Research Article

The growth of yeast and fungi, the formation of β-glucan, and the antibacterial activities during soybean fermentation in producing tempeh

Samsul Rizal*, Maria Erna Kustyawati*, Murhadi*, Udin Hasanudin*

[1] Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung; Jalan Sumantri Brojonegoro No. 1 Bandar Lampung, Lampung 35145. Indonesia. Email: samsul.rizal@fp.unila.ac.id

* Correspondence should be addressed to: samsul.rizal@fp.unila.ac.id;

Received: date; Accepted: date; Published: date

Abstract

Generally, the microorganism involved in soybean fermentation for the production of tempeh is *Rhizopus oligosporus*. However, *Saccharomyces cerevisiae*, a type of β-glucan-producing yeast, is known to be present and grow in the fermentation process. This study aimed to investigate yeast and fungi growth dynamics, the β-glucan formation, and antibacterial activity against *Escherichia coli* during the fermentation after adding *S. cerevisiae* as an inoculum. The Randomized Complete Block Design (RCBD) was applied with two treatments and three replications. Three kinds of starter culture are *S. cerevisiae, R. oligosporus*, and the combination of both. The second treatment was fermentation time at room temperature (30±2ºC) for 0, 8, 16, 24, 32, and 40 hours. The dynamics were observed every eight hours. The results explained that yeast grew during this process from a single *S. cerevisiae* culture and a mixture of *R. oligosporus* and *S. cerevisiae*, but not from *R. oligosporus* alone. The yeast grew during and until the end of fermentation and decreased after 32 hours in the mixed cultures. The β-glucan formed in tempeh with all types of inoculum, but the antimicrobial activity against *E. coli* increased with fermentation. The highest β-glucan content and antibacterial activity of tempeh are from the mixed culture. In conclusion, the addition of *S. cerevisiae* and *R. oligosporus* in soybean fermentation produced tempeh with the highest β-glucan content and antibacterial activity against *E. coli*. The presence of β-glucans suggests to higher health benefits of tempeh.

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

Introduction

Tempeh is a traditional Indonesian fermented food produced from soybeans by using *Rhizopus* sp. This healthy functional food is due to bioactive compounds such as isoflavones. It has nutritional advantages, unique textures, and pleasant flavors (Kustyawati *et al*., 2014). The quality of tempeh depends on the raw material and type of inoculum or starter culture

used. The kind of inoculum plays a vital role in making tempeh because it affects the tempeh's quality.

Generally, tempeh uses an inoculum containing *R. oligosporus* (O'Toole, 2016). Other important microorganisms involved in fermenting soybean to form tempeh are *R. oryzae* and *R. stolonifer* (Bintari *et al.*, 2017). All three microorganisms ferment soybeans into tempeh. *Rhizopus oligosporus* retains most of the nutrients in soybeans and increases protein digestibility (Nout *et al*., 2005). *R. oligosporus* synthesizes more protease enzyme, whereas *R. oryzae* favors the α-amylase enzyme (Triwibowo, 2011).

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

In this study, *S. cerevisiae* was added intentionally to the soybean fermentation process to produce tempeh with high β-glucan content and, therefore, to improve the functional properties as a healthy food. *S. cerevisiae* cell wall is composed of β-(1,3) and β-(1,6)-glucan, mannan, chitin (1-2%), and mannoproteins, comprising about 20-30% of the dry weight of the cell wall (Naruemon *et al*., 2013). β-Glucan is a polysaccharide with health benefits, one of which as a biological response modifier (Corno *et al*. 2020), has anti-aging effects, and antibiotic activity against bacteria, fungi, viruses, and parasites (Hetland *et al*. 2013).

The yeast could grow alongside fungi during soybean fermentation when a carbon source was added, thus resulting in β-glucans in the tempeh produced (Rizal *et al*. (2020). In this study, *S. cerevisiae* was added to the soybean fermentation without carbon sources. It was essential to examine if the fungi addition without any carbon source in the fermentation could give yeast and β-glucan in tempeh. Also, the presence of β-glucan due to yeast addition might add the health benefits, including antibacterial activity. Therefore, this study aimed to observe the effect of *S. cerevisiae's* addition to the growth dynamics of yeast and fungi, the β-glucan formation, and the antibacterial activities against *Escherichia coli* during soybean fermentation to produce tempeh**.**

Materials and Methods

This study used pure cultures of *R. oligosporus FNCC 6010*, *S. cerevisiae* FNCC 3012, *E. coli*, soybeans (brand "Soybean USA No. 1"), Nutrient Broth (NB), Nutrient Agar (NA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA). The experimental analysis employed a Factorial Randomized Block Design with three replications. The first factor was the three levels of cultures: *S. cerevisiae* (negative control), *R. oligosporus* (positive control), and the mix of both microorganisms (the primary treatment). The second factor was the fermentation time of six levels: 0, 8, 16, 24, 32, and 40 hours. During fermentation, we observed the microbial population, the β-glucan content, and the antibacterial activity toward *E. coli* in (0, 8, 16, 24, 32, and 40 hours) of fermentation time.

Preparation of *S. cerevisiae* culture

The *S. cerevisiae* was cultured in a sterile Malt Extract Agar (MEA) medium using a sterilized inoculating loop needle with a scratchplate, then incubated for 24 to 48 hours at 28°C to form colonies. The colonies were harvested by adding 5 or 10 mL of distilled water into the plate disk. The fungus 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 discarded, and the remaining solids were diluted with 25 to 30 mL of distilled water. The cells were transferred into a test tube containing 9 mL of

physiological saline solution and then homogenized using a vortex. The number of cells was calculated using a hemocytometer. The required concentration was 10^7 cells/mL.

Preparation of *R. oligosporus* culture

R. oligosporus from a tilted agar was cultured in a sterile medium of Potato Dextrose Agar (PDA) using a sterilized inoculating loop needle and a scratchplate. The yeast was 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

After removing the husks, 300 g soybean were soaked at room temperature overnight, boiled in three times their water weight for 30 minutes, drained, cooled to the ambient temperature, and 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 perforated for ventilation, then incubated at 32ºC for 40 hours, and observed every eight hours.

Enumeration of Microorganisms

The microorganisms were enumerated by culturing on PDA for the fungi and MEA for the yeasts. Immediately at 0 hours, then at 8, 16, 24, 32, and 40 hours, consecutively, 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 formation was analyzed every eight hours during fermentation following the Rizal *et al*. (2020). One gram of sample and 30 mL of 0.7 N NaOH was hydrolyzed for six hours at 75°C and 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, and centrifuged again at 10,000 rpm and 25°C for 30 minutes. This process was repeated three times. The precipitated material was 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. Afterward, the sample was diluted with 10 mL of sterile distilled water and shaken with an orbital shaker. 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 under 490 A wavelength.

Assessment of Antibacterial Activities

Preparation of E. coli

Pure *E. coli* (20 µL) was grown on Mac Conkey Agar (MCA) media and incubated at 37°C for 24 hours. The bacteria was taken with an inoculating loop needle from the MCA media and put into the Nutrient Broth (NB) media and incubated at 37°C for 24 hours. One mL of the bacterium was diluted in 9 mL of physiological NaCl 0.85% in a sterile test tube and homogenized using vortex for 15 seconds.

Antibacterial Testing

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

Results and Discussion

Growth of Yeast and Fungi during Fermentation

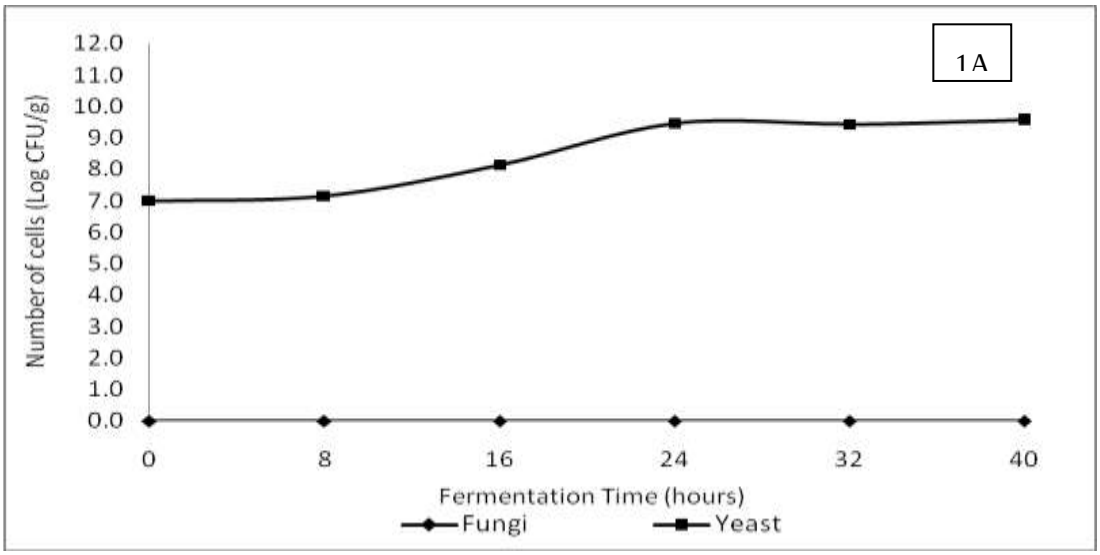
Figure 1 shows the growth of yeast and fungi in various types of cultures used in fermentation of soybeans to tempeh. During fermentation of soybean with only *R. oligosporus* culture, there was no increase in the amount of yeast (Figure 1A), whereas during fermentation using only *S. cerevisiae* alone, the fungus did not grow (1B). In contrast, both fungi and yeast reproduce well during the fermentation of soybeans with mixed cultures of R. oligospors and S. cerevisiae (Figure 1C).

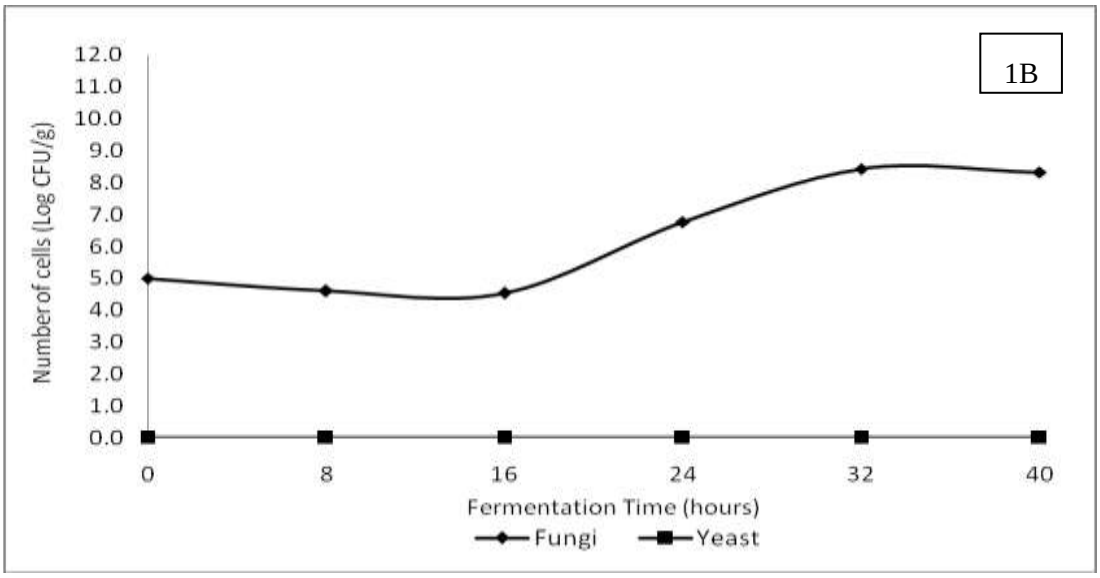
Figure 1A shows the growth curve of *S. cerevisiae* in tempeh inoculated with only *S. cerevisiae*. The adaptation phase occurred in zero up to 8 hours of fermentation with a population of 10^7 CFU/g. For comparison, Sugoro *et al*. (2006) stated that the adaptation phase in a modified 1% tapioca solution medium containing 10.21% glucose was at the sixth hour of fermentation. Kusmiati *et al*. (2011) reported that in media with glucose as a carbon source, this fungus' adaptation phase was four hours. On YNB medium containing 30% of glucose, Ishmayana *et al*. (2012) had it at six hours of fermentation. Our adaptation phase in this experiment was delayed than those of Sugoro *et al*. (2006), Kusmiati *et al*. (2011), and Ishmayana *et al*. (2012) because there was no carbon source on the substrate as needed for the growth during fermentation.

Figure 1A also shows that after eight hours of fermentation, the yeast experiences a sharp increase in the number of cells from 1.73×10^8 CFU/g at 16 hours of fermentation to 3.33×10^9 CFU/g at 24 hours of fermentation. This increase indicated that yeast (*S. cerevisiae*) entered an exponential phase after eight hours. Kavanagh (2005) stated that in the exponential phase, the yeast reproduced by budding. The maximum specific growth rate (μ_{max}) of yeast is 0.012 cells/hour based on the exponential phase. Furthermore, the yeast experienced a

stationary phase from 24 hours to 40 hours, with a population of 4.82×10^9 CFU/g. The death phase of yeast 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. *S. cerevisiae* as a sole culture (without the addition of the primary tempeh fungus) in 40 hours of fermentation does not form tempeh (Figure 2A). To make tempeh, an inoculum is needed. Otherwise the soybeans will simply decay. *S. 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.





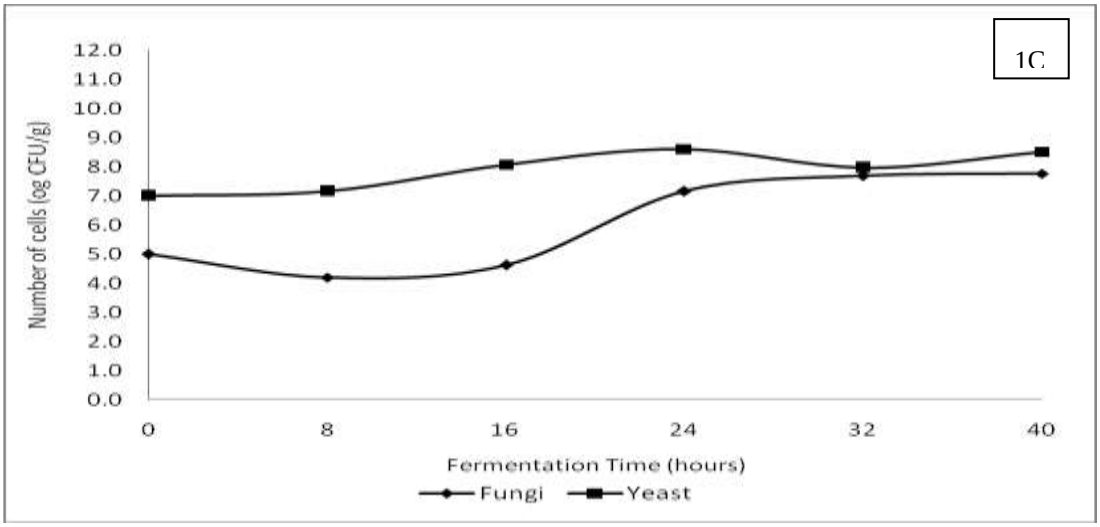


Figure 1. The growth curves of yeast and fungi during soybean fermentation inoculated with a single culture of *S. cerevisiae* (1A), a single culture of *R. oligosporus* (1B), and a mixed culture of both microorganisms (1C).

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 yeast in tempeh could only be seen when the 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 yeast's adaptation phase occurs between zero and eight hours while the adaptation phase of fungi occurs between zero and 16 hours. In this sample, both microorganisms grew simultaneously and continued increasing until the end of the experiment at 40 hours of fermentation. The appearance of tempe inoculated with mixed cultures of R. oligosporus and S. cerevisiae during fermentation showed that there was no significant fungal growth from 0 to 16 hours of fermentation and soybeans were still intact (Figure 2C). After 16 hours of fermentation, fungi entered the 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 yeast growth pattern in this treatment was similar to that of soybean inoculated with *S. cerevisiae* alone. It 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 helpful symbiosis in 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.*

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 both microorganisms (2C) during tempeh fermentation.

Formation of β-Glucan

All types of starter cultures increased the β-glucan content of tempeh over time (Figure 3). The β-glucan content of tempeh was higher (0.05% w/w), compared to that without inoculum. *S. cerevisiae* and the mixed inoculum produce higher β-glucan content in the resulting tempeh than soybeans without inoculum.

β-Glucan can be taken from S. cerevisiae's cell wall by alkaline extraction, but further purification is needed (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 depends on the addition of *S. cerevisiae* (Kusmiati, 2007) because its cell wall contains β-(1,3) and β-1,6) glucans (Naruemon *et al*., 2013).

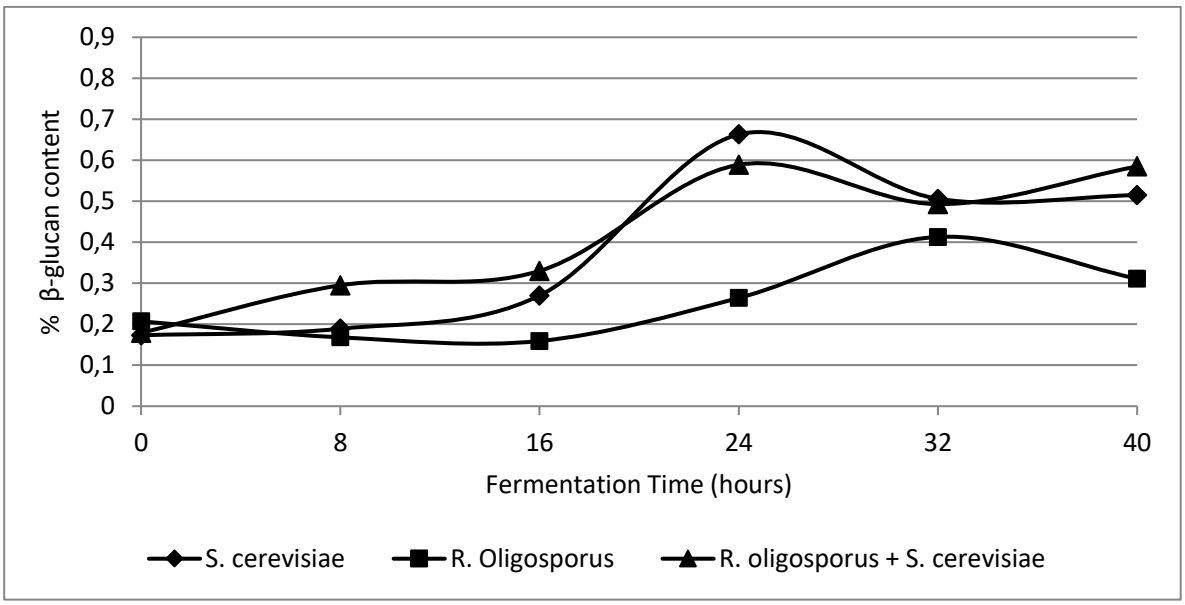


Figure 3. The β-glucan content of soybean fermented using three kinds of starter culture.

Soybeans inoculated with *S. cerevisiae* contained more β-glucan than those without *S. cerevisiae* (Figure 3). These results agreed with Thontowi *et al*. (2007) that the β-glucan content of *S. cerevisiae* in cultures with N peptone sources tended to increase along with fermentation time and was relatively constant by the end of fermentation time (84 hours). Kusmiati *et al*. (2011) also reported an increase in β-glucan production using 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. The 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.

Figure 3 shows that the β-glucan content of tempeh (0.578%) from this study is higher than that of Rizal *et al*. (2018) with 0.076%. 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) got 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 was observed 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 the 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 fermentation time of 40 hours (0.578% w/w) (Figure 3).

Antimicrobial Activities of Tempeh during Fermentation

Antibacterial activity testing was carried out during the fermentation process of soybeans added with various cultures (soybeans + *S. cerevisiae*, soybeans + *R. oligosporus*, soybeans + *S. cerevisiae* + *R. oligosporus*). In this study, tempeh's antibacterial activity was determined by measuring the inhibitory zone's diameter in the form of a clear area around the disc paper. The results showed that tempeh's antibacterial activity increased along with fermentation time for all treatments of starter culture types. The highest inhibitory zone appeared in tempeh fermented by the mixed starter culture at 40 hours of fermentation time, 25.98 ± 0.56 mm. Meanwhile, the lowest inhibition area diameter was in soybeans without

added inoculum with 7.68 ± 0.39 mm. The diameters of the tempeh inhibition area against *E. coli* are different in various cultures and fermentation times (Figure 3).

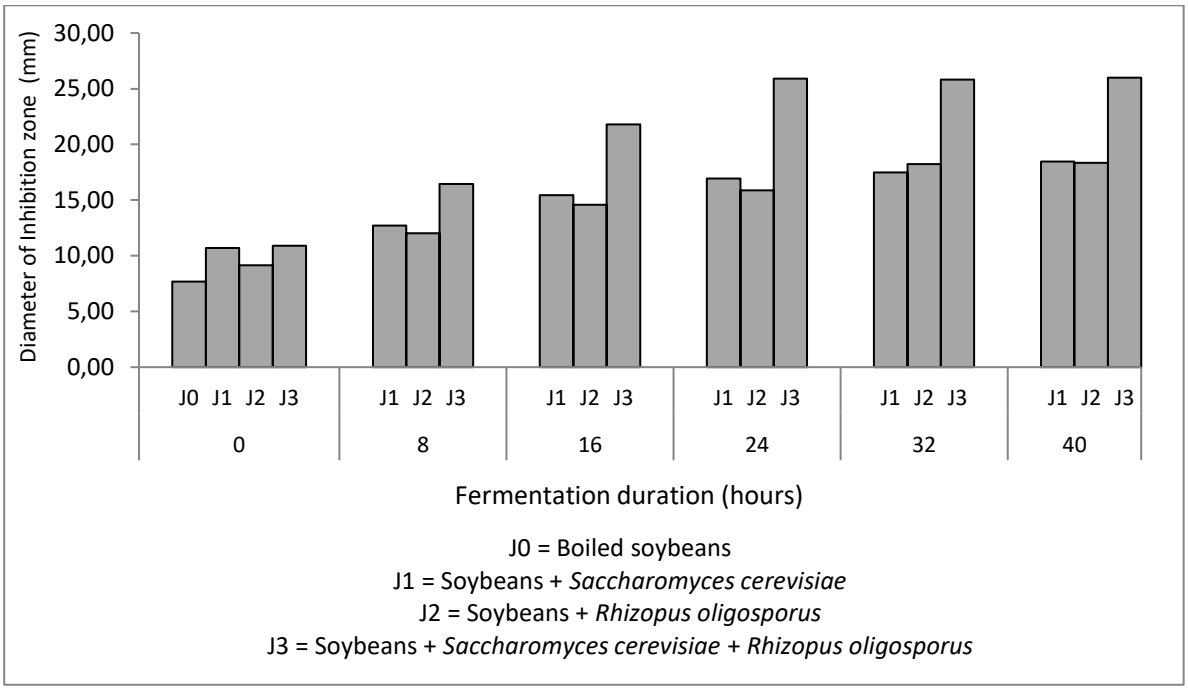


Figure 4. Antibacterial activities of soybean inoculated by a culture of *Saccharomyces cerevisiae*, a culture of *Rhizopus oligosporus*, and a mixed culture of both during tempeh fermentation.

Figure 4 shows that the boiled soybeans without any starter culture addition could still inhibit the growth of *E. coli* with an 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 also show antibacterial activity against several pathogens such as *Listeria monocytogenes* and *Pseudomonas aeruginosa*.

The addition of soybeans with all three types of starter cultures caused an improvement 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 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 (Figure 3). These results are consistent with research conducted by Rizal *et al*. (2020) that increasing the number of these two microorganisms escalates the β-glucan content, 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 increasing 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 approximately 225 and 465, respectively. Increasing the amount of isoflavones increases the inhibitory activity against bacteria because isoflavones act as antimicrobials (Mambang *et al*., 2014).

Conclusions

The addition of *S. cerevisiae and R. oligosporus* as mixed inoculums in tempeh's fermentation resulted in higher growth of yeast and fungi, forming beta-glucan and antibacterial activity of tempeh than without the addition of yeast. Therefore, the tempeh can have better functional properties as healthy food due to the health benefits of β-glucan. *In vivo* studies need to be done to prove the effect of adding both microorganisms on improving tempeh's functional properties in mice.

Acknowledgments: Our deepest gratitude goes to Fatimah and Lia Dahlia Pratiwi, alumnae of the Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung, Indonesia, for their assistance and cooperation in conducting this research. We would also like to thank the Integrated Laboratory of the University of Lampung, and the Institute for Research and Community Service, University of Lampung, Indonesia, for their contributions to this work, and The Ministry of Research, Technology, and Higher Education, Indonesia.

Conflicts of Interest: All authors declare no conflict of interest. No involvement of the funder in the design of the experiment; in the collection, analyses, data interpretation, writing, and deciding to publish the results".

References

1. Kustyawati, M.E.; Pratama, F.; Saputra, D.; Wijaya, A. The modification of color, texture, and aroma of tempeh processed with supercritical carbon dioxide. Jurnal Teknologi dan Industri Pangan. 25, 168-175. ISSN 1979-7788, 2014.
2. O'Toole, D.K. Non-Wheat Foods: Soy-Based Fermented Foods. Reference Module in Food Sciences. Elsevier Ltd, 6, 174-185, 2016.
3. Bintari**,** S.H.; Widyastiti**, N.**Y**.;** Putriningtyas **N.**D**.;** Hapsari R**.;** Nugraheni**,** K**.** Development and Properties of Tegurt, a Yogurt-like Tempe Product. Pakistan Journal of Nutrition. 16 (4): 221-226. 2017
4. Nout, M.J.R. and J.L. Kiers. Tempe fermentation, innovation and functionality: Update into the third millenium. J. Applied Microbiol., 98: 789-805. 2005.
5. Triwibowo, R. Chemical study of stakiosa and essential fatty acids in soybean tempeh (glycine max) during the fermentation process. Universitas Sebelas Maret. Surakarta. 2011.
6. Seumahu, C.; Suwanto, A.; Rusmana; Solihin, D.D. Bacterial and fungal communities in tempeh as reveal by amplified ribosomal intergenic sequence analysis. J. Hayati of Biosci., 20, 65-71, 2013.
7. Efriwati; Suwanto, A.; Rahayu, G; Nuraida, L. Population dynamics of yeast and lactic acid bacteria (lab) during tempeh production. Hayati J. Biosciences, 20, 57-64. DOI: 10.4308/hjb.20.2.57, 2013.
8. Kustyawati, ME.; Subeki; Murhadi; Rizal, S.; Astuti, P. Vitamin B12 production in soybean fermentation for tempeh. AIMS Agriculture and Food. 5, 262–271, 2020.
9. Kustyawati, M.E.; Nawansih, O; Nurdjannah, S. Profile of aroma compounds and acceptability of modified tempeh. International Food Research Journal. 2, 734-740, 2016.
10. Pengkumsri, N.; Sivamaruthi, B.S.; Sirilun, S.; Peerajan, S.; Kesika, P.; Chaiyasut, K.; Chaiyasut, C.T. Extraction of B-glucan from *Saccharomyces cerevisiae*: Comparison of different extraction methods and *In vivo a*ssessment of immunomodulatory effect in mice. J. of Food Sci. Technol, 37, 124-130. DOI: http://dx.doi.org/10.1590/1678-457X.10716, 2017.
11. Naruemon, M.; Romanee, S.; Cheunjit, P.; Xiao, H.; McLandsborough, L. A.; Pawadee, M. Influence of additives on *Saccharomyces cerevisiae* β-glucan production. International Food Research Journal, 2013, 20, 1953-1959.
12. Thontowi, A.; Kusmiati; Nuswantara, S. Production of β-glucan *Saccharomyces cerevisiae* in different nitrogen source media in fermenter-lift water. Biodiversitas J. Bio. Diversity, 8, 253-256, 2007.
13. Javmen, A.; Grigiškis, S.; Gliebute, R. β-glucan extraction from *Saccharomyces cerevisiae* yeast using *Actinomyces rutgersensis* 88 Yeast Lysing Enzymatic Complex. Biologija. 2, 51–59, 2012.
14. Sukardi, W.; Purwaningsih, I. The trial of the use of tempeh inoculums from mold *Rhizopus oryzae* with rice and cassava flour substrate in the tempeh production unit of Sanan Kodya Malang. Jurnal Teknologi Pertanian. 9, 207-215, 2008.
15. Kusmiati, A.; Tamat, S.R.; Nuswantara, S.; Isnaini, N. Production and determination of β-glucan levels from three *Saccharomyces cerevisiae* strains in molasses containing media. Jurnal Ilmu Kefarmasian Indonesia, 5, 7-16, 2007.
16. Hetland, G.; Johnson, E.; Eide, D.M.; Grinde, B.; Samuelsen, A.B.C.; Wiker, H. G. Antimicrobial effects of β-glucans and pectin and of the *Agaricus blazei* Based Mushroom Extract, AndoSan^{TM}. Examples of Mouse Models for Pneumococcal, Fecal Bacterial, and Mycobacterial Infections. Microbial Pathogens and Strategies for

Combating Them: Science, Technology and Education (A. Méndez-Vilas, Ed.). Formatex. 889-898, 2013.
17. Ishmayana, S.; Djajasoepana, A.S.; Rachman, S.D.; Safari, A. Fermentation Performance of Saccharomyces cerevisiae Yeast in VHG Media with Concentration Variation of Yeast Extract as a Nitrogen Source for Bioethanol Production. (Conference Paper). Padjadjaran University. Bandung. 2012.
18. Kavanagh, K. Fungi Biology and Applications. John Wiley and Sons Ltd, England. 2005.
19. Kim, S.P.; Park, S.O.; Lee, S.J.; Nam, S.H.; Friedman, M.A. Polysaccharide isolated from the liquid culture of *Lentimus edodes* (*shiitake*) mushroom mycelia containing black rice bran protects mice against salmonellosis through upregulation of the th1 immune reaction. Journal Agriculture and Food Chemistry. 62, 2384–2391, 2014.
20. Kusmiati; Thontowi, A.; Nuswantara, S. Effect of the different carbon sources on β-glukan production by *saccharomyces cerevisiae* on lift water fermenters. Jurnal Natur Indonesia. 3, 138-145, 2011.
21. Kustyawati, M.E. Study on the role of yeast in tempeh production. Jurnal Agritech, 2009, 29, 64-70.
22. Kustyawati, M.E., Pratama, F., Saputra, D., Wijaya, A. Viability of Molds and Bacteria in Tempeh Processed with Supercritical Carbon Dioxides during Storage. Intern. J. Food Sci. 7 pages, 2018.
23. Rizal, S.; Kustyawati, M.E.; Murhadi; Hasanudin, U.; Marniza. Effect of *Saccharomyces cerevisiae* Concentration on the Ash Levels, Protein Levels, Fat, and Beta-Glucan Content of Tempeh. Proceeding National Seminar of Anniversiry of Universitas Sebelas Maret. p. 96-103, 2018.
24. Rizal, S.; Murhadi; Kustyawati, M.E.; Hasanudin, U. Growth optimization of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* during fermentation to produce tempeh with high β-glucan content. Biodiversitas J. Bio. Diversity, 2020, 6, 2667-2673.
25. Shokri, H.; Asadi, F; Khosravi, A.R. Isolation of β-glucan from the cell wall of *Saccharomyces cerevisiae.* Natural Product Research. 22, 414-421. DOI: 10.1080/14786410701591622, 2008.
26. Sugoro, M.R.; Pikoli. Pertumbuhan khamir pada tapioka iradiasi. A Scientific Journal for The Applications of Isotopes and Radiation. 2, 1907-0322, 2006.
27. Varelas, V., Tataridis, P., Liouni, M. and Nerantzis, E. T. Application of different methods for the extraction of yeast β-glucan. e-Journal of Science & Technology (e-JST). 2016.
28. Wahono,S.K.; Damayanti, E; Vita,T.R; Evi, I; Sadyastuti. *Saccharomyces cerevisiae* Growth Rate in the Fermentation Process of Formation of Bioethanol from Sorghum Seeds (Sorghum bicolor l.). Conference: Proceedings of National Seminar on Chemical Engineering and Processes. Diponegoro University, Semarang, Indonesia, 2011.
29. Corno MD, Gessani S, Conti L. Shaping the innate immune response by dietary glucans: Any role in the control of cancer?. Cancers (Basel) 12 (1): 155. DOI: 10.3390/cancers12010155, 2020.
30. Dhayakaran RPA.; Neethirajan, S.; Xue, J.; Shi, J. Characterization of antimicrobial efficacy of soy isoflavones against pathogenic biofilms. Food Sci. Technol. 63, 859-865.
31. Mambang, D.E.P.; Rosidah; Suryanto, D. Antibacterial activity of tempe extract against *Bacillus subtilis* and *Staphylococcus aureus* bacteria. J. Teknol. Ind. Pangan, 25, 115-118, 2014.