdough bread (WSB), but there is no significance different. The addition of pineapple starter can fasten the fermentation time of sourdough and made the bread less brittle than CB.*

Keywords: *bread texture; lactic acid bacteria; pineapple starter; rice flour bread; wheat sourdough*

1. Introduction

Bread is a food that is widely consumed by people in Indonesia, with the average consumption in 2020 being 0.34 small packs per capita per week (Badan Pusat Statistik, 2021). Bread is made with wheat flour and other ingredients, fermented and baked. Wheat flour in bread can be replaced but can cause changes in the physical properties of the resulting bread. Rice flour, with other ingredients such as starch, hydrocolloids, or proteins, can imitate the elasticity and compactness of gluten-containing dough (Khoury *et al*, 2018). Rice flour with cassava starch (Sigüenza-Andrés *et al*., 2021) or potato flour (Liu *et al*., 2016) can improve the texture and specific volume of bread.

Sourdough is made by the spontaneous fermentation of a mixture of flour and water by lactic acid bacteria (LAB) and yeast (Arora *et al*., 2021). The addition of sourdough can improve bread's nutritional and physical properties, such as softer crumb, a higher acidity, and better shelf life than commercial fermented bread (Calvert *et al*., 2021; Yu *et al*., 2018). In general, sourdough can produce more LAB than yeast in a ratio of 100:1; this ratio also can change depending on the

Received February 1, 2023; Accepted June 6, 2023; Published August 25, 2023
https://doi.org/10.55043/jaast.v7i3.130

sourdough ingredients and environmental conditions (Calvert *et al*., 2021).

Sourdough can be made by adding fruit, thus providing the right environment for microbiota in sourdough and resulting in significant changes to the stability and strength of the final sourdough (Vilanova *et al*., 2015). The addition of some fruits in sourdough was performed by researchers such as Gordún *et al*. (2015), in which grapes with two days of fermentation and apple with five days of fermentation had different specific microbial. Grape sourdough also needs more time than apple and yogurt sourdough to reach suitable pH. The addition of pear sourdough and navel orange sourdough in a study by Yu *et al*. (2018) made a greater acidity and specific volume than control bread or bread with instant yeast. In a study by Comasio *et al*. (2021), lemon juice and apple juice enhanced the production of lactic acid, acetic acid, acetoin, and diacetyl in sourdough. The study by Zaidiyah *et al*. (2020) used pineapple as a starter for sourdough. Pineapple was also chosen in this research because pineapple juice is more acidic than other fruit juice, and the addition of pineapple can lower the pH of the starter.

The difference between this study and the research by Zaidiyah *et al*. (2020) is pineapple in that research was cooked and fermented until the tenth day, and the bread also had a proximate composition, pH, and crude fiber analysis. This study analyzed the number of microbiotas produced from sourdough with the addition of pineapple and its effect on the texture of rice flour bread. The purpose of this study was to determine the amount of microbiota such as Lactic Acid Bacteria (LAB) and yeast produced from sourdough with pineapple addition and its effect on the texture of rice flour bread.

2. Methods

2.1. Materials

The material used in this study was Pineapple of Honi variety (Sunpride, Indonesia) obtained from the traditional market in Indonesia. Pineapple, sugar, and water were bought from a traditional market in Indonesia. Wheat flour (Indofood Sukses Makmur Inc, Surabaya) and water are used in sourdough making. The main ingredients for bread were rice flour, tapioca flour, and potato flour. Hydroxypropyl methylcellulose (HPMC), Psyllium Husk, sugar, milk, margarine, salt, and commercial yeast are also added to the bread making. The materials for Lactic Acid Bacteria and yeast analysis were yeast extract, peptone, dextrose, agar, peptone water, and MRS broth (Merck, Germany).

2.2. Pineapple Starter Making

The pineapple starter was made by peeling the pineapple and washed with water to remove excess dirt. The washed pineapple was then cut and weight 100 g. The cut pineapple was mixed with 250 g water and 20 g sugar (Yu *et al*., 2018). The mixture was kept in an incubator at 30°C for 48 hours. The resulting liquid was used as a starter in sourdough making. The pineapple starter was taken every 12 hours for analysis.

2.3. Sourdough Making

The Pineapple Sourdough (PS) was made by mixing 30 g of pineapple starter with 30 g of wheat flour. The mixture was fermented for 12-24 hours inside the incubator at 30°C (Comasio *et al*., 2021). The fermented mixture or sourdough weight 30 g, mixed with wheat flour and water, with a ratio of wheat flour and water of 1:1. Sourdough was refreshed twice a day by combining the previous sourdough, wheat flour, and water with a ratio of 1:1:1. After three days of propagation, PS was used for Pineapple Sourdough Bread (PSB) making. Wheat Sourdough (WS) was made with wheat flour and water, with the same ratio as PS. WS was fermented until six days before Wheat Sourdough Bread (WSB) making. WS and PS were taken every day to analyze until the eighth day for WS and the fifth day for PS.

2.4. Bread Making

With some adjustments, the bread was made using the method by Yu *et al*. (2018) and Sigüenza-Andrés *et al*. (2021). The main ingredients for bread were 70 g of rice flour, 20 g of tapioca flour, and 10 g of potato flour. The other ingredients for bread were 2 g of HPMC, 2 g of psyllium husk, 7 g of sugar, 3 g of milk powder, 8 g of margarine, 1 g of salt, 30 g of egg, and 97.8 g of water. In WSB and PSB, 55 g of sourdough was added, while for Control Bread (CB), 1 g of commercial yeast was added. All of the bread ingredients were mixed for 10 minutes using a mixer. The dough was put into the bread pan for fermentation inside the incubator at 30°C. The dough for WSB and PSB was fermented for 4 hours, while CB was fermented for 45 minutes. The dough was baked for 40 minutes and cooled for around 1-2 hours. The bread was then wrapped in plastic and sealed for further analysis.

2.5. Analysis

2.5.1. Determination of titratable acidity and pH

Pineapple starter and sourdough were analyzed for titratable acidity (TA) and pH. TA and pH were measured with 10 g of pineapple starter or sourdough in 100 mL of distilled water (Yu *et al*., 2018). The TA was analyzed by putting 25 mL sample solution into Erlenmeyer and giving three drops of phenolphthalein indicator, until the sample solution changed to pink. TA was shown by the amount of NaOH in mL (Yu *et al*., 2018). The pH of the sample solution was analyzed with a digital pH meter (Filox, Pavan International, India).

2.5.2. Enumeration of total LAB and yeast

LAB and yeast were counted by homogenizing one gram of pineapple starter, WS or PS, with 9 mL of peptone water. The sample was diluted 2-9 times for lactic acid bacteria and 2-8 times for yeast. One milliliter of diluted sample and around 10-15 mL of suitable agar medium were added to the petri dish. Total LAB was counted on MRS agar medium, and yeasts were counted on YPD agar medium (Ripari *et al*., 2016). The Petri dish was incubated at 30 °C for 48 hours after the diluted sample mixture, and suitable agar became solid. The number of colonies was estimated as colony-forming units per gram.

2.5.3. Specific volume of bread

The specific bread volume is obtained by dividing the volume and weight of the bread. The bread volume was measured using rapeseed displacement (Chinma *et al*., 2016). The bread's weight was measured using a digital scale

2.5.4. Microstructure of bread

Microstructural bread analysis was performed by a slice of bread photographed and analyzed with Image J Software (Zhou *et al*., 2022). The microstructure of bread included the cell density and the mean cell area (Puerta *et al*., 2021). Cell density can be calculated from the number of cells divided by the mean cell area (Scheuer *et al*., 2015).

2.5.5. Texture analysis of bread

Bread texture was measured using a Texture Analyzer TA-XT2i with a 3 cm probe. The bread is compressed to 50% of its original high at a crosshead speed of 1.5 mm/s. The result for texture analysis in bread showed hardness in N, gumminess in N, chewiness in N, springiness, cohesiveness, and resilience (Yu *et al*., 2018).

2.5.6. Statistical analysis

Data were analyzed using the statistical package SPSS 22.0. The significance was determined through a one-way analysis of variance (ANOVA). Significant differences were tested using Duncan's multiple-range test. Two replications were used for all analytical determinations.

3. Results and Discussion

3.1. Titratable acidity and pH

Pineapple starter were analyzed every 12 hours, while WS and PS were analyzed every day. The titratable acidity and pH of the pineapple starter are shown in Figure 1a. The initial pH of the pineapple starter was 5.24 and continued to decrease until 72 hours. The initial TA of the pineapple starter was 0.05 mL NaOH and continued to increase until 48 hours. A similar result was also found in pear and navel orange starter research by Yu *et al*. (2018), which has the decrease of pH and increase of TA after fermentation. After 48 hours, TA showed a decrease, but that has not significant after 36 hours of fermentation.

The value of TA and pH from WS and PS is shown in Figure 1b. WS had an initial TA and pH of 0.38 mL NaOH and 6.09, respectively. The initial TA and pH for PS are 1.08 mL NaOH and 4.91, respectively. WS had lower initial pH and also higher TA than PS. The higher TA in PS, maybe because of the contribution of LAB in pineapple starter, which produced more lactic acid. LAB can convert sugar into lactic acid during fermentation (Calvert *et al.*, 2021).

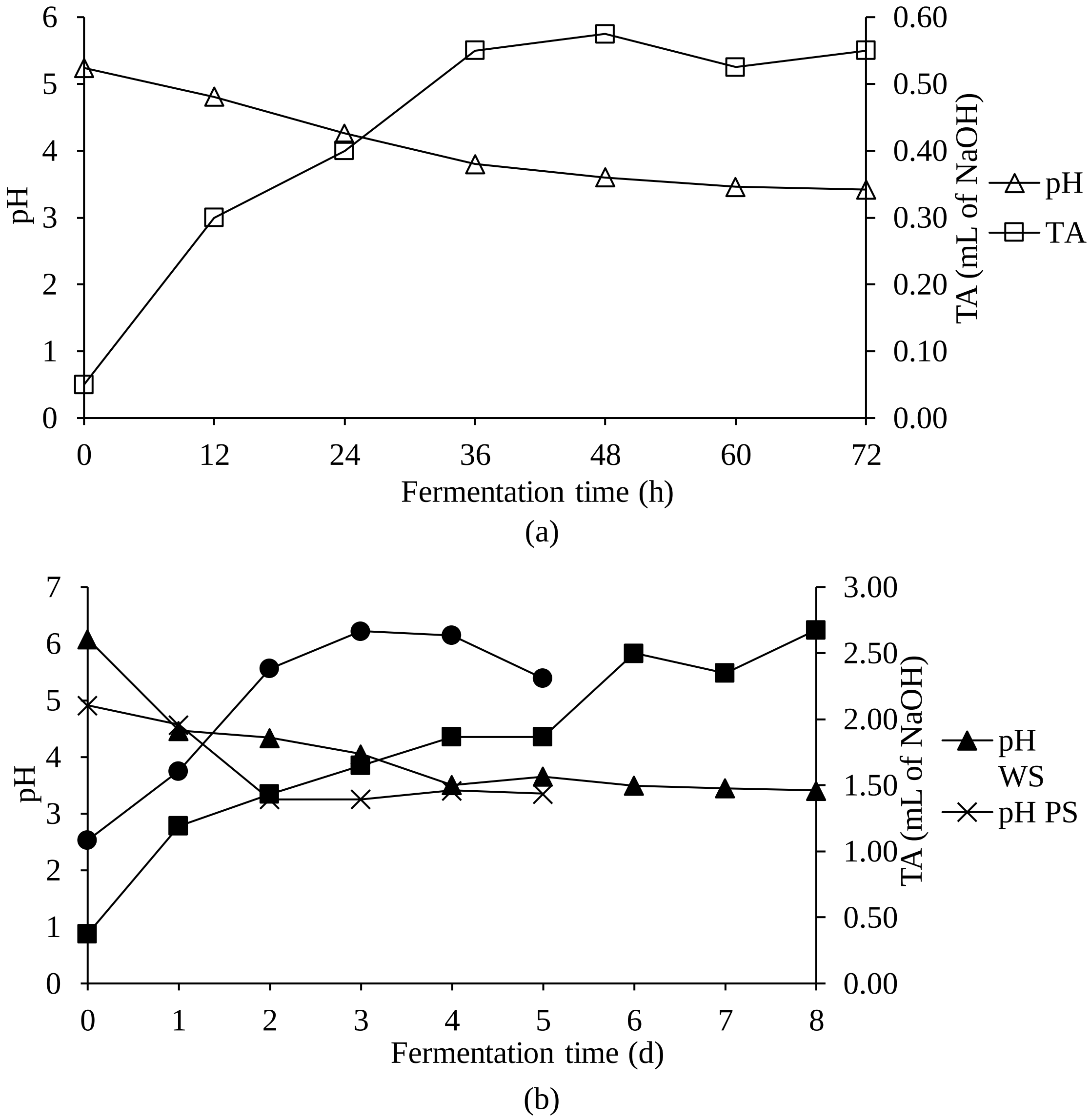
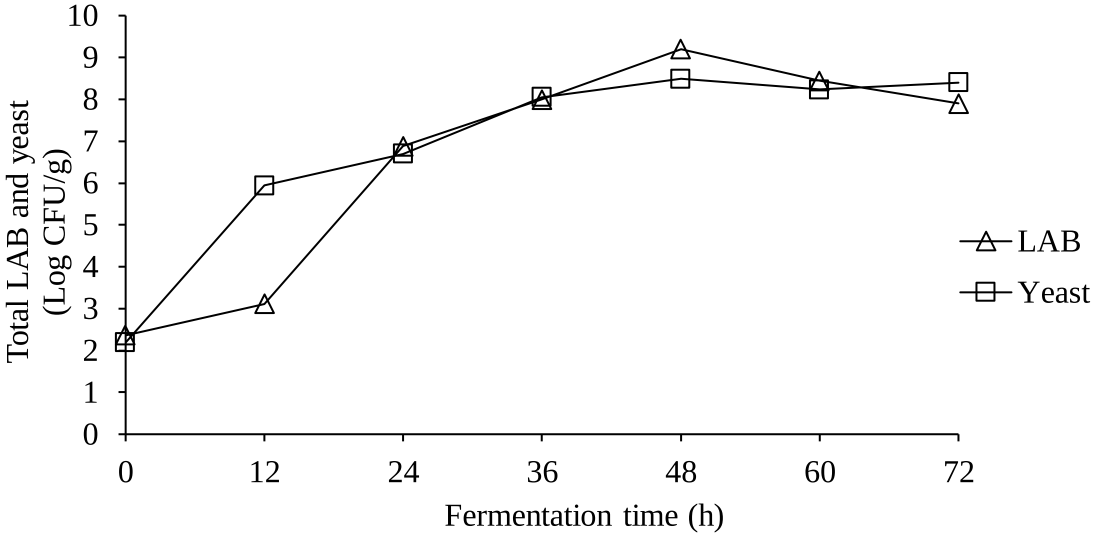
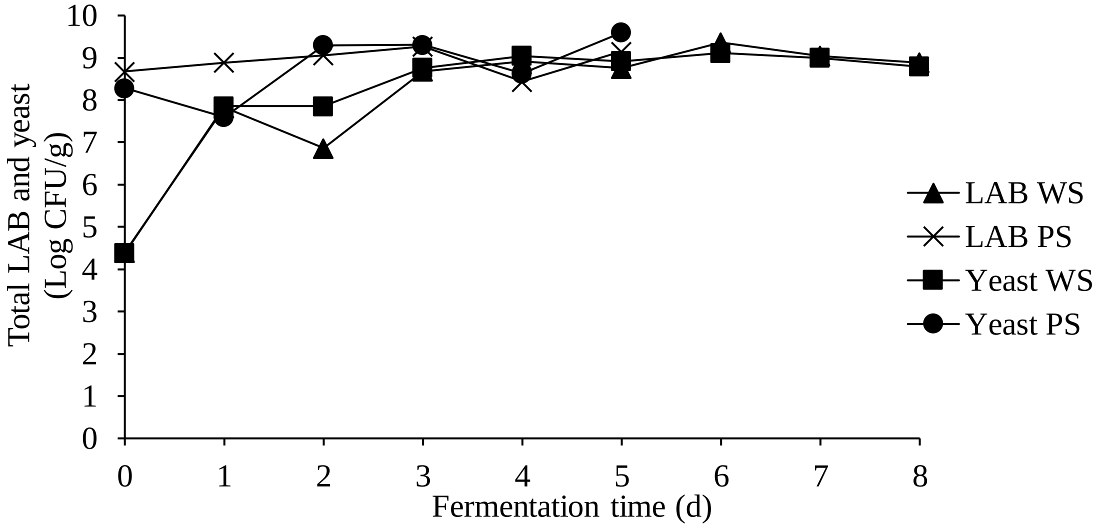


Figure 1. Titratable acidity (TA) and pH of Pineapple starter and Sourdough
(a) Pineapple starter, (b) Sourdough
WS: Wheat Sourdough; PS: Pineapple Sourdough

The TA and pH of PS reached their peak on the third day, while WS reached their peak on the sixth day, but there is no significance different. The pH value for mature sourdough is 3.5-4.3(Aplevicz *et al.*, 2014). TA of PS and WS increased due to the substrate in sourdough which can be used by LAB. Based on Gänzle (2015) in Calvert *et al.* (2021), metabolic process of LAB can produce lactic acid, acetic acid, and CO_2, which can increase the acidity. The TA and pH result in PS fermentation is similar to research by Yu *et al.* (2018), where fermentation can increase TA and decrease pH in the starter of pear and orange. The addition of pear and orange starter resulted in a higher total titratable acidity than sourdough without the addition of fruit starter. Another similar study was conducted by Gordún *et al*. (2015) by adding apples and grapes as a starter in sourdough. The highest TA was reached on the third day of sourdough fermentation. Adding lemon juice and apple juice can also increase the total TA in sourdough (Comasio *et al*., 2021).



(a)



(b)

Figure. 2 Total lactic acid bacteria (LAB) and Yeast of Pineapple starter and Sourdough
(a) Pineapple starter, (b) Sourdough
WS: Wheat Sourdough; PS: Pineapple Sourdough

3.2. Microbiota Analysis

Pineapple starter was analyzed every 12 hours, WS and PS were analyzed daily. The results of microbiota analysis in the pineapple starters are shown in Figure 2a. The initial amount of LAB and yeast in the pineapple starter was 2.36 Log CFU/g and 2.19 Log CFU/g. The LAB and yeast of the pineapple starter continued to increase until reaching their peak at 48 hours. After 48 hours of fermentation, the pineapple starter is used in PS making. The peak time of pineapple starter was 48 hours of fermentation, because it contains simple carbohydrate such as glucose and fructose from fresh pineapple and sugar, which can use as substrate for LAB and yeast. Other studies use fruit starter to make sourdoughs, but different fermentation time was used for their fruit starter. In the research of Yu *et al.* (2018), pear and orange starters were fermented for eight days. The difference in fruit fermentation time report in the research by Gordún *et al.* (2015) is that the apple starter was fermented for five days, while the grape starter was fermented for 2 days. This research used the highest total LAB and yeast in pineapple starters as the reason for using 48 hours of fermentation time.

The LAB and yeast in WS and PS are shown in Figure 2b. The total LAB and yeast increased due to the addition of wheat flour twice a day. This flour contains carbohydrate which can be used as substrate for LAB and yeast. The initial amount of LAB and yeast in WS was 4.40 Log CFU/g and 4.38 Log CFU/g and continued to increase until the sixth day of fermentation. The initial amount of LAB in PS was 8.89 Log CFU/g, while the yeast was 7.60 Log CFU/g. PS reached the highest LAB and yeast on the third day of fermentation. PS had a higher amount of LAB and yeast, and also reached the peak faster than WS. This result may be obtained due to the addition of pineapple starter, which already has LAB and yeast.

The increase in the total amount of LAB and yeast on the first day of PS fermentation may indicate that adding fruit starters produces a suitable environment for LAB and yeast in sourdough (Vilanova *et al*., 2015). The addition of pineapple starter in this study can increase total LAB and yeast in sourdough. In the research of Yu *et al.* (2018), the addition of pear starter can produce yeast of 8.67 Log CFU/g, higher than sourdough without the addition of fruit starter. In Gordún *et al.* (2015) research, adding apple and grape starter resulted in total yeast up to 9.9 Log CFU/g and 8.0 Log CFU/g, respectively.

In general, sourdough can produce more LAB than yeast, in a ratio of 100:1. This ratio also can change depending on the sourdough ingredients and environmental conditions (Calvert *et al*., 2021). Room temperature or incubator temperature also can affect yeast growth. Temperature above 27 °C can accelerate the fermentation; however, yeast will be easily contaminated at a temperature above 30 °C (Sanggramasari *et al*., 2018).

3.3. Specific volume of bread

The different kind of sourdough can affect the properties of the bread. The result of a specific volume of CB, WSB, and PSB is shown in Table 1. In this study, wheat sourdough and pineapple sourdough were used in bread, and commercial yeast were used only in control bread. The addition of wheat sourdough, the specific volume of the bread was higher than the control bread because LAB could increase the metabolic activity of yeast to generate CO_2, and the acidic environment could enhance gluten retention (Gobbetti, 1995, in Yu *et al*., 2018). PSB which had the lowest specific volume, may be due to the presence of bromelain enzymes that disrupt gluten bonds (Sanggramasari *et al*., 2018). The dough strength may be less strong and have smaller air holes, and the volume of the bread is also small compared to the weight of the PSB.

PSB had the lowest specific volume compared to CB and WSB. A similar result was obtained by Scarton *et al*. (2021) in their study of bread containing lime juice. Adding lime juice decreased the specific volume of bread without lime juice. The research by Yu *et al*. (2018) showed that adding orange sourdough resulted in a lower specific volume than pear sourdough bread, but it was not significantly different. WSB had the highest specific volume but was not significantly different from CB. The study by Savkina *et al*. (2019) also showed a similar result: the specific volume of bread with the addition of sourdough is higher than control bread.

3.4. Microstructure of bread

The result of the microstructure of CB, WSB, and PSB is shown in Table 1. PSB had the highest cell density, then CB and WSB. PSB also had the lowest mean cell area then others sourdough bread. Cell density corresponded to the number of air cells in area (cm) of the crumb (Puerta *et al*., 2021).

High cell density in PSB may be because PSB had small and many air cells. Bread containing many small particles or small air cells in the crumb was less firm and more elastic than bread containing large air cells (Puerta *et al*., 2021). WSB has the lowest cell density, probably due to the larger air cells, because it captures more CO_2 from yeast fermentation. Cell density affects the mechanical characteristics of bread crumbs and also can be affected by the content of fat substitutes in bread, such as margarine or butter (Scheuer *et al*., 2015).

Table 1. Specific volume and microstructure of bread

Sample	Specific volume (mL/g)	Cell density (u/cm^2)	Mean cell area (mm^2)
CB	1.89 ± 0.02^b	4.36 ± 0.77^b	23.58 ± 4.48^a
WSB	1.91 ± 0.03^b	2.23 ± 0.56^a	47.22 ± 11.50^b
PSB	1.68 ± 0.11^a	5.86 ± 0.42^c	17.137 ± 1.15^a

CB, control bread; WSB, wheat sourdough bread; PSB, pineapple sourdough bread. Data are expressed as mean ± standard deviation (n=6). Different letters in the same column indicate significant differences ($p<0.05$)

This study used rice flour, tapioca flour, and potato flour as the main ingredients for making bread. In the research by Liu *et al*. (2016), the addition of potato flour decreases the specific volume of steamed bread, due to potato flour diluting the gluten network. The addition of hydrocolloids, such as HPMC and psyllium husk, was also used to imitate the elasticity of gluten and improve the dough's rheological characteristic (Aldughpassi *et al*., 2020; Khoury *et al*., 2018). In the research of Belorio & Gómez (2020), the addition of psyllium husk and HPMC to wheat flour-based bread, produced bread with a good crumb appearance and fairly high specific volume.

Table 2. Texture analysis of bread

Sample	Texture parameters					
	Hardness	Gumminess	Chewiness	Springiness	Cohesiveness	Resilience
CB	20.07 ± 2.00[a]	12.08 ± 1.42[a]	10.82 ± 1.43[a]	0.89 ± 0.02[a]	0.60 ± 0.02[a]	0.52 ± 0.02[a]
WSB	21.58 ± 0.67[a]	12.78 ± 1.07[a]	11.43 ± 0.90[a]	0.89 ± 0.01[a]	0.59 ± 0.03[ab]	0.51 ± 0.03[a]
PSB	26.04 ± 3.04[b]	16.35 ± 1.88[b]	14.73 ± 2.19[b]	0.90 ± 0.01[a]	0.63 ± 0.01[b]	0.54 ± 0.01[a]

CB, control bread; WSB, wheat sourdough bread; PSB, pineapple sourdough bread. Data are expressed as mean ± standard deviation (n=6). Different letters in the same column indicate significant differences ($p<0.05$)

3.5. Texture analysis

The texture analysis of bread is shown in Table 2, with texture parameters hardness, gumminess, chewiness, springiness, cohesiveness, and resilience. PSB had the highest hardness, gumminess, and chewiness than CB and WSB. CB with the lowest hardness had high springiness, which was also related to crumb brittleness (Puerta *et al*., 2021). PSB which had the highest springiness, may perceive a softer and spongier crumb. PSB's springiness, cohesiveness, and resilience were also the highest but had no significant difference.

The sourdough used in this research is made from wheat flour, but the gluten in sourdough is less than the use of wheat flour directly. Adding wheat sourdough may play a role in strengthening the bonds in the bread dough, resulting in a denser but softer loaf. A higher specific volume generally results in lower hardness (Yu *et al*., 2018). This also showed that PSB had the highest hardness value and the lowest specific volume in this study

The ingredients of bread may influence the texture of CB, WSB, and PSB. The addition of pineapple core flour increases the hardness and gumminess of “Mantou” or steamed bread in the research by Shiau *et al*. (2015). In the study by Liu *et al*. (2016), adding potato flour increased the hardness value of steamed bread. Hardness was also significantly related to chewiness, which increased the value of chewiness. In the research of Belorio & Gómez (2020), bread made from rice flour with the addition of HPMC has a higher hardness value. This may be related to the change in the gel properties of HPMC when the bread is cooled, thereby increasing the crumb firmness after baking.

4. Conclusions

The addition of pineapple starter increased the titratable acidity, total LAB, and total yeast and decreased the sourdough’s pH. Adding a pineapple starter produces a fairly large hardness value, but bread with sourdough tends to be softer. CB, without adding sourdough, is more brittle and tends to dry out. Further research is needed to determine the effect of pineapple starter addition in PS, such as the change in gluten in wheat sourdough with the addition of fruit starter, and further microbiota analysis. The effects of fermentation in bread, such as volume and CO_2, sensory and flavour analysis in bread are also needed for further research.

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