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Antifungal Activity of Lactic Acid Bacteria as A Biopreservation in Sourdough Production

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ABSTRACT

Bread is a food made from flour and yeast. Bread made using conventional yeast only has limited shelf life because it is susceptible to the growth of fungi such as *Aspergillus niger* and *Rhizopus stolonifer*. One way to increase the shelf life of bread is to use Lactic Acid Bacteria (LAB) as a starter for making bread. This research aims to determine the ability of LAB yakult and yoghurt as an antifungal and to test the organoleptic and shelf life of sourdough with LAB formulation. The method used in this research is an experimental methode. The parameters observed were the antifungal activity of yakult and yoghurt with LAB formulation, organoleptic test of sourdough with LAB formulation, and shelf life test of sourdough with LAB formulation. The result showed that yoghurt LAB had antifungal activity, while yakult LAB did not have antifungal activity. Based on organoleptic test, sourdough with the additional of LAB yoghurt has better organoleptic test result compared to white bread without the additional of LAB yoghurt, and sourdough with the addition of LAB yoghurt has a longer shelf life compared to white bread without the additional of LAB yoghurt.

ABSTRAK

Roti adalah makanan yang terbuat dari tepung dan ragi. Roti yang dibuat menggunakan ragi konvensional hanya memiliki daya simpan terbatas karena rentan terhadap pertumbuhan jamur seperti *Aspergilllus niger* dan *Rhizopus stolonifer*. Salah satu cara untuk meningkatkan daya simpan roti adalah dengan menggunakan Bakteri Asam Laktat (BAL) sebagai starter pembuatan roti. Penelitian ini bertujuan untuk mengetahui kemampuan BAL yakult dan yoghurt sebagai antijamur dan uji organoleptik serta daya simpan sourdough dengan formulasi BAL. Metode yang digunakan dalam penelitian ini adalah metode eksperimental. Parameter yang diamati adalah aktivitas antijamur yakult dan yoghurt dengan formulasi BAL, uji organoleptik sourdough dengan formulasi BAL, dan uji daya simpan sourdough dengan formulasi BAL. Hasil penelitian menunjukkan bahwa BAL yoghurt memiliki aktivitas antijamur, sedangkan BAL yakult tidak memiliki aktivitas antijamur. Berdasarkan uji organoleptik, sourdough dengan penambahan BAL yoghurt memiliki hasil uji organoleptik yang lebih baik dibandingkan dengan roti tawar tanpa penambahan BAL yoghurt, dan sourdough dengan penambahan BAL yoghurt memiliki daya simpan yang lebih lama dibandingkan dengan roti tawar tanpa penambahan BAL yoghurt.

1. INTRODUCTION

Bread is a food product made with wheat flour and uses yeast which functions to develop dough by converting sugar molecules into CO_2 (Carbon dioxide) gas (Ramadhani *et al.,* 2020). Yeast has a function as a shaper of aroma and taste in bread. Around 300 BC, yeast was made from natural ingredients using a mixture of water and fermented flour (Ko, Sanjim., 2014). However, the use of yeast on bread does not affect the length of shelf life of bread (Rumeus and Turtoi, 2013). Additional chemicals in yeast can also have allergic side effects on the body (Warnock and Richardson, 2018). The use of bread yeast also causes an increase in antinutritional substances, namely phytic acid, so that the nutrients in bread cannot be absorbed optimally (Yousif and Faid, 2014). Indonesian consumption patterns that tend to pay more attention to health increase the production of healthier and organic bread (Ariyana *et al.,* 2018). The quality of bread depends on the quality of the dough which regionally involves yeast (Sevgili *et al.,* 2021). Lactic acid bacteria (LAB) is an

example of a natural ingredient that has a major role in preserving food ingredients by producing lactic acid, acetic acid and ethanol (Usman *et al.,* 2018), so it has the potential to be a substitute for yeast in bread and is expected to increase the shelf life of bread. One example of the application of LAB in bread making is to involve LAB as a starter for *sourdough* bread making.

Sourdough is an ancient fermentation method by grinding grains such as cereals and legumes with the addition of water to become dough (Bunning *et al.,* 2022). Sourdough is made traditional by mixing flour, water and salt followed by natural fermentation (Torrrieri *et al.,* 2014). Sourdough is a fermented dough that contains microflora such as LAB. The microflora of this dough serves in the process of acidification and development of bread. According to Ganzle, (2014), the use of LAB microflora in dough can improve the nutrition, taste, and aroma of bread and can improve the texture of bread and increase the shelf life of bread. This is because the LAB contained in sourdough is able to hydrolyze carbohydrates, lipids, and proteins. Lipid metabolism that occurs during the fermentation time of the dough can increase the activity of antioxidants, anti-mold, and some flavor-forming compounds so that the taste of bread is better and more powerful to keep bread longer. Sourdough contains LABs such as *Leuconostoc*, *Weissella*, *Pediococcus, Lactococcus, Enterococcus*, *Streptococcus*, and *Lactobacillus*. *Lactobacillus* strains still dominate sourdough. *Lactobacillus* contained in sourdough is able to produce exopolysaccharides which form biofilms that can prevent *stalling* in bread (Lau *et al.,* 2021).

The application of LAB to wholemeal bread helps the fermentation process as well as improves the taste quality and texture of bread, delays hardening and stalling, increases nutrient availability, and increases resistance to microbial damage (Aplevicz *et al*., 2013). This research aims to determine the ability of Yakult and yogurt Lactic Acid Bacteria (LAB) as antifungal and organoleptic tests as well as the storability of sourdough with LAB formulations. This study seeks to enhance the safety and shelf life of sourdough in daily consumption, providing a healthier alternative to chemical preservatives.

2. METHODS

Isolation of Lactic Acid Bacteria

LAB from Yakult and yogurt were isolated using trypton soy agar (TSA) media using the pouring dish technique. Observations were made after 2x24 hours after incubation at a temperature of 37ºC. The results of isolation were then inoculated on TSA media using the scratch dish technique.

Isolation of Fungi on Bread

Bread fungus isolates (IJR1 and IJR2) were isolated using potato dextrose agar (PDA) media and incubated at a temperature of 25ºC for 4x24 hours.

Antifungal Activity Test

The isolated LAB was made into a suspension with 9 ml of physiological NaCl plus isolate LAB to turbidity according to McFarland's comparison. A total of 200 µl LAB bacterial suspension was poured into a petri dish and then poured 15 mL of TSA media for each petri dish. Then semi-solid Yeast malt (YM) media and injected 20 µl of bread mushroom suspension (IJR1) and (IJR 2). Observations are made after 5x24 hours after incubation at a temperature of 25ºC.

Sourdough and Bakery Starter Making

a) Sourdough Making

Wheat flour plus LAB suspense was fermented for 24 hours. Wheat flour is added LAB culture and warm water in a ratio of 1:1 each, then the mixture is incubated for 24 hours at room temperature. The mixture was added with substrate and warm water in a ratio of 1: 0.5: 0.5. The same treatment was repeated until the 4th day. Furthermore, if the volume of sourdough has expanded 2-3 times the original volume and there are air bubbles and fresh sour aroma, then the sourdough is ready for use.

b) Bread Making

The ingredients of bread making were weighed according to a predetermined composition. Flour, sugar, and milk were mixed using *a mixer* and added yeast and *sourdough* that have been dissolved in warm water. The dough was added with egg yolks, salt, and butter, then stirred until smooth. Next, the initial fermentation was carried out by rounding the dough and covering it for 30 minutes. After 30 minutes, the dough then deflated until the air escapes and closes again for 30 minutes. After rising, the dough was deflated and weighed by 20 gr and then rounded and put into a cup. Dough roasting is done in the oven at 180°C for 20 minutes.

Organoleptic Test

Organoleptic tests were performed with appointed 30 respondents at random and provided bread samples as well as organoleptic test papers with parameters of taste, texture, color, and smell.

Shelf-life Test

Bread was stored at room temperature with tight packaging. The test is carried out by looking at the length of the bread resistance period until the onset of mold.

Data Analysis

Data analysis of antifungal activity results was carried out descriptively and presented in the form of pictures and tables while data analysis of organoleptic test results was calculated with Microsoft Excel. Data analysis of sourdough shelf-life testing results is carried out descriptively and presented in the form of a table.

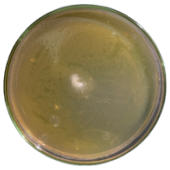
3. RESULT AND DISCUSSION

Antifungal activity

Testing the antifungal activity of yogurt using two test fungi, namely *Aspergillus niger* and *Rhizopus stolonifer.* Testing of antifungal activity was carried out by the agar diffusion method. The results of antifungal activity testing can be seen in Table 1.

Table 1. Antifungal activity test results of LAB.

No	Picture	Information
1		*Aspergillus niger* Antifungal Activity Testing against BAL yogurt no mold growth

2		Testing of Antifungal Activity of *Rhizopus stolonifer* against BAL yogurt no mold growth
3		*Aspergillus niger* Antifungal Activity Testing against BAL yakult there is mold growth of fungi
4		Testing of Antifungal Activity of *Rhizopus stolonifer* against BAL yakult there is mold growth of fungi

The test results of LAB yogurt dan Yakult with diffusion method prove that LAB Yoghurt has antifungal activity while LAB Yakult does not. Yoghurt is a processed product derived from milk which is made into a fermented sour drink made from lactic acid starter (Suharyono and Kurniadi, 2010), while yakult is a probiotic fermented milk drink product containing good bacteria originating from Japan. LAB is a promising alternative to replacing chemical preservatives due to its ability to produce antifungal compounds. LAB antifungal compounds are metabolites in the form of organic acids, protein compounds, and low molecular weight compounds (less than 1000 Da).

Antifungal compounds are extracellular metabolite products produced during the LAB growth process in fermentation media. Compounds known to have antifungal activity are organic acids produced by LAB during the fermentation process. Antifungal compounds in the form of mixed compounds have greater antifungal activity than purer forms of individual compounds. Antifungal agents work by damaging cell walls, causing changes in cell permeability, changing protein and nucleic acid molecules, and inhibiting the action of enzymes (Cahyaningrum, 2018). Lactic acid has antifungal activity by lowering the pH of the medium and the lipolytic properties of the undissociated form (Damayanti *et al*., 2015).

Organoleptic Test

Organoleptic test or sensory test or sensory test is a way of testing by using the human sense as the main tool for measuring the acceptability of the product. Organoleptic testing has an important role in the application of quality. The results of the organoleptic test can be seen in Table 2.

Table 2. Organoleptic test results

Treatment	Organoleptic test			
	Color	Aroma	Texture	Taste
Flour+water	3.73±0.94	3.97±0.67	3.53±0.68	3.90±0.66
Flour+yogurt LAB	4.33±0.55	4.53±0.63	4.50±0.63	4.50±68

Color

The panelists' liking level for the color of the bread added starter LAB 3.73-4.33 which means like. So this shows that the panelists liked and accepted the color of the bread added by the LAB starter. The addition of LAB to the breadmaking process can increase the panelists' liking for the color of the flour produced.

Aroma

The panelists' favorability for the aroma of the bread to which the LAB starter was added ranged from 3.97 to 4.53 which means like. This shows that panelists like the aroma of bread added with LAB starter. The addition of LAB to the breadmaking process can increase the panelist's liking for the aroma of the bread produced. This is because the fermentation treatment in making bread can give the impression of a distinctive aroma on the bread. The characteristic aroma in bread can be obtained through a fermentation process that produces acidic compounds. According to Setiarto *et al*. (2017) fermentation can increase reducing sugar because lactic acid bacteria produce the enzyme amylase so that glucose and maltose are produced.

Texture

The panelists' liking level for the texture of the bread to which the LAB starter was added ranged from 3.53 to 4.40 which means likes. So this shows that the panelists still like the texture of the bread added with the LAB starter. The addition of lactic acid bacteria to the breadmaking process can increase the panelists' liking for the texture of the resulting bread. The use of LAB affects the texture of bread caused by the fermentation of LAB isolates producing extracellular enzymes that can improve the texture of flour.

Taste

The panelists' liking level for the taste of the bread added starter LAB 3.90-4.50 which means like. So this shows that the panelists liked and accepted the color of the bread added by the LAB starter. The addition of LAB to the breadmaking process can increase panelists' liking for the color of the flour produced.

Effect of *LAB Starter* Addition on Sourdough Shelf Life

Based on Table 3 it can be seen that in bread samples without the addition of *LAB Yoghurt,* there was no fungal growth on day 1 to day 6, while on day 7 there was a slight growth of fungi. Bread with the method of *sourdough* application and the addition of *LAB Yoghurt*, no fungal growth was found on the 1st to the 7th day. Hendarto *et al.* (2019) said that the shelf life of yoghurt is higher than the shelf life of fresh milk. This is because yoghurt contains lactic acid which can provide durability. LAB that live in yoghurt also produce the enzyme lactase which is used to digest the remaining milk sugar in yoghurt.

Table 3. Test results of the addition of BAL starter to bread storage power

Treatment	D0	D1	D2	D3	D4	D5	D6	D7
Flour + water	-	-	-	-	-	-	-	+
Flour + yogurt LAB	-	-	-	-	-	-	-	-

The sign (-) means that there is no fungal growth on the bread, while the sign (+) means that there is a fungal growth on the bread.

The method of making bread using LAB is not a common method in Indonesia, but in some countries, especially in Europe, the bread produced contains ingredients

that have sensory qualities because of their excellent quality and a longer shelf life. *Sourdough* is a mixture of flour and water fermented with LAB. LAB that develops in the dough can come from certain natural impurities from flour or starter cultures containing one or more WATTs of a known type. The ability of LAB to increase resistance to microbial damage is due to the production of antibacterial, antifungal, and *antiropinees* compounds (Saeed *et al*., 2014). The addition of LAB to bread through *sourdough* applications is expected to improve the microbiological quality and shelf life of bread. The results of the observation of bread shelf life can be seen in Table 3.

This improvement in quality and shelf life is associated with the presence of compounds resulting from LAB metabolism, such as lactic acid, acetic acid, exopolysaccharides, and antimicrobials such as bacteriocins. The application of LAB in *Sourdough* causes several effects such as accelerating the fermentation and ripening process, improving the taste quality and texture of bread, delaying the onset of hardening, deterioration, and increasing the availability of nutrients. The ability of LAB to increase resistance to microbial damage is due to the production of antifungal compounds. This shows that the addition of LAB can improve the microbiological quality and shelf life of *sourdough.* The use of *sourdough* not only inhibits the growth of bacteria but also inhibits the growth of damaging microorganisms, namely fungi (Ariyana *et al.,* 2018).

4. CONCLUSION

The conclusion obtained from the research that has been carried out is that LAB yogurt has antifungal activity while LAB Yakult does not have antifungal activity. Sourdough with the addition of LAB yogurt has better organoleptic test results compared to ordinary bread without the addition of LAB yogurt. Sourdough with the addition of LAB yogurt has a longer shelf life compared to regular bread without the addition of LAB yogurt.

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