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Improving the bread quality and endophytic yeast of Salak Pondoh (*Salacca edulis* Reinw.) growth by addition of phosphate source

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ABSTRACT. Baker's yeast products which used in Indonesia are imported products. Isolation of endophytic yeasts from Salak Pondoh fruit (*Salacca edulis* Reinw.) were obtained in 3 isolates that potentially to develop bread. Large amounts of yeast will be needed by adding nutrients in phosphate source as KH_2PO_4 on yeast peptone glucose (YPG) media. The research objective is to determine the effects of nutrient enrichment of phosphate in the growth of isolates YIS-3, YIS-4, and YIS-7 and knowing the quality of bread which include of volume, color, texture, and taste of bread fermented using yeast YIS-3, YIS-4 and YIS-7 results from the addition of KH_2PO_4. This research is an experimental approach used a quantitative approach by calculating cell growth which included biomass and cell number. Measuring the volume of bread for 12 hours every 30 minutes and organoleptic tests which include of characteristics of color, texture, aroma and taste with 30 panelists. The results of the growth study showed that YIS-3 with 0.01% phosphate addition had the highest number of cells and YIS-7 with 0.01% phosphate addition had the heaviest biomass. The highest bread volume is produced by YIS-3 with 0.01% phosphate addition and the organoleptic test of color characteristics YIS-4 with 0.01% phosphate treatment has the most preferred while the texture, aroma, and taste of YIS-3 0.01% phosphate treatment is most favored by the panelists. It can be concluded that the addition of a phosphate source gave the best effect on yeast growth and bread quality.

Keywords: baker's yeast; bread quality; fermentation time; phosphate nutrition; yeast growth

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INTRODUCTION

Yeast is a unicellular fungus that can ferment sugar into alcohol and carbon dioxide (Bitrus *et al*., 2020) which has been widely used as a leavening agent in producing bread. Yeast in large quantities will be needed in accelerating the fermentation process so that a quality product is produced. Optimal cell growth is needed to produce yeast cell biomass and the number. A small amount of yeast will affect the low fermentation rate (Zohri *et al*., 2017). Yeast biomass contains the products of cell metabolism and cell material (Heitmann *et al*., 2018.). Increasing the number of yeast cells can be done by adding nutrients to the growth medium (Kolouchová *et al*., 2016). Sufficient nutritional needs will optimize the fermentation that occurs (Fitria & Lindasari, 2020).

The media needed for yeast growth must include macronutrients in the form of carbon sources, nitrogen sources, and mineral sources (Walker & Stewart, 2016). Yeast Peptone Glucose (YPG) media is a commonly used working medium in laboratory for yeast growth. Carbon sources and nitrogen sources have been met in YPG media, so additional substances are needed in the fermentation media in the form of mineral sources. One of the mineral sources used is phosphate (Samyn & Persson, 2016). Phosphate is needed by yeast for nucleotide biosynthesis (Yadav *et al*., 2016). One of the main skeletons of nucleotides consists of alternating phosphate and sugar groups. The use of phosphate used Potassium dihydrogen Phosphate (KH_2PO_4) which is a type of phosphate that is commonly found in nature. In the study of Nicolas *et al*., (2017) the addition of 0.01% phosphate, was able to increase the biomass of yeast *Arachniotus* sp. NIBGE., and *Candida utilis* NIBGE, up to 3 g/L.

The success of the fermentation process could be seen by measuring the increase in the volume of the fermented dough due to the results of CO_2 while the presence of alcohol can be assessed from the aroma and taste formed from the bread by hedonic testing (liking) involving panelists (Sitepu, 2019). In previous studies, the results of yeast isolation from Salak Pondoh fruit *(Salacca edulis* Reinw.*)* which is a native fruit of Indonesia, was tested for its potential as a bread developing agent but have less ability to produce biomass and without measuring the acceleration of bread dough fermentation time (Sari, 2020). This research was carried out by analyzing the amount of biomass and the number of cells with additional nutrients, namely phosphate nutrients and its application to bread quality which included the percentage of bread dough development, bread volume after baking, and organoleptic analysis (aroma, taste, color, and texture). The resulting biomass will affect the yield of bread. So that in the future it can be used as baker's yeast on an industrial scale which is original from Indonesia scale but needs to be applied in yeast cell encapsulation to make it more durable in storage and easy to use, because without encapsulation, the use of yeast as a baker's yeast will require rejuvenation every time it is used.

MATERIALS AND METHODS

Rejuvenation of yeast isolates. Rejuvenation of yeast isolates was carried out by aseptic method in Laminar Air Flow (ESCO). There were 8 yeast isolates identified macroscopically and microscopically but after testing in developing bread, only 3 isolates had the ability to develop bread like commercial yeast. It's called YIS-3, YIS-4 and Yis-7. One yeast colony each isolate was inoculated on Yeast Malt Extract Agar (YMEA) medium (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L, agar 20 g/L) with a streak plate. The cultures were incubated for 48 hours at room temperature of 28 °C. After being incubated for 48 hours, two colonies of yeast isolates grown on YMEA media were propagated on Yeast Malt extract Broth (YMB medium (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L). Then incubated on a shaker (Oregon KJ-201BD) at a speed of 140 rpm at 33 °C for 24 hours (Zohri *et al*., 2017).

Addition of nutrients to growth media. Additional nutrients were used in the form of potassium dihydrogen phosphate (KH_2PO_4) 0.01% (Nicolas *et al*., 2017). Each nutrient was added to 300 ml of YPG liquid medium. The media was sterilized by autoclave (ALP, Mc 30s) at 120°C for 15 min. After the media has cooled, then it is inoculated with 10% or 30 mL inoculum of aseptic propagated yeast in Laminar Air Flow (ESCO). Next, it was incubated on a shaker (Oregon KJ-201BD) at 140 rpm for 48 hours at 33 °C (Zohri *et al*., 2017).

Determination of yeast biomass. Biomass measurements were carried out on samples consisting of 30 ml of propagated yeast, 270 ml of Yeast Peptone Glucose (YPG) media (yeast extract 3 g/L, peptone 5 g/L, glucose 20 g/L) with the addition of nutrient phosphate 0.03 grams/300 ml, then separated the media with yeast pellets using a centrifuge (Thermo Scientific) at 4000 rpm for 30 minutes. Then the supernatant was discarded and the pellet was weighed (Karki *et al*., 2017).

Weighing biomass can be obtained with the following formula:

$$B = B2 - B1$$

Notes:
B = biomassa obtained g/mL
B2 = Eppendorf containing biomass
B1 = Eppendorf empty

Determination of the number of live yeast cells. Determination of the number of cells was carried out based on the modified Atanasova *et al*., (2019). Take 100 µL of yeast inoculum and put it in 1.5 ml tube (OneMed). Add 100 µL of methylene blue and dilute with sterile distilled water to final volume of 1000 µL. The yeast suspension that has been stained and diluted is then homogenized with vortex (Thermo Maximix). The yeast suspension is taken 20 µL and slowly introduced into the chamber on the Haemocytometer. Cells were counted under a computer microscope (Nikon) at 400× magnification. Cell observations were carried out in 5 medium boxes. After getting the number of cells in 5 medium boxes, the calculation is carried out using the formula (Pratikno *et al*., 2019):

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

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

$$\text{number of cells } \left(\frac{\text{cell}}{\text{ml}}\right) = \text{the average numbers of} \frac{\text{cells}}{\text{boxs}} \times \text{diluent factor } \times 10^4$$

Notes:
10^4 = 0.1 L conversion in 1 ml.
0,1 µL = volume in medium box.

Bread quality analysis. Percentage of bread dough development, bread volume after baking, and organoleptic analysis (aroma, taste, color, and texture). Bread dough making method used is in accordance with the modified research of Watanabe *et al*., (2016). The 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 yeast, and 70 ml of water. All ingredients are mixed and kneaded until it becomes a smooth dough. Maryam *et al*., (2017) stated, this test was equipped with positive control and negative control. As a positive control, commercial yeast (Fermipan) was used. While the negative control did not add a leavening agent (yeast) to the bread dough. Each sample of dough is weighed as much as 300 grams and put in a measuring cylinder or mold. The dough was incubated at a temperature of ± 30 °C and an increase the volume of the dough was observed (Karki *et al*., 2017). Measurement of the increase in bread dough volume or breading power referring to the modified Houngbédji *et al*.,(2018), was carried out by comparing the height of the dough after being incubated every 30 minutes for 600 minutes with the height of the initial dough. The process is carried out in a mold whose size has been known. The height of the dough is measured manually using a ruler, then the dough volume is calculated using the formula:

$$V = \pi \times r2 \times t$$

Notes:
V= Volume
π= 22/7
r = radius
t = height

After knowing the dough volume from each time, a calculation of the percentage of bread dough development is carried out which refers to modified Houngbédji *et al*., (2018), the formula used is:

$$\%\ \text{development} = \frac{\text{volume of final dough} - \text{volume of initiali dough}}{\text{volume of initiali dough}} \times 100\%$$

Measurement of bread volume after baking bread. Bread dough that had been incubated for 600 minutes was then baked at 150 °C for 30 minutes (Karki *et al*., 2017). Measure the height of the bread using a ruler, then calculate the volume of bread after baking using the formula:

$$V = \pi \times r2 \times t$$

Notes:
V= Volume
π= 22/7
r = jari-jari
t = tinggi

Testing of aroma, taste, color, and texture on bread was carried out by organoleptic hedonic methods, namely testing the level of preference involving 30 panelists or consumers. In this hedonic method, panelists are asked to give an assessment based on their level of preference using scoring. The score used is 1 = dislike very much, 2 = dislike, 3 = somewhat like, 4 = like and 5 = like very much (Agustina *et al*., 2021).

Data analysis. Based on data for the increasing volume of bread dough and yeast growth data which included biomass and cell number were analyzed using *Microsoft Excel* and presented in the form of tables and bar charts with descriptive analysis. Meanwhile, the data from organoleptic test results in the form of non-parametric data were analyzed using the Kruskal-Wallis test. If there is a significant difference, then the Mann-Whitney further test was carried out with a significance level of 5%. The data is processed with the help of the SPSS (Hariadi, 2020).

RESULTS AND DISCUSSION

Yeast growth. The first parameter observed was the biomass produced by YIS-3, YIS-4, and YIS-7. The formation of biomass can be indicated that yeast cell growth has occurred so that measurements are needed (Nicolas *et al*., 2017). Biomass yield was calculated by looking at the average value and standard deviation. The results showed that all isolates with the addition of 0.01% phosphate nutrition in the medium growth produced more biomass than those with 0% phosphate treatment (Table 1). This is because phosphate is needed in the biosynthesis of nucleic acids, phospholipids, and ATP. The phosphate content in yeast is about 3-5% of the dry weight which will take a part as a substrate and effector of enzymes (Walker & Stewart, 2016). The addition of phosphate to the growth medium will induce osmotic stress. Yeast growth and survival depend on the ability of each genus to withstand osmotic pressure conditions (Avila-Reyes *et al*., 2016).

Table 1. Yeast growth with treatment of phosphate 0.01% and phosphate 0%.

Type of yeast	Biomass (gr/300 mL)	Numbers of yeast (cell/mL)
YIS-3P	2.9±0.032	$13.50x10^6$±8.0
YIS-4P	2.8±0.26	$10.18x10^6$±14.2
YIS-7P	3.81±0.029	$10.34x10^6$±20.3
YIS-3K	2.72±0.034	$9.14x10^6$±7.2
YIS-4K	2.780±0.028	$12.34x10^6$±12.7
YIS-7K	3.5±0.034	$12.16x10^6$±8.5
Control +	unobserved	$24.72x10^6$±11.1

Notes: Yeast growth includes biomass and numbers of cell. YIS-3P = YIS-3 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-4P = YIS-4 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-7P = YIS-7 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-3K = YIS-3 Yeast Isolate without the addition of phosphate nutrients; YIS-4K = YIS-3 Yeast Isolate without the addition of phosphate nutrients; YIS-7K = YIS-3 Yeast Isolate without the addition of phosphate nutrients.

The next parameter to determine the growth of yeast is the number of cells (Table 1). The calculation of the number of cells was carried out with the help of a haemocytometer. The counting chamber used with a hemocytometer can identify live and dead yeast cells through the staining mechanism with methylene blue. Cells with blue color indicate yeast cells have died. According to Suryaningsih *et al*., (2018) methylene blue will produce color when oxidation and reduction reactions occured. Living yeast cells can reduce methylene blue so that a faded color will be produced. Dead yeast cells are unable to reduce methylene blue, so methylene blue is oxidized to give a blue color.

The results of yeast cells showed that the amount of biomass was not in line with the number of cells. This is could being an evidence in the study of Roberts *et al*., (2020) that the optimization of the media carried out only increased the number of higher cells without being followed by an increase in cell biomass. YIS-3 with 0.01% phosphate medium, the number of living cells was 13.50×10^6 cells/mL higher than YIS-3 phosphate 0%, 9.14×10^6 cells/mL. This was inversely proportional to the number of YIS-4 and YIS-7 cells, where YIS-4 and YIS-7 0% phosphate had higher cell numbers (12.34×10^6 cells/mL and 12.16×10^6 cells/mL) compared to YIS-4 and YIS-7 phosphate 0.01% (10.18×10^6 cells/mL and 10.84×10^6 cells/mL).

Bread quality includes an assessment of the increase in bread dough volume, bread volume after baking, and bread organoleptic. The increase volume of bread dough was based on research, that each isolate treated with 0.01% phosphate addition showed a different development time. At the beginning of the development time from the 30th minute to the 60th minute, the positive control expanded higher and faster than others. These was because the number of cells belonging to the positive control was

higher than the number of cells in the 0.01% phosphate and 0% phosphate treatments. This is in accordance with Akbar *et al*., (2019) that the number of cells growing on the test media is closely related to the fermentation process carried out by yeast. The more fermented yeast, the more CO_2 gas will be produced so that the volume of bread will increase. The increase in yeast concentration also causes faster CO_2. So, the bubbles will reach saturation faster. As for the comparison with the negative control, all the treated isolates showed better results. The negative control dough did not have a swelling agent so the fermentation process did not occur. The results in Fig. 1 explain the calculation of the percentage of dough expansion compared to the initial dough volume showing the same pattern of expansion between 0.01% phosphate and 0% phosphate treatments, but at different times. The 0.01% phosphate treatment had a slower time compared to 0% phosphate. This can be seen in YIS-3 phosphate 0.01% which can expand since the 30th min. Meanwhile, all isolates with 0% phosphate treatment were able to expand from the 30th min.

The baking process of bread dough can also increase the volume of the bread. This is in accordance with Miś *et al*., (2016) yeast will continue to produce CO_2 during the roasting process. The higher the baking temperature, the solubility of CO_2 in the dough will decrease which in turn CO_2 will evaporate. At the same time, the saturated water vapor pressure will increase widely which causes the gases to expand as well, thus encouraging the dough to stretch and expand. On further roasting, there will be a process of protein coagulation and starch gelatinization, this causes the formation of bread crust and breadcrumb structure. At the end of baking at a temperature of about 72 °C, the bread dough loses the ability to hold CO_2, the gas will come out of the dough, so the dough isn't rising again.

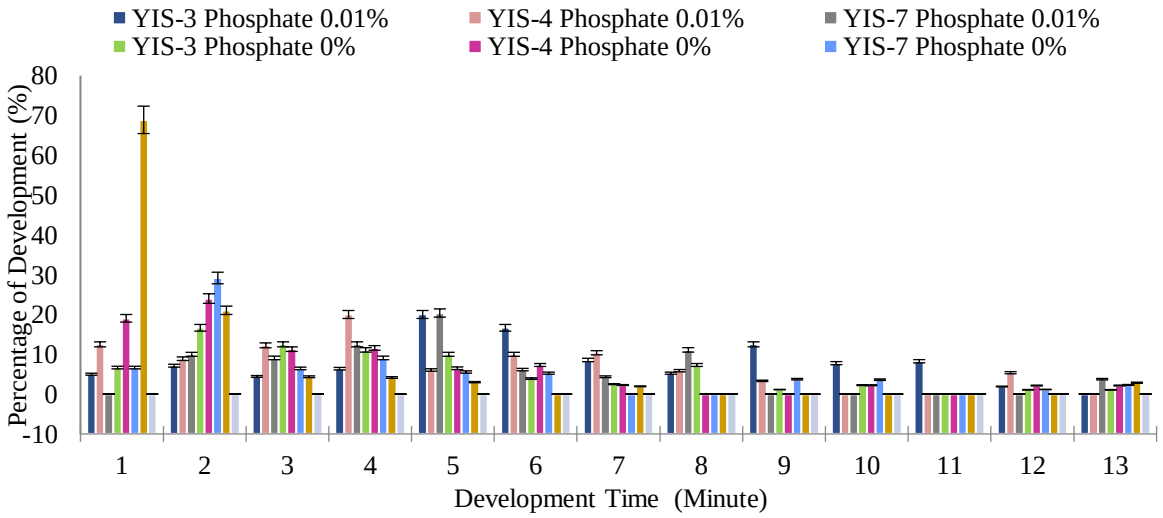

Fig. 1. Percentage of dough development; 0.01% phosphate treatment and 0% phosphate.

The volume of bread after baking affects the assessment of the quality of the bread. The results of bread after baking can be seen as the difference in volume. With the same diameter and weight, it can be compared with the height of each bread with different types of yeast (Figure 2). Yis-3 phosphate 0.01% isolates produced a higher volume of bread compared to the volume of bread with 0% phosphate treatment, positive control, and negative control. YIS-3 phosphate 0.01% was 813.89 cm^3. While in the positive control the volume of bread produced was 734.76 cm^3. Fermipan only requires a fermentation time of 30-45 minutes. In this study, the fermentation time that used was 600 minutes. The large number of living cells will cause high fermentation yields. This is in accordance with Akbar *et al*., (2019) that the number of growing cells is closely related to the fermentation process carried out by yeast. This is because more enzymes are produced. Yeast, water, and flour when combined, the enzymes present in the flour during the fermentation process will break down the starch content, so the volume of the bread will decrease (Shabrina, 2017).

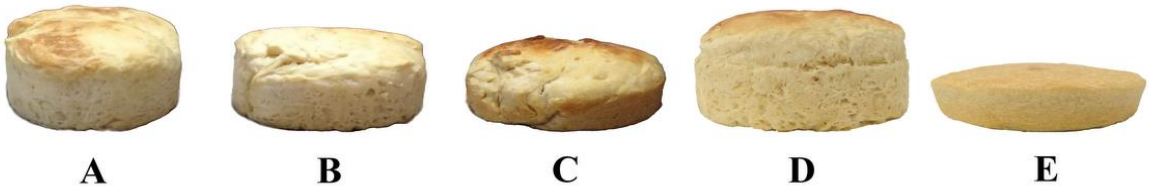

Fig. 2. Bread after baking. (a) YIS-3 phosphate 0.01%, (b) YIS-4 phosphate 0.01%; (c) YIS-7 phosphate 0.01%; (d) positive control; (e) negative control (Personal Doc.)

Organoleptic testing of bread is carried out on bread characteristics which include color, aroma, taste, and texture. In this study, hedonic test was conducted to determine the assessment of each panelist on the quality of bread fermented by yeast isolates with the addition of phosphate media. Responses to preferences are carried out by giving a certain score according to the specified scale range. The assessment based on a certain score can give an idea of the acceptance of the product by consumers (Moore *et al*., 2020).

Visually, color is very important in determining indicators of maturity, whether or not mixing or the processing is marked by an even color(Saepudin *et al*., 2017). The level of preference for the color of bread based on the results of the variance showed that the difference in the color of bread with the type of yeast treated with phosphate media had a significant effect on the level of preference of the panelists. Panelists chose bread with the use of the YIS-4 phosphate 0.01% yeast type with the highest average value. The color of the bread produced may have a color that is following the preferences of the panelists. YIS-4 phosphate 0.01% yeast has a brighter color compared to other yeast-made bread. The brown color of the bread is due to the Maillard and the caramelization of sugar during baking. Reaction Maillard is a reaction that occurs between the amine groups present in amino acids and reducing sugars, causing a brownish color (Andragogi *et al*., 2018).

The texture is an important component in determining the quality of bread. This affects the taste when chewing the material, which has the taste of real food which consists of 3 components, namely odor, taste, and oral stimulation (Saepudin *et al*., 2017). The addition of phosphate treatment on yeast media increased the number of cells that affected the ability of yeast to develop bread. Based on the results of data analysis (Table 2), it is known that the highest average texture value is YIS-3 phosphate 0.01% yeast with a value of 4.07 and the lowest average is a negative control, which is 2.17. Panelists like soft and elastic bread (Pusuma *et al*., 2018). The level of panelists' preference for bread texture is influenced by the softness of the bread (Anggarawati *et al* ., 2019). Pusuma *et al*., (2018) added that the panelists liked soft and elastic white bread. An increase in the volume of bread will have an impact on increasing the softness of the bread (Nur'utami *et al*., 2020). Guardado-Félix *et al*., (2020) added that bread products with a cavity structure will have a soft and elastic texture so that it can be seen that bread without the addition of yeast/ negative control has a hard and stiff texture because the resulting volume does not increase.

The results of the analysis of variance (Table 2) showed that the difference in the aroma of bread with yeast type treated with phosphate media significantly affected the panelists' preference level. Panelists chose bread with YIS-3 phosphate 0.01% yeast as the highest mean value. This is because the aroma produced is under the preferences of the panelists. The bread with the lowest average aroma selected by the panelists was the bread using positive control yeast. Some panelists considered that the bread smelled very sour compared to other breeds. According to Sitepu (2019), the greater the amount of yeast given, the more ingredients are fermented so that it affects the formation of compounds such as acids, aldehydes, and esters.

Table 2. Man Whitney Advanced Test Results Bread Organoleptic Treatment of Phosphate 0.01% and Phosphate 0%

Type of yeast	Color	Texture	Aroma	Taste
YIS-3P	3.47±1.008^{ab}	4.07±0.740^{a}	3.70±0.877^{a}	3.53±1.008^{a}
YIS-4P	3.50±0.777^{b}	3.03±0.809^{bc}	3.50±0.820^{ac}	3.33±0.959^{ab}
YIS-7P	3.43±0.935^{ab}	2.83±0.950^{c}	3.23±0.971^{abcd}	3.37±0.765^{bcd}
YIS-3K	2.97±1.033^{a}	3.03±0.765^{b}	3.20±0.887^{ce}	3.03±0.809^{c}
YIS-4K	3.47±0.571^{ab}	3.33±0.922^{b}	3.13±0.776^{be}	2.83±0.913^{bc}
YIS-7K	3.13±0.937^{b}	3.10±0.803^{b}	2.90±0.803^{de}	3.07±0.740^{cd}
Control +	2.33±0.606^{c}	3.67±0.959^{a}	2.00±0.788^{f}	2.37±0.964^{e}
Control -	2.43±0.898^{d}	2.17±1.020^{c}	3.03±1.033^{g}	2.20±0.887^{f}

Notes: YIS-3P = YIS-3 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-4P = YIS-4 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-7P = YIS-7 Yeast Isolate with the addition of 0.01% phosphate nutrition; YIS-3K = YIS-3 Yeast Isolate without the addition of phosphate nutrients; YIS-4K = YIS-3 Yeast Isolate without the addition of phosphate nutrients; YIS-7K = YIS-3 Yeast Isolate without the addition of phosphate nutrients. Figures with different superscripts between lines indicate a significant difference at the 95% confidence level ($p<0.05$) based on the Kruskal Wallis test, followed by the Mann Whitney test.

Taste is an important quality in bakery products. Based on the aspect of taste, it can be seen (Table 2) that the taste of bread with yeast type treated with phosphate media has a significant effect on the level of preference of the researcher. The highest average taste is YIS-3P. This could be because the YIS-3 phosphate 0.01% yeast fermented bread has a taste that is by preferences regarding the taste of bread. The taste of the bread produced is not only caused by the taste of the ingredients in the manufacture of bread, it is also caused by the waste generated during the dough fermentation process such as alcohol, acid, and esters which are the result of carbohydrate fermentation (Saepudin *et al*., 2017). This is reinforced by Birch *et al*., (2013) secondary fermentation products *nonvolatile* such as esters, phenolic compounds, and lipids.

CONCLUSION

The addition of phosphate nutrients increased YIS-3, YIS-4, and YIS-7 yeast cell biomass. The amount of cell biomass is not the same as the number of cells obtained. Only YIS-3 with 0.01% phosphate had a higher number of cells than yeast cells without 0.01% phosphate. The quality of bread produced shows the largest volume, the best value for aroma, taste, and texture characteristics is the result of bread fermentation using YIS-3 with the addition of 0.01%. The best value for color characteristics is the result of bread fermentation using YIS-4 with the addition of 0.01%. For future studies, it is expected to have the same number of cells so that the comparison is more balanced.

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