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Effect of Nitrogen Source on Growth Endophytic Yeast from *Salacca edulis* Reinw. and Bread Quality Analysis

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Abstract. *Yeast biomass is often used in the fermentation of bread dough. Dough fermentation can be maximized by adding a nitrogen source. This study used yeast isolates from salak pondoh (YIS-3, YIS-4, and YIS-7). The purpose of this study was to determine the effect of nitrogen addition on yeast growth and bread quality. This study used an experimental approach. The results of the growth study showed that all isolates treated with 0.05% urea produced higher biomass and cell counts than those treated with control. The highest biomass was produced by YIS-7, which was 3.81 g/300mL, while the highest number of cells was produced by YIS-3, which was 29.02x106 cells/mL. The percentage of proofing results showed that all yeast isolates treated with 0.05% urea needed a longer time to achieve the highest proofing. However, the volume of bread after baking showed better results than those treated with control. The largest volume of bread produced by YIS-3, was 972.14 cm3. The results of the organoleptic test showed that P<5%, which means that all treatments had a significant effect on the taste, aroma, color, and texture of the bread. Overall, the panelists gave good acceptance of the bread fermented by YIS-3 with 0.05% urea treatment. So it can be concluded that the addition of urea with a concentration of 0.05% in YIS-3 gave the best effect on the yeast growth and bread quality.*

Keywords: *bread quality, nitrogen, yeast growth*

Citation
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INTRODUCTION

Yeast is a single-celled eukaryotic microorganism that has the ability to ferment so it has been widely used in the fermentation industry since 700 BC (Jach & Serefko, 2018). Today's modern industry requires yeast that can produce large amounts of biomass to produce quality products and speed up the fermentation process. According to Pérez-Torrado et al. (2015), yeast biomass is needed in large quantities for bread dough proofing. The biomass content includes all parts of yeast cells including organelles and also the results of cell metabolism (Halász & Lasztity, 2017). When the biomass produced is small, it will affect the low fermentation rate and long fermentation time (Zohri et al., 2017). The low biomass produced can be increased by adding macronutrients to the yeast growth media (Walker & Stewart, 2016).

Yeast requires macronutrients during the growth process. These nutrients generate efficient fermentation (Cueto-Rojas et

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al., 2017). There are several macronutrient elements needed for the growth of microorganisms, such as carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). The growth medium used was YPG (yeast extract, peptone, glucose) media containing nitrogen in the form of peptone. According to Zohri et al. (2017), about 50% of yeast cells are composed of nitrogen, thus the synthesis of yeast biomass depends on the nitrogen content in the growth medium. When the nitrogen content in the growth media is limited, it can inhibit the growth of yeast (Broach, 2012). One type of nitrogen source that is used in the industrial sector and which can support yeast growth is urea (Cueto-Rojas et al., 2017). According to Putri & Restuhadi (2016), urea is an inorganic compound containing 46% nitrogen. The addition of urea as a nitrogen source can improve the performance of yeast and can help cell membrane biosynthesis. The urea used is a food-grade type of urea, which means that it is safe if it is added to food.

Murad et al. (2019) used nitrogen source to produce biomass in the yeast *Saccharomyces cerevisiae*. The yield of biomass obtained is 8.25 g/L. Hesham et al. (2020) in their research used 2 different yeast species, namely *Candida tropicalis* and *Pichia kluyveri*. Both isolates were treated by adding various types of nitrogen sources, one of them was urea. The yield of biomass obtained on the yeast *Candida tropicalis* was 3.43 g/L and the yeast P*ichia kluyveri* was 4.26 g/L. In this study, urea with a concentration of 0.05% was used. This is in accordance with the preliminary tests that have been carried out that the use of 0.05% urea with an incubation time of 48 hours is able to produce more biomass than urea with a concentration of 0.06%. These results are in accordance with the research by Putri & Restuhadi (2016), that the addition of urea to the growth medium of the yeast *Saccharomyces cerevisiae* FNCC 3049 with a concentration of no more than 0.06% was able to produce a cell number of $1.0x10^9$ cell/mL. The use of urea as an additional nutrient in yeast must be in sufficient concentration. If the concentration given is too excessive it will be toxic to the yeast and can inhibit the yeast growth.

The addition of macronutrients to increase biomass was limited to the amount of biomass obtained (Nicolas et al., 2017). Based on preliminary research, the number of live yeast cells in fermipan and yeast living cells in biomass can affect the speed of fermentation time for bread dough. Therefore, in this study, yeast biomass with known living cells can be used as an agent for bread dough fermentation. Fermentation occurred in a bread dough is a type of alcoholic fermentation. The results of fermentation are carbon dioxide (CO_2), alcohol and several other metabolites that affect the volume, texture, taste and aroma of bread (Maicas, 2020). Thus, in this study, besides analyzing the biomass and the number of live yeast cells, an analysis on the quality of bread dough was also carried out which included the percentage of dough proofing, bread's volume after baking, and organoleptic test (aroma, taste, color and texture).

MATERIALS AND METHODS

This research is an experimental type that was carried out from March-August 2021. This research was conducted at the Microbiology Laboratory of Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Research Material

The materials used in this study were yeast isolates (YIS-3, YIS-4, YIS-7) isolated from the salak pondoh fruit (*Salacca edulis* R.), originating from the Jombang area, East

Java Indonesia (Sari, 2020), Yeast Malt Broth (YMB), Yeast Malt Extract Agar (YMEA), Yeast Peptone Glucose (YPG), Sodium DL-Lactose, urea ($(NH_4)_2SO_4$), sugar (sucrose), salt, wheat flour, butter, and commercial yeast (Fermipan).

Preparation of Yeast Isolat

The preparation of yeast isolates was carried out by aseptic method in Laminar Air Flow (LAF). One yeast colony was inoculated on YMEA media with streak plate. The isolates were incubated for 48 hours at room temperature. After being incubated for 48 hours, two colonies of yeast isolates were propagated on 180 mL YMB media. Then, it was incubated on a shaker at 140 rpm at 33 °C for 24 hours (Zohri et al., 2017).

Addition of Nutrients to Growth Media

Additional nutrients used are urea ($(NH_4)_2SO_4$) with a concentration of 0.05% (Putri & Restuhadi, 2016). 0.15 gram of urea was added to 300 ml of YPG medium. The media was sterilized by autoclaving at 121 °C for 15 minutes. After the media has cooled, then it is inoculated with 10% yeast inoculum in Laminar Air Flow (LAF). Then, it was incubated on a shaker at 140 rpm for 48 hours at 33°C (Zohri et al., 2017).

Yeast Growth Analysis

The analysis of yeast growth was determined based on biomass and the number of live yeast cells used the counting chamber method.

Yeast Biomass Determination

Measurement of biomass was carried out on a sample consisting of 30 mL yeast, 270 mL YPG media, and added nutrients (urea 0.15 gram/300 mL). The sample wasseparated the media using a centrifuge at 4,000 rpm for 30 minutes. Then the supernatant was discarded and the pellet was weighed (Karki et al., 2017). The Weight of the obtained biomass was calculated using the formula:

$$B = B2\text{-}B1$$

where:
B = Biomass obtained (gram/mL).
B2 = Eppendorf containing biomass.
B1 = Eppendorf is empty.

Determination of The Number of Cells

Determination of the number of living yeast cells was carried out based on the modified Atanasova et al. (2019). 100 µL of yeast inoculum was taken and poured into a 1.5 ml tube. Then,100 µL of methylene blue was added and diluted with 800 µL of sterile distilled water. The yeast suspension was then homogenized with a vortex. 20 µL yeast suspension and placed into the chamber on the Hemocytometer. Cells were counted under a computer microscope at 400x magnification. Cell observations were carried out in 5 medium boxes. After getting the number of cells in 5 medium boxes, the calculation was carried out using the formula (Mahardika et al., 2018):

$$\text{the average number of cells/box} = \frac{\text{number of living cells}}{\text{5 boxes}}$$

$$\text{diluent factor} = \frac{\text{final volume of suspension}}{\text{inoculum volume}}$$

$$\text{number of cells } \left(\frac{\text{cell}}{\text{ml}}\right) = \text{the average number of cells/box x diluent factor x } 10^4$$

Description:
10^4 = 0.1 . conversion µL in 1 ml.
0.1 µL = volume in a medium box.

Bread Dough Making

The method used was based on the modified method of Watanabe et al. (2016). The

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ingredients for making bread consist of 200 grams of flour, 3 grams of salt, 15 grams of sugar, 16 grams of butter, 1.2% (2.4 grams) of yeast pellets, 2.4 grams of commercial yeast, and 70 ml of water. All ingredients are mixed and kneaded until it becomes a smooth dough. Maryam et al., (2017), stated vthat this type of test should equipped with a positive control and a negative control. As a positive control, commercial yeast (Fermipan) was used. While the negative control without the addition of a proofing agent (yeast). As much as 300 grams of dough was put into the mold. The dough was incubated at a room temperature and observed the increase in the volume of the dough (Karki et al., 2017).

Bread Quality Analysis

Bread quality analysis was determined based on the percentage of bread dough proofing (bread dough volume), bread volume after baking, and organoleptic test (aroma, taste, color, and texture).

Bread Dough Volume Measurement

Measurement of the increase in bread dough volume refers to a modified study by Houngbédji et al., (2018), which was carried out by comparing the height of the dough after being incubated every 30 minutes for 600 minutes from the original dough height. The process was carried out in a mold. The height of the dough was measured manually using a ruler and then the dough volume was calculated using the formula:

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

where:
V = volume (cm^3)
π = phi (3.14)
r = radius (cm)
t = height (cm)

After knowing the dough volume from each predetermined time, the calculation of the percentage of bread dough proofing was carried out by referring to the modified research of Houngbédji et al., (2018) using the formula:

$$\%\ \text{of proofing} = \frac{\text{final dough volume} - \text{initial dough volume}}{\text{initial dough volume}} x100\%$$

Bread Dough Making

The height of the bread was measured using a ruler, then the volume of the bread after baking was calculated using the formula:

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

where:
V = volume (cm^3)
π = phi (3.14)
r = radius (cm)
t = height (cm)

Bread Organoleptic

The organoleptic test of bread refers to a modified method by Radiastuti et al., (2020), the test was carried out on aroma, color, texture, and taste by using the hedonic method. The hedonic method is testing a product based on the panelist's preference level with a scoring assessment. In this test involved 30 panelists. The score used is 1 = dislike very much, 2 = dislike, 3 = neutral (neither like nor dislike), 4 = like and 5 = like very much.

Data Analysis

Data on the percentage of bread dough proofing and yeast growth, including biomass and cell number, were analyzed using Microsoft Excel and presented in a bar chart with descriptive analysis. Meanwhile, the results of the organoleptic test in the form of non-parametric data were analyzed using the Kruskal-Wallis test. If there is a significant,

then the Mann-Whitney further test with a significance level of 5%. Finally, the data is processed with the SPSS program (Ilmi & Aulia, 2020).

RESULTS AND DISCUSSION

Yeast Growth Analysis

Yeast Biomass

The first observed indicator was to determine yeast growth was the biomass. According to Nicolas et al., (2017), the formation of biomass can indicate the occurrence of growth in yeast. Based on the results of the study in Figure 1, shows that all isolates treated with 0.05% urea were able to produce higher biomass, respectively YIS-3, YIS-4, YIS-7 (3.66 g/300mL; 3.71 g/300mL; 3.81 g/300mL) when compared with 0% urea treatment (3 g/300mL; 3.04 g/300mL; 3.35 g/300mL). This is because the hydrolysis of urea in yeast growth media produces ammonia which can be used as a nitrogen source for yeast growth. According to Linder, (2019), yeast can use a variety of nitrogen-containing substrates by involving several catabolic steps, in which nitrogen is previously released in the form of ammonia so that it can be incorporated as biomass. Walker & Stewart, (2016), also stated that yeast growth involves transport and assimilation of nutrients followed by the integration of yeast into numerous cellular components, so that cells biomass increase.

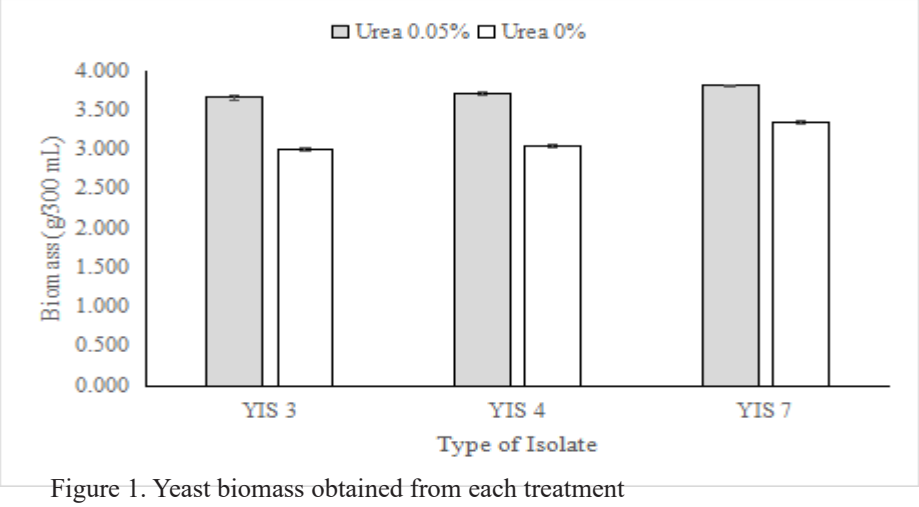

Figure 1. Yeast biomass obtained from each treatment

In the mechanism, urea is catabolized by several enzymes into ammonia (NH_3) and carbon dioxide (CO_2) which can be re-injected into cell metabolism. The first step in the utilization of urea is the conversion of urea to ammonia catalyzed by two different enzymes, namely urease and urea amidolyase (UA). UA consists of urea carboxylase (UC) and allophanate hydrolase (AH) domains. In its mechanism, UC converts urea to allophanate, and AH converts allophanate to ammonia (Zhao et al., 2018). Meanwhile, the urease enzyme catalyzes the independent hydrolysis of ATP and NADPH from urea into ammonia and carbamic acid (H_2N-COOH) which are hydrolyzed spontaneously by carbonic acid (H_2CO_3) and a second ammonia molecule (Veaudor et al., 2019). According to Linder, (2019) that hydrolysis of urea can produce ammonia, which is then converted into ammonium then l-glutamate, and l-glutamine. The compound is used as an amino donor group in anabolic transamination reactions.

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The Number of Live Yeast Cell

The second observed indicator to determine yeast growth was the number of cells. The calculation of the number of live yeast cells was carried out using the counting chamber method. The advantage of this method is the ability to differentiate between living and dead cells through the methylene blue stain. Yeast cells that show a transparent color are living cells as they are able to reduce the color of methylene blue. Meanwhile, yeast cells that are blue in color indicate the dead cells as they oxidized methylene blue (Figure 2) (Atanasova et al., 2019). The results of the number of yeast cell presented in Figure 3.

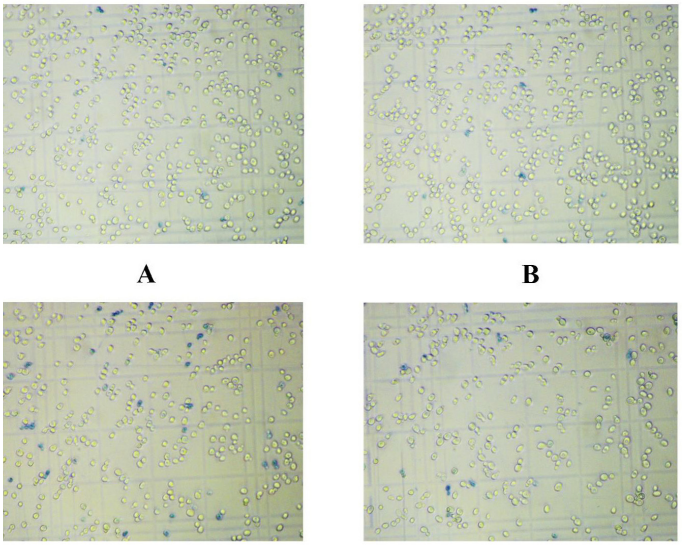

Figure 2. Number of yeast cell; A) positive control; B) YIS-3 urea 0.05%; C) YIS-4 urea 0.05%; D) YIS-7 urea 0.05%.

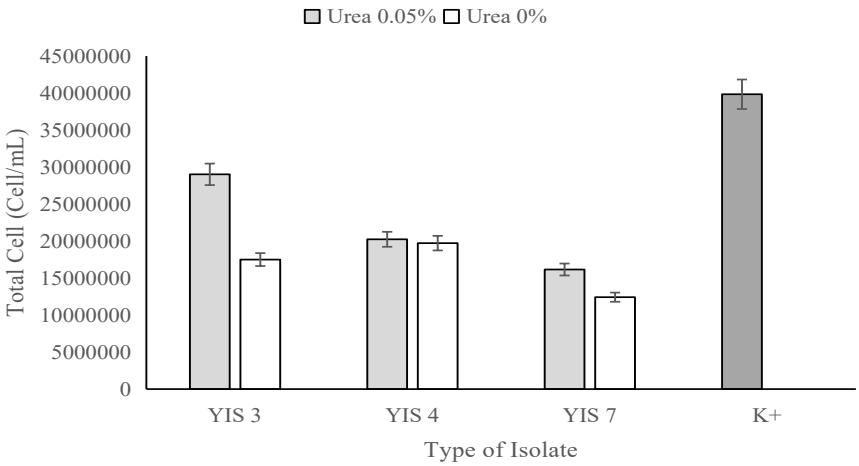

Figure 3. Number of yeast cells in each treatment

Based on the research data in Figure 3, the number of yeast cells in the positive control showed the highest yield (39.84 x 10^6) when compared to the number of cells in the treatment with urea 0.05% and urea 0%. This is because in all treatment groups, the sample used in calculating the number of cells is in the form of biomass. The biomass used is in the form of wet weight which still contains water. While the sample used in calculating the number of cells in the positive control was in the form of dry weight in which there was no water content. Therefore, in the same sample size, there are different numbers of cells. This is in accordance with Matovic, (2011) who stated that commercial yeast that is often used in the industrial sector is the yeast in the form of biomass which has removed the water molecules with an increase in temperature.

The number of yeast cells from all isolates treated with 0.05% urea resulted higher number of cells, respectively YIS-3, YIS-4, YIS-7 (29.02x10^6 cells/mL; 20.24 x10^6 cells/mL; 16.16 x10^6 cells/mL) when compared with 0% urea treatment (17.50 x10^6 cells/mL; 19.72 x10^6 cells/mL; 12.42 x10^6 cells/mL). From the results above, it can be seen that each yeast isolates produced a different number of cells with the same concentration. The YIS-3 and YIS-4 isolates were able to produce cell numbers ranging from 20 million cells/mL. While in YIS-7 the number of cells produced was below 20 million cells/mL. According to Lleixa et al., (2016) yeasts of Saccharomyces and non-Saccharomyces grew on media with the same composition will produce different growth. According to Irmayuni et al., (2018), the yeast Saccharomyces has several advantages, including fast breeding, relatively stable, have the ability to adapt to high temperatures, and their environment. In this study, YIS-3 and YIS-4 yeasts were suspected from the genus of Saccharomyces. While yeast YIS-7 from the genus of Pichia.

According to Hafsan, (2011) most yeast cells multiply or reproduce by budding. However, there are also a small number of yeasts that reproduce by splitting or binary fission. Linder, (2019), also stated that ammonia is one of the nitrogen substrates that can be used by yeast during budding. Ammonia is a product of the catabolism of several types of nitrogen compounds, including urea. At the time of budding reproduction, the occurrence of lysis of the cell wall in certain areas begins. The absence of a cell wall in this area causes pressure from the contents of the cell to form a sphere surrounded by the parent cell wall. Then this part enlarges, the nucleus divides by mitosis, and the nucleus which is the result of the division moves to the round bud. Thus, new shoots are formed which are equipped with a nucleus and can continue their growth. When the growth is complete, the shoot will break away from the parent cell and the replication cycle is complete. Thus, there will be multiplication or an increase in the number of cells (Hafsan, 2011).

The presence of more biomass and cell numbers in the 0.05% urea treatment proved that the addition of urea as a nitrogen source could increase yeast growth. According to Dewi (2018) nitrogen source is one of the important factors influencing growth. During the growth phase, nitrogen is required for the synthesis of proteins and nucleic acids. Putri (2016), also stated that in microorganism cells, nitrogen has several important functions including regulating the pH balance in the body, accelerating the recovery process, forming enzymes, and compiling 50% of the dry weight in the form of macromolecules, and as an energy source. Broach, (2012), also stated that the availability of a nitrogen source that is easily metabolized can suppress the autophagy process, in which cells send cyto-

plasmic macromolecular components to vacuoles for proteolytic recycling of other component parts. Thus, nitrogen accessibility can regulate metabolism, growth, transcription, post-transcriptional protein sorting, and protein turnover in yeasts.

Bread Quality

Bread Dough Volume

Bread quality testing can be assessed based on the increase in the volume of bread dough and the volume of bread after baking, as well as the organoleptic properties of the bread. Figure 4 showed the percentage of the increase in the volume of bread dough during the incubation period.

Based on the results of the study, it was known that isolates treated with urea showed different proofing times. At the beginning of the 60th minute of proofing time, the positive control expanded higher and faster by 41.51%. This was because the number of cells contained in the positive control was higher than the number of cells in all treatments. The number of positive control cells used as a bread dough proofing agent was 39.84 x 10^6 cells/ml. While the number of cells in all treatments ranged from 12.42 x 10^6 cells/ml to 29.02 x 10^6 cells/ml. This is in accordance with Akbar et al., (2019), which stated that the number of cells contained in the growth media is closely related to the fermentation process carried out by yeast. According to Kim et al., (2020), an increase in yeast concentration causes an increase in the rate of CO_2 gas production, resulting in a higher CO_2 mass transfer rate. As a result, the bubbles expand faster to reach saturation. Meanwhile, when compared with the negative control, all isolates treated with 0.05% urea and 0% urea showed better results. This is because in the negative control there was no proofing agent added so the bread dough fermentation process did not occur, which resulted in 0% percentage of proofing from the 30th minute to the 390th minute.

Based on Figure 4, all isolates treated with 0.05% urea needed a little longer time to reach the highest proofing when compared to the 0% urea treatment. One of them was proven in YIS-3 urea 0.05%, where the highest expansion (28.33%) occurred at the 90th minute, while YIS-3 urea 0% the highest expansion (23.08%) occurred at the 60th minute. This is because the yeast has its own mechanism to utilize its preferred nitrogen source during the fermentation process. According to Brice et al., (2018), yeast can take advantage of all nitrogen-containing compounds that are able to influence growth and fermentation. Thus, the nitrogen source can be classified as a preferred nitrogen source and one of the unfavorable nitrogen sources is urea. In the mechanism, urea will be hydrolyzed into ammonia which is used by yeast cells as a nitrogen source and then converted into ammonium (Riza, 2016). When several types of nitrogen sources are available in a growth medium or fermentation medium, the yeast will first utilize the preferred nitrogen source except for ammonium. This is because the absorption or consumption of ammonium by yeast can begin when the preferred nitrogen source has been used first. However, the availability of the substrate and the strain used is the main factors that affect nitrogen utilization during the fermentation process. This is related to the results of the differential regulation of the permease involved in the absorption of these molecules (Brice et al., 2018).

In this study, the observation of bread dough volume expansion was carried out for 10 hours or 600 minutes. However, after the 360th minute or starting from the 390, all treatments showed a decrease in the percentage of proofing to 0%. This is because the longer

the fermentation time, there will be various pressure conditions that can affect the ability of yeast to ferment. One of these pressures is the higher alcohol content. In accordance with Azizah et al., (2012), the fermentation time will affect the alcohol content produced. The longer the fermentation time, the higher the alcohol produced, so this can adversely affect the growth of yeast. Pongcharoen et al., (2018), added that an increasing alcohol concentration can negatively affect yeast cell metabolism and growth and can damage cell walls, decrease specific growth rates, increase cell death or alter plasma membrane permeability and transport systems.

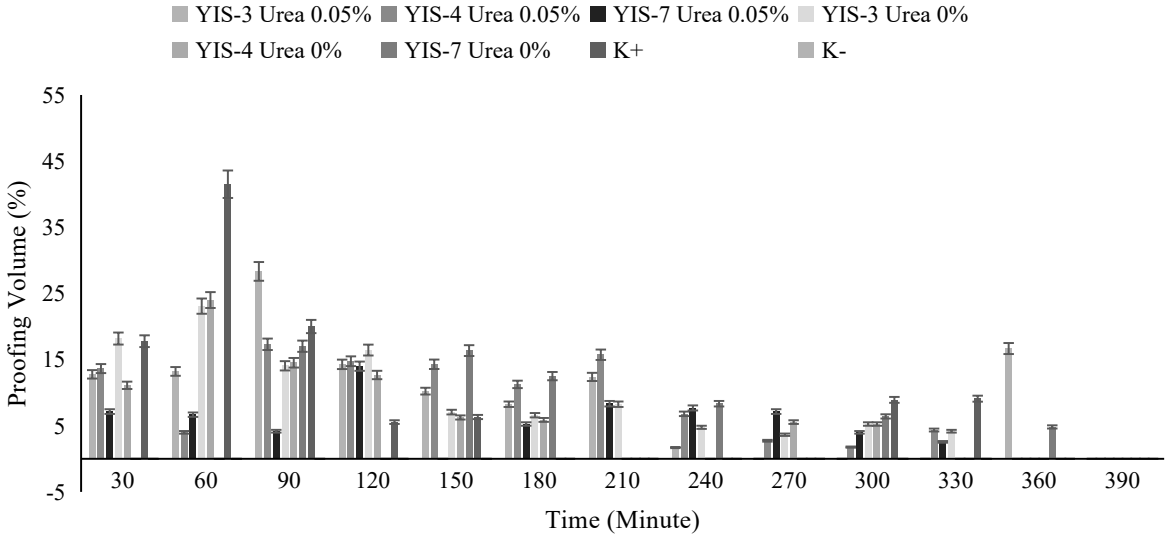

Figure 4. Percentage of bread dough proofing

The Volume Bread After Baking

The baking process on bread dough can also increase the volume of the bread. This is in accordance with Miś et al., (2016) during the baking process, yeast will continue to produce CO_2 gas. The higher the baking temperature, the solubility of CO_2 in the dough will decrease, then the CO_2 will evaporate. At the same time, the saturated water vapor pressure will increase widely which causes the gas to expand, thus pushing the dough to stretch and expand. As the baking occurs, the process of protein coagulation and starch gelatinization happens. This causes the formation of bread crust and bread crumbs. At the end of baking at a temperature of about 72ºC, the bread dough loses its ability to hold CO_2, so the gas escapes from the dough and the dough does not rise further. The results of baking bread as shown in Figure 5.

Based on the results of research on the volume of bread after baking, all isolates treated with 0.05% urea produced a higher volume of bread when compared to the volume of bread treated with 0% urea, positive control and negative control. The highest volume of bread was produced by YIS-3 urea 0.05% at 972.14 cm^3. While in the positive control, the volume of bread produced was 734.76 cm^3. This is because the rules for using commercial yeast only require 30-45 minutes of fermentation time. However, in this study, the fermentation time used was 600 minutes. According to Shabrina (2017), excessive fermentation time causes the dough to become sour. When yeast, water, and flour are combined, the enzymes attached to the flour during the fermentation process will break down

the starch content, so the volume of the bread will also decrease.

Previous research stated that YIS-3 is the yeast from the genus Saccharomyces (Sari, 2020). According to Lahue et al., (2020), most of the microorganisms that contribute to the fermentation of bread dough are from the Saccharomyces group. In the process of making bread, yeast *S. cerevisiae* is needed to utilize complex sugars efficiently and be able to tolerate osmotic stress. Zhen et al., (2020), stated that gluten strength was influenced by nitrogen compounds, so that by adding nitrogen compounds it was able to increase the amount of all gliadin and glutenin proteins which resulted in an increase in bread volume.

Figure 5a. Bread volume after baking; A) YIS-3 urea0.05%; B) YIS-4 urea 0.05%; C) YIS-7 urea 0.05%; D) positive control; E) negative control.

Figure 5b. Bread volume after baking; A) YIS-3 urea 0%; B) YIS-4 urea 0%; C) YIS-7 urea 0%; D) positive control; E) negative control.

Organoleptic Test

Organoleptic testing was carried out on all bread attributes including texture, color, aroma, and taste of bread. Based on the results of Mann Whitney's further test in table 1, all treatments (YIS-3 urea 0.05%, YIS-4 urea 0.05%, YIS-7 urea 0.05%, YIS-3 urea 0%, YIS-4 urea 0%, and YIS-7 urea 0%) showed significant differences which were indicated by the formation of different notations in the positive and negative controls. This means that each treatment has a significant effect on the response of the panelists on all attributes (color, aroma, texture, and taste). The average organoleptic value ranges from 2 to3 which indicates the response of the panelists between likes and dislikes. Panelists prefer bread fermented by YIS-3 urea 0.05% in all attributes.

The color attribute is the first criterion that consumers can visually judge. The distinctive color produced by bread is the result of the Maillard reaction. According to Starowicz & Zieliński (2019), in the final stage of the Maillard reaction, the condensed carbonyl and amine groups will form melanoidin compounds that give the bread a brownish color.

The aroma produced by bread is the result of the presence of compounds produced during the fermentation process (Heitmann et al., 2017). According to Xu et al., (2019), the metabolic processes carried out by yeast during fermentation will produce compounds such as acetic acid, lactic acid, alcohol, esters, carbonyls, ketones, and aldehydes. All these compounds contribute to the formation of the aroma of bread after baking.

According to Mildner-Szkudlarz et al., (2017), through the Maillard reaction, free amino acids and reducing sugars interact to produce several compounds that form flavors including alcohols, aldehydes, esters, ethers, ketones, acids, furans, hydrocarbons, lactones, pyrazine, pyrroline, and sulfur compounds. In addition, the taste that arises in bread is caused by the ingredients of the bread and the process of making bread. Heitmann et al., (2017) added that one of the enzymes that have an important role in the formation of taste is the lipase enzyme. Lipase activity is responsible for the production of short-chain fatty acids, so this induces changes in lipid composition that contribute to taste changes.

According to Starowicz & Zieliński, (2019), the Maillard reaction also contributes

to the formation of texture through several mechanisms, including the formation of protein cross-links, the formation of emulsion properties, and the formation of protein-polysaccharide conjugates. However, the most affecting the texture of the bread is protein cross-linking. This is because the protein bonds formed are related to the formation of gluten.

The treatment that is not liked was the negative control. This is because in the negative control there was no bread proofing agent (yeast) added so that the fermentation process does not occur and causes the bread to have a pale color, flour aroma, bland taste and hard tough texture.

Table 1. Results of organoleptic testing of bread

Attribute	The Mean Value of the Sample Hedonic Test							
	YI-3 urea 0.05%	**YIS-4 urea 0.05%**	**YIS-7 urea 0.05%**	**YIS-3 urea 0%**	**YIS-4 urea 0%**	**YIS-7 urea 0%**	**positive control**	**Negative control**
Color	3.97 ± 0.718^{a}	3.43 ± 0.728^{b}	3.67± 0.884^{ab}	3.43 ± 0.626^{b}	3.40 ± 0.770^{b}	3.57± 0.728^{b}	2.90 ± 0.995^{c}	2.13 ± 1.008^{d}
Aroma	3.70 ± 0.750^{a}	3.60 ± 0.932^{a}	3.47 ± 0.973^{ab}	3.07 ± 0.980^{bc}	2.90 ± 0.923^{c}	3.03 ± 0.964^{bc}	2.40 ± 1.192^{d}	1.80 ± 0.847^{e}
Texture	3.83 ± 0.747^{a}	3.57 ± 0.935^{ab}	3.47 ± 0.973^{abc}	3.30 ± 0.837^{bc}	3.00 ± 0.788^{c}	3.13 ± 1.042^{bc}	2.63 ± 0.809^{d}	1.67 ± 1.124^{e}
Flavor	3.90 ± 0.885^{a}	3.43 ± 0.858^{ab}	3.83 ± 0.791^{a}	3.23 ± 0.728^{b}	3.17 ± 1.020^{b}	3.07 ± 0.944^{b}	2.27 ± 0.980^{c}	1.73 ± 0.785^{d}

Numbers followed by the same letter show no significant difference at the 5% significance level from the Mann Whitney follow-up test.

CONCLUSION

The addition of nitrogen in the form of 0.05% urea can increase the growth of endophytic yeasts of salak pondoh fruit (*Salacca edulis* Reinw.) and can improve the quality of bread based on the measurement of dough volume, bread volume, and organoleptic testing using hedonic methods. However, endophytic yeasts need a long time to carry out the fermentation process. Therefore, the observation of environmental factors from the beginning to the end of the fermentation needs to be evaluated. Through this research, it is necessary to further analyze the biomass content in the form of primary and secondary metabolites in order to directly identify the components that have an important role during the fermentation process.

AUTHOR CONTRIBUTION

N.F.I.Z. collected and analyzed the data and wrote the manuscript. U.U. and N.K., design the research and supervised all the process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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