Saccharomyces cerevisiae

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Research Article

The effect of *Saccharomyces cerevisiae* on the growth of yeast and fungi, β-glucan formation, and antibacterial activities during soybean fermentation for tempeh

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Abstract

Generally, the microorganism involved in tempeh fermentation is *Rhizopus oligosporus*. However, *Saccharomyces cerevisiae*, a type of β-glucan-producing yeast, is known to be present and able to grow during tempeh fermentation. We observed the dynamics of yeast and fungi growth and β-glucan production during tempeh fermentation after adding *Saccharomyces cerevisiae* as an inoculum. This study used a Factorial Randomized Complete Block Design (RCBD) with two factors and three replications. The first factor was types of starter cultures as follows: *Saccharomyces cerevisiae*, *Rhizopus oligosporus*, and *Saccharomyces cerevisiae* + *Rhizopus oligosporus*. The second factor was fermentation time at room temperature (30±2°C) as follows: 0, 8, 16, 24, 32, and 40 hours. The growth of yeast and fungi, the formation of β-glucan, and antibacterial activity to *Escherichia coli* during soybean fermentation were observed every eight hours. The results showed that yeast grew during soybean fermentation from a single culture of *Saccharomyces cerevisiae* and a mixed culture of *Rhizopus oligosporus* and *Saccharomyces cerevisiae*, but not from *Rhizopus oligosporus* culture alone. During fermentation, the growth of yeast increased until the end of the fermentation period. However, in the soybean fermentation with a mixed culture of *Rhizopus oligosporus* and *Saccharomyces cerevisiae,* yeast growth decreased at 32 hours of fermentation time. β-glucan formed in tempeh with all types of inoculum. In all kinds of cultures, the antimicrobial activity of tempeh to *Escherichia coli* increased along with fermentation time. The highest β-glucan content and antibacterial activity of tempeh came from the mixed culture of *Rhizopus oligosporus* and *Saccharomyces cerevisiae*.

Keywords: soybean tempeh; yeast growth; *Saccharomyces cerevisiae*; *Rhizopus oligosporus*; β-glucan production; fermentation time.

Introduction

Tempeh is a traditional Indonesian food made from soybeans fermented by *Rhizopus* sp. Tempeh is classified as a functional food because it contains bioactive compounds such as isoflavones which are good for the health of the body. They have nutritional advantages, unique textures, and pleasant flavors (Kustyawati et al., 2014). The quality of tempeh is influenced by the raw material and type of inoculum or starter culture used. The type of

inoculum plays an important role in making tempeh because it affects the quality of the tempeh produced.

Generally, tempeh uses an inoculum containing *Rhizopus oligosporus* (O'Toole, 2016). Other important microorganisms involved in fermenting soybean to form tempeh are *R. oryzae* and *R. stolonifer*. All three microorganisms ferment soybeans into tempeh. *Rhizopus oligosporus* retains most of the nutrients contained in soybeans, increases protein digestibility, and increases the levels of some vitamins from the vitamin B family (Muchtadi, 2010). *Rhizopus oligosporus* synthesizes more protease enzyme, whereas *R. oryzae* favors α-amylase enzyme (Triwibowo, 2011).

Previous researches showed that the microflora in tempeh were not just fungi. Seumahu *et al.*, (2013) and Efriwati *et al.* (2013) found lactic acid bacteria (BAL) and yeasts in tempeh. Besides *rhizopus oligosporus*, yeast and bacteria are also involved during fermentation and contribute significantly in producing functional metabolites (Kustyawati et al. 2020). One type of yeast found in tempeh fermentation was *Saccharomyces cerevisiae* (Kustyawati *et al.*, 2016), which was known as a β-glucan producing microorganism (Pengkumsri *et al.*, 2017).

The *S. cerevisiae* cell wall is composed of β-(1,3) and β-(1,6)-glucan, mannan, chitin (1-2%), and mannoproteins composing about 20-30% of the dry weight of the cell wall (Naruemon *et al.*, 2013). β-glucan is a polysaccharide compound that has health benefits, one of which as a biological response modifier (Corno et al. 2020). β-glucans have anti-aging effect and antibiotic activity against bacteria, fungi, viruses, and parasites (Hetland *et al.* 2013). The presence of β-glucans in tempeh is expected to improve the functional properties of tempeh as a healthy food.

A research conducted by Rizal et al. (2020) showed that yeast could grow alongside fungi during soybean fermentation when a carbon source was added for yeast growth, thus resulting in β-glucans in the tempeh produced. In this study, *S. cerevisiae* was added intentionally to the soybean fermentation process without the addition of carbon sources to produce tempeh with high β-glucan content. It was important to examine if the addition of *Saccharomyces cerevisiae* without any carbon source in soybean fermentation could produce yeast and β-glucan in tempeh. In addition, the presence of β-glucan in tempeh produced by the addition of yeast was expected to provide added value for tempeh due to its health benefits including antibacterial activity. Therefore, this study aimed to observe the effect of adding *Saccharomyces cerevisiae* to the growth dynamics of yeast and fungi, β-glucan formation, and antibacterial activities during soybean fermentation to produce tempeh.

Materials and Methods

The main ingredients used in this study were pure cultures of *Rhizopus oligosporus FNCC 6010* and *Saccharomyces cerevisiae* FNCC 3012, *Eschericia coli,* soybeans (brand "Soybean USA No. 1"), Nutrient Broth (NB), Nutrient Agar (NA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA). The study was arranged in a Factorial Randomized Block Design with three replications. The first factor was the addition of three levels of inoculum: *S. cerevisiae* (negative control), *R. oligosporus* (positive control), and *R. oligosporus* + *S. cerevisiae* (the main treatment). The second factor was fermentation time with six levels: 0, 8, 16, 24, 32, and 40 hours. During fermentation, samples are analyzed for microbial content (number of *S. cerevisiae* and *R. oligosporus*), β-glucan content and antibacterial activity to *E. coli* after each level (0, 8, 16, 24, 32, and 40 hours) of fermentation time.

Preparation of *S. cerevisiae* culture

Isolate of *S. cerevisiae* was cultured into sterile Malt Extract Agar (MEA) medium using a sterilized inoculating loop needle with a scratch plate, then incubated for 24 to 48 hours at 28°C to form colonies. The colonies were then harvested by adding 5 or 10 mL of distilled water into the plate disk. *S. cerevisiae* cells were harvested and poured into a 50 mL centrifuge tube. The tube was weighed and spun at 3000 rpm for 10 minutes to obtain a separate solid from the supernatant. The supernatant was then discarded and the remaining solids were diluted with 25 to 30 mL of distilled water. The *S. cerevisiae* cells were transferred into a test tube containing 9 mL of physiological saline solution and then homogenized using a vortex. The number of *S. cerevisiae* cells was calculated using a hemocytometer. The required concentration was 10^7 cells/mL.

Preparation of *Rhizopus oligosporus* culture

Rhizopus oligosporus from tilted agar was cultured onto a sterile medium of Potato Dextrose Agar (PDA) using a sterilized inoculating loop needle and a scratch plate. It was then incubated for five to seven days at 30 to 35°C to obtain pure colonies, harvested in the same way as the *S. cerevisiae*. The required concentration was 10^5 cells/mL, 100 times less than *S. cerevisiae*.

Production of Soybean Tempeh

Three hundred grams of soybeans were soaked at room temperature overnight. The soybean husks were removed. Soybeans were then boiled in three times their weight of water for 30 minutes, drained, and cooled until they reached 32°C (ambient temperature) and were ready to be inoculated.

Three separate 100 g samples of boiled soybeans received these inoculums:
(1) 1 mL suspension of 10^5 spores/mL of *R. oligosporus*,
(2) 1 mL suspension of 10^7 cells/mL of *S. cerevisiae*, and
(3) 1 mL suspension of 10^5 spores/mL of *R. oligosporus* + 1 mL suspension of 10^7 cells/mL of *S. cerevisiae*.

The samples were packaged in plastics that had been perforated for ventilation then incubated at 32°C for 40 hours, observed every eight hours.

Enumeration of Microorganisms

Microbiological analysis followed Lay (1994), culturing on Potato Dextrose Agar (PDA) for fungi and Malt Extract Agar (MEA) for yeasts. Immediately at 0 hour, then at 8, 16, 24, 32, and 40 hours, each tempeh was sampled and diluted following the method of Kustyawati et al. (2009). Ten grams of sample and 90 mL of peptone water were homogenized with a stomacher paddle blender for five minutes, then diluted into the concentration series. One mL of each dilution was planted with the appropriate surface plate calculation method on the media. Incubation continued for 24 to 48 hours at 32°C to grow fungi and 30°C to grow yeast.

Analysis of β-Glucan

The β-glucan analysis every eight hours during fermentation followed Kusmiati *et al.* (2007). One gram of sample and 30 mL of NaOH 0.7 N was hydrolyzed for six hours at 75°C then centrifuged at 10,000 rpm at 25°C for 30 minutes. The supernatant was removed and the

residue was washed with 30 ml of 0.5 M acetic acid solution, then centrifuged again at 10,000 rpm and 25°C for 30 minutes. This process was repeated three times. The obtained residue was then twice-washed with 20 mL of water and centrifuged at 5,000 rpm for 10 minutes.

The residue with 20 mL of ethanol was centrifuged at 5,000 rpm for 10 minutes, resulting in wet β-glucan (crude). This biomass was dehydrated at 45°C oven for 24 hours and weighed to obtain the dry weight of β-glucan (crude). The dry residue with 4 mL of 1M NaOH was left for one hour. The sample was then diluted with 10 mL of sterile distilled water and shaken with an orbital shaker. After that, the sample was added with 2 mL of Pb-Acetate and left to stand for 30 minutes. Finally, one gram of sodium oxalate clears the solution, and two mL of it with 0.5 mL of phenol 5% and 2.5 mL of sulfuric acid 5N was tested using a sugar free content spectrophotometer with a wavelength of 490 A.

Assessment of Antibacterial Activities

Preparation of Target Bacteria (E. coli)

Twenty µl of pure *Escherichia coli* was grown on Mac Conkey Agar (MCA) media, then incubated at 37°C for 24 hours. From the MCA media, *Escherichia coli* was taken with an inoculating loop needle and put into the Nutrient Broth (NB) media and incubated at 37°C for 24 hours. One mL of *E. coli* grown in Nutrient Broth for 24 hours was then diluted in 9 mL of physiological NaCl 0.85% in a sterile test tube, then homogenized using vortex for 15 seconds.

Antibacterial Testing

A total of 100 µL of target bacteria (*E. coli*) was poured evenly on the surface of the Nutrient Agar (NA) medium using the spread plate method, then let dry. A total of 2 grams of the sample from each treatment was dissolved in 8 mL of sterile distilled water. Disc paper with a diameter of 5.5 mm was inserted into each of these treatments, then allowed to stand for 10 minutes. After that, disc paper was placed on the surface of the NA medium containing the target bacteria, then incubated at 37°C for 24 hours. After 24 hours, the diameter of the inhibitory area that formed around the disc paper was measured using a slide. The sample's antibacterial activity was expressed by the diameter of the inhibition zone that formed in the clear area around the disc paper.

Results and Discussion

Growth of Yeast and Fungi during Fermentation of Tempeh

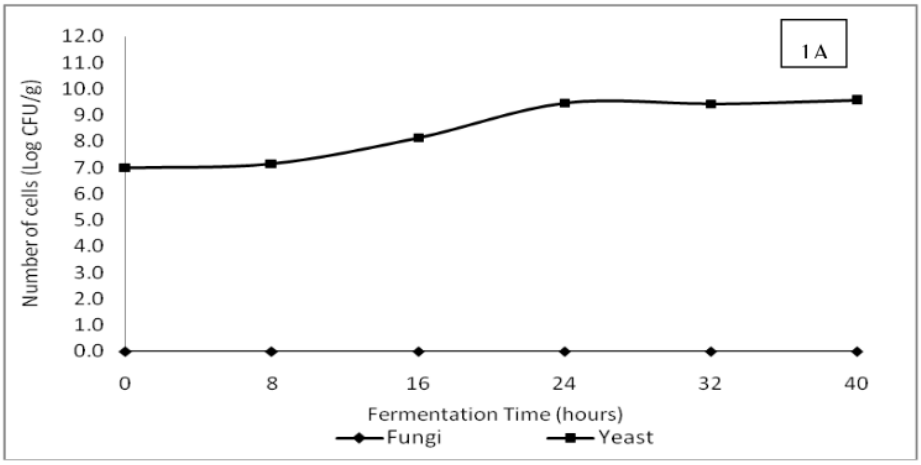
Results showed that yeast (*S. cerevisiae*) grew during soybean fermentation with *S. cerevisiae* culture and mixed culture of *R. oligosporus* and *S. cerevisiae*, but did not grow during soybean fermentation with *R. oligosporus* culture alone. Meanwhile, fungi grew during soybean fermentation with *R. oligosporus* inoculum and mixed culture of *R. oligosporus* and *S. cerevisiae* but did not grow during soybean fermentation with *S. cerevisiae* culture alone. See Figure 1.

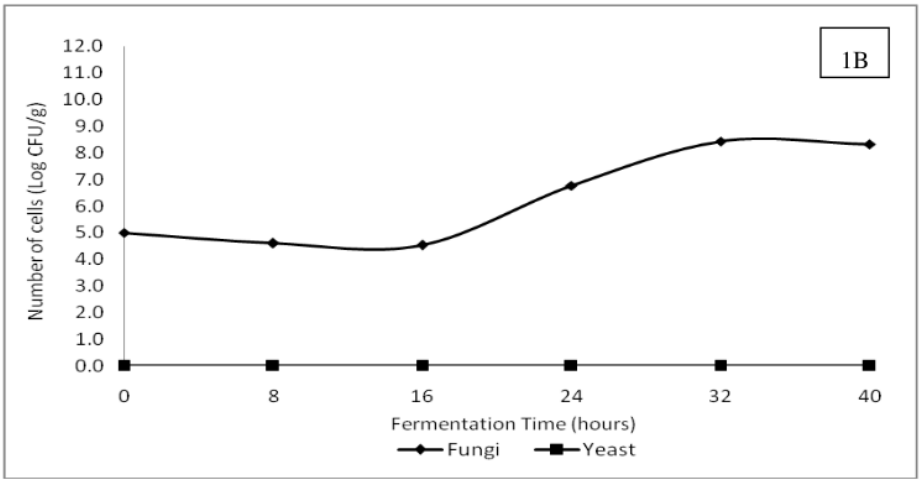
Figure 1A shows the growth curve of *S. cerevisiae* in tempeh inoculated with only *S. cerevisiae*. The adaptation phase occurred in zero up to 8 hours of fermentation with a population of 10^7 CFU/g. For comparison, Sugoro *et al*. (200616) stated that the adaptation phase of *S. cerevisiae* in modified 1% tapioca solution medium containing 10.21% glucose was at the sixth hour of fermentation. Kusmiati *et al*. (2011) in media using carbon sources of glucose had the adaptation phase of *S. cerevisiae* at four hours of fermentation. On YNB medium containing 30% of glucose, Ishmayana *et al*. (2012) had it at six hours of

fermentation. Our adaptation phase of *S. cerevisiae* in this research lasted longer than those of Sugoro *et al*. (2006), Kusmiati *et al*. (2011), and Ishmayana *et al*. (2012) because in this study, there was no carbon source on the substrate, which was needed for the growth of *S. cerevisiae* during fermentation.

Figure 1A also shows that after eight hours of fermentation, yeast experiences a sharp increase in the number of cells from 1.73 x 10^8 CFU/g at 16 hours of fermentation to 3.33 x 10^9 CFU/g at 24 hours of fermentation. This increase indicated that yeast (*S. cerevisiae*) entered an exponential phase after eight hours of fermentation time. Kavanagh (2005) stated that in the exponential phase, yeast reproduced by budding. Based on the exponential phase, the maximum specific growth rate (μmax) of yeast was 0.012 cells/hour. Furthermore, yeast experienced a stationary phase from 24 hours to 40 hours of fermentation time with a population of 4.82 x 10^9 CFU/g. The death phase of yeast (*S. cerevisiae*) appeared to occur after 40 hours of fermentation time.

Yeast can grow during the fermentation process of soybeans inoculated with only *S. cerevisiae* even though tempeh is not formed. *Saccharomyces cerevisiae* as a sole culture (without the addition of the main tempeh fungus) in 40 hours of fermentation does not form tempeh (Figure 2A). According to Sarwono (2004), to make tempeh, a tempeh inoculum is needed. Without an inoculum of tempeh (*R. oligosporus*), soybeans will simply decay. *Saccharomyces cerevisiae* increases, but there is no presence of *R. oligosporus* unless it is inoculated. This result is in line with Wahono *et al*. (2011), who reported that during the fermentation of sorghum seeds in bioethanol production, there was an increase in the growth rate of *S. cerevisiae*. Yeast can grow by utilizing the nutrients present in the soybean substrate. According to Kustyawati (2010), almost all foods provide sufficient nutrition to support yeast growth.





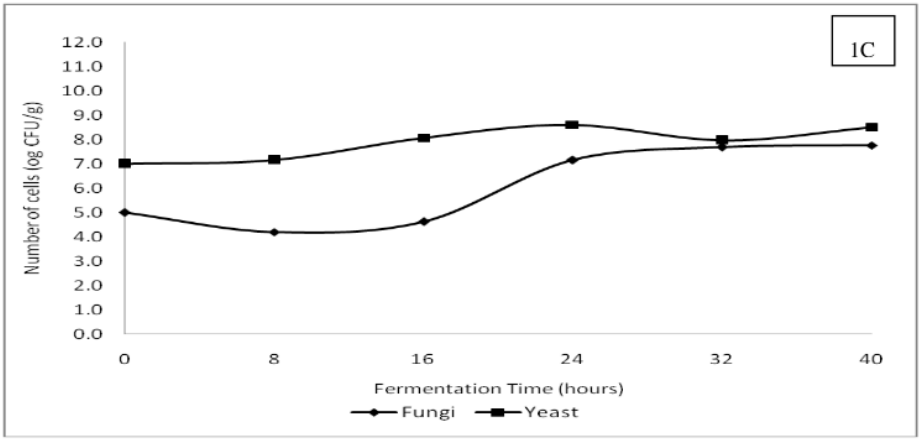


Figure 1. The growth curves of yeast and fungi during soybean fermentation inoculated with a single culture of *S. cerevisiae* (1A), single culture of *R. oligosporus* (1B), and mixed culture of *R. oligosporus* and *S. cerevisiae* (1C).

Figure 1B shows no growth of yeast during tempeh fermentation with *R. oligosporus* as a single inoculum. Unless *S. cerevisiae* is inoculated, there will be no yeast growth. In soybean fermentation using *R. oligosporus* as a single culture, there was no yeast growth, but tempeh was still formed due to hyphae from *R. oligosporus* (Figure 2B). This result was in line with Kustyawati (2009), which stated that yeast was not found during tempeh fermentation using *R. oligosporus*. Thus, this study revealed that the presence of yeast in tempeh could be found only if fermented soybeans were added with yeast.

The growth dynamics of yeast and the appearance of soybean during tempeh fermentation inoculated with the mixed culture of *R. oligosporus* and *S. cerevisiae* are presented in Figure 1C dan Figure 2C. Figure 1C shows that the adaptation phase of yeast occurs between zero and eight hours while the adaptation phase of fungi occurs between zero and 16 hours. In this sample, *S. cerevisiae* and *R. oligosporus* grew at the same time and continued growing until the end of the experiment at 40 hours of fermentation time. The appearance of tempeh inoculated with a mixed culture of *R. oligosporus* and *S. cerevisiae* during fermentation showed that between zero and 16 hours, there was no significant fungi (*R. oligosporus*) growth, as the soybeans remained intact (Figure 2C). After 16 hours of fermentation, fungi entered exponential growth phase marked by an increase in the number of *R. oligosporus* spores up to 7.67×10^6 CFU/g at 24 hours of fermentation time and 2.73×10^7 CFU/g at 32 hours of fermentation time.

This growth pattern is in line with the growth pattern of *S. boulardi* which was inoculated together with *R. oligosporus* for tempeh fermentation in a study conducted by Kustyawati (2009). The growth pattern of yeast in this treatment was similar to that of soybean inoculated with *S. cerevisiae* alone. This indicates that *S. cerevisiae* utilizes the nutrients present in soybeans for growth and there is a mutually beneficial symbiosis between *R. oligosporus* and *S. cerevisiae* during fermentation. According to Kustyawati (2009), there may be a mutually beneficial symbiosis in terms of nutrient availability between *R. oligosporus* and *S. cerevisiae* during tempeh fermentation to achieve synergistic growth. *Rhizopus oligosporus* breaks down carbohydrate, fat, and protein into simple forms, and *S. cerevisiae* absorbs the elements C, H, O, and N from them. In turn, enzymatic activity by *S. cerevisiae* benefits *R. oligosporus* (Kustyawati, 2009).

2A

2B

2C

Figure 2. The appearance of soybean inoculated with a single culture of *S. cerevisiae* (2A), a single culture of *R. oligosporus* (2B), and mixed culture of *S. cerevisiae* and *R. oligosporus* (2C) during tempeh fermentation.

Formation of β-Glucan during Tempeh Fermentation

All types of starter cultures increased β-glucan content of tempeh over time (Table 1). The β-glucan content of tempeh was higher than that – 0.05% (w/w) – of soybean without inoculum. *Saccharomyces cerevisiae* and mixed *R. oligosporus* and *S. cerevisiae* inoculum produced higher β-glucan content in resulting tempeh than soybeans without inoculum.

β-glucan can be taken from the cell wall of *S. cerevisiae* through alkaline extraction, but further purification is needed (Lee *et al.*, 2001; Javmen *et al.*,2012). Commercial tempeh inoculum contains not only *R. oligosporus* but also other microorganisms and fillers such as rice flour (Sukardi and Purwaningsih, 2008). The β-glucan content as a result of the addition of *S. cerevisiae* increases with the concentration of *S. cerevisiae* (Kusmiati, 2007), because the cell wall of *S. cerevisiae* contains β-(1,3) and β-(1,6) glucans (Naruemon *et al.*, 2013).

Based on careful measurement, it was found that the highest β-glucan content was found in tempeh inoculated with a mixed cultures of *R. oligosporus* and *S. cerevisiae* at 40 hours of fermentation time – 0.578% (w/w).

Table 1. The β-glucan content of soybeans inoculated with various starter cultures during tempeh fermentation.

Fermentation time (hours)	% β-glucan content (w/w) ± DS of tempeh on various types of starter culture		
	S. cerevisiae	*R. oligosporus*	*R. oligosporus* + *S. cerevisiae*
0	0.173± 0.001	0.207± 0.013	0.179± 0.002
8	0.189± 0.004	0.168± 0.005	0.295± 0.006
16	0.227± 0.008	0.159± 0.006	0.330± 0.008
24	0.663± 0.006	0.264± 0.008	0.593± 0.005
32	0.506± 0.004	0.413± 0.019	0.486± 0.010
40	0.515± 0.008	0.311± 0.009	0.578± 0.010
Soybean without inoculum		0.050±0.021	

Soybeans inoculated with *S. cerevisiae* contained more β-glucan than those without the addition of *S. cerevisiae* (Table 1). Our results agreed with Thontowi *et al.* (2007), who stated that the β-glucan content of *S. cerevisiae* in cultures with N peptone sources tended to increase along with fermentation time and was relatively fixed by the end of fermentation time (84 hours). Kusmiati *et al.* (2006) also reported an increase in β-glucan production with the use of different carbon sources, for example by utilizing sugar mill waste (molasses) as a fermentation medium. Increased β-glucan production follows the increasing number of *S. cerevisiae* cells. Formation of β-glucans continues until *S. cerevisiae* reaches a stationary growth phase. Kim *et al.* (2014) reported that the β-glucan content of polysaccharides in black rice bran fermented by *L. edodes* increases with time.

From Table 1, β-glucan content in this study is higher than that of Rizal *et al.* (2018) with 0.578% and 0.076% respectively. Shokri *et al.* (2008) obtained β-glucan from *S. cerevisiae* cell walls using NaOH with 27.5% of β-glucan, whereas Varelas *et al.* (2016) obtained 40% of β-glucan. Meanwhile, our percentages ranged from 0.05% to 0.663%, significantly lower than the numbers previously mentioned. This difference was caused by the different methods used to extract β-glucan. In this study, the β-glucan content was investigated from the resulting fermented soybean flour, while the β-glucan content studied by Shokri *et al.* (2008) and Varelas *et al.* (2016) was directly isolated from the cell wall of *S. cerevisiae*.

This study showed that the addition of *S. cerevisiae* in making of tempeh could increase yeast growth and β-glucan content of tempeh. The highest content of β-glucan was found in tempeh which was made by adding a mixed culture of *R. oligosporus* and *S. cerevisiae* inoculum at 40 hours of fermentation time with 0.578% (w/w) (Table 1).

Antimicrobial Activities of Tempeh during Fermentation

Antibacterial activity testing was carried out during the fermentation process of soybeans added with various types of culture (soybeans + *S. cerevisiae*, soybeans + *R. oligosporus*, soybeans + *S. cerevisiae* + *R. oligosporus*). In this study, the antibacterial activity of tempeh was determined by measuring the inhibitory zone's diameter in the form of

a clear area around the disc paper. The results showed that the antibacterial activity of tempeh increased along with fermentation time for all treatments of starter culture types. The highest inhibitory zone was shown in tempeh with the addition of *R. oligosporus* and *S. cerevisiae* mixed starter culture at 40 hours of fermentation time, which was 25.98 ± 0.56 mm. Meanwhile, the diameter of the lowest inhibition area was shown in soybeans without added inoculum with 7.68 ± 0.39 mm. The diameters of the tempeh inhibition area against *E. coli* in various types of culture at different stages of fermentation time are shown on Figure 3.

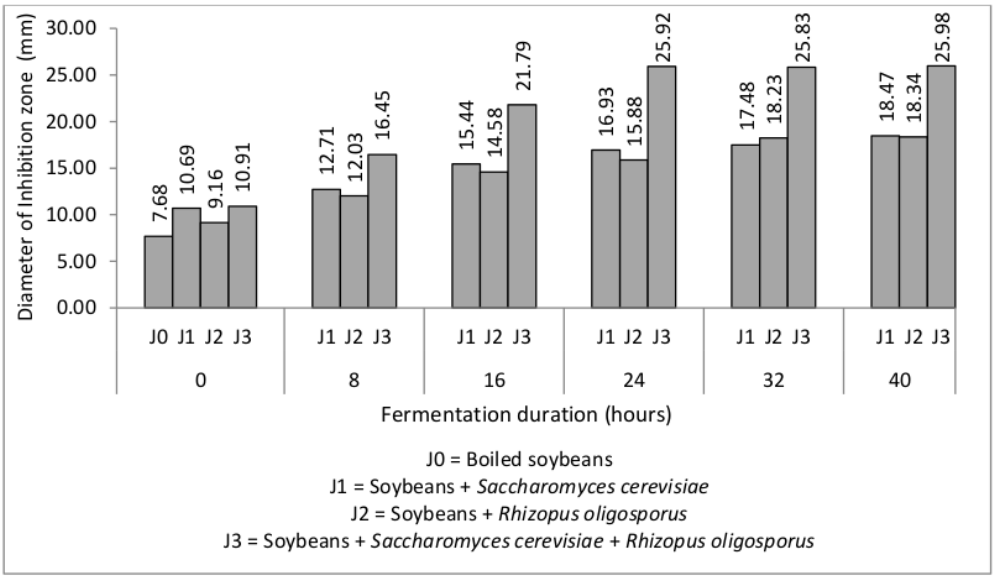


Figure 3. Antibacterial activities of soybean inoculated by a culture of *Saccharomyces cerevisiae*, a culture of *Rhizopus oligosporus*, and a mixed culture of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* during tempeh fermentation.

Figure 3 shows that the boiled soybeans without any starter culture addition could still inhibit the growth of *Escherichia coli* with inhibitory area diameter of 7.68 ± 0.39 mm. The content of isoflavones in soybeans causes the antibacterial activity of soy. According to Kustyawati (2009), antibacterial activity happens because soybeans alone contained isoflavones in the form of genistein (0.25 ± 0.60) and daidzein (0.69 ± 0.20). Additionally, according to Dhayakaran et al. (2015), soy isoflavones shows antibacterial activity against several pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

The addition of soybeans with all types of starter cultures (*S. cerevisiae*, *R. oligosporus*, and a mixed culture of *S. cerevisiae* and *R. oligosporus*) caused an improvement in antibacterial activity during fermentation that continued to increase along with fermentation time. Both *S. cerevisiae* and *R. oligosporus* contribute to improving antibacterial activity during tempeh fermentation. The highest antibacterial activity was found in tempeh added with mixed cultures of *S. cerevisiae* and *R. oligosporus* after 40 hours of fermentation. The increase in tempeh antibacterial activity during soybean fermentation by *S. cerevisiae* and *R. oligosporus* was related to tempeh β-glucan content, which also increased (Table 1). These results are consistent with research conducted by Rizal et al. (2020). According to Rizal et al. (2020), increasing the number of *S. cerevisiae* and *R. oligosporus* in tempeh can cause tempeh β-glucan content to increase, thus increasing the

antibacterial activity of tempeh. As stated by Hetland et al. (2013), β-glucans are compounds that are antagonistic to several microorganisms including bacteria, mold, yeast, and viruses.

The increased antibacterial activity of tempeh during fermentation is also caused by the increase in the number of soy isoflavones. Kustyawati et al. (2020) showed that soybean added with *S. cerevisiae* and *R. oligosporus* contained daidzein and genistein of 224.37 ± 0.20 and 465.12 ± 0.90, respectively. Increasing the amount of isoflavones can increase the inhibitory activity against bacteria because isoflavones act as antimicrobials (Mambang et al., 2014).

Conclusions

This study showed that the addition of *S. cerevisiae* in tempeh fermentation resulted in growth of yeast and fungi, production of beta-glucans, and increase in the antibacterial activity of tempeh. The highest content of β-glucan and diameter of inhibition (antibacterial activity to *E. coli*) was found in tempeh with the addition of a mixed culture of *R. oligosporus* and *S. cerevisiae* at 40 hours of fermentation time, with 0.578% (w / w) and 25.98 mm, respectively. Tempeh with the addition of *S. cerevisiae* inoculum has the potential of added functional properties due to the health benefits of β-glucan. Future research is needed to see the effect of tempeh with additional *S. cerevisiae* inoculum on body resistance in vivo.

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