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Changes of nutritional composition of tempeh during fermentation with the addition of *Saccharomyces cerevisiae*

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Abstract. *Rizal S, Kustyawati ME, Suharyono AS, Suyarto VA. 2022. Changes of nutritional composition of tempeh during fermentation with the addition of* Saccharomyces cerevisiae. *Biodiversitas 23: 1553-1559.* This study aimed to determine the effect of the addition of *Saccharomyces cerevisiae* on changes in nutritional composition during tempeh fermentation. The study used a Completely Randomized Block Design with two treatments and two replications. The first treatment was the type of tempeh inoculum with 4 levels, namely 0.2% commercial inoculum (Raprima), 1% *Rhizopus oligosporus* inoculum, 1% *S. cerevisiae* inoculum, and a mixture of 1% *R. oligosporus* inoculum, and 1% *S. cerevisiae* inoculum. The second treatment was the length of fermentation with 4 levels, namely 0 hours, 15 hours, 30 hours, and 45 hours. Observation parameters include fat, protein, ash, moisture, carbohydrates, and β-glucan content during fermentation of tempeh. The data obtained were analyzed statistically using the Barlett and Tukey test then continued with the ANOVA test and the Orthogonal Polynomial-Orthogonal Comparison (OP-OC) test at the 5% level. The results showed that the addition of *S. cerevisiae* as an inoculum in making tempeh had an effect on changes in nutritional content during tempeh fermentation. The fat content, ash content, and moisture content of tempeh increased during fermentation, while the fat content and carbohydrate content decreased. The addition of 1% *S. cerevisiae* to tempeh fermentation by 1% *R. oligosporus* at 45 hours of fermentation resulted in the best nutritional content of tempeh with 17.40% protein content, 8.23% fat content, 65.74% moisture content, 1.33 ash content, 7.30% carbohydrate content, and 0.13% β-glucan content.

Keywords: β-glucan, fermentation time, *Rhizopus oligosporus*, *Saccharomyces cerevisiae*, tempeh

INTRODUCTION

Tempeh is a food product that is fermented with *Rhizopus oligosporus* and has a high nutritional composition. Tempeh contains 46.68-52.70% protein, 6.57-6.12% carbohydrates, 2.01-2.47% ash content, and 6.21-6.77% crude fiber (Astawan et al. 2013). Several basic ingredients can be used to make tempeh, but the ingredient that is often used is soybeans (Indriyani et al. 2010). According to Radiati and Sumarto (2016), fungi that grow during the soybean fermentation process will produce several enzymes such as protease, lipase, and amylase enzymes which can break down complex compounds like protein, fats and carbohydrates into simpler compounds. This will cause the content in tempeh to be more easily digested, absorbed, and utilized by human body compared to ordinary soybeans (Jayanti 2019).

According to Efriwati et al. (2013), fungi were involved in the tempeh fermentation process, and other microorganisms were found, namely Lactic Acid Bacteria (LAB) and yeast. The presence of these yeasts indicates that yeasts are able to grow and interact with other microflora contained in tempeh so it is possible that yeasts have a role in improving the nutritional quality and taste of tempeh (Kustyawati 2009). One of the yeasts that can play a role in the tempeh fermentation process is *Saccharomyces cerevisiae* (Rizal and Kustyawati 2019).

The utilization of yeast *S. cerevisiae* is quite extensive. *Saccharomyces cerevisiae* is widely used in various industries, especially in fermented products, one of them is food industry. *Saccharomyces cerevisiae* can produce several enzymes such as lipase enzymes (Treichel et al. 2010) and amylase enzyme (Kustyawati 2018) which might have an important contribution to improvement of the tempeh quality. The addition of *S. cerevisiae* in this study is expected to improve the nutritional quality of other tempeh when compared to tempeh without the addition of *S. cerevisiae*. As in infertile egg results reservations, the addition of *S. cerevisiae* can reduce fat content (Hatijah et al. 2018) and in tapioca products, the addition of *S. cerevisiae* can increase the protein content (Kustyawati et al. 2013).

In addition to the type of inoculum, the fermentation time can also affect the nutritional quality of the tempeh produced. Increasing the fermentation time to a certain extent can improve the quality of the tempeh produced, such as protein, moisture, and ash content which will increase. However, if the fermentation is carried out for too long, it can cause the tempeh to become soft, and an unpleasant odor will arise and the nutritional composition will decrease. As in the research of Sawitri and Santoso (2014), it was reported that the protein content of durian seed tempeh increased up to 48 hours of fermentation with the highest protein content at the 48 hours fermentation time, which was 3.378%, then decreased at 60 hours of fermentation to 2.554%. In a study conducted by Raharjo et al. (2019), the fat content of tempeh gude would decrease along with the length of fermentation. At 0 hours of fermentation the fat content was 3.3%, then at 72 hours of fermentation would decrease to 1.26%.

The contribution of *R. oligosporus* and *S. cerevisiae* which are able to grow and interact during fermentation might also improve nutritional quality such as protein content, fat content, moisture content, ash content, and carbohydrate content of tempeh. This needed to be investigated to determine the effect of increasing *S. cerevisiae* on changes in the nutritional composition of the tempeh produced. In addition, the length of fermentation also affected the nutritional composition of tempeh. Based on this, it was suspected that there was an interaction between the utilization of the type of inoculum and the duration of fermentation on the nutritional composition of tempeh. Therefore, it was necessary to study the effect of the addition of *S. cerevisiae* and the duration of fermentation along with the interaction of the two on changes in the nutritional composition of the tempeh produced.

MATERIALS AND METHODS

The materials used in this research include tempeh yeast with the trademark Raprima, pure culture of *R. oligosporus* FNCC 6010 and *S. cerevisiae* FNCC 3012, imported soybeans with the trademark Soybean USA no. 1 obtained from Gunung Sulah in Bandar Lampung, Indonesia. This study used a Randomized Complete Block Design (RCBD) with two treatments and two repetitions. The first treatment was the type of tempeh inoculum with 4 levels, namely commercial inoculum (Raprima, 0,2%) (T1), 1% single inoculum of *R. oligosporus* (T2), 1% single inoculum of *S. cerevisiae* (T3), and a mixture of *R. oligosporus* and *S. cerevisiae* (1%:1%) (T4). The second treatment was the length of fermentation with 4 levels, namely 0 hours (F1), 15 hours (F2), 30 hours (F3), and 45 hours (F4).

Preparation of *Saccharomyces cerevisiae* culture

The *S. cerevisiae* was cultured in a sterile Malt Extract Agar (MEA) medium using sterilized inoculating loop needle with a scratchplate. Then it was incubated for 48 hours at 32ºC to obtain pure *S. cerevisiae* in the form of colonies. Colonies that have grown are harvested by adding 5-10 mL of sterile distilled water into the plate disk. The yeast cells were harvested and poured into a 50 mL centrifuge tube. Then the tube spun at 3000 rpm for 10 minutes to separate the pure culture and the supernatant. The supernatant in the centrifuge tube was discarded and a pure culture pellet of *S. cerevisiae* was obtained. Furthermore, pure pellets of *S. cerevisiae* were diluted in a test tube using saline solution. The number of *S. cerevisiae* cells was counted using a haemacytometer to obtain 10^7 cells/mL of *S. cerevisiae*.

Preparation of *Rhizopus oligosporus* culture

The *R. oligosporus* was cultured in a sterile Potato Dextrose Agar (PDA) using sterilized inoculating loop needle with a scratchplate. Then it was incubated for 7 days at 25ºC to obtain pure colonies, and then harvested in the same way as the *S. cerevisiae*. The required concentration was 10^5 spores/mL.

Production of soybean tempeh

The process of making tempeh follows the procedure of Kustyawati (2009). A total of 400 g of soybeans were soaked in clean water at room temperature overnight and removed the husk. Furthermore, the soybeans were boiled using clean water with a ratio of 1: 3 (soybean: water) for 30 minutes after boiling. Drained it and then aerated until it reaches room temperature. The fermentation stage was carried out by mixing every 100 g of boiled soybean with tempeh inoculum according to the treatment. Four separate 100 g samples of boiled soybeans received these inoculums: (i) 0.2 g RAPRIMA, (ii) 2 mL suspension of 10^5 spores/mL of *R. oligosporus*, (iii) 2 mL suspension of cells/mL of *S. cerevisiae*, and (iv) 1 mL suspension of 10^5 spores/mL of *R. oligosporus* + 1 mL suspension of cells/mL of *S. cerevisiae*. After thoroughly mixed, each of the 100 g of the sample was packed in PE (polyethylene) plastic packaging which has been perforated and incubated at 32°C for 45 hours by observing at 0, 15, 30, and 45 hours.

Observations

Observations made in this study included the moisture content of the Gravimetric method (AOAC 2016), the ash content of the Gravimetric Method (AOAC 2016), the fat content of the Soxhlet Method (AOAC 2016), the protein content of the Gunning method (Sudarmadji et al. 2010), carbohydrate content by different method (Andarwulan et al. 2011). Analysis of tempeh β-glucan content was conducted for the best treatment (Thontowi et al. 2007). The data obtained were analyzed statistically using the ANOVA test and then further analyzed with orthogonal polynomial-orthogonal comparison (OP-OC) test at the 5% level.

RESULTS AND DISCUSSION

Fat content

The results of the analysis of variance showed that the use of the type of inoculum and the duration of fermentation had a significant effect on the fat content of tempeh and there was no interaction between the two factors. The results of the further test of orthogonal polynomial-orthogonal contrast at 5% level showed that the fat content of tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum was significantly different from the treatment with the addition of tempeh yeast inoculum and *S. cerevisiae* inoculum. Based on the further test of orthogonal-contrast polynomials, it showed that the fermentation time in T1, T2, and T3 had a significant linear effect on the fat content of tempeh, while T4 had a significant quadratic effect on the fat content. The response of the fermentation time to the fat content of tempeh in each type of inoculum can be seen in Figure 1.

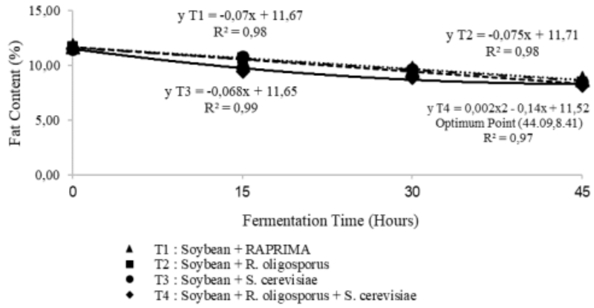

Figure 1. The changes in the fat content during tempeh fermentation in each type of inoculum

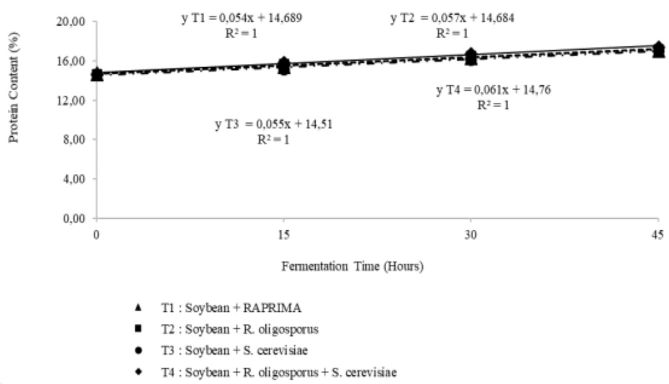

Figure 2. The changes in the protein content during tempeh fermentation in each type of inoculum

Fat content of tempeh continued to decrease along with the length of fermentation. This decrease was due to the activity of the lipase enzyme produced by the fungus *R. oligosporus* which was able to hydrolyze triacylglycerol into glycerol and free fatty acids during fermentation. These free fatty acids will be used as an energy source for the growth of *R. oligosporus* during tempeh fermentation (Asmoro 2016).

The treatment with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum had the lowest fat content at 45 hours, which was 8.23% and already complied with SNI 3144:2015, which contained a minimum fat content of 7%. The fat content of tempeh in this treatment is lower than that Asmoro (2016) did, who reported that soybean tempeh had a fat content of 8,69%. The addition of *S. cerevisiae* in tempeh can decrease fat content of tempeh. The low fat content in this was due to the interaction between *R. oligosporus* and *S. cerevisiae* during fat breakdown in soybeans. During fermentation, *R. oligosporus* can produce lipase enzymes that are able to hydrolyze the fat contained in soybeans into free fatty acids. *S. cerevisiae* can grow with *R. oligosporus* during fermentation by utilizing carbon and nitrogen sources from soybeans as well as free fatty acids produced by *R. oligosporus* (Kustyawati and Pujiastuti 2018). These free fatty acids can also be used by *R. oligosporus* as nutrients for its growth (Asmoro 2016).

Protein content

The results of the analysis showed that the use of the type of inoculum didn't significantly affect the protein content of tempeh, while the length of fermentation had a significant effect on the protein content of tempeh. The interaction between the type of inoculum treatment and fermentation time showed no significant effect on the protein content of tempeh. The results of the orthogonal polynomial-orthogonal contrast further test showed that the protein content of tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum was not significantly different from the treatment with the addition of tempeh yeast inoculum (RAPRIMA), *R. oligosporus* inoculum and *S. cerevisiae* inoculum. Based on the further test of orthogonal polynomial-orthogonal contrast showed that increasing the fermentation time could increase the protein content of tempeh linearly. The response of the fermentation time factor to the tempeh protein content in each type of inoculum can be seen in Figure 2.

Tempeh protein content increased along with the length of fermentation. This increase was due to the *R. oligosporus* fungi metabolizing during its growth and producing protease enzymes that were able to break down proteins into free amino acids (Affandi et al. 2010). These free amino acids contain an N group so that the protein content increases (Dewi et al. 2014).

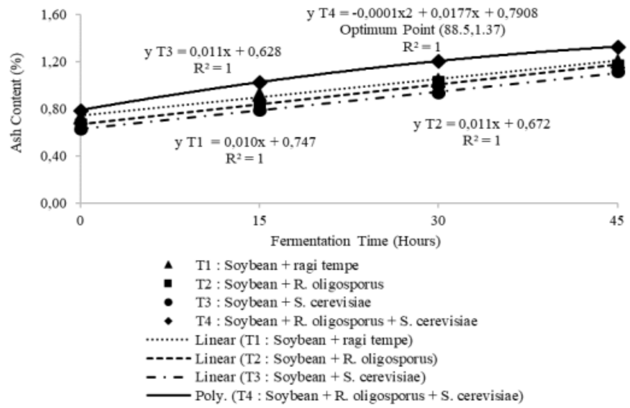

Figure 3. The changes in the ash content during tempeh fermentation in each type of inoculum

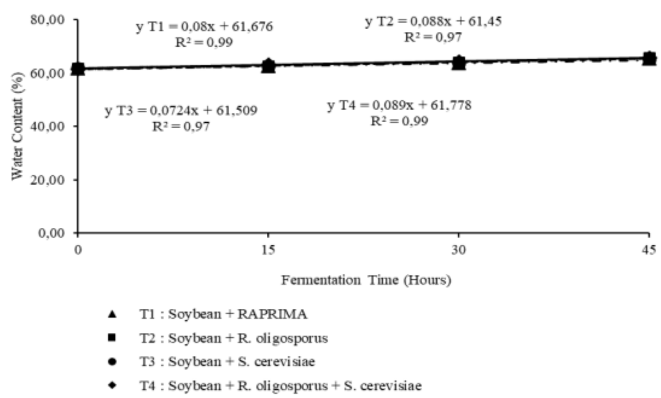

Figure 4. The changes in the moisture content during tempeh fermentation in each type of inoculum

The highest protein content was found in tempeh with the addition of a mixed inoculum of *R. oligosporus* and *S. cerevisiae*, with 45 hours of fermentation, which was 17.4% and had complied with SNI 3144:2015 which contained minimal 15%. The protein content of tempeh in this treatment is lower than that Dewi et al. (2014) did, who reported that soybean tempeh had a protein content of 28,875%. In this study, the addition of *S. cerevisiae* in tempeh can increase protein content of tempeh if compered with treatment without addition of *S. cerevisiae*. The high protein content was due to the interaction between *R. oligosporus* and *S. cerevisiae*. It is because not only *R. oligosporus* can produce protease enzymes but also yeast *S. cerevisiae* is also able to produce protease enzymes during its growth (Seredynski et al. 2016). Protease enzymes are able to hydrolyze proteins into amino acids, so that protein levels will increase. In addition, the growth and development of *S. cerevisiae* during fermentation can increase the mass of protein-rich microbes (Malianti et al. 2019).

Ash content

The results of the analysis showed that the use of the type of inoculum and the duration of fermentation had a significant effect on the ash content of tempeh. The interaction between the type of inoculum treatment and fermentation time showed a significant effect at the 5% level on the ash content of tempeh. The results of the orthogonal polynomial - orthogonal contrast further test showed that the ash content of tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum was significantly different from the treatment with the addition of tempeh yeast inoculum (RAPRIMA), *R. oligosporus* inoculum and *S. cerevisiae* inoculum. Based on the further test of orthogonal polynomial - orthogonal contrast showed that the increase in fermentation time in T1, T2, and T3 could increase the ash content linearly, while T4 could increase the ash content quadratic. The response of the fermentation time factor to the ash content of tempeh in each type of inoculum can be seen in Figure 3.

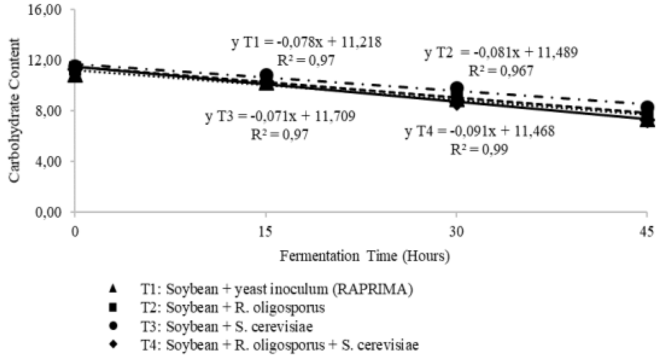

Figure 5. The changes in the carbohydrate content during fermentation in each type of inoculum

Figure 3 shows that the longer the fermentation time, the higher the ash content. This is in accordance with Dewi et al. (2014), who reported that the ash content in soybean tempeh increased during the fermentation time. The increase in ash content in this study was caused by the formation of vitamin B12 during tempeh fermentation so it could cause the amount of ash in tempeh to increase from cobalt (Co in vitamin B12) contained in the vitamin B complex (Sine and Soetarto 2018).

The treatment with the highest ash content was found in the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum with 45 hours of fermentation, which was 1.33%, lower than that of Dewi et al. (2014) who reported that soybean tempeh had an ash content of 1.433%. The high ash content in this treatment was due to the formation of vitamin B12 in the resulting tempeh. The addition of *S. cerevisiae* in tempeh can increase ash content of tempeh. It is because *S. cerevisiae* is one of the yeasts that can produce vitamin B12 and is rich in chromium (Fakruddin et al. 2017). Based on research conducted by Kustyawati and Pujiastuti (2018), it was reported that tempeh inoculated with a mixture of *R. oligosporus* and *S. cerevisiae* had the highest vitamin B12 content at 3.15 mg/100 g even when compared to tempeh inoculated with a mixture of *R. oligosporus* and *Klebsiella* sp. In addition to being naturally fermented in tempeh, vitamin B12 can be formed. The presence of vitamin B12 produced by *S. cerevisiae* was thought to increase the vitamin B12 content in tempeh, so that the ash content will also be higher.

Moisture content

The results of the analysis of variance showed that the use of different types of inoculum and fermentation time had a significant effect on the moisture content of tempeh. The interaction between the type of inoculum treatment and fermentation time showed no significant effect on the moisture content of tempeh. The results of the further test of orthogonal polynomial - orthogonal contrast at the 5% level showed that the moisture content of tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum was significantly different from the treatment with the addition of *S. cerevisiae* inoculum. Based on the further test of orthogonal polynomial - orthogonal contrast, it showed that increasing the fermentation time could increase the moisture content linearly. The response of the fermentation time factor to the moisture content of tempeh in each type of inoculum can be seen in Figure 4.

The 45 hours fermentation time in this study resulted in a fairly high moisture content of tempeh, which ranged from 65 to 65.74%. The longer the tempeh is fermented, the higher the moisture content produced. The increase in moisture content during tempeh fermentation in this treatment was caused during the microbial fermentation process to digest the substrate and produce water, carbon dioxide, and a certain amount of energy (ATP) (Lelatobur and Dewi 2016). Fermentation time is one of the most important factors causing the increase in moisture content so that with increasing fermentation time the moisture content will also increase (Qomariyah and Utomo 2016).

The highest moisture content in this study was found in tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae*, which was 65.74%, higher than Astawan et al. (2013) did, who reported tempeh soybean had a moisture content ranging from 57,98-61,42%. The moisture content value didn't meet SNI 3144:2015 which states that the maximum moisture content in tempeh was 65%. The addition of *S. cerevisiae* in tempeh can increase moisture content of tempeh. The high moisture content in this treatment was probably due to the interaction between *R. oligosporus* and *S. cerevisiae* in tempeh fermentation. With the availability of oxygen during tempeh fermentation, the yeast *S. cerevisiae* could also carry out respiration which was able to convert sugar into carbon dioxide (CO_2) and water (Kustyawati 2018). In addition to water produced by fungi, water produced by yeast respiration was also thought to be able to contribute moisture content to tempeh.

Table 1. The recapitulation of determining the best tempeh treatment with the addition of the type of inoculum

Inoculum types and fermentation time	Protein content (%; min. 15)	Fat content (%; min 7)	Moisture content (%; 66%)	Effectiveness value	Yield value
Raprima; 0 hours	14.69	11.82	61.80	1.01	0.72
Raprima; 15 hours	15.49	10.45	62.79	1.43	1.13
Raprima; 30 hours	16.34	09.70	63.87	1.67	1.42
Raprima; 45 hours	17.11	08.77	65.44	1.82	1.63
Rhizopus oligosporus, 0 hours	14.58	11.69	61.69	1.03	0.72
Rhizopus oligosporus, 15 hours	15.65	10.75	62.53	1.46	1.17
Rhizopus oligosporus, 30 hours	16.47	09.18	63.87	1.87	1.58
Rhizopus oligosporus, 45 hours	17.14	08.47	65.65	1.86	1.67
Saccharomyces cerevisiae, 0 hours	14.63	11.52	61.73	1.08	0.77
Saccharomyces cerevisiae, 15 hours	15.17	10.81	62.39	1.31	1.01
Saccharomyces cerevisiae, 30 hours	16.17	09.65	63.44	1.73	1.44
Saccharomyces cerevisiae, 45 hours	17.06	08.52	65.00	1.98	1.74
R. oligosporus + *S. cerevisiae*, 0 hours	14.64	11.61	61.66	1.08	0.77
R. oligosporus + *S. cerevisiae*, 15 hours	15.82	09.51	63.31	1.68	1.37
R. oligosporus + *S. cerevisiae*, 30 hours	16.70	08.93	64.44	1.88	1.62
R. oligosporus + *S. cerevisiae*, 45 hours	17.40*	08.23	65.74	2.00	1.80*

Carbohydrate content

The results of the analysis showed that the use of different types of inoculum didn't significantly affect the carbohydrate content of tempeh, while the length of fermentation had a significant effect on the carbohydrate content of tempeh. The interaction between the type of inoculum and the length of fermentation showed that the results didn't significantly affect the carbohydrate content of tempeh. The results of the orthogonal polynomial-orthogonal contrast further test showed that the carbohydrate content of tempeh with the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum was not significantly different from the treatment with the addition of tempeh yeast inoculum (RAPRIMA), *R. oligosporus* inoculum and *S. cerevisiae* inoculum. Based on the further test of orthogonal polynomials - orthogonal contrast showed that increasing fermentation time could decrease carbohydrate content linearly. The response of the fermentation time factor to the carbohydrate content of tempeh in each type of inoculum can be seen in Figure 5.

The longer the fermentation time, the carbohydrate content would also decrease. This is because carbohydrates have been widely used by microbes as a source of nutrition for their growth during the fermentation process (Dewi et al. 2014). In addition, the decrease in carbohydrate content was due to the activity of enzymes produced by the fungus *R. oligosporus* during the fermentation process. Fungi is able to digest carbohydrates so that the main changes will occur in the form of loss of hexoses and stachyose which undergo slow hydrolysis (Damanik et al. 2018).

The lowest carbohydrate content was found in the treatment with the addition of a mixture of *R. oligosporus* and *S. cerevisiae*, which was 7.3%, higher than Astawan et al. (2013) did, who reported tempeh soybean had a moisture content ranging from 6,57-7,12%. In this study, the addition of *S. cerevisiae* in tempeh can decrease protein content of tempeh if compared with treatment without addition of *S. cerevisiae*. Decreased carbohydrate levels during fermentation can occur because carbohydrates will be used by microbes such as *R. oligosporus* and *S. cerevisiae* as a source of nutrition for their growth (Dewi et al. 2014). In addition to fungi that can break down carbohydrates, *S. cerevisiae* is also able to break down polysaccharides into simple sugars due to the amylase enzyme produced by *S. cerevisiae* so that the carbohydrate content will decrease.

The best treatment

Determination of the best treatment of tempe inoculated with various types of inoculum was carried out using the effective index method with a weighting procedure (De Garmo 1984) on each parameter that contained in SNI 3144:2015 standards. The parameters contained in SNI 3144:2015 include protein content, fat content, and moisture content. The ash content and carbohydrate content are not contained in SNI 3144:2015, so these two parameters are not included in the basis for determining the best treatment. Table 1 shows the recapitulation of the results of the analysis of the nutritional content of tempeh with the addition of different inoculums and the results of the effectiveness test on the treatment of inoculum type and fermentation time using the effectiveness index method. Determination of the best treatment with the effectiveness index method is indicated by the effectiveness value and the result value. Table 1 shows that the T4F4 treatment has the highest effectiveness values and yield values, which are 2.00 and 1.80, respectively, compared to other treatments. Therefore, the T4F4 treatment, which is a combination inoculum of *R. oligosporus* and *S. cerevisiae* with 45 hours of fermentation, was declared as the best treatment. The T4F4 treatment resulted tempeh which had a protein content of 17.4%, a fat content of 8.23% and a moisture content of 65.74% (Table1).

β-glucans content

The best tempeh from the research was further analyzed to determine its β-glucan content. Based on the highest protein content, mixed inoculum of *R. oligosporus* and *S. cerevisiae* was named the best treatment. Based on the analysis of the β-glucans content of tempeh produced with a mixed inoculum of *R. oligosporus* and *S. cerevisiae*, it was found that the tempeh contained 0.15% β-glucan. The amount of β-glucans was higher than the amount of β-glucans in unfermented soybeans, which was 0.13%, but still lower than the results of research by Rizal et al. (2021). Rizal et al. (2020) found β-glucans content of 0.578% in soybean tempeh which was given a mixed inoculum of 1.5% *R. oligosporus* and 1.5% *S. cerevisiae*. The presence of β-glucan content in the resulting tempeh causes the tempeh to have the potential as a food ingredient that can increase body resistance, according to the benefits of β-glucans itself on health (Del Corno et al. (2020).

In conclusion, during tempe fermentation, the protein content, the ash content, and the moisture content increased, while the fat content and the carbohydrate content decreased. The best treatments to produce tempeh is addition of a mixture inoculum of 1% *R. oligosporus* and 1% *S. cerevisiae* with 45 hours of fermentation. The treatment produced tempe with the nutritional composition value in the form of protein content of 16.7%, fat content of 8.93%, moisture content of 64.44%, ash content of 1.21%, carbohydrate content of 8.73%, and β-glucan content 0.13%.

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