The effect of molasses and yeast extract concentration on yeast growth as leavening agent for bread

The effect of molasses and yeast extract concentration on yeast growth as leavening agent for bread

U Utami, R A Nadiya* and L Harianie

Biology Department, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Gajayana street Malang, Indonesia

*Email: rizqianadiya28@gmail.com

Abstract. Yeast is a microorganism that is widely used in food and industrial products, such as a fermentation agent in the production of bread dough from yeast biomass. The goal of this study to see how variation concentrations of molasses and yeast extract affect yeast growth parameters biomass and the live cells, as well as the quality of bread fermented by the yeast *Candida tropicalis*. The treatment media was repeated three times with a 50 ml mixture. Sugarcane molasses concentrations are 0.8, 0.9%, and 1% (w/v), while yeast extract concentrations are 0% and 0.75%. The ANOVA test results showed that the combination of molasses and yeast extract media had a significant effect on yeast biomass but had no effect on living cells number. The highest biomass gain of 0.47 g/50 ml was obtained with a combination treatment of molasses 8% and yeast extract 0.75%. However, the 0.9% molasses and 0% yeast extract treatment had the most cells, 12,24 x 10^6 cells/ml, and thus the isolate was used to make bread. Bread dough with C.*tropicalis* increased higher and faster than fermipan isolate. Based on organoleptic properties, the panelists liked the taste of bread dough with C.*tropicalis* as much as dough with fermipan.

Keyword: Bread quality, *Candida tropicalis*, molasses, yeast, yeast extract

1. Introduction

Yeast is a single-celled eukaryotic microorganisms that has the capacity to ferment. In the field of industrial microbiology, the process of mass cell growth occurring both aerobically and anaerobically is referred to as fermentation [1]. One application for yeast is as a fermentation agent in the production of bread dough utilising yeast biomass. Yeast is widely employed in food and industrial products. Around the world, the industrial sector produces 400 million kilogrammes of yeast biomass annually. Profitable factory scale procedures have been optimised, but it still doesn't give the best yeast biomass, thus the process is empirically optimised by lowering costs and raising biomass production to get the highest yields [2].

The optimal medium selection is essential for the fermentation process to encourage the synthesis of the desired product, the media must be designed. The media must fulfil a number of basic needs, including those for water, energy, carbon, nitrogen, mineral elements, and vitamins [1]. YPG (Yeast Peptone Glucose) is a common media for yeast growth. YPG media satisfies the fundamental criteria that must be met in fermentation media in order to promote the synthesis of the desired product. It does this by providing the macro- and micronutrients that yeast need. However, despite YPG media's widespread availability in the industrial sector, its use is still inefficient. Pure media are simpler to use on a small laboratory scale, but they won't work for larger-scale fermentation operations with more

expensive expenditures [1]. Peptone media is the most expensive component per kilogram [3]. In industrial fermentation media, the carbon supply in the form of glucose is typically not freely available. Sucrose, which is present in molasses, is the more prevalent carbon source [4].

Formulating yeast growth media with carbon and nitrogen sources derived from natural biological materials is a strategy to improve biomass production efficiency and the number of living cells. Molasses, a waste product or byproduct of the sugar industry, nevertheless has 50% sugar by volume. Molasses is commonly utilised as a raw material for bread-making yeast generation and fermentation due to its widespread availability [5]. A cheap alternative carbon source appropriate for medium processing is sugarcane molasses [6]. Due to its nutritional content, which is rich in sugar, protein, amino acids, and microelements including Mg, P, and K [7], molasses has emerged as a viable substitute. Additionally, molasses has a number of vitamins and other minerals that can help yeast growth [8]. Molasses has a lot of sugar, which makes it a good substrate for industrial biotechnological activities [9]. In addition to the fact that they are simple to get, organic materials like molasses can be used as a carbon source and yet meet the requirements for a medium [1]. In order to optimise yeast growth media that can satisfy the requirements for commercial fermentation media, molasses may be a alternative choice.

The primary carbon source is molasses, which also supplies some of the necessary nitrogen as well as sources of energy for growth [10]. Yeast can typically use some of the amino acids found in molasses as nutrition, although this necessitates the addition of additional nitrogen sources [11]. Yeast extract is one kind of nitrogen source that is frequently utilised in the food sector [12]. Yeast extracts are frequently employed as a crucial component in media as a supplement for the development of microorganisms because they contain growth-simulating chemicals [12]. Yeast extract, which is used as a nitrogen source because yeast extract is inexpensive per kilogram, it can be used on a large scale. [13]. In contrast to the nitrogen supply in the form of peptone found in YPG media, which may cost millions of rupiah per kilogramme [3], the pricing of yeast extract is still reasonable if employed on an industrial scale.

Previously study optimized culture media for the growth of several yeast strains, including *Candida tropicalis*, by varying the concentration of the carbon source molasses and supplementing with the nitrogen source yeast extract. In that study, yeast growth parameters are measured indirectly using turbidity or Optical Density (OD) values [3]. So, living yeast cells cannot be determined. While, in this study will take direct measurements of wet biomass and count live yeast cells. Aside from that, previous research didn't know the potential of yeast as a leavening agent, so research on varying concentrations of molasses and yeast extract as a medium to increase the growth of C.*tropicalis* yeast applied to bread dough has never been carried out and is required to increase the growth of C.*tropicalis* and bread quality.

Molasses concentrations of 0.8, 0.9, and 1% were employed. 0 and 0.75% of different amounts of yeast extract will be put in to the molasses media for yeast growth [14]. The wet biomass metrics and the quantity of live cells show yeast growth. To carry out the fermentation process, bread dough will be given the treatment with the greatest number of living cells. The number of cells that grow is very closely related to the yeast fermentation process [56]. The larger the dough produced, the more live cells there are [58]. Volume, texture, colour, scent, and taste of bread are all evaluated. Therefore, the purpose of this study is to ascertain how different molasses and yeast extract concentrations affect the growth of the *Candida tropicalis* yeast and the quality of bread made through its fermentation.

2. Method

2.1. Yeast isolate

The yeast is a *Candida tropicalis* H346 C strain that was isolated from sweet corn (*Zea mays* var. saccharata sturt), and it has been tested for its potential as a bread enhancer in earlier studies [15]. According to reports, C.*tropicalis* can ferment on cellulose and hemicellulose carbon sources, which are unavailable to commercial yeasts like S.*cerevisiae* [14].

2.2. Tool and material sterilization

Petri dishes and other glass-related tools are wrapped with paper before use, while the media-containing blender tube is placed in Petromax plastic. The tools and materials are placed in an autoclave and sterilised for 15 minutes at 121 °C.

2.3. Media preparation

A mixture of 3 g/L malt extract, 3 g/L yeast extract, 5 g/L peptone, 10 g/L glucose, and 20 g/L agar are dissolved in 1000 ml of distilled water to create Yeast Malt Extract Agar (YMEA) media. To ensure that the media is homogeneous, all of the ingredients are placed in an Erlenmeyer flask and cooked on a hot plate with a magnetic stirrer. 120 l of the antimicrobial antibiotic sodium DL-lactose were added. The YMEA media is then added to a petri plate and let to solidify. Petri dishes containing YMEA media are not opened until they are used in order to avoid contamination [16].

Yeast Extract Malt Broth (YMB) medium made with a mixture of 5 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L glucose were dissolved in 1000 ml of distilled water in an Erlemender flask [17]. The media is then homogenised by heating it on a hot plate while being stirred by a magnetic device. Antimicrobial antibiotic sodium DL-lactose were added 120 µl [16]. The media are kept in an Erlenmeyer flask until they are ready to be used.

The ingredients for Yeast Peptone Glucose (YPG) media are 5 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, and 20 g/L agar. In an Erlemenyer flask, the components were dissolved in 1 L of distilled water [18]. On a hotplate, the media were stirred to homogeneity. 9 ml of YPG medium should be added to an Eppendorf tube. The Eppendorf tube containing the YPG media should not be opened until it is prepared for use in order to avoid contamination.

2.4. Yeast rejuvenation and multiplication

Yeast isolates are revitalised aseptically under laminar air flow (LAF). On YMEA solid media, a single yeast colony was injected using a streak plate. 48 hours were spent incubating the culture at 28 ^{o}C, room temperature. Two loops from each yeast isolate that had grown on YMEA medium were taken and placed in sterile YMB liquid media after being incubated for 48 hours. The culture was incubated on a rotating shaker for 24 hours at a temperature of 33 ^{o}C and a speed of 140 rpm [20].

2.5. Sugarcane preparation

Three concentrations of sugarcane molasses were prepared, namely 0.8, 0.9, and 1.0% (w/v) [14]. The treatment media was repeated three times with a 50 ml mixture. Weighing 0.4 ml of molasses three times and adding it to 1.5 L of distilled water produced a concentration of 0.8%. Weighing 0.45 ml of molasses three times and adding it to 1.5 L of purified water produced a concentration of 0.9%. Weighing 0.5 ml of molasses three times and adding it to 1.5 L of distilled water produced a concentration of 1.0%. Each Erlenmeyer flask was filled with each molasses solution.

2.6. Yeast extract addition

Yeast extract was added to molasses media at quantities of 0 and 0.75% [14]. While the 0.75% concentration is generated by weighing 0.375 g of yeast extract three times and then adding the molasses solution to each blender, the 0% concentration is the molasses medium without the addition of yeast extract. The media was then stirred together on a hotplate. 9 ml of the homogenised media should be placed in an Eppendorf tube. The next step was to aseptically inoculate 1 ml of yeast inoculum into each Eppendorf containing sterile treatment media based on the results of the propagation on YMB media. The culture was incubated on a rotary shaker for 48 hours at 28 ^{0}C and 140 rpm per minute [19].

2.7. Yeast biomass production

Measurement of yeast biomass from calculating the weight of the wet biomass in the cells. The yeast suspension was separated in a treatment medium that contained an Eppendorf tube. utilising a centrifuge for 4 minutes at a speed of 4,500 rpm to separate the medium from the yeast biomass [20]. To calculate

biomass production, the supernatant was removed, and the pellet was weighed on an analytical balance [19]. Until just the biomass precipitate was left, the supernatant remaining at the end of the Eppendorf tube was removed using a sterile yellow tip and a 20-200 µl micropipette. The following formula is used to calculate the weight of yeast biomass: [21].

$$B = E1\text{-}E2$$

Note :
B = Weight of biomass cell (g/50 ml)
E1 = The obtained pellets are kept in Eppendorf
E2 = Empty Eppendorf

2.8. Live cells count

The number of living yeast cells was counted using a hemocytometer and the direct microscopic count technique. Before counting the cells, 1000 µl of distilled water was added to each Eppendorf containing pellet, which was then homogenised before the yeast pellets were removed using a blue tip and a 100–1000 µl micropipette. Transfer the pellets to a different Eppendorf so they can be mixed with the appropriate treatments.

The hemacytometer and cover glass were first sterilized using 70% alcohol. Cover the hemacytometer chamber with the glass. Methylene blue and distilled water are combined at a 1:9 ratio, 100 µl of methylene blue dye should be diluted until the total volume was 1000 µl or 1 ml. Put 100 µl of yeast inoculum in a 1.5 ml tube., methylene blue dye that had been diluted to 100 µl and distilled water were added. Use a vortex to homogenise the yeast suspension. The yeast suspension was collected and added 20 µl to the hemacytometer chamber after homogenization. Under a microscope with a 400x magnification, calculations were performed on 5 medium boxes that were situated in the centre (Figure 1) of the image.

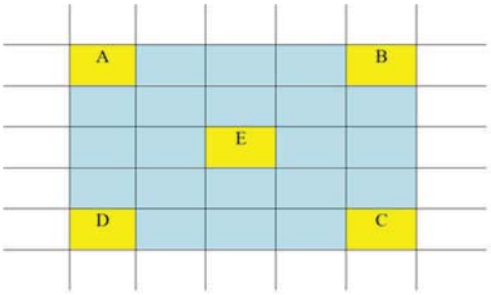

Figure 1. Pattern of calculations on the hemocytometer box

The formula is calculated using the number of living cells that can be seen in the box after getting that information :[24].

$$\text{Average of live cells} = \frac{Live\ cells}{5\ of\ box}$$

$$\text{Dilution factor} = \frac{Final\ volume\ after\ diluent\ addition}{Volume\ after\ diluted\ inoculum}$$

$$\text{Live cells count } (\frac{cell}{mL}) = \text{Average of live cells x Dilution factor x } 10^4$$

Note:
104 = Conversion 0,1 µL in 1 ml
0,1 µL = Volume on medium box

2.9. Bread dough preparation

Bread dough was made by combined of 100 g of wheat flour, 7 g of sugar, 1.4 g of salt, 2 g of skim milk, and 7 g of butter. Up to 67 ml of water should be added slowly [22]. Then, at a concentration of 2% (2 g), the yeast pellet with the greatest number of cells from the best treatment obtained after centrifugation was added. To activate, yeast pellets are dissolved in a small amount of sugar. The flour mixture is well combined with the activated yeast. Put the dough in an 8 cm diameter mica tube once it has been thoroughly smoothed out.

The dough for the bread is fermented at room temperature. Up until the dough reaches its maximum expansion, keep track of the dough's volume of expansion. After the incubation period, the dough is baked for 20 minutes at 180°C [23]. A commercial yeast isolate (Fermipan) with the same concentration as the treated yeast pellets 2% was utilised in the positive control test. Bread dough that didn't have yeast pellets added served as the negative control.

2.10. Bread dough volume measurement

Volume calculating by measuring the bread dough's length, width, and height. By putting the dough into a mould and then measuring it with a ruler, measurements are made [24]. The rising power of the bread dough is then determined once the bread's volume has been determined. For 300 minutes, the bread's rising capacity was assessed every 30 minutes. The following formula [25] is used to determine the bread dough proofing.

$$\% \text{ Bread dough proofing} = \frac{V1-V0}{V0} \text{ x } 100\%$$

Note :
V1 = Volume after incubation
V0 = Volume before incubation

2.11. Organoleptic test

Organoleptic testing tries to assess a product's degree of acceptability so that it can be used by consumers or panellists. A hedonic scale with five scores—very like = 5, like = 4, neutral = 3, don't like = 2, truly don't like = 1—is used to conduct the assessment [26]. In this test, 30 panellists rated the items according to how much they liked them. [27]. The bread's colour, aroma, texture, and flavour are all measured [25].

2.12. Data analysis

The SPSS programme was used to do an ANOVA (Analysis of Variance) analysis on the biomass and cell number data related to yeast growth. Continue the DMRT (Duncan Multiple Range Test) further test if there is a large difference. Using the Microsoft Excel programme, bread quality data in the form of bread dough expansion volume were analysed and presented graphically with descriptive analysis. statistics on bread quality from organoleptic tests that are scored are ordinal or scale statistics that include bread's texture, colour, taste, and scent characteristics.

Bread organoleptic data was analysed using the Microsoft Excel programme and presented in the form of a spider diagram to determine which treatment was most preferred for each criterion. The hedonic test, also known as a liking test, is conducted by calculating the median of the data. For ordinal data or multilevel data, the panellists' favourite treatment is identified by the median value, which represents the data's centre [28]. As a result, hedonic testing is conducted using the median value, or middle value, of the panellists' ratings for each parameter.

3. Result

3.1. Yeast Biomass

The results showed that yeast biomass was significantly impacted by the different molasses and yeast extract concentrations. This is predicated on getting a value of Fcount > Ftable with a significance level

of less than 0.05, indicating that the concentration of yeast extract and molasses, the treatment variables, have an impact on the biomass of *Candida tropicalis* 2. The effect of molasses and yeast extract treatment media on yeast biomass can occur because the molasses growth media primarily contain a carbon source (C), namely carbohydrates in the form of sugar, the main ones being sucrose, a type of disaccharide, glucose, and fructose, which are monosaccharides, as well as an additional source of nitrogen in the form of yeast extract. Yeast extract at a preset concentration serves as the media.

Between 65% and 75% of molasses is made up of sugar, mostly sucrose [29]. Higher yields may be supported by the media's complex nutrients. Because microbial cells are mostly composed of these elements, carbon and nitrogen sources are crucial in the medium [14]. Carbohydrates, which provide energy and carbon, are the most crucial nutrients for yeast. [30]. The invertase enzyme, which transforms sucrose into glucose and fructose as invert sugar, is one of the enzymes needed by yeast to carry out the process of metabolising different types of sugar, such as sucrose [31].

Table 1 of Duncan ANOVA test showed that no significant differences existed between either of the molasses and yeast extract concentration treatments for the C.*tropicalis* growth. The average yeast biomass obtained at each concentration, which was an average of 0.4 g/50 ml, was not significantly different. These outcomes could be the result of the molasses and yeast extract concentration ranges being too narrow and low to sustain healthy yeast growth. A greater molasses content of up to 2% can be added to enhance yeast growth. The initial molasses concentration has an impact on the growth rate of Candida species, including *Candida tropicalis*, in the range of 1.0-20.0 g/L, or 0.1-2 g/100 ml with a concentration range of 0.1-2% (w/v), The study's findings demonstrated that yeast's specific growth rate rose as initial sugar concentration in molasses grew up to 20.0 g/L, or 2 g/100 ml, or 2% molasses content [32]. To enable cells to create the lipid size needed by yeast, molasses media with greater sugar contents are utilised, which can improve high biomass and fermentation efficiency [4].

Table 1. Duncan ANOVA test of varying treatments on yeast biomass yield

Isolate	Treatment	Biomass average (g/50 mL)
***Candida tropicalis* H346 C**	Molase 0,8% *Yeast extract* 0%	0.39[a]
	Molase 0,8% *Yeast extract* 0,75%	0.47[a]
	Molase 0,9% *Yeast extract* 0%	0.34[a]
	Molase 0,9% *Yeast extract* 0,75%	0.42[a]
	Molase 1% *Yeast extract* 0%	0.42[a]
	Molase 1% *Yeast extract* 0,75%	0.43[a]
	YPG	0.63[b]

The maximum average biomass was found at molasses concentrations of 0.8% and yeast extract concentrations of 0.75%, or 0.47 g/50 ml for C.*tropicalis* isolates,. In all molasses concentration treatments, the addition of 0.75% yeast extract can boost the output of yeast biomass. To stimulate yeast development, add yeast extract to the culture medium. Increasing the concentration of yeast extract, which serves as a nitrogen source for yeast growth, can also improve these outcomes. The addition of yeast extract at various doses, ranging from 0.1-2 g/100 ml for yeast growth media, improves molasses media. The outcomes demonstrated that the addition of 2 g/100 ml yeast extract, or a concentration of 2%, is optimal for the growth of the yeast Candida sp. [14]. Yeast extract is extracted from yeast cells

which consists of a complete amino acid composition such as aspartic acid, glutamic acid, leucine, lysine, alanine etc. In addition, it includes a number of vitamins, including vitamins B1, B2, B6, B12, and E [8].

There is a noticeable difference between YPG media and all treatment media, which is supported by study on the yield of C.*tropicalis* biomass on YPG media as a control medium, which reached 0.63 g/50 ml. Yeast extract as a nitrogen source has been shown to boost yeast biomass. The Duncan test findings are shown in table 1 with (notation b). YPG medium is made out of a mixture of glucose, peptone, and yeast extract. These results may have occurred, because the concentration of the yeast extract employed in the YPG media in this research was 20 g/L, or the equivalent of 1 g/50 ml (2% concentration), [53]. This composition has a higher concentration (0.375 g/50 ml) than the yeast extract used in the treatment, the amount of biomass produced is similarly higher. A popular yeast growing medium used in labs is called YPG media. A nutritional medium with abundant deposits of amino acids and carbohydrates is thought to be Media YPG [54]. Glucose, a carbohydrate, may be effectively absorbed by yeast as a carbon source and is immediately employed as an energy source in metabolic activities to build large amounts of biomass [55].

3.2. Live cells count

Microscope with 400x magnification was used to view live yeast cells while they were stained with methylene blue. The results of watching living cells are displayed in Figure 2. There are two different sorts of cells, each of which has a different colour. The first form of cell is clear and transparent, signifying that the yeast cell is still alive. The second type of cell is blue, indicating that the yeast cell has passed away. When observed under a microscope, the blue colour of the methylene blue dye appears to dominate the entire cell because dead yeast cells are unable to control the methylene blue dye that enters the cell through the cell membrane. Dehydrogenase, an active enzyme, participates in the cell's chemical reduction of the blue dye in living yeast cells. Methylene blue is oxidised and becomes blue to black because dead yeast cells cannot decrease methylene blue [32].

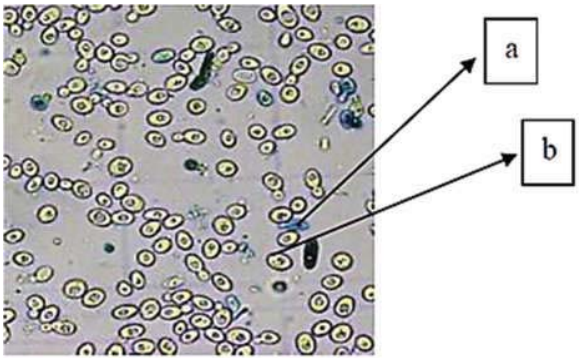

Figure 2. Yeast cells with methylene blue staining at 400x magnification. Dead cell (a), live cell (b).

The result of research showed that the quantity of yeast cells was not significantly impacted by differences in the concentration of molasses and yeast extract. The significance of the treatment > 0.05 and the value of Fcount Ftable serve as indicators of this. These outcomes might be the result of the molasses or yeast extract concentration range not being broad enough or high enough to enable ideal yeast growth. The trea tment media with varying concentrations of molasses and yeast extract produced 10^6 living yeast cells, indicating that the yeast that developed there has the necessary characteristics to carry out alcoholic fermentation and has the potential to enhance bread. Fermentation of alcohol in food involves 10^5–10^7 yeast cells [33].

There are differents in the number of cells between treatments, as shown in Figure 3 by the acquisition of the number of live *Candida tropicalis* yeast cells in 0.5 g of biomass. The results showed

that the 0.9% molasses concentration of 0% yeast extract produced 12.24 x 10^6 more cells on average per millilitre than other treatments. The ideal concentration for the growth of the yeast *Candida tropicalis* is 0.9% molasses treatment without the addition of a nitrogen source in the form of yeast extract. *Candida tropicalis* yeast growth best with a total sugar content of 9 mg/ml, or 0.9% [14].

It may be claimed that the 0.9% molasses concentration treatment contains a sugar concentration that is neither too low nor too high, allowing for the achievement of optimal cell numbers even without the addition of yeast extract. In order to prevent the Crabtree effect, yeast must grow in media with a low initial sugar concentration [34]. This allows for the production of larger quantities of yeast cells with more cellular components. The crabtree effect happens when yeast has an overabundance of glucose and tends to use glycolysis to break it down [35]. Catabolic suppression is one mechanism that the crabtree effect causes. When the medium's sugar concentration is too high, catabolic repression may take place. The crabtree effect, which can result in enhanced amino acid absorption in yeast cells, is thought to not occur when treatment with molasses concentrations of 0.9% or above [36].

The number of yeast cells on media with molasses concentrations of 0.8% and 1% can be increased by adding 0.75% yeast extract. This shows the nutrients in yeast extract can be effectively exploited by yeast as a source of nitrogen and as a direct energy source during metabolic processes, which resulted in the production of a large number of cells. Yeast may utilise nitrogen sources to produce functioning cell structure components and as a source of energy [37].

The concentration of 0.9% molasses and 0% yeast extract used in the treatment produced more live cells than the Fermipan brand commercial yeast isolate (7.72 x 10^6 cells/ml) and the C.*tropicalis* isolate grown on YPG control media. Consequently, 10.35 x 10^6 cells/ml were generated. As opposed to fermipan, whose average biomass is 0.50 g/50 mL, these results are not directly correlated with the average biomass obtained for C.*tropicalis*, which is 0.34 g/50 mL. This result showed that a large amount of biomass does not always include a large amount of live cells. Microorganism cells' weight can increase by enlarge the storage sac for the yeast's metabolic byproducts instead of multiplying the cells [38].

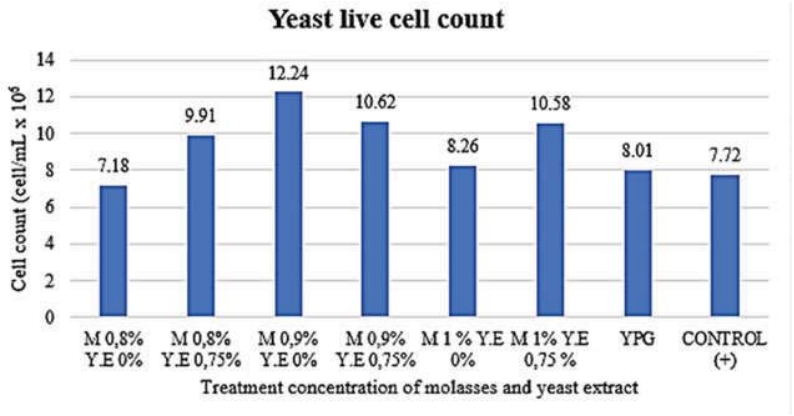

Figure 3. Result of yeast live cells count

3.3. Bread quality

3.3.1. Dough volume. The incubation period for dough containing both a Candida tropicalis isolate and a Fermipan isolate as a positive control was 12 hours, according to the research findings shown in figure 4. Both loaves of bread kept rising until the 690th minute. It was showed that the dough volume drastically decreased at the 720th minute because the dough with the addition of Candida tropicalis had reached its maximal growth and ceased rising at that time. When Fermipan isolate was added to dough,

these outcomes also happened. This has to do with how long the dough is allowed to ferment, which can have an impact on the yeast's capacity to do so. Alcohol content pressure is one of the stresses that yeast may encounter. The alcohol level increase as yeast ferments for longer periods of time [39]. High alcohol concentrations can be hazardous to yeast cell because can make membrane damage so that prevent yeast cells from growing and developing normally [40].

Bread dough containing C.*tropicalis* yeast increased higher and faster than fermipan isolate, accounting for 15% of the initial dough volume at the start of the 30th minute. Meanwhile, the dough had not yet expanded after the addition of fermipan isolate, resulting in 0% swelling power. This is because the *Candida tropicalis* isolate from the best treatment results had a higher cell number in 0.5 g of biomass than the fermipan isolate. The number of cells that grow is very closely related to the yeast fermentation process [56]. The rate of CO^2 formation in bread dough is related to the amount of yeast [57]. Yeast cells consume fermentable sugar in the dough and produce CO^2, so the more living cells there are, the more dough is produced [58].

The largest proofing was produced by the *Candida tropicalis* isolation at the 360th minute, namely 23.53%, while the fermipan proofing isolate was 13.95%, according to the percentage proofing of the bread dough in Figure 5. In contrast, if compare the volume of the bread dough at 360 minutes, the dough containing the Fermipan isolate had a larger volume than the C.*tropicalis* isolate. The volume of the dough produced by the addition of C.*tropicalis* was 211.01 cm^3, but the volume produced by the addition of fermipan isolate was 241.15 cm^3. The rate of fermentation and the degree of bread dough formation are significantly influenced by the number of cells active during the fermentation process in each yeast strain employed. Due to the influence of other elements, including the ability of yeast isolates to create CO^2 gas during the fermentation process of dough, this cannot be regarded as a complete reference. The isolate utilised as a positive control was obtained through isolation from instant yeast under the Fermipan brand, which primarily consists of the yeast *Saccharomyces cerevisiae*. Compared to other forms of yeast, *Candida tropicalis* yeast is less capable of fermenting bread dough [41]. *Candida tropicalis* is a yeast with good texture and flavour but a poor capacity to rise bread dough [23].

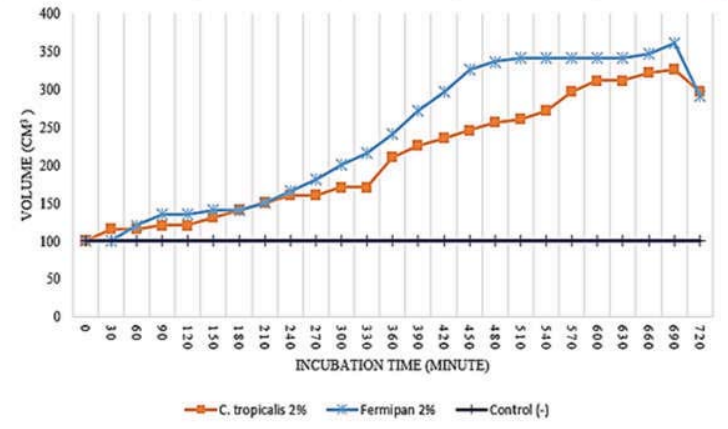

Figure 4. Bread dough volume

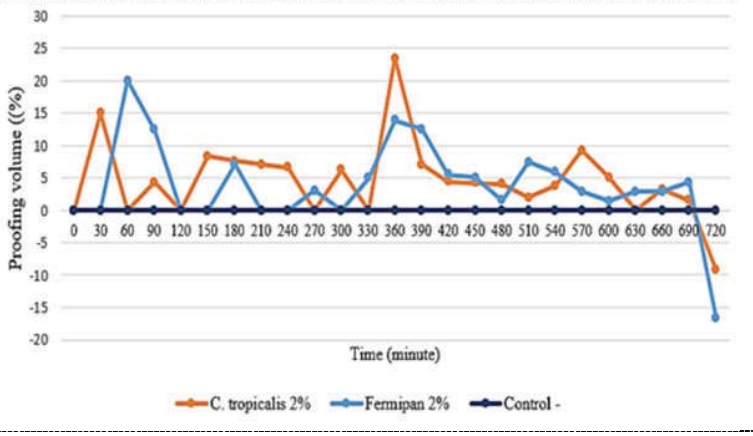

Figure 5. Percentage of bread dough proofing

3.4. Organoleptic test

3.4.1. Color. According to the spider diagram in figure 7, the colour of bread treated with fermipan is preferred over the colour of bread with C.*tropicalis*. Compared to the colour of bread with C.*tropicalis*, which has a median value of 3, which is neutral, the colour of bread with K+ is on the outermost line with a value of 4, which signifies like it. The panellists favoured the colour of K+ bread over that of C.*tropicalis* bread because the bread crumbs from the fermipan isolate were white while those from the C.*tropicalis* yeast fermentation were a light shade of brown. This is so that the C.*tropicalis* yeast used to make bread can be obtained from wet pellets that still have molasses in them. The findings revealed that panellists favoured bread crumbs that appeared whiter. A higher whiteness index is the preferred bread quality [42]. Higher scores were given to bread with whiter and brighter crumb color [43]. Differences in the isolate used during fermentation can also affect the color formed in the bread. Different types of yeast also impact the color of bakery products due to their ability to ferment different sugars as well as different metabolic by-products [44].

The browning reaction, commonly known as the Maillard reaction, and caramelization, which include protein components and reducing sugars, can also account for the variation in colour between the bread isolated from C.*tropicalis* and K+. This is plausible considering that the key components for producing bread employed in this study are high protein wheat flour and either added sucrose sugar or those found in the leftover molasses medium in the wet pellets, which also contain glucose and fructose. In the presence of protein and when heated, the reducing sugars glucose and fructose can produce a Maillard (brownish) reaction. Additionally, many kinds of sugar can result in heat-induced caramelization, which can add to the brown colour [45].

3.4.2. Texture. According to the spider diagram in figure 8, the texture of the bread treated with fermipan is preferred above the texture of the bread treated with *C.tropicalis,* which has a median value of 3, or neutral. This is possible as a result of the final volume of the K+ dough with a yeast concentration being higher than the dough having a C.*tropicalis* concentration. The dough with the addition of fermipan concentration had a greater final volume, demonstrating the dough's good ability to contain gas both during baking and until the end of the incubation period. In order to serve as a structural basis for bread and be able to create vast volumes, gluten must be able to hold onto the CO^2 gas produced by yeast [46]. Bread's pore structure develops as a result of volume resistance [47]. The panellists' preferred bread texture is created as a result, which is soft and characterised by evenly distributed pores, as seen in figure 6. Bread with consistent pore size, smoothness, and softness receives higher marks [43]. Therefore, there is a correlation between the final dough volume and the bread's texture.

The negative control bread, which had no additional yeast, had a low median score of 1.5, as seen in Figure 8. This result demonstrates that the panellists did not enjoy the bread's negative control's texture. This has to do with how yeast functions during the fermentation of the dough. Because yeast creates carbon dioxide gas during the fermentation process, which is then absorbed by the gluten in the flour, the concentration of yeast used in baking impacts the texture of the bread [26].

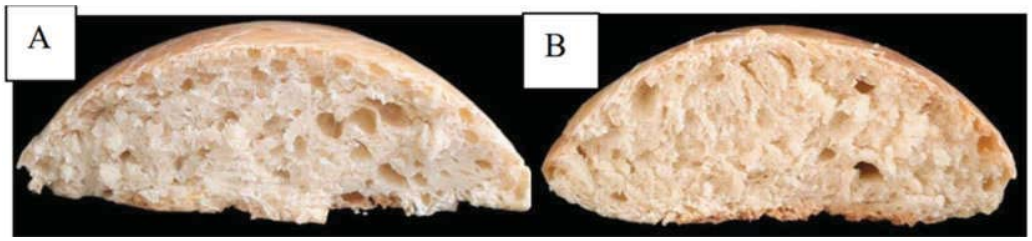

Figure 6. Bread texture with C.*tropicalis* (A) and fermipan (B)

3.4.3. Aroma. The spider diagram in figure 9 showed that the panellists preferred the bread with fermipan isolate added to it over the bread with C.*tropicalis* isolate. This is demonstrated by the outside line, which has a score of 4, and bread with C.*tropicalis* isolate added, which has a score of 3. Because more ethanol is created in the form of volatile chemicals when fermipan isolate is added to bread than when C.*tropicalis* isolate is, panellists prefer the aroma of bread with this addition. Because of the action of yeast during the fermentation of bread dough, which creates a collection of chemicals, bread has a characteristically fermented smell.

Consumer approval of bread is significantly influenced by its scent [49]. This is due to the fact that aroma is produced when the sense of smell is stimulated, and since the mouth is also stimulated when the sense of smell is stimulated, scent is one of the elements that affects how good a dish is. The usage of yeast, which has a characteristic aroma and can add aroma and improve the taste of the bread, has an impact on the aroma of bread. Compared to isolate bread, fermipan isolate bread has a better aroma. This indicates that the panellists valued the scent of bread treated with S.*cerevisiae*-containing Fermipan isolate. This is possible because the various yeasts utilised might have an impact on the aroma the bread produces. The scent of bread is due to the conversion of chemicals in the dough into secondary metabolite compounds produced by yeast, such as aldehydes and esters [48]. Yeast creates secondary components, a class of volatile molecules that alter the aroma of bread during the proofing process in addition to CO^2 gas [45]. One of the volatile chemicals produced during fermentation that positively affects the final aroma of bread is a 3-Methyl-1-Butanol [49]. Changes in the bread's fragrance profile are mostly caused by variations in the genes found in S.*cerevisiae* strains [50]. S.*cerevisiae* creates the necessary scent as a result [51].

3.4.4. Flavor. According to the spider diagram, the bread that included *C.tropicalis* isolate and had yeast concentration is on the outer line with a score of 4, indicating that the panellists thought the bread tasted good. This also occurred in bread that had fermipan isolate added, which was given a score of 4. Consumer perception of the flavour of bread is correlated with the production of volatile chemicals. The synthesis and release of volatile compounds during baking may account for the residual volatiles left in the bread crumb or crust at the conclusion of baking, which may affect how consumers perceive the flavour [49].

Bread with more yeast isolate has a better flavour than bread without additional yeast. Figure 10 demonstrates that the panellists disliked the taste of the negative control bread the least. This is possible as a result of yeast's significant contribution to the dough fermentation process, which includes the conversion and production of chemicals that can alter the flavour of bread. When baking bread or while the dough is fermenting, flavour might develop. The conversion of wheat flour's starch and amino acids is one of the many chemical and biological processes the dough goes through during fermentation. The

bread's flavour may be enhanced by this method. Amylolytic enzymes convert the fermentable starch in flour into monosaccharides, which are then converted into carbon dioxide, alcohol, organic acids, and flavor-precursor molecules during the bread fermentation process [43]. In addition to starch molecules, the conversion of amino acid compounds also affects how bread tastes. The alcohol and metabolites produced during secondary fermentation can directly contribute to the flavour of bread depending on how many amino acids and simple sugars are present in goods fermented by non-industrial or commercial yeast [52]. Therefore, the metabolic process of yeast can give bread crumbs a highly appealing flavour.

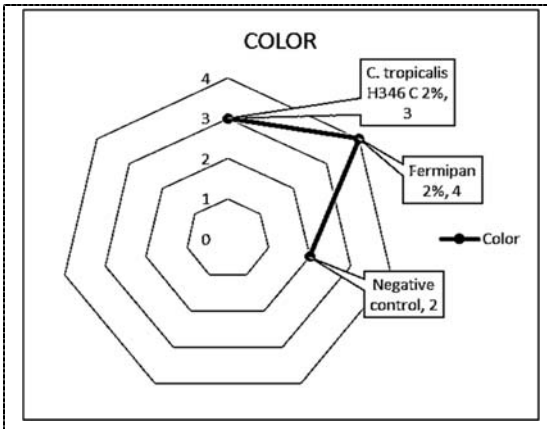

Figure 7. Parameter of color

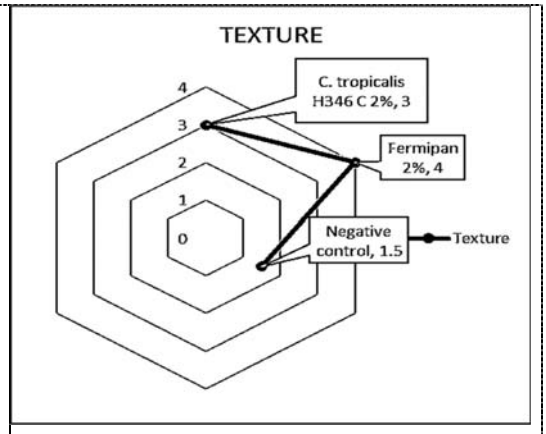

Figure 8. Parameter of texture

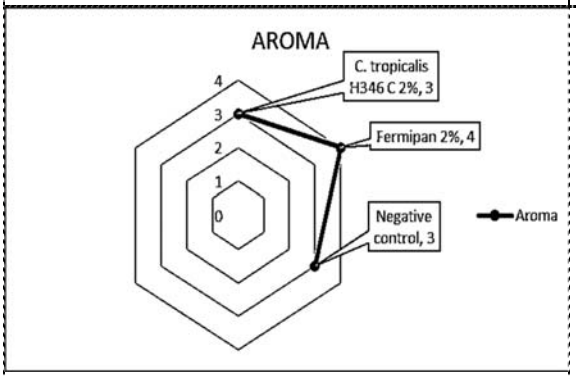

Figure 9. Parameter of aroma

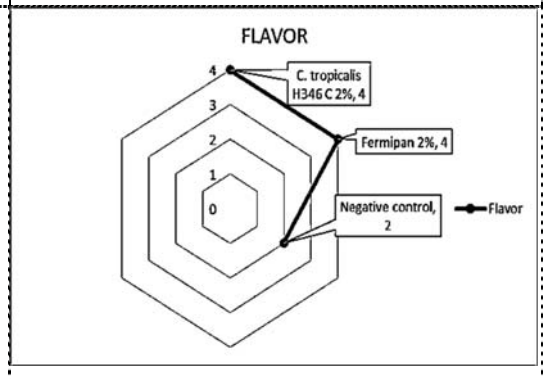

Figure 10. Parameter of flavor

4. Conclusion

The combination of molasses and yeast extract media had a significant effect on yeast biomass but not significant differences existed between varying concentration of molasses and yeast extract with each other on C.*tropicalis* biomass yield. The average yeast biomass obtained at each concentration, which was an average of 0.4 g/50 ml, was not significantly different. The 0.8% yeast extract 0.75% molasses treatment had the highest mean biomass value of 0.47 g/50 ml. The lowest biomass was obtained at a molasses concentration of 0.9% and 0% yeast extract, but this treatment had the highest number of living cells, namely an average of 12.24 x 10^6 cells/ml, so it was chosen for bread quality testing. The number

of living cell was not significantly impacted by differences in the concentration of molasses and yeast extract. The treatment 0.9% molasses without the addition of a nitrogen source in the form of yeast extract is ideal concentration for the growth of the yeast *Candida tropicalis*.

The dough volume and organoleptic test was used to determine bread quality parameters. Organoleptic test in terms of taste, color, texture, and aroma. Bread dough containing *C. tropicalis* yeast increased higher and faster than fermipan isolate, accounting for 15% of the initial dough volume at the start of the 30th minute. Meanwhile, the dough had not yet expanded after the addition of fermipan isolate, resulting in 0% swelling power. The largest proofing of the bread dough was produced by the *Candida tropicalis* isolation at the 360th minute, namely 23.53%, while the fermipan proofing isolate was 13.95%. But, the volume of the bread dough at 360 minutes, the dough containing the Fermipan isolate had a larger volume than the C. *tropicalis* isolate. The volume of the dough produced by the addition of *C. tropicalis* was 211.01 cm^3, but the volume produced by the addition of fermipan isolate was 241.15 cm^3. Based on the results of organoleptic tests, the panelists preferred bread with fermipan isolate over *C. tropicalis* in terms of color, aroma, and texture. Meanwhile, bread with *C. tropicalis* and fermipan were liked by the panelists in terms of taste.

Acknowledgments
This article is dedicated to all parties who have supported the author in compiling and solve it, especially parents who have cared for, educated, provided motivation and support until this writing was finished. My supervisors have facilitated this research and guided me. My school friends who always support me and my micro biology laboratory colleagues, especially the bread team, who have fought together.

5. References

[1] Nurhadianty V, Cahyani C, Nirwana W O C and Dewi L K 2018 *Pengantar Teknologi Fermentasi Skala Industri* (Malang : Universitas Brawijaya Press)
[2] Pérez-Torrado R, Gamero E, Gómez-Pastor R, Garre E, Aranda A and Matallana E 2015 *T. Food Sci. Technol.* **46** 2 167–175
[3] Vázquez J A, Durán A I, Menduíña A and Nogueira M 2020 *Biomol.* **10** 1184
[4] Joseph R and Bachhawat A K 2014 *E. Food Microbiol.* **3**
[5] Agustina D K, Sulitiana D and Anggraini D P 2019 *Bioteknologi Mikroba: Tinjauan Umum dan Aplikasi* (Banten: CV. Aa Rizky)
[6] Xie Y 2017 *Amb. Express* **7** 1 1-9
[7] Acosta-Cárdenas 2018 Dyna **85** 206 220-5
[8] Ogoshi R C, Zangeronimo, M G, Dos Reis J S, França J, Santos J P, Pires C P and Saad F M 2014 *J. Ani. Sci.* **85** 5 555-561
[9] Marques W L, Raghavendran V, Stambuk B U, and Gombert A K 2016 *FEMS. Yeast Res.* **16** 1
[10] Priya K, and Kaur J 2018 *Int. J. Microbiol. Res.* **10** 12 1429-30
[11] Sokchea H, Thi Hang P, Dinh Phung L, Duc Ngoan L, and Thu Hong T 2018 *J. Vet. Ani. Res*. **1** 104.
[12] Mahazar N H 2017 *Pakist J. Biol. Sci*: *PJBS*. **20** 3 154-9
[13] Krull S *et al* 2020 *Ferment.* **6** 4 97
[14] Sarlin P J and Philip R 2013 *Int. J. Res. Mar. Sci*. **2** 39-44
[15] Fauziah H N 2021 (Skripsi: Universitas Islam Negeri Maulana Malik Ibrahim Malang)
[16] Biomedical Engineering 2015 (Netherlands: Endhoven University of Technology)
[17] Chauhan A and Jindal T 2020 *Spring. Nat.*
[18] Murad H A 2019 *Biotechnol*. **18** 2 55-56
[19] Zohri A 2017 *J. Egypt. Sugar* **9** 57-66
[20] Damtew, Wondimagegn S A E, and Aber A B 2012 *Appl. Sci. Res.* **4** 5 1938-48
[21] Nengsih I A 2020 *J. Coastal Ocean Sci.* **1** 1 68-73
[22] Mahardika G R and Pratikno H 2018 *J. Teknik ITS* **7** 2 G145-G9
[23] Mitsuiki S, Fukinbara A, Oonishi T, Nakayama S, Oba T and Sato H 2015 *Japan. J. Brew. Soc.*

110 11 775–9
[24] Karki T B 2017 *Int. Biotechnol. Res.* **11**
[25] Pusuma 2018 *J. Agroteknol.* **12**29-42
[26] Sitepu K M 2019 *J. Agrokom.* 71-77
[27] Saepudin L, Setiawan Y and Sari P D 2017 *AGROSCI.* **7** 1 227-243
[28] Sari N and Ratna W 2015 (Yogyakarta: CV. Budi Utama)
[29] Gómez-Pastor 2011 *Tech. Rijeka* 202-222
[30] Türker M 2014 *Proc. 27th VH Yeast Conf.* 1-26
[31] Bhalla T C, Thakur N, and Thakur N 2017 *LWT* **77** 178-185
[32] Suryaningsih V, Ferniah R S, and Kusdiyantini, E. 2018 *J. Akademik. Biol.* **7** 1 18-25
[33] Suyadi S 2012 *J. Anim. Agric.* **1** 2 246-257
[34] Ginovart M 2018 *Front. Microbiol.* **8** 2628
[35] Vieira É D 2013 *J. Brazil. Microbiol.* **44** 551-8
[36] Hothersall J S and Ahmed A 2013 *J. Amino Acids*
[37] Yurliasni and Zakaria Y 2013 *Bionatura-J. Ilmu Hayati dan Fisik* **15** 1 56
[38] Mahreni M and Sri Suhenry S 2011 *Pros. Sem. Nas. Tek. Kim.* "Kejuangan" 2011 D03-1 Jurusan Teknik Kimia, Fakultas Teknik Universitas Diponegoro, Semarang
[39] Sutanto R 2013 J. *Keilmuan dan Terapan Teknik Mesin* **3** 2
[40] Maryam B M, Mohammed S S D and Ayodeji O A 2017 *J. Int. Microbiol. and Biotechnol*. **2** 1 12.
[41] Boboye B and Owoyeni I D 2009 *J. Appl. Sci.* **9** 4 771-6
[42] Swami S B, Thakor N J and Murudkar P R 2015 J. *Food Res. and Technol.* **3** 4 131-141
[43] Borsuk Y, Bourré L, McMillin K, Sopiwnyk E, Jones S, Dyck A and Malcolmson L 2021 *Appl. Sci.* **11** 21 10203
[44] Dendegh T A 2018 *J. Asian Food Sci*. **3** 4 1-10
[45] Andragogi V, Bintoro V P, and Susanti S 2018 *J. Teknol. Pangan* **2** 2 163-7
[46] Nur'aini Arista 2011 (Skripsi: Universitas Sebelas Maret Surakarta)
[47] Arwini N P D 2021 *J. Ilmiah Vastuwidya* **4** 1 33-40
[48] Zhou N, Semumu T and Gamero A 2021 *Ferment.* 7 102
[49] Pico J, Khomenko I, Capozzi V, Navarini L and Biasioli F 2020 *Foods* **9** 10 1498
[50] Heitmann M, Zannini E and Arendt E K 2015 *J. Cereal Sci.* **63** 49-56
[51] Lahue C, Madden A A, Dunn R R and Smukowski Heil, C. 2020 *Front. Gen.* **11** 584718
[52] Alba C, *et al* 2017 *J. Czech Food Sci.* **35** 6 496-506
[53] Murad H A, *et al* 2019 *Biotechnol.* **18** 2 55-56
[54] Dang T D, Yong C C, Rheem S and Oh S 2021 *J. Anim. Sci. Technol.* **63** 3 603
[55] Listyawati Agusniar Furkani 2018 *Jurnal Ilmiah Kedokteran Wijaya Kusuma* **5** 2 29-32
[56] Akbar G P, Kusdiyantini E and Wijanarka W 2019 *Berkala Bioteknologi* **12**
[57] Verheyen C, Albrecht A, Elgeti D, Jekle M and Becker T 2015 *Food Res. Int.* **76** 860-866
[58] Struyf N E V, der Maelen S, Hemdane J, Vespreet K J, Verstrepen and C M Courtin 2017 *Comp. Rev. Food Sci. Food Safety* **16** 4 1-2