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Exploring the potential of fermented papaya as a functional ingredient for sourdough bread: a study on fermentation time and quality of sourdough donuts

Potensi fermentasi pepaya sebagai bahan fungsional pembuatan roti sourdough: kajian waktu fermentasi dan kualitas donat sourdough

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ABSTRACT

Sourdough bread is known to have health benefits due to its fermentation process using lactic acid and wild yeast. It can improve the taste, texture, shelf life, and nutritional content of bread as a functional food. In this study, sourdough was made from fermented papaya with varying fermentation times of 4, 5, and 6 days. The substrate was fed five times to strengthen the sourdough, resulting in a robust enough mixture to develop the dough. The resulting sourdough was used to create sourdough bread with a 30% sourdough concentration. The fermentation time impacted the quality of the sourdough, with results showing that sourdough became increasingly active as fermentation time progressed. The best sourdough was obtained from a six-day fermentation period, with a pH of 3.60 and a total plate count of 1.70 $\times 10^4$ CFU/ml. The best donuts were produced using the sourdough that had been fermented for six days, with an almost perfect score of 3.9 out of 4 for all aspects, including color, aroma, taste, and texture. Using this sourdough resulted in a significantly longer shelf life; no mold appeared until the eighth day of storage at room temperature. The moisture content of the donuts was measured at 4.57%. This study demonstrates the potential benefits of using sourdough made from fermented papaya to improve the quality and shelf life of sourdough bread while also providing potential health benefits.

ABSTRAK

Roti sourdough dikenal memiliki manfaat kesehatan karena proses fermentasinya menggunakan asam laktat dan ragi alami. Hal ini dapat meningkatkan rasa, tekstur, umur simpan, dan kandungan nutrisi roti sebagai makanan fungsional. Pada penelitian ini, sourdough dibuat dari air fermentasi pepaya dengan waktu fermentasi yang bervariasi selama 4, 5, dan 6 hari. Pemberian substrat dilakukan lima kali untuk memperkuat sourdough dan menghasilkan campuran yang dapat mengembangkan adonan. Sourdough digunakan untuk membuat roti sourdough dengan konsentrasi sourdough 30%. Waktu fermentasi memengaruhi kualitas sourdough, dengan hasil menunjukkan bahwa sourdough menjadi semakin aktif seiring berjalannya waktu fermentasi. Sourdough terbaik diperoleh dari waktu fermentasi selama enam hari, dengan pH sebesar 3.60 dan TPC 1.70 $\times 10^4$ CFU/ml. Donat terbaik dihasilkan dari sourdough yang difermentasi selama 6 hari, dengan skor 3.9 dari 4 pada semua aspek, termasuk warna, aroma, rasa, dan tekstur. Penggunaan sourdough dapat menghasilkan donat yang memiliki umur simpan yang lebih lama; tidak ada jamur muncul sampai hari-8 penyimpanan pada suhu ruang. Kandungan air donat diukur sebesar 4.57%. Penelitian ini menunjukkan potensi manfaat penggunaan sourdough yang dibuat dari air fermentasi pepaya untuk meningkatkan kualitas dan umur simpan roti sourdough serta memberikan manfaat kesehatan potensial.

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INTRODUCTION

Bread is a baked product made primarily from flour, water, and yeast (Barbarisi et al., 2005). During bread production, several stages of biochemical and chemical-physical processes occur, including kneading, fermentation, and baking, which can affect various constituents (Cappelli et al., 2021). Fermentation, which is an anaerobic biological process, is crucial in bread making. Yeast or bacteria convert carbohydrates, such as sugars and starches, into carbon dioxide, alcohol, or acid, which determine bread expansion and mechanical properties (Maicas, 2020). To achieve desired color, flavor, and aroma, other ingredients like salt, sugar, milk, butter, chocolate, cheese, raisins, and dried fruit can be added (Kusnedi, 2021). Saccharomyces cerevisiae is the most common yeast used in bread making, often referred to as baker's yeast or simply "the yeast" (Parapouli et al., 2020). Commercial yeast, typically obtained from the S. cerevisiae strain, is commonly used as a leavening agent in fermentation by bread manufacturers. This type of yeast is resistant to drying processes, high sugar concentrations, and other fermentation process inhibitors such as propionate content in dough (Hidalgo & Brandolini, 2016).

Bread production typically involves the use of milk and its derivative products, such as butter, cheese, and skimmed milk, to enhance the bread's quality by providing additional nutrition, enhancing color, and imparting a unique taste and flavor (Sitepu, 2019). However, lactose intolerance is a health concern for consumers who cannot tolerate lactose-containing ingredients. Lactose intolerance is characterized by clinical symptoms such as bloating, abdominal pain, vomiting, redness of the anus, and diarrhea that occur due to lactose not being hydrolyzed due to lactase deficiency. Low lactase production is common in children over five years of age, with 3% prevalence in Northern Europe and nearly 80% in East Asia (Almon et al., 2013). To address this issue, an alternative material to commercial yeast is required. Sourdough can be used as an alternative for lactose intolerance consumers. Sourdough is a natural yeast obtained by mixing flour and water that is spontaneously fermented by lactic acid bacteria (LAB) originating from flour, other dough ingredients, fruits, or vegetables, which is also known as wild yeast (Arora et al., 2020; Suo et al., 2021). Lactic acid bacteria in sourdough can break down lactose in milk into lactic acid (Kumalasari et al., 2013), improve the quality of bread (Mert et al., 2014), and enhance taste, texture, shelf life, and nutritional content (Hati et al., 2013; Wolter et al., 2014; Mokoena, 2017; Wang et al., 2021). Sourdough bread can be a functional bakery product because previous studies have shown that it can improve the quality of dietary fiber complexes, release bioactive peptides, boost mineral and vitamin absorption, lower the glycemic index of bread, and produce nutritionally beneficial chemicals through microbial metabolism, such as peptides, functional amino acid derivatives, and prebiotics (Chis et al., 2019; Paculary-Burada et al., 2020; Rashmi et al., 2020).

Riana et al. (2021) investigated the use of natural yeast obtained from pepino fruit (Solanum maricatum) extract in the production of Indonesian honeycomb cake. The pepino fruit was hardened using a 1:1 ratio of fruit and water for 24 hours, and the resulting brewed water was given a substrate of wheat flour and water in a 1:1 ratio every 24 hours for two days. The organoleptic tests revealed that sourdough made an appealing cake with a texture that could substitute commercial yeast in Indonesian honeycomb cake. Another study (Putra et al., 2018) explored the use of natural yeast derived from yogurt in the production of sweet bread with various concentrations. The study recommended a natural yeast concentration of 30% as the best treatment for producing sweet bread with a pH range of 3.60-3.65, moisture content of 29-33% w/w, expansion volume of 193.53%, and a total lactic acid bacteria count of 9.08 log CFU/g. Zaidiyah et al. (2020) developed natural yeast from pineapple fruit juice, which was heated and fermented for ten days, and then used as a yeast mixture in bread making. The research findings showed that the optimal natural yeast ratio of 4:1 exhibited the best bread-making quality. Studies examining the effect of fermentation time on the production of natural yeast are not yet well-established. The choice of papaya as a natural yeast source is attributed to its immense potential in Indonesia. Despite its limited utilization as a derivative product, there is still limited studies or findings of using papaya as natural source to obtain wild yeast. In this study, sourdough was prepared using fermented water from papaya, and fermentation time variations were evaluated (4, 5, and 6 days). The resulting sourdough was then utilized to produce donuts with a 30% concentration.

MATERIALS & METHODS

This research was conducted in multiple stages. Firstly, chemicals and materials were prepared, followed by pre-treatment in the form of grinding and heating of papaya. The papaya was then fermented for 4, 5, and 6 days with variations in fermentation time. The resulting fermented water was then given a substrate five times to form sourdough, which would be used as a natural yeast to ferment donuts or bread dough.

Chemicals and materials

Honeydew papaya (Carica Papaya L.) was obtained from the local market in Pekanbaru, Indonesia, and used to prepare papaya juice. Commercial high-protein wheat flour with 12% protein content (Bogasari Flour Mills, Indonesia) was used as the substrate to prepare sourdough, while medium-protein flour (10% protein content) was used to make donuts. All chemicals used in this study were of analytical grade.

Fruit preparation and fermentation

The fermentation process began with the sterilization of the jars, which were washed until clean and then heated in a pan for 5 minutes (Zaidiyah et al., 2020; Ko, 2012). After boiling, the jars were removed and allowed to cool. The papaya was peeled, cleaned, and mashed with a blender (Philips Daily Juicer HR 1811, Indonesia) for 5 minutes, then filtered. The papaya fruit juice was then heated in a pan for 10 minutes. After heating, 500 g of papaya juice was put into a sterile jar, distilled water (1000 g), and sugar (75 g) were added and stirred thoroughly. The jar was not closed too tightly to prevent it from exploding or breaking due to the large amount of carbon dioxide gas produced during fermentation. Fermentation was carried out for 4, 5, and 6 days at room temperature.

Substrate addition

The substrate was added to the sourdough as a food source for the bacteria contained within it. Periodic substrate supplementation can maintain bacterial activity and enable sourdough to perform fermentation. Referring to the study conducted by Putra et al. (2018), the substrate was added five times to the fermented papaya water to obtain sourdough. The first substrate was added by mixing high-protein flour with fermented papaya water to form a sourdough starter. The second to fifth substrate provisions were carried out by adding high-protein flour and water to the sourdough starter, which was formed from providing the previous substrate.

Dough preparation and donut production with sourdough

Referring to the previous study (Putri & Windiana, 2019), donut production with sourdough began by mixing several ingredients, including high-protein flour, medium-protein flour, sugar, milk, eggs, and water, then stirring until blended for 5 minutes. The initial dough was rested for 3 hours for the autolysis process (Nurfatihayati et al., 2022). Autolysis aims to give gluten formation time ideally. After the autolysis stage, sourdough was added and kneaded until well blended for 10 minutes. The dough was then left for 45 minutes. The remaining ingredients (salt and butter) were added and stirred until smooth and rested for 45 minutes. Then, the dough was rested until it expanded two times its initial volume. The donuts were then fried and topped. In this study, three variations of donuts were made: donut A (using sourdough fermented for 4 days), donut B (using sourdough fermented for 5 days), and donut C (using sourdough fermented for 6 days).

Sourdough analysis

The pH of the fermented papaya water was tested using a pH meter (Yinmik EZ-9901, China) in accordance with SNI 01-2982-1992. The bacterial culture test was performed using the pour plate method (A'yuni et al., 2020). Samples of fermented papaya juice were diluted with sterile NaCl and inoculated on Nutrient Agar (NA) medium, then incubated at 37 °C for 24 hours. The colonies growing on the NA medium were counted using a colony counter (Suntex 570, Taiwan). After obtaining the number of colonies in the sample (CFU/ml), the total number of bacterial colonies (Total Plate Count) was calculated using the following formula:

$$\text{Total Plate Count (TPC)} = \text{Colonies Number} \times \frac{1}{\text{Dilution Factor}} \quad (1)$$

A sourdough activity test was conducted at each substrate application, following the standard method of SNI 01-2982-1992 with modifications in the amount of materials and observation time. Initially, pH and TPC tests were performed thrice, and the average values were analyzed by ANOVA followed by the Scheffe method using IBM SPSS Statistic 27 software. The naturally occurring yeast was transferred to a sterilized jar and mixed with water and flour in a 1:1:1 ratio, stirred well, and left for 3-5 hours. The volume expansion of the yeast in the jar (maximum height before shrinkage and time required to reach maximum volume) was observed and recorded. The sourdough activity was calculated using the following equation:

$$\text{Sourdough Activity} = \frac{\text{Final Volume}}{\text{Initial Volume}} \quad (2)$$

Analysis of donuts with sourdough

Organoleptic tests were conducted to determine consumer preference for the color, taste, aroma, and texture of the produced donuts. Following Lamusu (2018), untrained panelists with an age range from 17 to 45 years old were asked to express their preference for the tested material's properties. The test was conducted using a scoring method, and 30 panelists were included. Each panelist was given three variants of donuts (donuts A, B, and C) to evaluate their level of preference for the four criteria, including color, taste, aroma, and texture (ease of biting and chewing). The samples presented were coded randomly to avoid a particular interpretation by the panelists. The criteria for evaluating the organoleptic test are presented in Table 1. An average score was calculated for each aspect from the organoleptic test data, and an average similarity test was conducted with ANOVA, followed by the Scheffe method to determine the significant difference in the produced donuts.

Table 1. Organoleptic test rating scale

Hedonic Scale				Numeric Scale
Color	**Taste**	**Aroma**	**Texture**	
Brown	Delicious/Sweet	Good/Bread	Soft	4
Pale Brown	Slightly Sour	Ordinary	Slightly Soft	3
Dark Brown	Sour	Slightly Rancid	Slightly Hard	2
Black	Bitter	Rancid	Hard	1

A mold visualization test was conducted to assess the ability of donuts made with natural yeast to resist mold growth, which could lead to spoilage of baked goods. The test protocol followed that of a previous study (Putra et al., 2018). Each donut sample was placed in a sterile container and left at room temperature. The emergence and growth of mold were monitored visually on a daily basis. A positive sign (+) was used to indicate the presence of mold.

RESULTS & DISCUSSIONS

pH analysis of fermented papaya

The pH of papaya juice during the fermentation process was measured daily using a pH meter. The test results are presented in Table 2. The results of the observations and variance analysis showed that the fermentation duration had a significant effect on the pH of the fermented water. The measurement data in Table 2 show that the pH of the fermented papaya juice decreased over time, indicating bacterial activity in processing glucose into lactic acid, carbon dioxide, and ethanol. This is in line with the previous study conducted by Winarno (2008) that described the fermentation reactions:

$$C_6H_{12}O_6 \rightarrow C_3H_6O_3 + CO_2 + C_2H_5OH$$

glucose → lactic acid + carbon dioxide+ ethyl alcohol (ethanol)

The studies conducted by Devita et al. (2019) and Oshiro et al. (2020) have reported a similar trend of decreasing pH during the fermentation process. This phenomenon is attributed to the production of acidic compounds resulting from glucose decomposition by lactic acid bacteria.

Table 2. Effect of time on the pH of fermented papaya water (n=3)

Fermentation time (Day)	pH	Std. Deviation	Sig.
1	5.28[a]	0,04359	1.00
2	4.74[b]	0,04041	1.00
3	4.29[c]	0,03215	1.00
4	3.81[d]	0,02082	0.27
5	3.73[d]	0,02646	0.27
6	3.54[e]	0,05292	1.00

Note: Numbers followed by the same letter in the same column show no significant difference at 5% significance.

Microbiological analysis

Microbiological analysis was performed by calculating the total bacterial colony, as shown in Table 3. The table illustrates an increase in the total number of bacterial colonies as the fermentation time progressed. Therefore, it can be assumed that the number of lactic acid bacteria also increased with prolonged fermentation time. This can happen because, during the fermentation process, lactic acid bacteria metabolize sugar (Primurdia & Kusnadi, 2014).

Table 3. Effect of fermentation time on TPC (n = 3)

Fermentation day	TPC (CFU/ml)	Sig.
4	6.00×10^{2} [a]	1.00
5	2.43×10^{3} [b]	1.00
6	1.70×10^{4} [c]	1.00

Note: Numbers followed by the same letter in the same column show no significant difference at 5% significance.

Table 3 shows that the duration of fermentation on the fourth, fifth, and sixth days had a significant effect on the number of bacteria present in the fermented water. The longer the fermentation time, the more glucose in the papaya juice would be metabolized by the bacteria. This result is consistent with the study by Hawashi et al. (2018), which showed that the bacterial count increased with the progression of the fermentation process. During fermentation, lactic acid bacteria can break down glucose into lactic acid and other simple sugars such as lactose, galactose, fructose, sucrose, and maltose. Lactic acid bacteria use fructose and glucose in fruit juice as a source of carbon and nitrogen to grow during fermentation (Al-Fahrozi et al., 2017).

Sourdough activity test

Fermented papaya water with varying fermentation times of four, five, and six days were used to create a sourdough starter by adding substrate five times. The sourdough was then used to ferment dough for the production of bakery products. Figure 1 shows the comparison of volume expansion for each substrate addition, while Figure 2 displays the time taken for the sourdough to reach maximum volume.

Figures 1 and 2 show that the volume of sourdough expands 2 to 3 times its initial volume after each addition of substrate. The inconsistent volume expansion is caused by the substrate application process carried out at room temperature, which leads to fluctuations in fermentation activity by lactic acid bacteria. The fermentation process runs faster at higher temperatures (Calvert et al., 2021). With increasing fermentation time, the addition of substrate to the four-day fermented papaya water resulted in growth acceleration on the second substrate addition, which lasted for three hours and then became stagnant until the fifth substrate addition. For the five-day fermentation, the sourdough experienced accelerated growth in the second substrate addition, with a growth time of three hours, while the fifth substrate addition resulted in a growth time of two hours. In contrast, for the six-day papaya water fermentation, the sourdough expanded on the second day, lasting for three hours, and the fourth substrate addition resulted in a growth time of two hours.

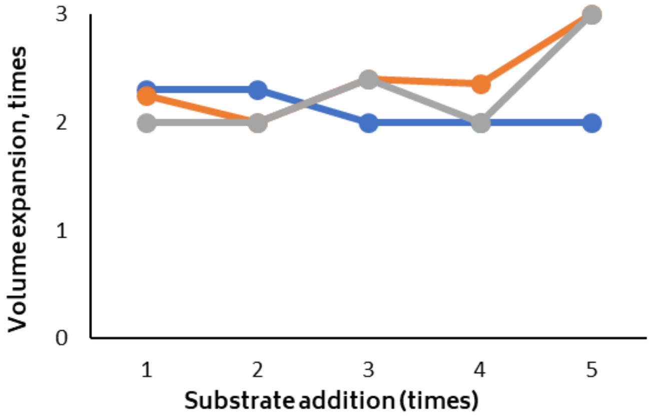


Figure 1. Volume expansion of sourdough after substrate addition in fermented papaya water for four days (blue), five days (orange), and six days (grey) fermentation time

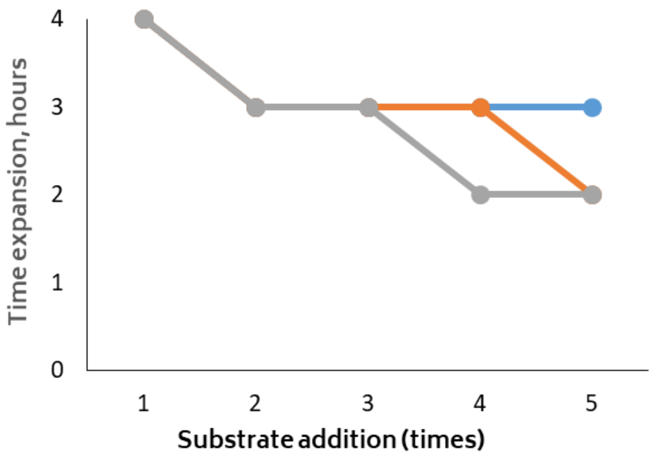


Figure 2. Time expansion of sourdough after substrate addition in fermented papaya water for four days (blue), five days (orange), and six days (grey) fermentation time

Table 4. Effect of yeast fermentation time on donuts quality

Donut	Color	Taste	Aroma	Texture
A	3.73	3.13[b]	3.80	3.60
B	3.80	4.00[a]	4.00	3.67
C	3.80	4.00[a]	3.87	3.87
Sig.	-	<0.01	-	-

Note: Numbers followed by the same letter in the same column show no significant difference at 5% significance.

Based on the results, it can be assumed that sourdough grew more substantially with the continuous addition of substrate, resulting in faster expansion of yeast. However, several factors could cause fluctuations in sourdough expansion, including room temperature conditions that tend to fluctuate, leading to irregular growth. Additionally, the immaturity of the sourdough can also affect its resistance to significant environmental changes (Carbó et al., 2020). A previous study (Calvert et al., 2021) has shown that fermentation time and temperature are two factors that influence yeast activity.

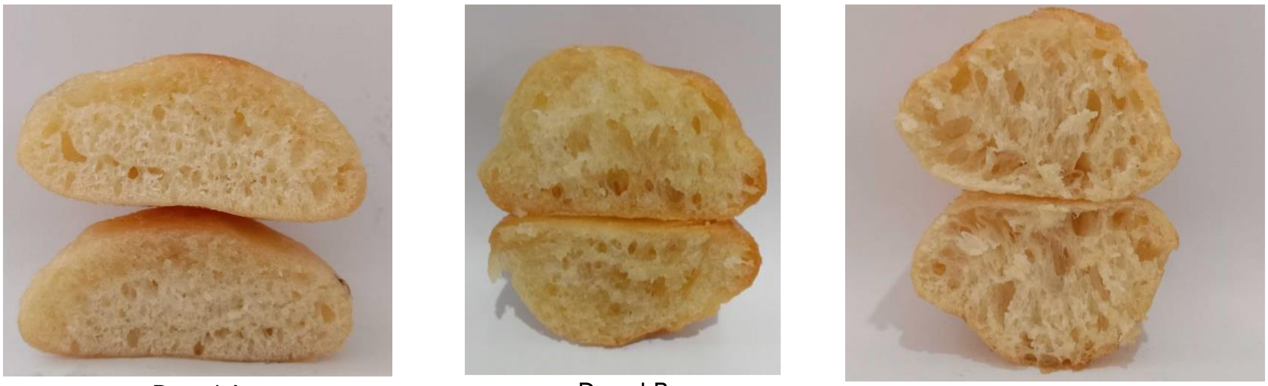
Organoleptic test of sourdough donut

After the preparation of the donuts, they were subjected to organoleptic testing by untrained panelists. The test results have been presented in Table 4 after the data analysis. From Table 4, it is evident that the difference in fermentation time significantly impacted the taste of the produced donuts. Donut A received a score of 3.13 for taste quality, indicating that the panelists found it slightly sour. In contrast, donuts B and C had a sweet taste as judged by the panelists. Hence, donut A did not meet the criteria of SNI 01-3840-1995 due to its sour taste. During the preparation of donut A, it was observed that the dough took 5.5 hours to double in volume and be ready for further processing. Sourdough with a fermentation time of 4 days is known to be dominated by homofermentative bacteria that produce primarily lactic acid, which imparts a more pungent sour taste. Homofermentative bacteria generate few by-products such as glycerol, ethanol, acetic acid, formic acid, and CO_2 during fermentation. The production of CO2 as a by-product of fermentation slowed down the dough expansion process. Homofermentative lactic acid bacteria oxidize glucose to 2 pyruvates through the EMP pathway, producing 4 ATP + NADH to reduce pyruvate to lactic acid (Bangun, 2009). The overall reaction is as follows:

$$C_6H_{12}O_6 + 2\ ADP + 2\ P \rightarrow 2C_3H_6O_3 + 2\ ATP$$

glucose + 2 adenosine diphosphate + 2 pyruvate → lactic acid +adenosine triphosphate

Donuts B and C expanded within three hours and did not taste sour, presumably because heterofermentative lactic acid bacteria were more dominant than homofermentative lactic acid bacteria. Heterofermentative bacteria produce both carbon dioxide and lactic acid, resulting in faster bread expansion. Additionally, Venturi et al. (2013) reported that changes in storage temperature could affect product characteristics. When the ratio of lactic acid bacteria to yeast was 10:1, the donuts expanded, and the sourness was acceptable to consumers. However, when the ratio was 100:1, the yeast concentration was insufficient, resulting in inadequate donut expansion and sourness. Concerning texture, most panelists agreed that donuts made with six-day fermented papaya sourdough or donut C were the softest. Figure 5 depicts the texture of the bread interior, where more voids led to a softer texture, such as that of donut C.



Donut A Donut B Donut C

Figure 3. Comparison of the texture of donuts using sourdough from fermented papaya in four days (donut A), five days (donut B), and six days (donut C) of fermentation time

Mold visualization

The results of the eight-day storage test are presented in Table 5. Donuts A and B showed mold growth on the seventh day after storage, while donut C did not show any mold growth until the eighth day. However, further observations revealed that donut C started to grow mold on the eleventh day. The shelf life of bread products made with natural yeast from papaya fruit was found to be longer than bread without preservatives made with commercial yeast, which typically only lasts for two days at room temperature, as reported in previous research (Rustanto et al., 2019). Mold growth on the donut was indicated by the appearance of white, black, or greenish spots, and the donuts began to harden. Storage was affected by several factors, including temperature, degree of acidity (pH), humidity, light, oxygen, and nutrients or food ingredients contained in the bread. Research conducted by Mohsen et al. (2016) showed that bread containing natural yeast had an extended shelf life of up to 8 days, while ordinary bread only lasted two days at room temperature before mold growth was observed. Therefore, it can be concluded that using sourdough could help maintain the quality and extend the shelf life of bakery products.

Table 5. Observation results of mold visualization on donuts during storage

Day	Mold visualization		
	Donut A	Donut B	Donut C
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	+	+	-
8	++	+	-

The use of sourdough fermented from papaya water as yeast in the production of bakery products was found to produce bread with higher preference among panelists in terms of quality (taste and texture) compared to bread made with sourdough based on tubers, as reported by Fadiati (2021). Additionally, sourdough from papaya fruit exhibited better fermentation ability as it was able to expand up to 3 times, in contrast to sourdough from pineapple, which could only expand a maximum of 1.5 times, as reported by Zaidiyah et al. (2020).

CONCLUSION

The present study successfully produced sourdough from fermented papaya using pre-treatment, fermentation, substrate addition, and bread/donut production. The quality of the sourdough was found to be affected by different fermentation time treatments, with increasing activity observed as the fermentation process progressed. The six-day fermentation process yielded the best sourdough yeast, with a pH of 3.54, the highest number of bacterial colonies (1.70×10^4 CFU/ml), and yeast volume development of 2 to 3 times with each addition of substrate. The yeast expanded maximally within 2 hours when the fourth and fifth substrates were added. Furthermore, the best donuts were obtained from the six-day fermented yeast, with an almost perfect rating in all aspects (color, aroma, taste, and texture) of 3.9 out of a scale of 4. These donuts can last up to 8 days without mold growth. Our findings suggest that the use of sourdough from fermented papaya fruit can improve the quality and extend the shelf life of bakery products, providing a promising alternative to traditional methods that rely on commercial yeast and preservatives.

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