

Potential of Endophytic Yeast *Candida sanyaensis* and *Candida* sp. from Nira Siwalan (*Borassus flabellifer* L.) as a Bread Dough Leavening Agent

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Abstract. The increasing global demand for bread has driven the bakery industry to seek alternative leavening agents to reduce dependence on commercial yeast imports. Endophytic yeasts isolated from local agricultural sources offer promising potential for sustainable bread production. This study aimed to evaluate the potential of endophytic yeast isolates *Candida sanyaensis* and *Candida* sp., previously isolated from Nira Siwalan (*Borassus flabellifer* L.), as bread dough leavening agents. The research assessed the tolerance of both yeast isolates to various temperatures (30°C, 37°C, and 45°C) and ethanol concentrations (10%, 13%, and 15%) by measuring optical density using UV-Vis spectrophotometry at 24 and 72 hours of incubation. Additionally, the quality of bread fermented by these isolates was evaluated through texture profile analysis (hardness, cohesiveness, adhesiveness, and gumminess) and color analysis (L, a, b* values) using a texture analyzer and color reader, respectively. Commercial instant yeast (*Saccharomyces cerevisiae*) served as a positive control, while dough without yeast served as a negative control. The results demonstrated that both *Candida sanyaensis* and *Candida* sp. were tolerant to high temperature (45°C) and high ethanol concentration (15%), as indicated by increased cell density from 24 to 72 hours of incubation. Bread fermented with both endophytic yeast isolates exhibited texture and color characteristics similar to those of bread fermented with commercial yeast, with a soft texture and a light brown color. Statistical analysis using ANOVA and DMRT at the 5% significance level revealed no significant differences in texture and color parameters between bread produced with the endophytic yeast isolates and the commercial yeast control. These findings suggest that *Candida sanyaensis* and *Candida* sp. isolated from Nira Siwalan (*Borassus flabellifer* L.) possess promising potential as alternative bread dough leavening agents, offering a sustainable approach to reducing dependency on imported commercial yeast in the bakery industry.

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INTRODUCTION

The most frequently consumed food product by commodity groups in Indonesia is bread. The per capita weekly consumption of white bread and sweet bread among Indonesians is 0.3 ounces and 1.1 ounces, respectively (BPS, 2022). The average per capita bread consumption in Indonesia in 2022 was 18,411 slices of white bread and 54,419 slices of sweet bread. Bread sales in Indonesia are among the highest in Southeast Asia, at 2.6 trillion. This is shown by the increase in the number of bread industries in Indonesia, reaching 600 units by April 2022. The

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rising demand for bread in Indonesia has increased the need for commercial yeast. Yeast plays a role in the fermentation process that produces carbon dioxide gas. Yeast helps convert starch into sugar, where the reducing sugar reacts with amine groups at high temperatures, producing the brown color in bread (Sitepu, 2019). The commercial yeast used is *Saccharomyces cerevisiae*. A decrease in the amount of commercial yeast will certainly make it difficult for the bakery industry. The dependence on commercial yeast can be overcome through research and development related to commercial yeast substitutes.

Nira Siwalan is a sweet liquid extracted from the flower clusters of the siwalan tree (*Borassus flabellifer* L.). *Candida sanyaensis* and *Candida* sp. isolated from Nira Siwalan have potential as bread dough developers. This is indicated by the ability of these yeasts in several tests, such as the carbohydrate fermentation test, glucose tolerance test, hydrogen sulfide test, and flocculation test. Yeast isolates *Candida sanyaensis* and *Candida* sp. ferment 5 types of carbohydrates, including fructose, sucrose, glucose, lactose, and maltose, and convert these sugars into carbon dioxide and ethanol, which play a role in the development of bread dough (Struyf et al., 2017). In addition, both yeast isolates tolerated high glucose concentrations (50%) and survived during bread dough baking (Karki et al., 2017; Maryam et al., 2017). The yeast isolates *Candida sanyaensis* and *Candida* sp. do not produce hydrogen sulfide and can form flocs (Stewart, 2018).

In this research, the temperature tolerance test, ethanol tolerance test, bread dough volume development test, bread texture test, and bread color test will be conducted. In the temperature tolerance test, this research uses temperature variations of 30°C, 37°C, and 45°C. The variation aims to determine the temperature tolerance of both yeast isolates during bread dough fermentation. A good temperature range for yeast growth in bread dough is 25°C–32°C. The highest temperature limit for bread dough developer yeast is 40 °C to 45 °C. The ethanol tolerance test in this research used concentrations of 10%, 13%, and 15%. This aims to ensure that yeast isolates survive and remain active during bread dough fermentation, enabling them to produce optimal dough volume and bread texture. Yeast grows well in the dough at ethanol concentrations between 10% and 13%, and the highest ethanol tolerance limit in bread dough developer yeast is 15% (Tsegaye et al., 2018).

The bread dough development volume test measures the level of bread dough development using *Candida sanyaensis* and *Candida* sp. The volume of bread dough development is determined by the ability of bread dough to retain carbon dioxide gas produced during the fermentation process. Texture and color tests on bread were conducted to determine the ability of yeast isolates *Candida sanyaensis* and *Candida* sp. to ferment bread dough. A good fermentation process can yield high-quality bread with a soft, chewy texture and a brown color typical of bread (Zainab & Azizah, 2022). This research aims to determine whether *Candida sanyaensis* and *Candida* sp. yeasts isolated from Nira Siwalan (*Borassus flabellifer* L.) exhibit good qualities as bread dough leavening agents.

MATERIALS AND METHODS

The research was conducted from May to July 2023. The endophytic yeast isolates *Candida sanyaensis* and *Candida* sp. were obtained from the Microbiology Laboratory collection of the Biology Program, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang. The instruments used in this research were a spectrophotometer (Thermo Scientific), Countess II FL, a Conica Minolta CR-10 color reader, and a Shimadzu texture profile analyzer (TPA EZ Test model SM-500N-168). The materials used in this research were Yeast Malt Extract Agar (YMEA), Yeast Peptone Glucose Broth (YPGB), Yeast Malt Broth (YMB), sodium DL-lactose, trypan blue, and bread dough (wheat flour, sugar, salt, butter, and water).

Preparation and Rejuvenation of Yeast Isolates

Endophytic yeast isolates *Candida sanyaensis* and *Candida* sp. isolated from Nira Siwalan (*Borassus flabellifer* L.) were collected from the Microbiology Laboratory collection of the Biology Study Program, Faculty of Science and Technology, State Islamic University Maulana Malik Ibrahim Malang. Instant yeast isolate (positive control) came from the isolation of *Saccharomyces cerevisiae* from Fermipan. *Saccharomyces cerevisiae* is done using multilevel dilution. Rejuvenation of yeast isolates was carried out aseptically in a LAF (Laminar Air Flow). One loop of yeast isolate was inoculated on YMEA (Yeast Malt Extract Agar) solid medium by the streak plate method (Figure 1). The culture was incubated at 28°C for 48 hours. Then, it is stored in a refrigerator with a temperature of 0°C-1°C.

Propagation of yeast isolates

Propagation of yeast isolates was done aseptically in LAF (Laminar Air Flow) by growing one ose of yeast isolate on 10 ml of YMB (Yeast Malt Broth) liquid media and incubating on a shaker at 140 rpm at 33°C for 24 hours (Zohri et.al., 2017).

Temperature Tolerance Test

The temperature tolerance test was performed by growing 1 ml of yeast isolate from 48-hour-old YMB (Yeast Malt Broth) media, then inoculating into 9 ml of YPGB (Yeast Peptone Glucose Broth) media. The sample was homogenized by shaking. Then, it was incubated at 30°C, 37°C, and 45°C for 72 hours with three replicates. The negative control used was YPGB media without yeast isolates. The concentration or number of yeast is determined by measuring the absorbance of 1 ml of yeast isolate from YPGB media in a cuvette tube at 3 temperatures. Then, place it in the UV-Vis spectrophotometer at 600 nm (Nasir et al., 2017).

Ethanol Tolerance Test

The ethanol tolerance test was conducted by inoculating 1 ml of 48-hour-old yeast isolate from YMB (Yeast Malt Broth) media into YPGB (Yeast Peptone Glucose Broth) media with various ethanol concentrations, including 10%, 13%, and 15%. The 10% ethanol solution was prepared by adding 1 mL of ethanol to 9 mL of YPGB media. The 13% concentration was prepared by adding 1.3 mL of ethanol to 8.7 mL of YPGB media. The 15% ethanol concentration was prepared by adding 1.5 ml of ethanol to 8.5 ml of YPGB media, and each ethanol concentration was repeated 3 times. Then incubated at 30°C. The negative control of this study was YPGB media without yeast isolates. The absorbance was measured by adding 1 ml of yeast isolate from the YPGB media with 3 ethanol concentrations to a cuvette tube. Then, place it in the UV-Vis spectrophotometer at 600 nm (Nasir et al., 2017).

Yeast Cell Count

Candida sanyaensis yeast isolate, *Candida* sp. yeast isolate, and *Saccharomyces cerevisiae* isolate were taken 100 µL and cultured in 15 mL Eppendorf tubes containing 10 mL of YPGB medium. Incubated for 48 hours at 30°C. Centrifuged for 15 minutes at 4000 rpm. The centrifuged supernatant was discarded, and the pellet was weighed. The number of cells was calculated by preparing the same biomass (2 g). Each yeast isolate was added to 1 ml of sterile distilled water and homogenized by pipetting up and down several times. The number of yeast cells was determined by adding 10 µL of yeast isolate cell suspension to 10 µL of 0.4% trypan blue dye and homogenizing by pipetting up and down several times. Then, it was stained and transferred into a semicircular sample slide container. The sample mixture was allowed to stand for 30 seconds. Then the slide was inserted into the slide port. The cell count of each yeast isolate will be displayed on the Countess II FL screen (Chadwick et.al., 2016) (Figure 2)

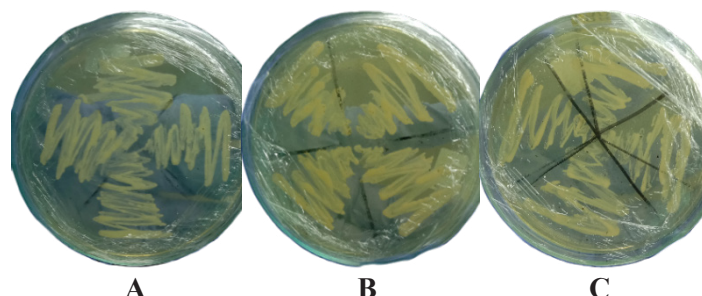


Figure 1: Typical morphological characteristics (A) *Candida sanyaensis*, (B) *Candida* sp., (C) *Saccharomyces cerevisiae*

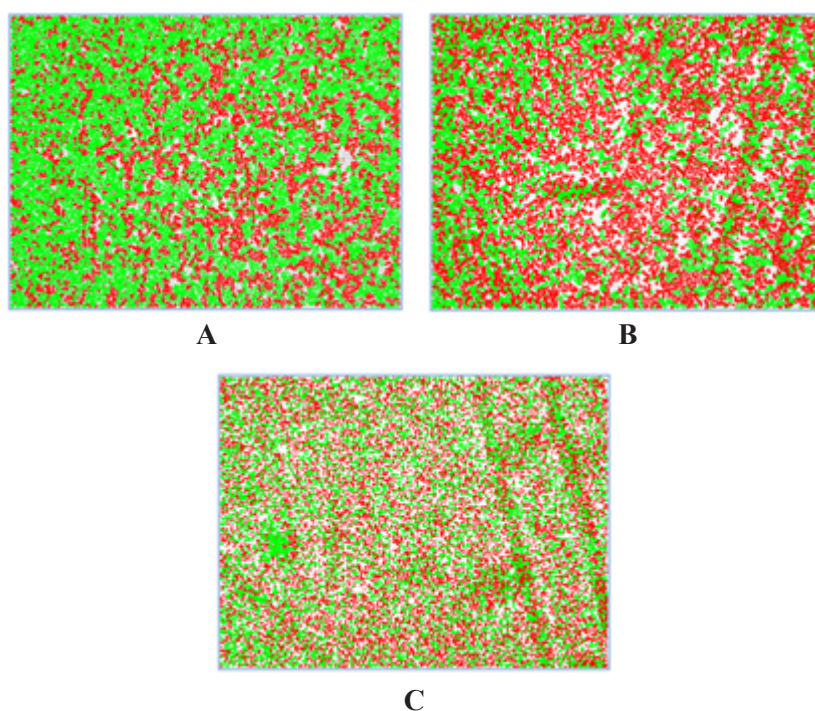


Figure 2: Number of live cells (green) and number of dead cells (red). (A) *Candida sanyaensis*, (B) *Candida* sp., (C) *Saccharomyces cerevisiae*

Yeast Biomass

Yeast biomass was obtained by growing 100 μ l each of *Candida sanyaensis* yeast isolates, *Candida* sp. yeast isolates, and *Saccharomyces cerevisiae* isolates from YMB medium into 15-ml Eppendorf tubes containing 10 ml of YPGB medium, for a total of 10 tubes. Incubated at 30°C for 48 hours. Centrifuged at 4000 rpm for 15 minutes. The supernatant from the centrifugation was discarded, and the pellet was weighed. Weighing of yeast biomass was done using the formula:

$$B = B_2 - B_1$$

Description:

B = Biomass obtained (gram)

B₂ = Eppendorf tube containing yeast biomass (gram)

B₁ = Empty Eppendorf tube (gram)

Bread Dough Making

The pellets used in bread dough have the same number of live cells. The pellet used is the wet weight of each yeast isolate (Table 1). The pellets were then activated for 5-10 minutes. Bread dough was made by mixing 100 g flour, 1.5 g salt, 7.5 g sugar, 8 g butter, 35 ml water, and yeast pellets (2.6: 4.4: 2). After the dough was smooth, it was put into a 9 cm jar and closed. The bread dough was incubated at 30°C for 2 times the initial volume of bread dough development in each yeast isolate. Then bake in the oven at 180°C for 20 minutes (Karki et al., 2017).

Table 1. Determination of live cell count and biomass (100 gram flour)

Yeast Isolate	Total Biomass	Number of Cells
<i>Candida sanyaensis</i> (CS)	2.6 gram	6.22 x 10 ⁷ cells/ml
<i>Candida</i> sp. (C)	4.4 gram	6.22 x 10 ⁷ cells/ml
<i>Saccharomyces cerevisiae</i> (K ⁺)	2.0 gram	6.22 x 10 ⁷ cells/ml

Bread dough development volume test

The bread dough development volume test was conducted by measuring the difference between the final and initial volume. The volume of bread dough was measured every 10 minutes over a 2 times development volume interval for each yeast isolate. Measurements were made using a ruler and calculated based on the tube volume formula (Satrianawati, 2015):

$$V = \pi r^2 t$$

Description:

V = volume of the tube

$\pi = 22/7$ or 3.14

r = radius of the tube

t = tube height

Bread texture test

The bread texture test was performed on bread baked with a texture profile analyzer. The bread texture test was conducted by preparing 20 bread samples across 4 treatments, with 5 repetitions per treatment. Bread texture test parameters are hardness, cohesiveness, adhesiveness, and gumminess. The texture profile analyzer and computer were turned on by pressing the button located at the back of the tool. The TA 18 probe was mounted on the probe holder. Then the probe and the object table were set at a distance so as not to hit the object with the probe located approximately ± 0.5 cm from the sample. The Texture ProLite program was opened by clicking the Define New Test section and entering the trigger point, test speed, target value, and probe type.

The sample is measured to be 4 mm thick using a ruler. The target test section is filled in, and the compression 'fill Texture Results' is selected according to the sample parameters. The primary calculation section is clicked on everything except the cycle 1 and 2 areas (for hardness sample measurements), then the secondary calculation menu is clicked on the work done to the hardness 1 section. After that, the additional calculations are clicked on the sample length section. Fill in the general results section. All standard results sections in the general results tab are clicked, except special results. The probe is left to calibrate first. Then the sample is placed back on the object table, and the run test button is pressed to run the texture measurement. Then press the view load/timchart button to see the entire measurement results. After the tool stops working, the texture data will be obtained, and the file will be saved in the folder (Johnson & Szczesniak, 2014).

Bread Color Test

The bread color test was performed on bread baked with a CR-10 color reader. The bread color test was conducted by preparing 20 bread samples across 4 treatments with 5 repetitions per treatment. Samples were prepared in transparent plastic or in PP (polypropylene). The lens cover was removed, and the color reader was turned on. Then the color measurement button was pressed, and the value indicated on the digital screen was recorded. The L* value indicates brightness with positive (+) values meaning bright, and negative (-) values meaning dark. The a* value indicates the degree of redness or greenness, with positive values (+) meaning red, and negative values (-) meaning green. The b* value indicates the degree of yellowish or bluish, with a positive value (+) meaning yellow, and a negative value (-) meaning blue (Konica Minolta, 2007).

Statistical Analysis

Data were analyzed using SPSS version 25. Research data from the temperature tolerance test, ethanol tolerance test, and bread quality test were analyzed by ANOVA at the 5% significance level and were followed by a DMRT.

RESULTS AND DISCUSSION

Temperature Tolerance

Candida sanyaensis yeast isolates and *Candida* sp. yeast isolates experienced an increase in cell density at temperature variations of 30°C, 37°C, and 45°C (Table 2). The lowest cell density at 30°C was observed in the positive control (*Saccharomyces cerevisiae*), which was 0.29 (after 24 hours) and 0.50 (after 72 hours). The highest cell density at 30°C was observed with the yeast isolate *Candida sanyaensis*, reaching 0.58 (after 24 hours) and 0.59 (after 72 hours). The lowest cell density at 37°C was observed with the yeast isolate *Candida* sp., which was 0.18 (after 24 hours) and 0.30 (after 72 hours). The highest cell density at 37°C was observed in the positive control (*Saccharomyces cerevisiae*), which was 0.25 (after 24 hours) and 0.33 (after 72 hours). The lowest cell density at 45°C was observed in the positive control (*Saccharomyces cerevisiae*), which was 0.23 (after 24 hours) and 0.37 (after 72 hours). The highest cell density at 45°C was observed with the yeast isolate *Candida sanyaensis*, reaching 0.27 (after 24 hours) and 0.40 (after 72 hours).

Gaglio et al. (2022) confirmed that indigenous yeast isolates exhibited temperature-tolerance profiles similar to those of commercial baker's yeast, supporting the feasibility of using local yeast resources for bread production. According to Struyf et al. (2020), yeast tolerance to high temperatures is essential for efficient bread dough fermentation, particularly during baking. This finding is consistent with recent studies on thermotolerant yeasts for food applications (Ma et al., 2020; Zotta et al., 2023). Similarly, Rashid et al. (2022) reported that thermotolerant yeast strains maintain metabolic activity at elevated temperatures, ensuring proper dough leavening. The thermotolerance capability observed in isolates aligns with the characteristics of potential baker's yeasts described by Karki et al. (2017) and Tsegaye et al. (2018). Both *Candida sanyaensis* and *Candida* sp. demonstrated the highest cell densities at 30°C, indicating their optimal growth temperature. In addition, the ability of *Candida sanyaensis* and *Candida* sp. to grow at 45°C demonstrates their thermotolerance, a desirable characteristic for baker's yeast applications. The ability to maintain growth at 45°C is particularly important for bread making, as it enables yeast to survive during the initial stages of baking and potentially accelerates fermentation (Tsegaye et al., 2018).

The increase in optical density values from 24 to 72 hours at all tested temperatures confirms that both isolates remain metabolically active during the exponential growth phase. According to Kurniawan et al. (2014), the exponential phase of yeast growth occurs between 24 and 72 hours,

during which cell numbers increase significantly due to continuous cell division. The optimal temperature range for baker's yeast fermentation is typically 25-32°C, with a maximum tolerance of 40-45°C. The thermotolerance mechanism in yeasts involves multiple cellular adaptations, including the production of heat shock proteins (HSPs), modifications in membrane lipid composition, and accumulation of compatible solutes such as trehalose (Choudhary et al., 2016). Trehalose acts as a membrane protectant, helping to maintain protein structure under heat stress. The comparable growth patterns between the endophytic *Candida* isolates and commercial *Saccharomyces cerevisiae* suggest that these isolates possess similar thermoprotective mechanisms.

Table 2. Temperature tolerance of endophytic yeast isolates *Candida sanyaensis* and *Candida* sp

Sample	30°C		37°C		45°C	
	24 hour	72 hour	24 hour	72 hour	24 hour	72 hour
<i>Candida sanyaensis</i> (CS)	0.58±0.34 ^b	0.59±0.12 ^b	0.20±0.04 ^b	0.33±0.05 ^b	0.27±0.11 ^b	0.40±0.10 ^b
<i>Candida</i> sp. (C)	0.46±0.11 ^b	0.56±0.21 ^b	0.18±0.05 ^b	0.30±0.05 ^b	0.23±0.21 ^b	0.37±0.02 ^b
<i>Saccharomyces cerevisiae</i> (K ⁺)	0.29±0.07 ^{ab}	0.50±0.15 ^b	0.25±0.07 ^b	0.33±0.05 ^b	0.23±0.01 ^b	0.37±0.01 ^b
without yeast isolate (K ⁻)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Note : Values followed by the same superscript letter within the same column are not significantly different according to DMRT at $\alpha = 0.05$.

There is a correlation between cell density and temperature tolerance. Temperature tolerance refers to a yeast strain's ability to survive, grow, and maintain metabolic activity across various temperature ranges (from optimal to stressful). Higher cell density indicates more living cells that survive and multiply. Lower cell density indicates more cells that die or fail to grow. Yeast with high-temperature tolerance and a higher live-cell density indicates that the yeast can maintain survival and fermentation activity despite suboptimal temperature changes, ensuring optimal dough development and producing a product with good texture. In this research, *Candida sanyaensis* was the yeast capable of tolerating a wide range of temperatures.

Ethanol Tolerance

Candida sanyaensis and *Candida* sp., yeast isolates, showed increased cell density at 10%, 13%, and 15% ethanol concentrations (Table 3). The lowest cell density at 10% ethanol concentration was observed in the positive control (*Saccharomyces cerevisiae*), which was 0.21 (after 24 hours) and 0.22 (after 72 hours). The highest cell density at 10% ethanol concentration was observed with the yeast isolate *Candida* sp., reaching 0.24 (after 24 hours) and 0.30 (after 72 hours). The lowest cell density at 13% ethanol concentration was observed in the positive control (*Saccharomyces cerevisiae*), with values of 0.18 (after 24 hours) and 0.19 (after 72 hours). The highest cell density at 13% ethanol concentration was observed with the *Candida* sp. yeast isolate, at 0.21 (after 24 hours) and 0.23 (after 72 hours). The lowest cell density at 15% ethanol concentration was observed in the positive control (*Saccharomyces cerevisiae*), which was 0.16 (after 24 hours) and 0.17 (after 72 hours). The highest cell density at 15% ethanol concentration was observed with the yeast isolate *Candida sanyaensis*, at 0.17 (after 24 hours) and 0.19 (after 72 hours).

The ability of both *Candida* isolates to grow in media containing up to 15% ethanol indicates their high ethanol tolerance, which is essential for bread dough fermentation. During fermentation, yeasts convert sugars to ethanol and CO₂, and the accumulating ethanol can become inhibitory if

the yeast lacks tolerance mechanisms. The higher ethanol tolerance observed in *Candida* sp. compared to *Candida sanyaensis* at 13% ethanol after 72 hours suggests species-specific differences in ethanol tolerance mechanisms. Ethanol tolerance in yeasts is conferred by multiple factors, including plasma membrane composition (particularly ergosterol content), H⁺-ATPase activity, and the presence of stress-responsive genes. Ethanol can disrupt membrane integrity, denature proteins, and inhibit glucose transport, underscoring the importance of tolerance mechanisms crucial for survival during fermentation. The cell wall also plays a critical role in ethanol tolerance. Yeast cell walls contain various enzymes (amylase, lipase, protease, and cellulase) responsible for regulating metabolism under extreme conditions, including high ethanol concentrations. The cell wall provides mechanical and thermal protection, preventing turgor pressure damage that could compromise the plasma membrane (Zhang et al., 2021).

The observed increase in OD values from 24 to 72 hours at all ethanol concentrations confirms that both isolates maintain metabolic activity despite ethanol stress. These results are supported by recent findings on ethanol-tolerant yeasts for industrial applications (Dalawai & Murthy, 2021; Zhang et al., 2021). Wang et al. (2022) emphasized that ethanol tolerance mechanisms in yeasts involve membrane adaptation and metabolic regulation. Furthermore, Iosca et al. (2023) demonstrated that *Candida* species possess remarkable tolerance to ethanol, making them suitable for various fermentation processes. The ethanol tolerance capability observed was comparable to that reported by Perpetuini et al. (2021) for non-*Saccharomyces* yeasts in baking applications. These findings are consistent with previous studies on endophytic yeasts. Tsegaye et al. (2018) reported that yeast isolates from Ethiopian fruits could tolerate ethanol concentrations up to 15%, with growth observed after 72 hours of incubation. Lakew (2022) similarly found that endophytic yeasts from various fruits exhibited tolerance to 15% ethanol, as indicated by increased optical density values.

There is a correlation between cell density and ethanol tolerance. Ethanol tolerance refers to the yeast's ability to survive, grow, and maintain metabolic activity. Yeast with higher cell density has more living cells that survive and multiply. Yeast with a lower cell density has fewer surviving cells. Yeast with high cell density and high ethanol tolerance maintains cell viability and continues fermentation without significant cell death, resulting in a more stable, higher-quality fermentation product. Based on this research, *Candida* sp. and *Candida sanyaensis* demonstrated superior ethanol tolerance compared to *Saccharomyces cerevisiae*, as evidenced by consistently higher live cell densities at all tested ethanol concentrations after 24 and 72 hours.

Table 3. Ethanol tolerance of endophytic yeast isolates *Candida sanyaensis* and *Candida* sp.

Sample	10%		13%		15%	
	24 hour	72 hour	24 hour	72 hour	24 hour	72 hour
<i>Candida sanyaensis</i> (CS)	0.24±0.03 ^b	0.26±0.04 ^b	0.19±0.01 ^b	0.21±0.02 ^{bc}	0.17±0.02 ^b	0.19±0.01 ^b
<i>Candida</i> sp. (C)	0.46±0.11 ^b	0.30±0.08 ^b	0.21±0.02 ^b	0.23±0.01 ^c	0.18±0.01 ^b	0.18±0.01 ^b
<i>Saccharomyces cerevisiae</i> (K ⁺)	0.24±0.04 ^b	0.22±0.03 ^b	0.18±0.03 ^b	0.19±0.03 ^b	0.16±0.01 ^b	0.17±0.02 ^b
without yeast isolate (K ⁻)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Note : Values followed by the same superscript letter within the same column are not significantly different according to DMRT at $\alpha = 0.05$.

Bread Dough Development Volume

Measurement of bread dough development volume quantifies the increase in bread dough volume during fermentation. The lowest average bread dough development volume is for the negative control (without yeast isolate), at 127.17 cm³. The highest average bread dough development volume is observed in the positive control (*Saccharomyces cerevisiae*), at 239.28 cm³ (Table 4 and Figure 3). Each treatment has the same initial volume of bread dough development (127.17 cm³), but has a different final volume. This depends on the incubation time, which reached 2 times the initial volume of bread dough development in each treatment. The positive control (*Saccharomyces cerevisiae*) had a final bread dough volume of 254.34 cm³ with an incubation time of 100 minutes. The *Candida sanyaensis* yeast isolate had a final bread dough volume of 260.6985 cm³ after 410 minutes of incubation. *Candida* sp. yeast isolate has a final bread dough volume of 260.6985 cm³ with an incubation time of 480 minutes (Table 5). Thus, it can be concluded that *Saccharomyces cerevisiae* is superior in fermentation speed, while *Candida sanyaensis* and *Candida* sp. are slower but achieve a marginally higher final dough volume. *Candida sanyaensis* and *Candida* sp. successfully doubled the dough volume, confirming their fermentative ability.

The volume expansion of bread dough is a critical parameter for evaluating yeast performance, as it directly affects bread texture and overall quality. All three yeast isolates (*Candida sanyaensis*, *Candida* sp., and *Saccharomyces cerevisiae*) successfully expanded the dough, although with different kinetics. The commercial *Saccharomyces cerevisiae* achieved the greatest volume increase in the shortest time (100 minutes), while *Candida sanyaensis* required 410 minutes and *Candida* sp. 480 minutes. The slower fermentation rate of the endophytic isolates compared to commercial yeast is expected, as *Saccharomyces cerevisiae* has been specifically selected and adapted for rapid dough fermentation over decades of industrial use. However, the final volumes achieved by *Candida sanyaensis* (260.70 cm³) and *Candida* sp. (260.70 cm³) slightly exceeded that of *Saccharomyces cerevisiae* (254.34 cm³), indicating that despite slower fermentation, these endophytic isolates have comparable or even superior gas production capacity.

The viability analysis revealed important differences among the yeast isolates. *Candida sanyaensis* showed the highest percentage of live cells (66%), followed by *Saccharomyces cerevisiae* (51%) and *Candida* sp. (44%). These viability differences explain the variations in fermentation performance and dough expansion rates observed in this study. The higher viability of *Candida sanyaensis* correlates with its faster fermentation time (410 minutes to reach double volume) compared to *Candida* sp. (480 minutes). This relationship between viability and fermentation performance is well-established; higher proportions of metabolically active cells lead to more efficient sugar conversion and CO₂ production (Sitepu, 2019). The biomass differences required to achieve equal viable cell counts (2.6 g for *Candida sanyaensis*, 4.4 g for *Candida* sp., and 2.0 g for *Saccharomyces cerevisiae*) reflect variations in cell size, density, and possibly the accumulation of storage compounds such as glycogen and polyphosphates. Biomass weight does not always correlate directly with cell number, as cells may enlarge due to the accumulation of metabolic products.

Table 4. Average of development volume of bread dough using endophytic yeast isolates *Candida sanyaensis* and *Candida* sp.

Parameter	Mean			
	<i>Saccharomyces cerevisiae</i> (K ⁺)	without yeast isolate (K ⁻)	<i>Candida sanyaensis</i> (CS)	<i>Candida</i> sp. (C)
Volume (cm ³)	239.28±34.38 ^c	127.17±0.00 ^a	179.20±51.02 ^b	165.45±43.21 ^b

Note : Values followed by the same superscript letter within the same row are not significantly different according to DMRT at $\alpha = 0.05$

The mechanism of dough expansion involves CO₂ production during alcoholic fermentation. Yeasts secrete enzymes, including invertase, maltase, and zymase, that convert sugars (sucrose, maltose, glucose, and fructose) into ethanol and CO₂ (Shabrina, 2017). The CO₂ gas is trapped by the gluten network in the dough, forming bubbles that expand during proofing and baking (Struyf et al., 2017). The gluten network, composed of glutenin and gliadin proteins, provides elasticity and extensibility, allowing the dough to retain gas and expand (Arif, 2019). The longer fermentation time required by endophytic isolates may relate to their adaptation to different ecological niches. As endophytes originally isolated from Nira Siwalan, these yeasts may have different sugar transport and metabolism kinetics compared to dough-adapted *Saccharomyces cerevisiae*. This is consistent with findings that *Saccharomyces cerevisiae* strains used in baking have undergone specific genetic and metabolic adaptations to dough environments, including specialized regulation of hexose transport genes and rapid transcriptional responses to the sugars present in dough (Cha et al, 2025). However, their ability to ultimately achieve good volume expansion suggests they possess the necessary enzymatic machinery for dough fermentation.

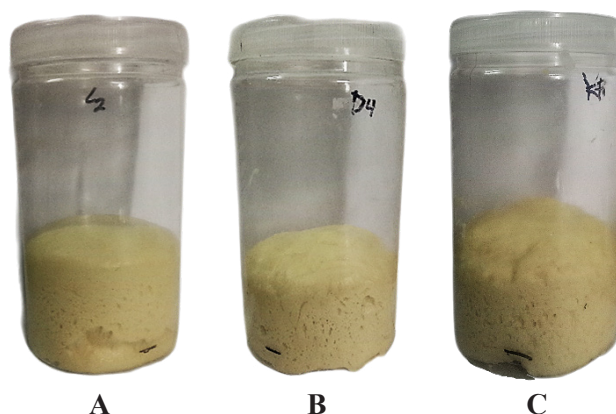


Figure 3: Bread dough development volume (A) *Candida sanyaensis*, (B) *Candida* sp., (C) *Saccharomyces cerevisiae*

Table 5. Dough volume development at two fermentation times using endophytic yeast isolates of *Candida sanyaensis* and *Candida* sp.

Time (min)	Volume (cm ³)			
	<i>Saccharomyces cerevisiae</i> (K ⁺)	without yeast isolate (K ⁻)	<i>Candida sanyaensis</i> (CS)	<i>Candida</i> sp. (C)
0	127.17	127.17	127.17	127.17
100	254.34	127.17	127.17	127.17
410	254.34	127.17	260.6985	127.17
480	254.34	127.17	260.6985	260.6985

Bread Dough Development Volume

Texture analysis revealed that bread fermented by *Candida sanyaensis* and *Candida* sp. exhibited desirable textural properties comparable to or better than commercial yeast-fermented bread. The lower hardness values of bread from *Candida sanyaensis* (12.64 g) and *Candida* sp. (16.79 g) compared to *Saccharomyces cerevisiae* (36.79 g) indicate a softer crumb texture. According to Haliza et al. (2012), lower hardness values correspond to softer bread, which consumers prefer. The negative control produced extremely hard bread (200.80 g), confirming the essential role of yeast fermentation in developing proper bread texture. The inverse relationship

between dough volume and hardness (Gomez et al., 2010) is evident in this study. The higher final volumes achieved by endophytic isolates (260.70 cm³) correlated with lower hardness values, while the lower volume of *Saccharomyces cerevisiae* bread (254.34 cm³) corresponded to higher hardness (Table 6).

The higher cohesiveness values of bread from *Candida sanyaensis* (0.47) and *Candida* sp. (0.47) compared to *Saccharomyces cerevisiae* (0.29) indicate stronger internal bonding within the crumb structure. Cohesiveness measures how well the bread structure holds together during chewing. Higher cohesiveness values suggest better structural integrity and resistance to crumbling. The absence of additional fibers or starches in our formulation enabled optimal gluten network development, resulting in higher cohesiveness. The positive adhesiveness value for *Saccharomyces cerevisiae* bread (0.0473 g·s) indicates slight stickiness to oral surfaces, while the negative values for endophytic yeast breads (-0.0012 to -0.0018 g·s) suggest minimal adhesiveness. According to Shaliha et al. (2017), higher adhesiveness corresponds to greater stickiness. The minimal adhesiveness of endophytic yeast bread may be advantageous for consumer acceptance, as excessive stickiness is generally undesirable. The lower gumminess values for *Candida sanyaensis* (5.92 g) and *Candida* sp. (7.89 g) bread compared to *Saccharomyces cerevisiae* (10.18 g) indicate that less energy is required to chew and break down these breads. Gumminess is calculated as hardness × cohesiveness, so the combination of low hardness and high cohesiveness in endophytic yeast breads resulted in optimal gumminess values (Table 6).

The texture analysis showed that bread fermented with *Candida sanyaensis* and *Candida* sp. had hardness, cohesiveness, and gumminess values similar to those of bread fermented with commercial yeast. These findings are consistent with recent studies on yeast impact on bread texture (Bartkiene et al., 2022). The textural properties of bread are influenced by multiple factors, including protein content, gluten strength, water absorption, and fermentation efficiency (Pusuma et al., 2018). Gluten strength, determined by the balance of glutenin and gliadin proteins, affects gas retention and final crumb structure. The superior textural properties of endophytic yeast-fermented bread suggest that these isolates produce enzymes that favorably influence gluten development and starch-protein interactions during fermentation. According to Cappelli et al. (2020), bread texture is influenced by yeast metabolic activity during fermentation, which affects gluten network development. Palla et al. (2022) reported that autochthonous yeasts can produce bread with desirable textural properties comparable to commercial strains. The low hardness values obtained in our study indicate good bread quality, as suggested by Rizzello et al. (2019) and Arora et al. (2021).

The texture analysis of *Candida sanyaensis* and *Candida* sp. produces bread with textural properties that are comparable to commercial yeast *Saccharomyces cerevisiae*. These endophytic yeasts yield softer, less sticky, more cohesive, and easier-to-chew bread, suggesting favorable enzymatic activity that enhances gluten development and starch-protein interactions during fermentation.

Table 6. Result of Bread Texture made using commercial yeast and isolated yeast of palm sap

Sample	Hardness (g)	Cohesiveness	Adhesiveness (gs)	Gumminess (g)
<i>Saccharomyces cerevisiae</i> (K ⁺)	36.79±31.64 ^{bc}	0.29±0.26 ^{ab}	0.0473±0.08 ^{ab}	10.18±16.14 ^{ab}
without yeast isolate (K ⁻)	200.80±101.08 ^d	0.32±0.19 ^{ab}	-0.027±0.05 ^{ab}	50.75±31.97 ^c
<i>Candida sanyaensis</i> (CS)	12.64±2.63 ^{ab}	0.47±0.21 ^{ab}	-0.0012±0.001 ^{ab}	5.92±1.15 ^{ab}
<i>Candida</i> sp. (C)	16.79±1.70 ^{ab}	0.47±0.23 ^{ab}	-0.0018±0.002 ^{ab}	7.89±1.001 ^{ab}

Note : Values followed by the same superscript letter within the same column are not significantly different according to DMRT at $\alpha = 0.05$.

Bread Color

Color parameters are usually described by the L^* value (lightness/brightness), a^* value (degree of redness/greenness), and b^* value (degree of yellowish/bluishness) (Gonzales et al., 2020). The lightness value indicates the change in brightness with positive (+) values meaning bright, and negative (-) values meaning dark. The a^* value indicates the chromatic color of the red-green mixture, with a positive value (+) meaning red, and a negative value (-) meaning green. The b^* value indicates the chromatic color of the blue-yellow mixture, with a positive value (+) meaning yellow, and a negative value (-) meaning blue. The highest lightness value was observed in the positive control (*Saccharomyces cerevisiae*), which was 63.7%. The lowest lightness value was obtained with *Candida* sp. yeast isolate, at 52.64%. The highest a^* value was obtained by *Candida* sp. yeast isolates, which was 10.28. The lowest a^* value was obtained by *Candida sanyaensis* yeast isolate, which was 11.66. The highest b^* value was obtained by the positive control (*Saccharomyces cerevisiae*), which was 24.32. The lowest b^* value was obtained by *Candida* sp. yeast isolate, which was 19.12 (Table 7 and Figure 4).

The color analysis revealed that bread fermented with *Candida sanyaensis* and *Candida* sp. exhibited similar lightness (L), redness (a), and yellowness (b^*) values compared to control bread. These results align with recent findings on yeast contribution to bread color formation (Birch et al., 2021; Caspani et al., 2022). The color of bread crust results from Maillard reactions and caramelization during baking. The Maillard reaction occurs between reducing sugars and amino groups of proteins at high temperatures, producing brown pigments and flavor compounds (Sitepu, 2019). Caramelization involves the thermal degradation of sugars above their melting points, which also contributes to the formation of brown color. All bread samples in this study showed positive L^* values (lightness), ranging from 52.64% to 63.7%, indicating acceptable color development. The positive control (*Saccharomyces cerevisiae*) produced the lightest bread ($L^*=63.7$), while *Candida* sp. produced the darkest ($L^*=52.64$). These L^* values are within the range reported by Putri et al. (2022) for sweet bread (30.29-79.41). The positive a^* values (10.28-11.66) indicate reddish tones in all bread samples, while positive b^* values (19.12-24.32) indicate yellowish tones. These color characteristics are typical of well-baked bread and result from the combination of Maillard reaction products and caramelization compounds.

The lower lightness values of endophytic yeast-fermented bread compared to *Saccharomyces cerevisiae* bread may indicate more extensive Maillard reactions, possibly due to differences in sugar utilization patterns or the production of additional reducing sugars through amylase activity. According to Birch et al. (2013), yeast isolates produce various enzymes, including α -glucosidase, β -fructosidase, and invertase, which influence sugar availability for browning reactions. The color component percentages (%X, %Y, %Z) provide additional insight into the color characteristics. The higher %X (redness) values for *Saccharomyces cerevisiae* (44.7%) and the negative control (41%) compared to endophytic yeast breads (30-33%) suggest differences in the balance of Maillard reaction products. These color variations, while statistically significant, may not be perceptible to consumers, and all samples exhibited acceptable bread color. Belleggia et al. (2023) explained that yeast fermentation influences Maillard reactions during baking, which are responsible for crust color development. Saez and Raya (2023) demonstrated that *Candida* species contribute to desirable color characteristics in fermented products. The color parameters obtained in our study are within the range reported by De Vuyst et al. (2023) for high-quality bread.

Table 7. The bread color was made using commercial yeast and isolated yeast from palm sap

Sample	Lightness (%)	a* value	b* value
<i>Saccharomyces cerevisiae</i> (K ⁺)	63.7±2.97 ^e	11.56±2.44 ^a	24.32±1.98 ^{bc}
without yeast isolate (K ⁻)	61.08±2.27 ^e	10.84±1.01 ^a	20.98±1.08 ^{bc}
<i>Candida sanyaensis</i> (CS)	54.86±9.07 ^d	11.66±2.26 ^a	21.76±4.75 ^{bc}
<i>Candida</i> sp. (C)	52.64±9.99 ^d	10.28±1.95 ^a	19.12±3.80 ^b

Note : Values followed by the same superscript letter within the same column are not significantly different according to DMRT at $\alpha = 0.05$.

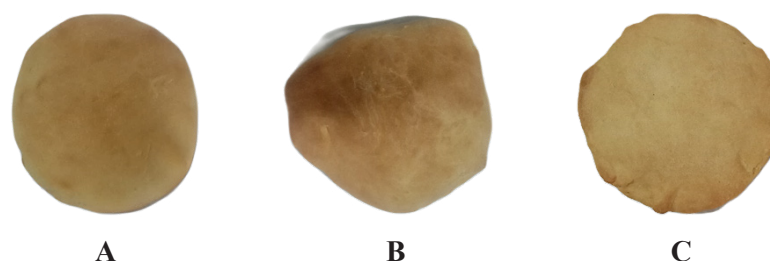


Figure 4: Bread color (A) *Candida sanyaensis*, (B) *Candida* sp., (C) *Saccharomyces cerevisiae*

CONCLUSION

Candida sanyaensis and *Candida* sp., endophytic yeasts isolated from Siwalan (*Borassus flabellifer* L.), demonstrated thermotolerance up to 45°C and ethanol tolerance up to 15%, fulfilling key requirements for baker's yeast applications. Both isolates successfully fermented bread dough, producing bread with textural properties (hardness, cohesiveness, adhesiveness, gumminess) comparable to commercial *Saccharomyces cerevisiae*. The bread exhibited acceptable color characteristics with light brown coloration. These findings support the potential of these endophytic yeast isolates as alternative leavening agents for the baking industry, enabling reduced dependence on imported commercial yeast while leveraging local biodiversity.

AUTHOR CONTRIBUTION

P.D.A. designed the research, collected and analyzed the data, wrote the manuscript, **U.U.** designed the research and supervised the process, **L.H.** designed the research and supervised the process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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