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Viability test of yeast encapsulation (*Candida tropicalis*) using sodium alginate polymer in bread production

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Abstract. This study explores sodium alginate encapsulation's impact on *Candida tropicalis* yeast cell viability and its influence on bread making. Encapsulation protects yeast cells from damage during drying and storage. The research used 10% and 15% sodium alginate for two isolates and one control. Statistical analysis, including Kruskal Wallis and Mann-Whitney tests in Microsoft Excel SPSS, showed significant effects. Sodium alginate encapsulation notably improved *Candida tropicalis* yeast cell viability and final bread swelling, especially at 15% concentration. Mann-Whitney tests on organoleptic parameters revealed panelist preferences: *Candida tropicalis* 2 with 15% sodium alginate for color, taste, and texture, while *Candida tropicalis* 1 with 15% sodium alginate for aroma. This study suggests sodium alginate's potential to enhance yeast viability and improve bread quality, offering insights for food processing and preservation applications. The research findings may contribute to developing effective encapsulation techniques for yeast-based products.

Keywords: bread quality,*Candida tropicalis*, encapsulation, endophytic yeast, fermentation, viability.

1. Introduction

Yeast is a unicellular eukaryotic microorganism that is widely used in aspects of life, one of which is in the food industry, namely processed cakes and bread. Yeast contains various enzymes, namely phosphatase, lipase, zimase and protease so that they play a role in the decomposition of organic compounds which can also be used in the bakery industry [18]. Bread is a bakery product that uses yeast microorganisms in the fermentation process [23]. The manufacture of bread and cakes utilizes yeast in its fermentation, namely in the formation of flavors and aromas. Cake or bread dough will experience an increase in volume so that it will produce a soft texture, this is due to the role of yeast which is able to break down sugar and form CO_2 and C_2H_5OH (ethanol) gases which result in carbon dioxide gas being bound and trapped [22].

Candida tropicalis is a type of yeast that can ferment and has a fairly good ability to develop bread dough. The presentation of *Candida tropicalis* bakers with commercial yeast is of equal value. Compared with commercial yeast, bread with *Candida tropicalis* developer has a better texture and taste test is more preferred by consumers. However, the aroma of bread from *Candida tropicalis* is the same as the aroma of bread produced by commercial yeast [4]. Commercial yeast that is often used is yeast in the form of biomass which has lost its water molecules by increasing the temperature. This treatment affected cell viability in commercial dry yeast [12]. The encapsulation method is a technique and method used to maintain cell viability.

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Encapsulation is often used in yeast cells to provide cell protection to keep it alive from external threats that have the potential to damage or kill cells. Encapsulation processes have been widely used in the chemical, pharmaceutical and food industries to protect active compounds from environmental conditions (oxygen, water, acids, interactions with other materials), which may affect stability during processing, to provide controlled release or to change physical properties, reduce stiffness during storage or transportation [17]. Several studies have been carried out with different active ingredients in the manufacture of encapsulation. The enzymes-galactosidase adenylate kinase and pyruvate kinase, are able to increase the permeability of the yeast encapsulation membrane but also reduce the activity of the enzymes in it [25].

All of the above methods still have drawbacks, namely the limited types of yeast tested. Yeast encapsulation methods are still limited to several types of yeasts such as *S. cerevisiae, S. bayanus , C. utilis, Cryptococcus curvatus, Kluyveromyces fragilis, Endomyces vernalis*, and *Torulopsis lipofera* . In addition, the use of active ingredients and enzymes is also considered too expensive, so it is necessary to update the encapsulation method used [15]. According to [5], sodium alginate is often used as the main polymer for making encapsulation because of its advantages that are cheaper and easy to apply. Sodium alginate when in contact with bivalent cations (eg Ca 2+) will form a network and usually take the form of a capsule or bead [6].

The yeast isolates used in this study were yeasts that had been successfully isolated from Sweet Corn (*Zea mays var. saccharata Sturt*) and Papaya Fruit (*Carica papaya L.)* which had been identified molecularly as *Candida tropicalis* yeasts with isolate code NJM1 (*Candida tropicalis* 1) and NJM2 (*Candida tropicalis* 2). Yeast isolation from corn and papaya samples yielded yeast isolates with the potential to serve as bread leavening agents. This potential was identified through biochemical tests, including H2S test, flocculation test, and fermentation test. The results of these biochemical tests serve as indicators that the yeast has the potential to be used as a bread leavening agent [1]. This is because the isolate has met the criteria as a yeast for bread dough leavening. *Candida tropicalis* has the ability to leaven bread dough quite well. The bread leavening percentage of *Candida tropicalis* is equal to the capability of commercial yeast [3].

This research has never been conducted in Indonesia. Therefore, based on the above background, this research needs to be carried out, with the hope that it can provide results according to the research objectives, namely obtaining good viability test results on *Candida tropicalis* which has been encapsulated with sodium alginate is able to improve the quality of bread.

2. Research Method

2.1. Research Materials

The research materials used in this study were *Candida tropicalis* yeast isolates, alcohol, aluminum foil, plastic, tissue, plastic wrap, paper, labels, sterile distilled water, acetic acid, Methylene blue staining reagent, Yeast Malt Broth (YMB) media, Yeast Media. Malt Extract Agar (YMEA), Yeast Peptone Glucose (YPG) medium, Sodium DL-Lactose, bread dough developer (sucrose, salt and wheat flour), yeast (Fermipan), distilled water, sodium alginate.

2.2. Research Procedure

2.2.1. Type and Research Design. This type of research is classified as experimental research. This type of experimental research is carried out by manipulating research variables and comparing the results with the control group or without manipulation [16]. The research was carried out by giving the encapsulation treatment using sodium alginate to the yeast *Candida tropicalis*. Yeast isolates with the highest number of living cells were applied to bread dough. Preparation of the *Candida tropicalis* capsule formula using sodium alginate with a concentration of 10% and 15% alginate + 22.5 g of isolate. The research design is non-parametric with the Kruskal Wallis test and the Mann-Whitney

follow-up test for bread quality parameters. Quantitative data analysis, namely the results of yeast viability and volume tests, will be carried out using one way ANOVA analysis using Microsoft Excel.

2.2.2. Research Variables. The independent variable in this study was the type of isolate used, namely *Candida tropicalis*. While the dependent variable in this study was the viability of encapsulated yeast cells and bread quality which included volume, color, aroma, texture and taste of bread.

2.3. Time and Place

This research was carried out from December 2021 to December 2022. This research was carried out at the Laboratory of the Department of Biology, Faculty of Science and Technology, State Islamic University of Maulana Malik Ibrahim Malang. Microencapsulation treatment using sodium alginate polymer with chitosan coating on *Candida tropicalis* yeast cells was carried out in the Microbiology Laboratory. While testing the quality of bread is carried out in the Food and Biochemistry Laboratory.

2.4. Yeast Rejuvenation

Rejuvenation of *Candida tropicalis* yeast isolates was carried out in an aseptic manner in Laminar Air Flow (LAF). One yeast colony was inoculated on YMEA solid media using the streak plate method. The culture was then incubated for 48 hours at room temperature 28 ºC. After 48 hours of incubation, two colonies of yeast isolates that grew on YMEA media were multiplied on YMB liquid media, incubated on a shaker at 140 rpm at 33 ºC for 24 hours [26].

2.5. Encapsulation Procedure with Sodium Alginate

2.5.1. Capsule Manufacturing. A sterile alginate solution containing *Candida tropicalis* suspension was put into a dropper pipette and then dripped precisely and slowly into a 0.5 M C6H10CaO6 solution, let stand for one hour until a solid encapsulation was formed, then the capsules formed were transferred to sterile distilled water and stirred slowly using a shaker for one hour to remove residual C6H10CaO6, then filter.

2.6. Yeast Viability Test (Number of Living Cells)

Calculation of the number of cells is carried out using the counting chamber method with minor modifications [11]. Sterilization was carried out on the Haemocytometer device and cover glass with 70% alcohol. The cover glass is placed on the Haemocytometer. The encapsulated yeast was dissolved and then 100 µL of yeast inoculum was taken and put into a 1.5 ml microtube. 100 µL of methylene blue dye was added and 1 ml of sterile distilled water was also added as a diluent. The suspension obtained was homogenized with a vortex. Take as much as 20 µL to be put into the Haemacytometer well. Cells were counted with the help of a 400x magnification microscope. After Haemocytometer calculations, the results obtained are entered into the following formula [10]:

$$\text{average number of cells/boxes} = \frac{\text{living cells count}}{\text{5 boxes}} \qquad (1)$$

$$\text{dilution factor} = \frac{\text{final suspension volume}}{\text{inoculum volume}} \qquad (2)$$

$$\text{cell number } \left(\frac{\text{cell}}{\text{ml}}\right) = \text{average number of cells/boxes x dilution factor x } 10^4 \qquad (3)$$

Information:
104 = 0.1 µL conversion in 1 ml
0.1 µL = volume in the medium box Haemocytometer

2.7. Making of Bread Dough

Making bread dough begins with weighing the ingredients to be used. The dosage of ingredients used consisting of 200 gr flour, 3 gr salt, 15 gr sugar, 16 gr butter, 1.2% (2.4 gr) encapsulated yeast, 2 .4 gr fermipan, and 70 ml of water. All the ingredients are mixed and kneaded until it becomes a smooth dough. Each dough sample is weighed as much as 300 gr and put in a measuring cylinder or mold. The dough was incubated at ± 30 ºC and the increase in dough volume was observed. The final stage of making bread is the baking process at 150 ºC for 30 minutes [10].

2.8. Bread Quality Testing

Testing the quality of this bread is based on research by [24], this test includes volume, taste, aroma, color and texture. Volume measurement is done by calculating the difference between the final volume and the initial volume of the bread dough. Measurements were made at 30 minute intervals for 1200 minutes. The calculation of the percentage of development is based on [21]:

$$\%\ \text{bread developing} = \frac{\text{final dough volume} - \text{starting dough volume}}{\text{starting dough volume}} \text{x } 100\% \qquad (4)$$

Testing the aroma, taste, color and texture characters was carried out using an organoleptic test involving 30 panelists. The assessment given to the quality of the bread being tested will be expressed in the form of a score. The scale used is scoring, with details of value 1 = really dislike; 2 = do not like; 3 = neutral; 4 = likes and 5 = likes very much [7].

2.9. Data Analysis

Data analysis was performed on both test parameters. Yeast viability and organoleptic data were analyzed using Kruskal Wallis with the SPSS program. The Mannwhitney follow-up test was carried out on organoleptic data with a significance level of 5% [19].

3. Results and Discussion

3.1. Yeast Viability

The difference in the percentage of the amount of sodium alginate used aims to determine the differences in the viability of encapsulated yeast cells. The use of 10% sodium alginate solution in a mixed suspension of sodium alginate + yeast solution is in accordance with [5], using a 10% solution of a mixture of yeast *Saccharomyces cerevisiae* and sodium alginate solution. Yeast encapsulation results can be seen in Figure 1.

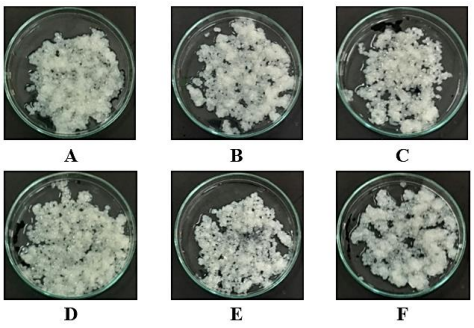

Figure 1. Results of *Candida tropicalis* isolate capsules. A) *Candida tropicalis* 1 + 10% Alginate , B) *Candida tropicalis* + 15% Alginate, C) *Candida tropicalis* 2 + 10% Alginate, D) *Candida tropicalis* 2 + 15% Alginate, E) K+1 + 10% Alginate and F) K+1 + 15% Alginate

Figure 1 shows the results of the yeast-suspended alginate capsule that has been formed. The spherical capsule is formed due to an ionic gelation process between alginate and calcium compounds in a calcium lactate solution used as an activation solution [14]. When calcium is exposed to sodium alginate solution, the solution will react with calcium lactate and cause an ionic gelation reaction [15]. Calcium diffuses through the matrix until it reaches an area where it is able to form non-covalent physical interactions with sodium alginate. Thus forming a capsule. Encapsulation can be used to increase yeast viability, surround stable nuclei from environmental impacts, thereby increasing stability, extending core shelf life, and sustaining and controlled release.

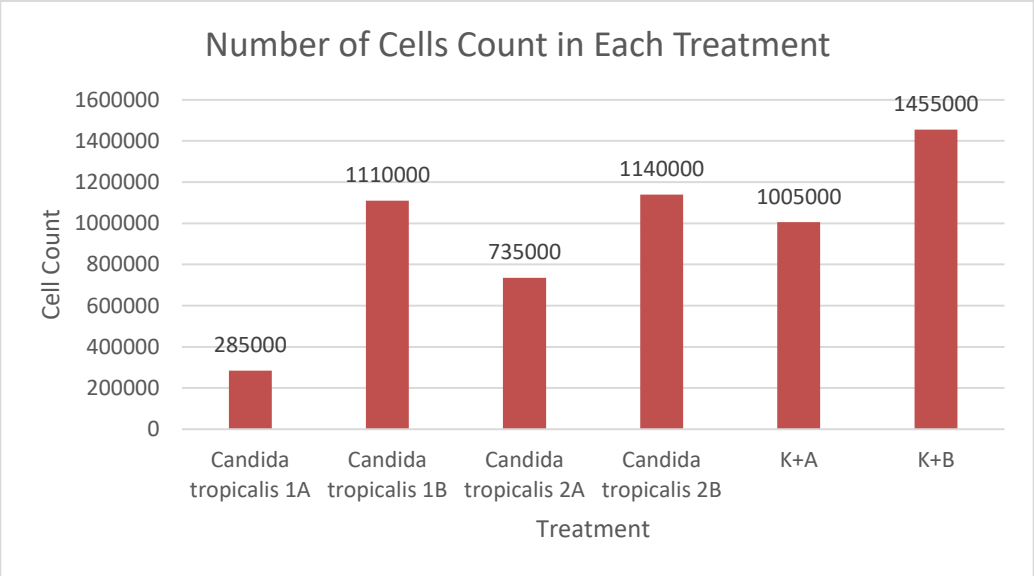

Figure 2. Number of cells in each treatment

The results of the study in Figure 2 show that the average number of yeast cells from each isolate that was treated with the addition of 15% sodium alginate concentration resulted in a higher number of cells when compared to the concentration treatment with the addition of 10% sodium alginate. In the K+ treatment with the addition of 15% sodium alginate it also showed significantly more cell numbers than the number of cells in K+ with the addition of 10% sodium alginate. This shows that a concentration of 15% sodium alginate + 2.25 g of yeast isolate optimally works more effectively in maintaining the number of living cells in yeast that has been treated than at a concentration of 10% sodium alginate + 2.25 g of yeast isolate. Statistical analysis proved that encapsulation with sodium alginate polymer had an effect (P =0.005<0.05) on the viability of *Candida tropicalis yeast cells*. The results of the best encapsulation concentration, then the isolate concentration was reduced and taken from 6 treatments to the 3 most significant treatments to test the quality of encapsulated yeast bread using organoleptic tests with volume and hedonic quality parameters of the bread formed.

3.2. Quality of Encapsulated Yeast Fermented on Bread

Figure 3 shows that each treated isolate showed a different development time. When compared with the positive control it can be seen that at the beginning of the development time of the 30th minute the positive control expands constantly until the 1200th minute. This is because a stable metabolic reaction occurs, so that development occurs constantly. The positive control experienced a continuous increase in volume starting from the beginning, while the other treatments experienced a constant phase before a continuous increase in volume occurred, but the positive control experienced a decrease in volume after the 1200th minute. The addition of bread is caused because during fermentation, the yeast Saccharomyces cerevisiae which is added to the bread-making process will produce CO_2 gas as a

result of glucose metabolism. The CO2 gas that is formed will increase the growth of air bubbles on the bread [20].

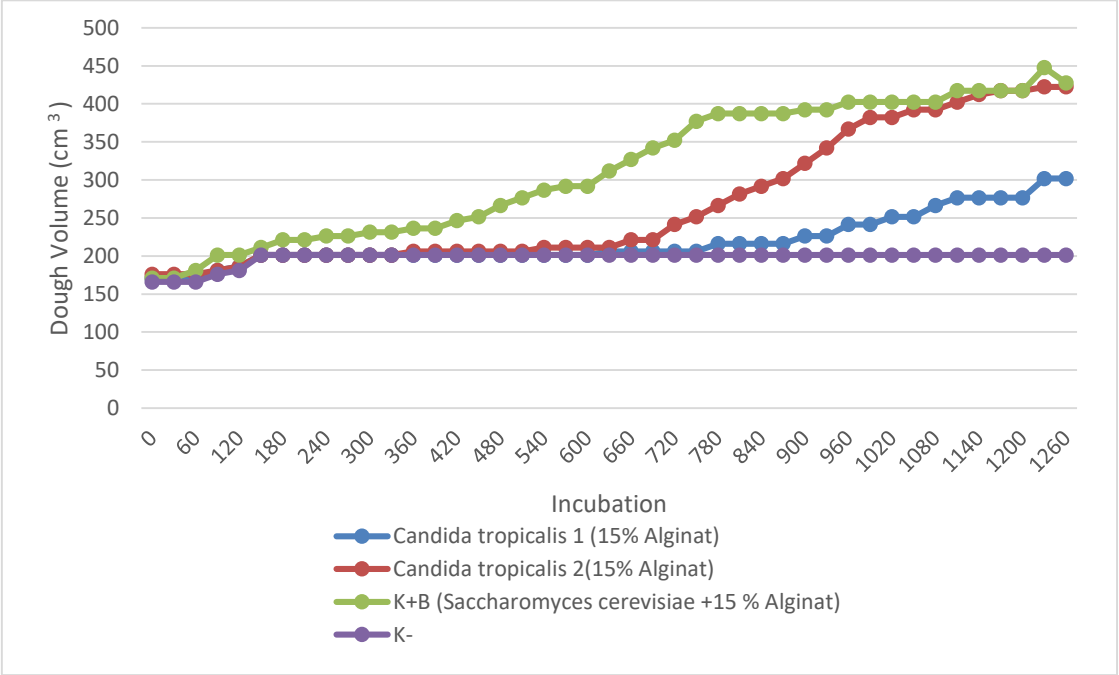

Figure 3. Effect of incubation time of *Candida tropicalis yeast isolates* on the volume of bread dough

The decrease in bread volume in the positive control in the 1200th minute was due to a decrease in yeast fermentation activity. The tendency for a constant and even decreased concentration of bioethanol is caused by reduced nutrients in the medium and the longer the fermentation process causes much of the bioethanol that has been produced to be oxidized into organic acids and CO_2 which are inhibitory compounds by microorganisms which cause death in microorganisms [2]. All treatments showed the same thing at the beginning of time, namely an increase in bread volume from the 90th to 150th minute before experiencing a constant phase. All treatments experienced an increase in bread volume again at different times. The more yeast that carries out the fermentation process, the more carbohydrates are broken down into alcohol and CO_2, so that the dough can expand quickly too [22]. Meanwhile, when compared with the negative control, all treatment groups showed better results. This is because in the negative control there is no swelling agent added so that the bread dough fermentation process does not occur which results in the percentage of swelling from the 150th minute to the 1200th minute showing 0%.

3.3. Bread Organoleptic Test Results using Encapsulated Yeast

Based on the results of the Kruskal-Wallis test, it showed P <0.05, meaning that H0 was rejected so that there was a significant difference in the treatment (*Candida tropicalis* 1+ 15% sodium alginate, *Candida tropicalis* 2+ 15% sodium alginate, *Saccharomyces cerevisiae* isolate + 15 % sodium alginate, and negative control without giving yeast) on aroma, color, texture, and taste attributes that have been encapsulated in yeast growth media (*Candida tropicalis* 1 with the addition of 15% sodium alginate, *Candida tropicalis* 2 with the addition of 15% sodium alginate, K+ with the addition of 15% sodium alginate, and K-). The average score for each treatment is shown in Figure 4.

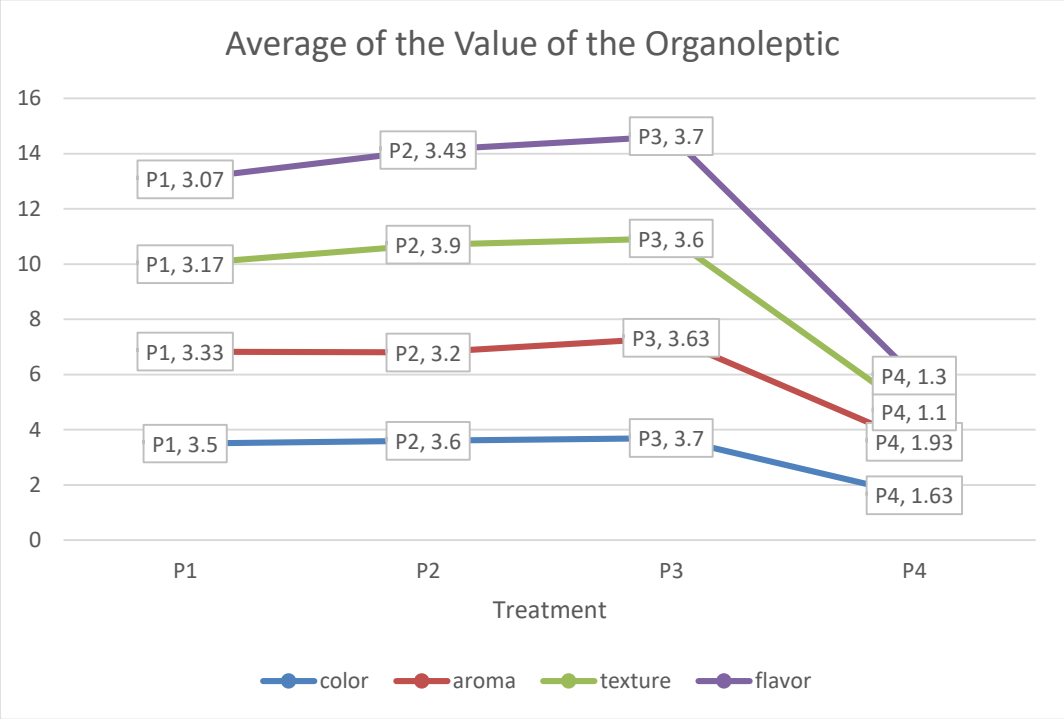

Figure 4. The curve of the average value of the organoleptic test

The Mann-Whitney follow-up test was used to determine the real effect between each treatment. The mean (mean) results of the Mann-Whitney follow-up test are shown in Table 1.

Table 1. The results of the Mann-Whitney test for several parameters

Parameter	**Hedonic Test**			
	P1	P2	P3	P4
Color	3.50±0.662 ^{a}	3.60±0.724 ^{b}	3.70±0.651 ^{a}	1.63±0.718 ^{d}
Aroma	3.33±0.802 ^{a}	3.20±0.610 ^{b}	3.63±0.615 ^{c}	1.93±0.980 ^{d}
Texture	3.17±0.791 ^{a}	3.90±0.662 ^{b}	3.60±0.563 ^{b}	1.10±0.305 ^{a}
Flavor	3.07±0.828 ^{ab}	3.43±0.728 ^{a}	3.70±0.750 ^{b}	1.30±0.535 ^{d}

Color parameters, based on the results of the Mann Whitney advanced test, the color parameters in Table 4.3 show that all treatments (*Candida tropicalis* 1+ 15% sodium alginate and *Candida tropicalis* 2+ 15% sodium alginate) were significantly different from the positive control (*Saccharomyces cerevisiae* isolate + 15% sodium alginate) and negative control. This means that each treatment has a significant effect on the panelist's response to the color attribute. The organoleptic test scores on the color attribute ranged from 1.63 to 3.70 which indicated the panelists' responses were between likes and dislikes. The highest average value is the positive control of *Saccharomyces cerevisiae isolates* with a value of 3.70 (likes). While the lowest average value is the negative control with a value of 1.63 (dislike). This is because in the negative control there is no yeast activity which ferments the bread

dough so that Maillard reactions and sugar caramelization do not occur which cause color formation. The concentration of yeast added to the dough is also a factor that affects the formation of color. This is because if the yeast concentration increases, the starch converted to sugar also increases, so that the Maillard reaction and sugar caramelization occur more quickly which results in the formation of an increasingly brown crust [22]

Aroma parameters, based on the results of the Mann-Whitney advanced test for aroma attributes in Table 4.1 with a significance level of 5%, showed a significant difference between the treatment groups (*Candida tropicalis* 1 with the addition of 15% sodium alginate and *Candida tropicalis* 2 with the addition of 15% sodium alginate) with the positive and negative control groups (*Saccharomyces cerevisiae isolate* with the addition of 15% sodium alginate and without the addition of yeast). The organoleptic test scores on the aroma attribute ranged from 1.93 to 3.63 which indicated the response of the panelists between likes and dislikes. The highest average value was obtained by *Saccharomyces cerevisiae isolates* with the addition of 15% sodium alginate, namely the positive control of *Saccharomyces cerevisiae isolates* with a value of 3.63 (likes). While the lowest average value is the negative control with a value of 1.93 (dislike) in the negative control there is no yeast activity that carries out the fermentation so that the aroma that is formed is like the smell of flour. The aroma that is formed on the bread is a result of the activity of yeast fermentation. During fermentation, yeast metabolizes by producing several types of recursive compounds 8such as 8recurs, aldehydes, esters and ketones. Compounds that play a role in the formation of aromas are produced by yeast activity during fermentation [9].

Texture parameters, based on the results of Mann Whitney's advanced test for texture attributes in Table 4.1 with a significance level of 5%, showed a significant difference between the treatment groups (*Candida tropicalis* 1 with the addition of 15% sodium alginate and *Candida tropicalis* 2 with the addition of 15% sodium alginate) with positive and negative control groups. The organoleptic test values for the texture attributes ranged from 1.10 to 3.90 which indicated the panelists' responses were between likes and dislikes. The highest average value was produced by *Candida tropicalis* 2 with the addition of 15% sodium alginate with a value of 3.90 (likes). When compared with the positive control, the panelists preferred the texture of the yeast *Candida tropicalis* 2 isolate with the addition of 15% sodium alginate. This is due to the concentration of yeast and the appropriate time, the fermentation will work optimally so that the resulting product will form perfect dough pores and produce a soft texture on the bread.

Whereas in the positive control the dough was *over proof* which made the dough soft and watery so that after baking, the pores formed on the bread were less than perfect. The lowest average value is produced by a negative control with a value of 1.10 (dislike). The texture formed on bread is the result of the presence of yeast fermentation products in the form of alcohol and CO_2 [7]. The CO_2 will be dispersed in the form of fine bubbles which are retained by the gluten due to mechanical treatment. Thus after roasting it will form fine pores on the bread and also a soft texture. The factors that affect texture are the use of flour and the proofing time of the bread. In this research the second proofing time used is 30 minutes.

Taste parameters, based on the results of the Mann Whitney advanced test for taste attributes in Table 4.1 with a significance level of 5%, showed a significant difference between the treatment groups (*Candida tropicalis* 1 with the addition of 15% sodium alginate and *Candida tropicalis* 2 with the addition of 15% sodium alginate) with positive and negative control groups. The organoleptic test scores on the taste attribute ranged from 1.30 to 3.70 which indicated the panelists' responses were between likes and dislikes. The highest average value was produced by *Saccharomyces cerevisiae isolates* with a value of 3.70 (likes). When compared with *Candida tropicalis* 2 isolates with the addition of 15% sodium alginate with a value of 3.43, the difference is only around 0.27. While the lowest average value is produced by a negative control with a value of 1.30 (dislike). The taste that forms on the bread is a result of the Maillard reaction and also the caramelization of the sugar. Apart from playing a role in the formation of color and aroma, Maillard reactions and caramelization of sugar also contribute to the formation of taste [22]. Through the Maillard reaction, free amino acids

and reducing sugars interact to produce several compounds including alcohols, aldehydes, esters, ethers, ketones, acids, furans, hydrocarbons, lactones, pyrazine, pyroline, and sulfur compounds [13].

4. Conclusion

The conclusion of this study is that there is an effect of encapsulation with sodium alginate polymer in increasing the viability of *Candida tropicalis yeast cells* with the best results (average highest number of cells) at concentration B (15% alginate) compared to concentration A (10% alginate) in each each isolate (NJM1, NJM2 and Control +). Meanwhile, the results of the final "percentage volume swelling test" on the concentration of NJM1B and NJM2B isolates showed a significant increase, with the treatment of the concentration of NJM2B isolates (140%) very close to the positive control (161.76%) compared to the treatment of the concentration of NJM1B isolates (76.47 %) after the 1200th minute. The results of Mann Whitney's advanced test on the "organoleptic test" parameter with color, taste, and texture attributes showed that the panelists preferred fermented bread from NJM2B, while the aroma attribute showed that panelists preferred fermented bread from NJM. This study highlights the potential of sodium alginate encapsulation, particularly at a 15% concentration, to significantly enhance Candida tropicalis yeast cell viability and improve the volume and organoleptic qualities of bread. The findings hold practical implications for the food industry, suggesting a promising approach for preserving yeast and enhancing bread quality through effective encapsulation techniques.

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