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Suitability of Endophite Yeasts from The Skin and Flesh of The Podang Mango (*Mangifera Indica* L.) as Leavening Agents for Bread

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Abstract. Yeast is a leavening agent used to aid the fermentation process in bread making, by producing ethanol, as well as carbon dioxide, to improve the aroma, and increase the dough's volume, respectively. In Indonesia, the yeast used in bread making is imported. Thus, there is a need for homegrown alternatives. This study, therefore, aims to obtain yeast isolates with the capacity to act as bread leavening agents from the skin and flesh of Mangifera indica var. Podang. In this study, a total of three endophytic isolates, YDM-1, and YDM-3, from the fruit's flesh, as well as YKM-1 from the skin, were obtained. YDM-1 and YKM-1 were identified macroscopically and microscopically as Ascomycota class and Hemiascomycetes sub-class. In comparison, YDM-3 was identified as Ascomycota class and Archiascomycetes sub-class. Subsequently, the isolates were subjected to carbohydrate fermentation, 50% glucose tolerance, flocculation, hydrogen sulfide compound, temperature tolerance, and ethanol tolerance analyses. The three isolates have met the criteria for leavening yeast. YDM-3 and YKM-1 are able to produce a similar increase in the volume of bread dough commercial yeast, with an incubation period of 12 hours. Meanwhile, YKM-1 produced the best aroma as well as the best potential in developing bread and is, therefore, suitable to be used as commercial yeast.

INTRODUCTION

Indonesia is a tropical country that has a variety of fruits with high carbohydrates and sweet taste [1]. Podang mango fruit (Mangifera indica L.) is fruit from Kediri East Java Indonesia that has a sweet taste, and widely developed horticultural commodity because it has a very good opportunity [2]. According to Yuliati and Kurniawati [3], podang mango fruit contains sugar 13.95% a higher than gadung mango and hampalan mango fruit. Fruit with high carbohydrates can be ideal habitat for yeast growth [4].

Few reports in the literature analyzed the diversity of yeasts associated with flesh of the mango fruit in Columbia reveal seven species of yeast they are *Meyerozyma caribbica, M. guilermondii*, *Clavispora natalensis*, *Aureobasidium pullulans*, *Pichia* sp. *Saturnispora diversa* dan *C. Jaronii* [4]. In addition, yeast isolates from mango fruit in Euthopia are *S. boulardii*, *S. cerevisiae* B., *Candida apicola*, dan *Zygosacharomyches fermentation* [5]. Yeast isolates are also isolated from the flesh and skin of the mango fruit Candida sp, *Kluyveromyces marxianus*, *Pichia kudriavzzevii*, and *Saccharomyces cerevisiae* [6].

Yeast has benefits in various industrial fields, one of which is as a bread developer. The process of making bread consists of mixing dough, fermenting dough, and baking [7]. Yeast fermentation in bread dough utilizes sugar to produce carbon dioxide (CO_2), ethanol, and amino acids, which function to increase volume, taste, and aroma in bread dough [8]. Yeast which is used for bread, has tolerance for glucose ethanol temperature [9]. Yeast isolates have been isolated from skin and flesh are *Candida* sp, *Kluyveromyces marxianus*, *Pichia kudriavzzevii*, dan *Saccharomyces cerevisiae* [6]. The test of the ability of endophytic yeast isolates from mangoes to increase the volume of bread dough

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has been published by a study in Malaysia. The yeast isolate was obtained from *Saccharomyces cerevisiae* which can grow at 37 °C, capable of fermenting carbohydrates, sucrose, maltose, lactose, fructose, and glucose [8]. While research on yeast isolation from the flesh and skin of mango podang fruit in Indonesia has not yet been carried out, so it is possible to find unique and competent isolates to be used as bread dough developers considering that Indonesia still uses imported baker's yeast. This study aims to isolate and analyze the potential of yeast from the flesh and skin of mango podang as a bread dough developer.

MATERIAL AND METHOD

The materials used in this study were flesh and skin of mango podang (*Mangifera indica* L.) from Mojokerto East Java Indonesia, sucrose, glucose, lactose, fructose, sugar, salt, flour, YMB (Yeast Malt Broth), YMA (Yeast Malt Extract Agar), YPG Broth (Yeast Extract Peptone Glycerol), Lead Acetate Medium, ethanol, carbohydrate fermentation medium, commercial yeast, and Sodium DL-Lactose.

Yeast Malt Extract Broth/Agar (YMB/YMA) Media

YMB media was made with reference to Kurtzman and Felt's 1998 book. The preparation of 1000 mL of YMB media was carried out using 10 grams glucose, 3 grams *yeast extract*, 5 grams peptone, and 3 grams *malt extract,* and YMA added 20 grams *microbial agar*. The media was sterilized using an autoclave at 121 °C for 15 minutes. Sterillized YMB was added with the antibiotic Sodium DL-Lactose as much as 120 µL when the media temperature was 50 °C [10] [11].

Isolation and Purification of Yeast Colonies

Samples of riped podang flesh and skin were cleaned with alcohol. The sample was cut crosswise and put into a 50 mL Eppendorf tube until it was full, then added Yeast Malt (YM) Broth and was incubated at 27 °C for 48 hours [11], [12]. The selected yeast colony was purified by taking 1 mL of the sample solution into a test tube containing 9 mL of sterile distilled water, and a serial dilution was carried out until 10^{-3}, then 200 µL of the sample was inoculated on Yeast Malt (YM) Agar using the spread plate method and then incubated at 27 °C for 72 h [13]. Yeast isolates were a subculture to obtain pure isolates.

The selected isolates were grown on YM Broth and shaken at room temperature for seven days, then it was inoculated on YM Agar with the spread plate technique and incubated at 27 °C for 48 h. Furthermore, culture was performed on YM Agar with streak quadrant plate. After the colonies were separated, they were inoculated on YM Agar slanted media [14].

Macroscopic and Microscopic Observation

The macroscopic observation was carried out following the book "The Yeast: A Taxonomy Study," covering texture, color, surface, elevation, and edge. Microscopic observation was carried out using a smear-dried technique. Isolates observance was carried out under light microscope Nikon Eclipse E 200 LED MV RS 4×10 until 100×10 magnification to observe the size, shape, and asexual reproduction of yeast cells.

Screening as Baker's Yeast

Carbohydrate Fermentation Test

The carbohydrate fermentation test was carried out by adding 100 µL yeast isolates aged 48 hours into a test tube containing 10 mL carbohydrate fermentation solution 10% (m/v). The carbohydrates used were fructose, glucose, lactose, and sucrose. Furthermore, incubation was carried out at 27 °C for seven days. Every day, the bubbles and changes in color were checked, and on the seventh day, the pH was checked using a pH meter. Production of gas trapped in Durham tube was taken as fermentation positive while only acid production was taken as carbohydrate assimilation [15]

Glucose Tolerance Test

A tolerance test for glucose was carried out according to the method from [16]. Yeast isolates grown on YMB for 48 hours as much as 100 µL were grown in 10 mL YPG containing concentration glucose 50% (m/v) and incubated at 30 °C for 48 h. The Optical density (OD) for UV absorbance was examined at 600 nm using UV-Vis spectrophotometer every 24 hours until 48 hours and the growth medium was blank.

Flocculation and Hydrogen Sulfide (H_2S) Test

Flocculation and hydrogen sulfide tests were carried out according to the method from[9]. 100 µL yeast isolates grown on YMB for 48 hours were grown in 10 mL YPG containing concentration glucose 50% (m/v) and incubated at 30 °C for 72 h. Then it was centrifuged for 60 minutes at 500 rpm. The formation of floc was characterized by the presence of deposits under the Eppendorf tube [17]. Yeast isolates aged 48 hours were grown on *lead acetate* with a stab method at the base of the media and incubated at 30 °C for 7 days. The black color base of the media indicated yeast contains hydrogen sulfide.

Temperature and Ethanol Tolerance Test

Temperature and ethanol tolerance tests were carried out according to the method [18]. Yeast isolates grown on YMB for 48 hours. A100 µL of yeast isolates were cultured in three different test tubes containing 10 mL YPG broth and incubated at 30, 37, and 45 °C for 72 hours. The Optical density (OD) for UV absorbance at 600 nm was examined using UV-Vis spectrophotometer every 24 hours until 72 h hours and the growth medium was blank.

100 µL of yeast isolates grown on YMB for 48 hours were grown in 10 mL YPG broth containing three different concentrations of ethanol of 10%, 13%, and 15% (v/v) and incubated at 30 °C for 72 h. Reading of Optical density (OD) for UV absorbance at 600 nm was carried out using UV-Vis spectrophotometer every 24 hours until 72 h hours and the growth medium as blank.

Yeast Application as Bread Developer

Yeast was put in a shaker for 72 hours and then centrifuged for 60 minutes at a speed of 500 rpm to take yeast pellets. After that, the bread dough was tested by preparing 17 grams of wheat flour mixed with 1% salt, and 6% sugar. It was dissolved in 17 mL of warm water and 0.2 grams of yeast pellet strain was inoculated into a sugar solution then waited for 2 hours for the cell activation process. All compositions were mixed and stirred. This experiment was compared with a positive control using fermipan and a negative control without bread yeast. The test was carried out by incubating the dough at room temperature for 12 hours for the dough development process then it was baked in a hots air oven at 180 °C for 20 minutes [9]. The observed parameters included bread dough volume, bread aroma, bread crust color, and bread texture after baking.

RESULTS AND DISCUSSION

Endophytic Yeast Flesh and Skin of Podang Mango Fruit

Isolation and purification of endophytic yeast from mango podang resulted in three isolates. From the flesh of the mango podang, two isolates were obtained which were coded YDM-1, and YDM-3, while from the skin of the mango fruit obtained one isolate with the code YKM-1. The three isolates had different morphological characters. The different morphological characters indicate the diversity of endophytic yeasts in the flesh and skin of the podang mango. The macroscopic characters of endophytic yeasts from the flesh and skin of mango podang are shown in Table 1.

TABLE 1. Microscopic and macroscopic characteristics of endophytic yeasts

Character	Yeast Isolates		
	YDM-1	YDM-3	YKM-1
Shape	Irregular	Circular	Circular
Texture	as creamy	as creamy	Mucoid
Colour	White	Cream	White
Elavation	Convex	Flat	Flat
Margin	Wavy	Entire	Entire
Surface	Dull	Glistering	Glistering
Asexual Reproduction	Ovoid	Engolated	Apiculate
Size Width × Lenght (µm)	Multiteral Budding	Fission	Monopolar Budding

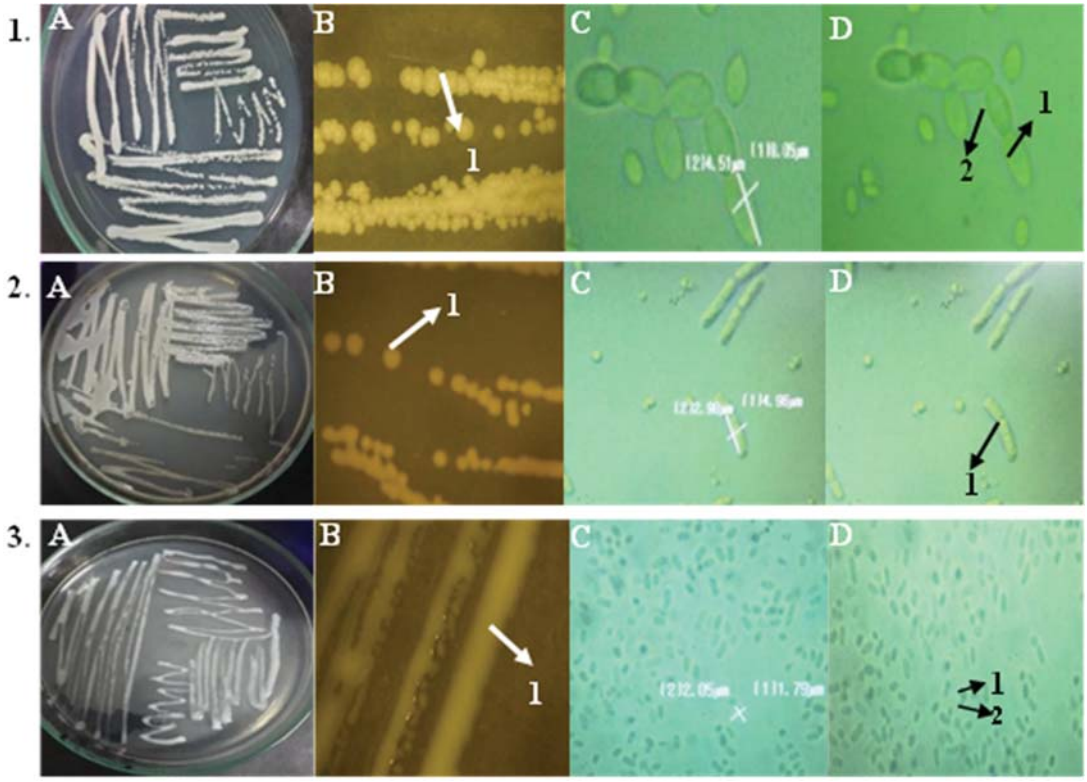

FIGURE 1. Isolate 1) YDM-1; 2) YDM-3; 3) YKM-1; 4) RYK2; 5) RSK1, a) Colony in YMEA media b) Colony 10× magnification, c) Cell shape 1000× magnification, d) 1. Stem cells 2. Stem cells, monolateral budding type.

Endophytic yeast isolates from mango flesh and skin are included in the Ascomycetes class because the obtained yeast isolates have white, bone-white, and cream colors (Figure 1). Yeast class Ascomycetes generally do not have color pigments like class Basidiomycetes [19]. Figure 1 shows the morphology of yeast obtained using a microscope (1000 × magnifications). Colony size ranges from 1.75 × 2.09 to 4.51 × 8.05. Yeast strain YDM-1 and YKM-1 show asexual reproduction by budding (Figure 1). Asexual reproduction by budding belongs to the subclass Hemiascomycetes [20]. Meanwhile, YDM-3 isolate reproduce asexually by fission. (Figure 1). According to Yamauchi *et al*. yeast cells that reproduce asexually by fission are included in the subclass Archiascomycetes [21].

Carbohydrate Fermentation Test

All the yeast isolates from podang mango flesh and skin visible of gas were trapped in the Durham's tube, except for YDM-1 isolates. No gas was trapped in the Durham tube on lactose and sucrose sugars. Yeast isolates have varying abilities to ferment carbohydrates, similar to the effect on the pH value (Table 2). The increase and decrease in pH

occur because the final product produced is different depending on the type of microorganisms involved. The carbohydrate fermentation test produces gas released by yeast isolates in the fermentation process.

TABLE 2. The ability of endophyte yeast isolate ferment the carbohydrate after 7 days incubation

Sugar Type	Isolate Name	Colour	Gas	pH
Sucrose	YDM-1	Red	-	7
	YDM-3	Pink	++	8
	YKM-1	Pink	++	8
	Negative Control	Red	-	7
Fructose	YDM-1	Pink	++	6
	YDM-3	Pink	++	8
	YKM-1	Pink	++	8
	Negative Control	Red	-	7
Glucose	YDM-1	Pink	+++	5
	YDM-3	Pink	++	8
	YKM-1	Pink	+++	8
	Negative Control	Red	-	7
Lactose	YDM-1	Red	-	7
	YDM-3	Pink	+	5
	YKM-1	Pink	+	5
	Negative Control	Red	-	7

Note: (-) no bubbles; (+) slight bubbles; (++) medium bubble; (+++) many bubbles.

According to Yumas and Rosniati, the end result of carbohydrate fermentation by yeast is the release of carbon dioxide, acetic acid, and ethanol [22]. Yeasts that produce acetic acid are characterized by a decrease in pH, while those that produce ethanol are characterized by an increase in pH. The decrease in pH in the media occurs due to the release of H+ ions during the fermentation process to produce organic acids and the increase in pH occurs due to an increase in OH- ions due to the yeast growth process [23].

During the fermentation process, yeast releases carbon dioxide which is used to develop bread dough [24]. The ability to produce gas is the most important criterion for yeast as it represents its high invertase activity and high gas production so that the yeast can be selected as bread expanders [9]. In the fermentation of carbohydrates, Yeast ability to release carbon dioxide is required for the development of the dough during the baking process [25]

Yeast ability test on 50% glucose

The isolates that experienced an increase in the value of optical density after incubating from 24 hours until 72 hours were YDM-1 and YDM-3 isolates, while YKM-1 isolate experienced a decrease in optical density values (Table 3). The increase in cell density indicates the growth of yeast cells in media with a 50% glucose concentration. High sugar concentrations cause osmotic pressure in yeast cells causing yeast cells to lose water in the cytoplasm so that it will inhibit yeast cell growth [26] [27].

TABLE 3. The optical density of cell yeast in 50% concentration glucose

Yeast Isolates	Time Hours	
	24	48
YDM-1	0.165	0.424
YDM-3	0.056	0.075
YKM-1	0.058	0.031
Negative Control	0.000	0.000

According to Ali and Khan, yeast isolates tested at 35% glucose concentration have optical density values below 0.2 [16]. This shows that YDM-1 isolate in this study is better than previous studies because at 50% glucose concentration it has a density value of 0.424. YDM-3 and YKM-1 isolates are also still able to grow at 50% glucose concentration during 24-hour incubation. Yeast endophytic flesh and skin of mango podang can be used as a bread

dough developer because they are still able to live at a glucose concentration of 50%. According to Asyikeen *et al*. yeast isolates with the qualitative tests are able to grow well at 20% glucose concentration, so that they can be used as bread developers [18].

According to Okafor yeast that can be used as a bread dough developer is able to tolerate the osmotic pressure of salt and sugar in the dough [28]. Yeast that is able to grow on the glucose tolerance test can be used for bread development because in the bread development process there are several types of sugar in the manufacture of bread dough. In dough, there are three sources of natural sugars, namely flour (glucose, sucrose, fructose, and maltose) and sucrose which can be added by bakers, and maltose that is released by the amylolytic breakdown of starch [29].

Flocculation and Hydrogen Sulfide Test

Endophytic yeast isolates from mango podang flesh and skin show flocculations formation and does not produce hydrogen sulfide compound (Table 4). YKM-1 and YDM-3 isolates have the most flocculation and do not produce hydrogen sulfide compounds. While the YKM-1 isolate produces a brown color and forms carbon dioxide so that the medium is lifted.

TABLE 4. Result of flocculation and hydrogen sulfide test of isolates yeast

Yeast Isolates	Flocculation	Hydrogen sulfide production
YDM-1	Yes	-
YDM-3	Yes	-
YKM-1	Yes	++
Negative Control	No	-

Note: (-) basic media fixed color; (+) basic media brown color; (++) basic media brown to black color; (+++) media basic black color

Yeast cells that are able to carry out the flocculation process due to the adhesion process between cells can be used for commercial yeast production, used as bread developer, and ethanol production. According to Asyikeen *et al*., Flocculation characteristics are determined by yeast cells that can be separated from the broth medium [18]. Floc formation in growth media shows high yeast cell density [9]. Floc formation can also accelerate commercial yeast products because it does not require filtration during precipitate formation, so that yeast cells can be easily separated from the growth medium. The purpose of floc formation in yeast is to survive under tense conditions, such as no nutrients so that it can be stored when there is no growth medium [30].

High ethanol production in bread dough is able to produce a good aroma. Hydrogen sulfide can give ascent, and taste that affects the quality of bread [18]. However, commercial yeasts used as bread developers produce hydrogen sulfide compounds and do not change the aroma of bread [9]. The content of hydrogen sulfide compounds in food should not go beyond 50-80 µg/L to not interfere the quality of the aroma [31]. Bread is a food that contains sulfates (> 10 µmol/g or 1 mg/g) [32]. Therefore, all yeast isolates from the flesh and skin of the mango podang can be used as bread yeast.

Temperature and Ethanol Tolerance Test

The temperature tolerance test in this study was carried out by incubation at 30, 37, and 45 ºC. YDM-1 isolate was able to grow at temperatures 30, 37, and 45 ºC as indicated by the increasing value of optical density. YDM-3 and YKM-1 isolates experience an increase in the value of optical density at temperatures 30 ºC and 37 ºC (Table 5). Yeast isolates that can be used to help ethanol fermentation are yeasts that are able to grow at temperatures 28-30 ºC [33]. Ethanol fermentation is a process used for the development of bread dough.

Commercial yeasts that have been used as bread dough developers and most yeasts are only able to grow at a temperature of 37 ºC [9]. Yeast which is able to grow at a temperature of 45 ºC has better tolerance to this temperature than commercial yeast. Yeast isolates that are able to tolerate high temperatures show that they can be used in bread making to accelerate the process of increasing carbon dioxide production, forming flavors and aromas [8]. Besides, yeast isolates that are tolerant to high temperatures can be used to speed up the baking process, and increase the production of carbon dioxide in bread making [5].

TABLE 5. Optical density of cell yeast at different temperature

Yeast Isolates	30 ºC		37 ºC		45 ºC	
	24 Hours	72 Hours	24 Hours	72 Hours	24 Hours	72 Hours
YDM-1	0.748	0.775	0.7495	1.176	0.134	0.435
YDM-3	0.767	1.6942	0.726	0.770	1.23	0.878
YKM-1	2.407	2.777	0.868	0.903	0.557	0.399
Negative Control	0.000	0.000	0.000	0.000	0.000	0.000

YDM-1 yeast cells are able to increase the density value from concentrations of 10%, 13%, and 15%. YDM-3 and YKM-1 isolates experience fluctuation in optical density values (Table 6). The fluctuating results are similar to a previous study [34] of yeast which was tested for ethanol content, resulting in increased and decreased optical density values at concentrations of 13% and 15%.

TABLE 6. Optical density of cell yeast in high concentration ethanol

Yeast Isolates	10%		13%		15%	
	24 Hours	72 Hours	24 Hours	72 Hours	24 Hours	72 Hours
YDM-1	0.056	0.108	0.020	0.083	0.055	0.526
YDM-3	0.085	0.265	0.073	0.015	0.052	0.672
YKM-1	0.004	0.03	0.010	0.008	0.037	0.544
Negative Control	0.000	0.000	0.000	0.000	0.000	0.000

The results of this study showed that the obtained yeast isolates have a better ability than the study [34] which produces yeast isolates capable of surviving only at 5-10% ethanol concentrations. The results of the study [9] explained that yeast isolates from commercial yeast are only tolerant at 10% ethanol concentration. This shows that yeast isolates from mango podang fruit and skin can be used as bread developers.

According to Asyikeen *et al*. high ethanol concentration can increase the aroma of bread [18]. Therefore, if yeast is able to grow in high ethanol concentrations, it will increase the aroma of the bread dough during the baking process. In addition, the yeast in bread making can also generate primary and secondary metabolites such as alcohol, and ester carbonyl compounds that contribute to aroma of the bread [35].

Yeast Isolate Application in Bread Dough Development

Commercial yeast is able to increase the volume of the best bread dough for six hours, while isolates YDM-1, YDM-2, and YKM-1 can not match commercial yeast (Figure 2). This is because the commercial yeast contains an emulsifier in the form of sorbitan monostearate E-491. Meanwhile, the YDM-1, YDM-3, and YKM-1 isolates are able to match the positive control bread dough volume for twelve hours. According to [36] the process of developing bread dough requires incubation at room temperature for twelve hours.

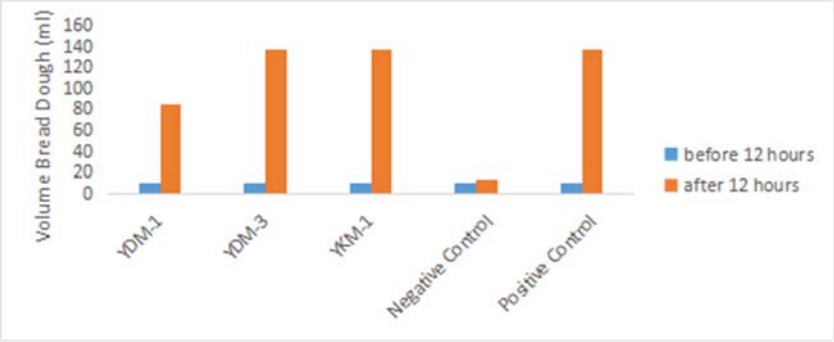

FIGURE 2. Yeast isolate test results on bread dough development

Sorbitan monostearate E491 has the function to increase the activity of yeast cells so that it can properly increase the volume of bread dough [37]. In addition to the addition of sorbitan monostearate E-491, *Lactobacillus bulgaricus* bacteria can also be added to bread dough. According to Lennox maximum bread volume development can be done by combining yeast with *Lactobacillus bulgaricus* in bread dough because it can help the yeast fermentation process and extend bread storage time [38].

The addition of isolates YDM-1, YDM-3, and YKM-1 in the bread dough after incubation can cause the formation of pores in the dough. The pores formed by bread dough can trap carbon dioxide produced by yeast fermentation [39]. According to Yano, the carbon dioxide that is the final outcome of yeast in bread dough fermentation are trapped by the gluten, causing development [40].

The aroma, texture, and color of baked bread dough with YDM-1, YDM-3, and YKM-1 yeast isolates are presented in Table 7. YKM-1 isolate produces the best aroma due to the high concentration of ethanol resulting from the formation of the final product of alcoholic fermentation. According to Birch *et al*., the aroma in bread making is influenced by the different compounds produced by yeast in the fermentation process [41]. Compounds that can form aroma in bread making are 3-methylbutanal, 23- butanedione, 3-methyl-1-butanol, and phenylacetaldehyde. These compounds are formed by secondary fermentation reactions [42]. Consumers generally prefer expanded bread with not too brown skin color, and has a crumb texture [43].

TABLE 7. Properties of bread dough after baking

Yeast Isolates	Color of Crust	Aroma	Texture
YDM-1	Bright yellow	+	Crumb
YDM-3	Bright yellow	++	Crumb
YKM-1	Bright yellow	+++	Crumb
Positive Control	Bright yellow	+++	Crumb
Negative Control	Bright yellow	-	Hard

Note: (-) unscented ; (+) less fragrance than commercial yeast; (++) smells almost the same as commercial yeast; (+++) smells the same as commercial yeast

Bread dough with all yeast isolates has a soft texture and pores after the baking process occurs (Figure 3). The baking process can cause the bread to form pores which indicate development [39]. The formation of bread texture is also influenced by alcoholic fermentation by yeast which produces CO_2. CO_2 production increases dough volume and is able to produce bread with a crumb texture [44].

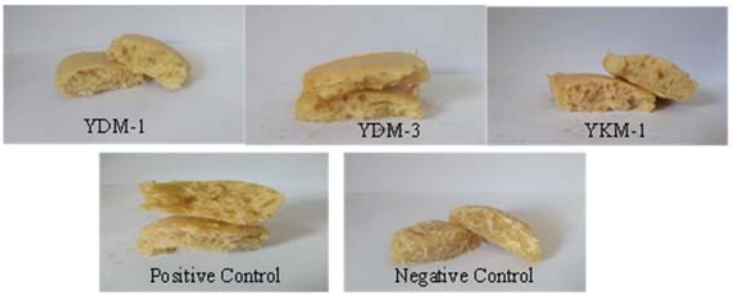

FIGURE 3. Baked Dough Different Isolates Yeast

The bread bright yellow color after baking produces is due to the caramelization of sugar. According to Cho and Peterson [7], the formation of color on the bread crust during baking is due to the Maillard reaction. Maillard reaction is a reaction between the carbonyl group, especially from reducing sugars with amino groups, especially from amino acids, peptides, and proteins. The sugar used in the caramelization process is a sugar residue that is not used by yeast in the fermentation process [43].

SUMMARY

Three prepared isolates met the criteria for leavening yeast. YDM-3 and YKM-1 are able to produce a similar increase in the volume of bread dough as commercial yeast, with an incubation period of 12 hours. Meanwhile, YKM-1 produces the best aroma as well as the best potential in developing bread, and is, therefore, suitable to be used as commercial yeast.

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