# Growth optimization of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* during fermentation to produce tempeh with high β-glucan content

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**Abstract.** *Rizal S, Murhadi, Kustyawati ME, Hasanudin U. 2020. Growth optimization of Saccharomyces cerevisiae and Rhizopus oligosporus during fermentation to produce tempeh with high β-glucan content. Biodiversitas 21: 2667-2673.* Saccharomyces cerevisiae grows and produces β-glucan during fermentation in tempeh production. The content of β-glucan in tempeh is influenced by the growth of *S. cerevisiae* throughout fermentation. The purpose of this study was to determine the effects of different types and concentrations of carbon sources on yeast growth, fungi growth, and β-glucan content in tempeh inoculated using *Rhizopus oligosporus* and *S. cerevisiae*. This study used a Factorial Randomized Complete Block Design (RCBD) with two factors and three replications. The first factor was the types of carbon sources, tapioca and wheat flour; the second factor was the concentrations of carbon source, 0.0%, 2.5%, 5.0%, 7.5% and 10.0% (w/w). Tempeh produced was investigated for yeast number, fungi number, β-glucan content, and pH value. The obtained data were tested using Tukey's Honestly Significance Difference (HSD) test. The results showed that the addition of various types and concentrations of carbon source significantly influenced the increase in yeast number, fungi number, β-glucan content, and pH in tempeh. The growth of yeast, fungi, and β-glucan content increased along with the increment of carbon source concentration. The amounts of yeast, fungi, and β-glucans in tempeh added with tapioca were higher compared to tempeh with wheat flour. The addition of 10% tapioca produced the highest amount of yeast with 9.505 Log CFU/g and the highest β-glucan content with 0.707% (w/w).

**Keywords:** β-glucan, carbon source, *Rhizopus oligosporus, Saccharomyces cerevisiae,* tempeh

## INTRODUCTION

Tempeh is a popular traditional food from Indonesia made of soybeans. It is beneficial as a source of fiber for human health (Soka et al. 2014). Tempeh is produced from fermented boiled soybeans through the enzymatic activity of *Rhizopus oligosporus* (Kustyawati et al. 2016). *Rhizopus oligosporus* is a fungus that plays a major role in producing tempeh. This fungus can maintain most of nutrients contained in soybeans and enhance the digestibility of protein and several types of vitamin B. According to Mo et al. (2013), Vitamin B12 and folate of tempeh were influenced by microbial activity during fermentation, whereas isoflavone aglycone content of tempeh was determined by bean variety.

At first, fungus was the only microbe that had the most role in tempeh fermentation. However, based on several studies, there was bacteria and yeast involvement in tempeh fermentation. According to Pangastuti et al. (2019), several types of microbes had been found in tempeh and during the immersion process of soybeans. The presence of yeast in this fermentation showed that yeast could grow and interact with other microflora and was thought to have a role in improving the flavor and nutrient quality of tempeh (Kustyawati 2009). One type of yeast found in tempeh fermentation was *Saccharomyces cerevisiae* (Kustyawati et al. 2016), a known source of β-glucan (Many and Vizy 2014; Pengkumsri et al. 2017).

β-glucan is a polysaccharide compound that has various biological activities: as a biological response modifier (Corno et al. 2020); as an anti-infectious agent against microorganisms including bacteria, fungi, viruses, and parasites (Hetland et al. 2013); as an anti-cytotoxic, anti-mutagenic, and anti-tumorigenic (Widyastuti et al. 2011); and as an enhancer of anticancer immunity responses (Vannucci et al. 2013). Research by Meena et al. (2013) also showed that β-glucan imparted immunity against various fish pathogens.

In this study, *S. cerevisiae* was intentionally added to the tempeh fermentation process to produce tempeh that contained β-glucan. Based on the research by Rizal et al. (2018), tempeh added with 3% *S. cerevisiae* in its fermentation produced the highest β-glucan content which was 0.076%. Meanwhile, Pratiwi (2018) explained that adding a mixed inoculum of *R. oligosporus* and *S. cerevisiae* in 40 hours of fermentation resulted in the highest β-glucan content in tempeh with 0.578% (w/w). However, the amount of β-glucan in tempeh produced from the study was considered to still be low because the growth of *S. cerevisiae* during fermentation was not optimal. According to Rizal (2018b), the highest amount of yeast in tempeh inoculated with *R. oligosporus* and *S. cerevisiae* was $1.31 \times 10^8$ CFU/g. The amount of yeast in tempeh may still be increased if the growth substrate contains enough nutrients for its growth. Yeast growth requires a high carbon source, whereas tempeh only contains 12.1%

carbohydrates (Cahyadi 2006). Therefore, to optimize the growth of *S. cerevisiae* during fermentation, it is necessary to add substrates that contain nutrients needed for the growth of *S. cerevisiae*, which is mainly carbon.

Tapioca and wheat flour are known to contain high carbohydrates. The carbon content in tapioca and wheat flour is thought to meet the nutritional needs for the growth of *S. cerevisiae* and *R. oligosporus* during tempeh fermentation. Therefore, the addition of tapioca and wheat flour to tempeh fermentation is expected to optimize the growth of *S. cerevisiae* and *R. oligosporus* which will produce β-glucans. The purpose of this study was to determine the type and concentration of carbon sources that increased the growth of yeast and fungi most optimally and produced tempeh with the highest β-glucan content.

## MATERIALS AND METHODS

### Materials

The materials used in this study were pure culture of *Rhizopus oligosporus* FNCC 6010 and *Saccharomyces cerevisiae* FNCC 3012 obtained from the Inter-University Center for Food and Nutrition, UGM Yogyakarta; imported soybeans with the trademark Soybean USA No. 1 obtained from Gunung Sulah in Bandar Lampung; wheat flour; tapioca; Oxoid Potato Dextrose Agar (PDA); Oxoid Malt Extract Agar (MEA); purified water; chloramphenicol; NaOH 0.7 N; phosphate buffer pH 4 and pH 7; Pb $(C_2H_3O_2)_2$; $H_2SO_4$; $C_6H_5OH$; $CH_3COOH$; $Na_2C_2O_4$; NaCl; 70% alcohol; and aluminum foil.

### Methods

This study used a Randomized Complete Block Design with two factors and three replications. The first factor was the types of carbon sources, tapioca and wheat flour. The second factor was carbon source concentrations, 0.0%, 2.5%, 5.0%, 7.5%, and 10.0% (w/w). Boiled soybeans were inoculated with pure *R. oligosporus* and *S. cerevisiae* inoculum then added with different carbon sources of different prescribed concentrations. Observation was made to record the number of fungi and yeast, pH value, and β-glucan content after a 36-hour fermentation at room temperature. Data were analyzed by ANOVA (analysis of variance) and tested further using Tukey's Honestly Significant Difference (HSD) test at the 5% level.

### Preparation of *S. cerevisiae* culture

Isolated *S. cerevisiae* was cultured into sterile Malt Extract Agar (MEA) medium using a sterilized inoculating needle with a scratchplate, then incubated for 24 to 48 hours at 28°C to form colonies. The colonies were harvested by adding 10 mL of distilled water into the plate disk. *Saccharomyces 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 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 (Kustyawati et al. 2009).

### 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 needle and a scratchplate, and then incubated for five to seven days at 30°C to 35°C to obtain pure colonies, harvested in the same way as the *S. cerevisiae*. The required concentration ($10^5$ cells/mL) was 100 times less than that of *S. cerevisiae* (Kustyawati et al. 2009).

### Tempeh production

The procedure of tempeh processing followed by Kustyawati et al. (2009). A total of 100 g of soybeans were soaked at room temperature overnight. Afterward, the soybean husks were removed manually. Soybeans were then boiled with a 1:3 ratio of soybeans to water for 30 minutes, drained, and aerated before they were ready to be inoculated. The inoculation stage was carried out by mixing every 100 g of boiled soybeans with 1 mL of $10^7$ spores/mL *R. oligosporus* + 1 mL of $10^7$ cell/mL *S. cerevisiae* suspension. Specific concentration of flour was then added to the microorganism-inoculated soybeans. Finally, the mixture was packaged with plastic with aeration holes and incubated for 36 hours at room temperature.

### Enumeration of microorganisms

Culturing was done on Potato Dextrose Agar (PDA) for fungi and on Malt Extract Agar (MEA) for yeast. 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, and then diluted into the concentration series. One mL of each dilution was planted with the appropriate surface plate calculation method on the media. Incubation happened for 24 to 48 hours at 32°C to grow fungi and at 30°C to grow yeast.

### Analysis of β-glucan

The β-glucan production was determined every eight hours during fermentation, following Kusmiati et al. (2007). One gram of sample and 30 mL of NaOH 0.7 N were hydrolyzed for six hours at 75°C and 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 and centrifuged again at 10,000 rpm and 25°C for 30 minutes. The supernatant was removed again and then washed with acetic acid three times. The obtained residue was then washed twice with 20 mL of water and centrifuged at 5,000 rpm for 10 minutes.

The residue and 20 mL of ethanol were centrifuged at 5,000 rpm for 10 minutes, resulting in wet β-glucan (crude wet β-glucan). This biomass was dehydrated using oven at 45°C for 24 hours and weighed, resulting in crude dry β-glucan. The dry residue with 4 mL of 1M NaOH was left

for one hour. The sample was then diluted with 10 mL of purified 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 cleared the solution. Two mL of the solution 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.

## RESULTS AND DISCUSSION

### Growth of yeast

*Saccharomyces cerevisiae* added in tempeh fermentation grew together with *R. oligosporus* to form tempeh. Figure 1 shows that carbon source concentration influenced the number of *S. cerevisiae* cells in tempeh. The higher the concentration of carbon source added to the tempeh fermentation process, the higher the number of *S. cerevisiae* cells in tempeh. Tempeh with 10% tapioca contained the highest number of *S. cerevisiae* cells with 3.9 x $10^9$ CFU/g, while the lowest number of *S. cerevisiae* cells was found in 0% wheat flour with 1.0 x $10^8$ CFU/g. Figure 1 displays the growth of *S. cerevisiae* in tempeh with different types and concentrations of carbon sources.

Based on Figure 1, the highest number of yeast cells was found in tempeh produced by adding 10% tapioca with up to 9.50 log CFU/g of *S. cerevisiae*. Overall, these results showed an increase in the number of yeast cells along with the increase of concentration of both carbon sources. These results proved that nutrients contained in added carbon source could be utilized by yeast cells to support their growth. Wheat contains 74.48% carbohydrates while

tapioca contains 86.9% carbohydrates (USDA 2014). According to Saini et al. (2017), *S. cerevisiae* was a potential amylase-producing organism. Therefore, *S cerevisiae* could break down carbohydrates in both types of carbon sources into simpler compounds. Melliawati et al. (2006) stated that amylolytic microorganisms, one of which was *S. cerevisiae*, could thrive in solid media containing 2% sago starch as a source of carbon and energy. Besides being able to grow well, these microorganisms could also break down starch into simpler compounds. Additionally, research results from Kustyawati et al. (2013) showed that the number of *S. cerevisiae* increased from 6.85 Log CFU/mL to 7.63 Log CFU/mL when producing tempeh with tapioca through fermentation by *S. cerevisiae* for 48 hours.

Statistical analysis showed that adding different carbon source types and concentrations during formation of tempeh had a significant effect on the number of *S. cerevisiae* cells with an average value ranging from 8.01 Log CFU/g to 9.50 Log CFU/g. Further investigation through HSD test regarding the effect of adding different types and concentrations of carbon source on the number of yeast cells is shown in Table 1.

Based on Table 1, the number of yeast (*S. cerevisiae*) cells in tempeh containing 10.0% tapioca was not significantly different from that with 10% wheat flour, but it was significantly different from those with other concentrations. The highest number was obtained in tempeh by adding 10% tapioca producing yeast cells up to 9.50 (± 0.12 SD) Log CFU/g. The number of yeast cells with the addition of the lowest wheat flour concentration (2.5%) was 8.18 (± 0.08) Log CFU/g, thus still higher than those without added carbon source.
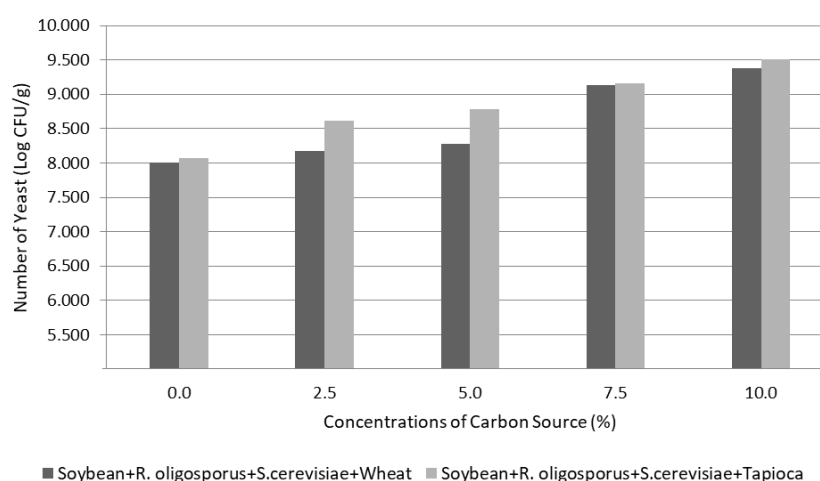


**Figure 1.** Growth of yeast cell in tempeh inoculated with *Rhizopus oligosporus* and *Saccharomyces cerevisiae* with the addition of different types and concentrations of carbon source
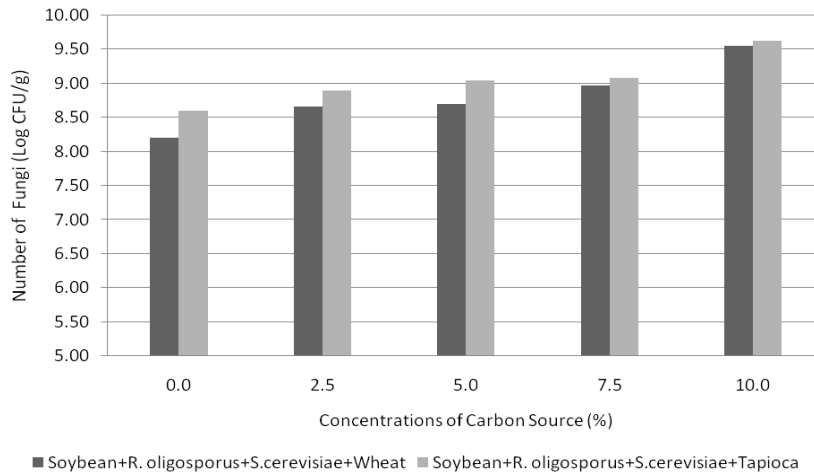
**Figure 2.** Graph of the growth of fungi cells in tempeh inoculated with pure cultures of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* with the addition of different types and concentrations of carbon source

According to Walker and Stewart (2016), most *S. cerevisiae* strains could grow if they were supplied with glucose, inorganic ions, ammonium salts, and a few other growth factors. Macronutrients need to be supplied at milli-molar concentrations, and these comprise of sources of carbon (i.e., sugars), oxygen, free amino nitrogen (amino acids, small peptides, and ammonium salts), phosphorus, sulfur, potassium, and magnesium. Suprihatin (2010) stated that the amount of nutrients in fermentation media might affect the number of cells produced. Since there was no significant difference in the number of yeast cells in tempeh with 10% tapioca and 10% wheat flour, it could be assumed that both carbon source types contained similar amount of those necessary nutrients. However, according to Rizal (data not shown), in the absence of fungi, *S. cerevisiae* could still grow during fermentation of soybeans inoculated with *S. cerevisiae* even though tempeh was not formed.

**Growth of fungi**

Growth of *R. oligosporus* increased with increasing concentrations of added carbon sources. The result showed that the highest number of *R. oligosporus* cells was obtained in tempeh produced by adding 10% tapioca with total cells up to $5.0 \times 10^9$ CFU/g, while the lowest number of *R. oligosporus* cells was found in tempeh produced by 0% wheat flour with $2.2 \times 10^8$ CFU/g. Statistical analysis proved that the addition of carbon source with different concentrations had a significant effect on the number of *R. oligosporus* cells. The addition of carbon source was intended to support the growth of yeast during fermentation, but the nutrient in carbon source was also used by fungi during fermentation. As carbon source concentration increased, the number of fungi cells increased as well. Overall, tapioca yields higher amount of fungi than wheat flour in any concentration. The graph showing the growth of fungi cells in tempeh inoculated with culture mixture of *R. oligosporus* and *S. cerevisiae* with the addition of different types and concentrations of carbon source is shown in Figure 2.

**Table 1.** The result of HSD analysis on the effect of adding different types and concentrations of carbon source on the number of *S. cerevisiae* in tempeh

| Treatments | Number of *S. cerevisiae* ± SD (Log CFU/g) |
|---|---|
| Tapioca 10.0% | $9.50 \pm 0.12^a$ |
| Wheat 10.0 % | $9.38 \pm 0.07^{ab}$ |
| Tapioca 7.5% | $9.16 \pm 0.04^{bc}$ |
| Wheat 7.5 % | $9.13 \pm 0.12^c$ |
| Tapioca 5.0% | $8.78 \pm 0.10^d$ |
| Tapioca 2.5% | $8.62 \pm 0.07^d$ |
| Wheat 5.0% | $8.27 \pm 0.10^e$ |
| Wheat 2.5 % | $8.18 \pm 0.08^{ef}$ |
| Tapioca 0.0% | $8.08 \pm 0.03^{ef}$ |
| Wheat 0.0 % | $8.01 \pm 0.05^f$ |

Note: Data presented in means ± SD. The mean value followed by the same letter indicates no significant difference in the 5% HSD test. ($\alpha = 0.231$)

**Table 2.** The results of HSD Test on the effect of adding different types of carbon source at different concentrations on the amount of *Rhizopus oligosporus* cells in tempeh

| Treatments | Number of *R. oligosporus* ± SD (Log CFU/g) |
|---|---|
| Tapioca 10.0% | $9.63 \pm 0.31^a$ |
| Wheat 10.0 % | $9.55 \pm 0.19^{ab}$ |
| Tapioca 7.5% | $9.08 \pm 0.09^{abc}$ |
| Tapioca 5.0% | $9.04 \pm 0.12^{abc}$ |
| Wheat 7.5 % | $8.96 \pm 0.02^{bc}$ |
| Tapioca 2.5% | $8.89 \pm 0.11^c$ |
| Wheat 5.0% | $8.69 \pm 0.10^{cd}$ |
| Wheat 2.5 % | $8.66 \pm 0.09^{cd}$ |
| Tapioca 0.0% | $8.59 \pm 0.31^{cd}$ |
| Wheat 0.0% | $8.20 \pm 0.45^d$ |

Note: Data presented in means ± SD. The mean value followed by the same letter indicates no significant difference in the 5% HSD test. ($\alpha = 0.63$)

Fungi have an important role in the processing of tempeh because they can retain most of the nutrients contained in soybean seeds and increase the digestibility of proteins and certain types of vitamin B. *Rhizopus oligosporus* produces proteases that can break down proteins into peptides, amylase enzymes that can break down starch into sugars, and lipase enzymes that can digest fats (Kobayasi et al. 1992). Similarly, Setiarto, et al. (2016) stated that during the fermentation process, *R. oligosporus* produced extracellular enzymes such as amylase and protease that degraded starch and protein contained in sorghum seeds into simple sugars and amino acids. Wang et al. (2012) reported that a decrease in carbohydrate content occurred in small amount in tempeh produced by a mixture of soybeans and wheat inoculated with *R. oligosporus* inoculum. The decrease in carbohydrates was caused by *R. oligosporus* using carbohydrates as a source of carbon for its growth. Research by Widiantara (2012) showed that there was a significant decrease in starch content in cassava fermented by *R. oligosporus*. This confirmed that *R. oligosporus* could break down starch into simpler compounds because these fungi contained the alpha-amylase enzyme (Kanti 2016).

Statistical analysis showed that the number of fungi cells was influenced by several carbon source types and concentrations as well as the interaction between the two. Further test results depicted that the types and concentrations of carbon sources had a significant effect on the number of *R. oligosporus* cells at the 5% level. HSD test results are shown in Table 2.

Based on Table 2, the number of *R. oligosporus* cells on tempeh with 10% tapioca was not significantly different from those with 10.0% wheat flour, 7.5% tapioca, and 5.0% tapioca; but it was significantly different from those with 7.5% wheat flour, 2.5% tapioca, 5.0% wheat flour, 2.5% wheat flour, 0.0% tapioca, and 0.0% wheat flour. The number of *R. oligosporus* cells on tempeh with 10.0% wheat flour was not significantly different from 7.5% tapioca, 5.0% tapioca, and 7.5% tapioca and wheat flour. These results were close to the research conducted by Aptesia et al. (2013) and they proved that tapioca could act as a nutrient substrate for growing *R. oligosporus* because it had high carbohydrate content of 84.2%, consisting of 17-23% amylose and 76-83% amylopectin. Research by Hermiati et al. (2011) also explained that tapioca had 96.06% starch and 20.47% amylose. The carbohydrate content in tapioca and wheat flour will be overhauled by *R. oligosporus* because it contains amylase enzyme. In aerobic conditions, *R. oligosporus* produces a lot of extracellular amylase enzymes (Crueger and Crueger 1984). The enzyme is produced to break down complex compounds into simpler compounds, so that they can be absorbed by cells and used for growth.

**β-glucan content**

Results showed an increase in β-glucan content along with the increase of carbon source concentration added in tempeh fermentation. The highest β-glucan content was found in 10% tapioca with 0.707% and the lowest with 0.12% (w/w) in 0% both carbon sources. The results indicated that the addition of carbon source during fermentation influenced increasing the content of β-glucan of tempeh (Table 3).

**Table 3**. The amount of β-glucan content in tempeh with the addition of different carbon source types at different concentrations

| Carbon source concentrations (%) | % β-glucan (w/w) ± SD in tempeh at different types of carbon source | |
|---|---|---|
| | Wheat flour | Tapioca |
| 0.0 | 0.118 ± 0.010 | 0.145 ± 0.006 |
| 2.5 | 0.253 ± 0.010 | 0.231 ± 0.002 |
| 5.0 | 0.288 ± 0.014 | 0.441 ± 0.026 |
| 7.5 | 0.445 ± 0.018 | 0.487 ± 0.007 |
| 10.0 | 0.676 ± 0.004 | 0.707 ± 0.029 |

Note: Data presented in means ± SD

**Table 4**. Results of HSD test on the effect of adding different types and concentrations of carbon source on the amount of β-glucan in tempeh

| Treatments | % β-glucan (w/w) ± SD |
|---|---|
| Tapioca 10.0% | 0.707 ± 0.029[a] |
| Wheat 10.0 % | 0.676 ± 0.004[a] |
| Tapioca 7.5% | 0.487 ± 0.007[b] |
| Wheat 7.5% | 0.45 ± 0.018[b] |
| Tapioca 5.0% | 0.441 ± 0.026[b] |
| Wheat 5.0% | 0.288 ± 0.014[c] |
| Wheat 2.5 % | 0.253 ± 0.010[cd] |
| Tapioca 2.5% | 0.231 ± 0.002[d] |
| Tapioca 0.0% | 0.155 ± 0.006[e] |
| Wheat 0.0% | 0.118 ± 0.010[e] |

Note: Data presented in means ± SD. The mean value followed by the same letter indicates no significant difference in the 5% HSD test. (α = 0.046)

**Table 5.** The results of HSD Test on the effect of adding different types of carbon source at different concentrations toward pH value in tempeh.

| Treatments | pH value ± SD |
|---|---|
| Tapioca 0.0% | 6.93 ± 0.03[a] |
| Wheat 2.5% | 6.87 ± 0.05[ab] |
| Wheat 0.0% | 6.83 ± 0.05[ab] |
| Wheat 10.0% | 6.78 ± 0.08[abc] |
| Wheat 5.0% | 6.77 ± 0.02[abc] |
| Wheat 7.5% | 6.76 ± 0.11[bc] |
| Tapioca 5.0% | 6.63 ± 0.03[cd] |
| Tapioca 7.5% | 6.56 ± 0.07[d] |
| Tapioca 2.5% | 6.52 ± 0.07[d] |
| Tapioca 10.0% | 6.48 ± 0.03[d] |

Note: Data presented in means ± SD. The average value followed by the same letter shows no difference in the 5% HSD test. (α = 0.164)

The increase in the β-glucan content in tempeh is caused by an increase in the amount of *S. cerevisiae* and *R. oligosporus*. As shown by Figures 1 and 2, increasing the concentration of carbon sources can increase the number of fungus and yeast cells. Increasing the number of fungus and yeast cells will automatically increase the β-glucan content in tempeh because β-glucan are found in the cell walls of both microbes. In previously published researches, Naruemon et al. (2013) stated that the cell membrane of *S. cerevisiae* contains β (1,3)-glucan and β (1,6)-glucan; Kusmiati et al. (2007) reported that the most optimal increment in β-glucan weights was obtained from the addition of 6% and 8% molasses, which resulted in β-glucan levels of 53.07 and 61.79%; and Thontowi et al. (2007) stated that the β-glucan content of *S. cerevisiae* in cultures with N peptone sources tended to increase within fermentation process and was relatively constant at the end of fermentation, with β-glucan levels at the end of fermentation of 933.33 µg/L.

Statistical analysis showed that carbon source types and concentrations significantly affected β-glucan content in tempeh with an interaction between the two. HSD test 5% level analysis results are presented in Table 4. Table 4 shows that β-glucan content in tempeh with 10% tapioca is not significantly different from the 10% wheat flour but it is significantly different from other concentrations. The content of β-glucan with the addition of either 10% tapioca or 10% wheat flour is higher than those with other wheat and tapioca concentrations. The highest β-glucan content was found in tempeh added with tapioca at 10% concentration.

β-glucan can be obtained by extracting *S. cerevisiae* cell membranes through base extraction. In this study, extraction was carried out through base extraction using NaOH which was based on its solubility in alkaline solutions (Lee 2001). The difference in β-glucan content produced in tempeh was caused by varying number of nutrients contained in different carbon sources at different concentrations, which affected the number of *S. cerevisiae* cells. β-glucan production increased along with the number of *S. cerevisiae* cells (Kusmiati et al. 2007). According to Cempaka and Aryantha (2014), formation of β-glucan increased along with the growth of the cell. The highest β-glucan content in tempeh in this study, 0.707% (w/w), was higher than that from a research conducted by Rizal et al. (2018) through the addition of 3% *S. cerevisiae* with 0.076% and by Pratiwi (2018) through the addition of a mixture of *R. oligosporus* and *S. cerevisiae* at 40 hours fermentation with 0.578% (w/w). However, it was lower than the research conducted by Shokri et al. (2008) with 27.5% and Varelas et al. (2016) with 40%. This significant difference might be caused by the different isolation processes of β-glucan where generally, β-glucan was obtained through direct isolation from *S. cerevisiae* cell membranes, whereas in study of Rizal et al. (2018) and Pratiwi (2018), β-glucan was obtained from tempeh flour extraction.

## Value of pH

Statistical analysis showed that the addition of different carbon sources at different concentrations had a significant influence on the pH value in tempeh. The result of pH measurement proved that tempeh with no tapioca (0%) had the highest pH value (6.9) while the lowest pH value was 6.4 at the mixture of 10% tapioca. Test results of pH value in tempeh are presented in Table 5.

Degree of acidity (pH) is an important factor affecting the tempeh fermentation. Azizah et al. (2012) explained that the pH value is influenced by the product of the fermentation process and there was a correlation between fungal growth and the rise of pH value. Kustyawati (2009) stated that during fermentation, there were to be no overhaul compounds that produced H to achieve non-acidic tempeh. Generally, tempeh increased in pH during the fermentation process (Handoyo and Morita 2006). As long as there was growth of fungi in the fermentation of soybean, the pH of medium change greatly (Handoyo and Morita 2006) due to water-soluble organic acids were produced from proteins (Sparringa and Owens 1999) and oligosaccharides (Rehms and Barz1995). As reported by Suprihatin (2010), the proteolytic activity of *R. oligosporus* in which protein degradation occurred caused an increase in concentration of dissolved nitrogen in tempeh, thus raising the pH value of tempeh. Fungi will actively hydrolyze proteins during tempeh fermentation. However, in this study, tempeh decreased in pH with increasing concentrations of carbon source used. During tempeh fermentation, carbohydrates in tapioca or wheat flour added to the fermentation process were degraded by the activity of enzymes produced by *R. oligosporus* and *S. cerevisiae* into simpler compounds such as disaccharides and monosaccharides. Cempaka and Aryantha (2014) stated that the decrease in pH of the medium during fermentation was probably caused by the formation of primary metabolites such as organic acids by *S. cerevisiae*. Finally, the formation of organic acids would reduce the pH value of the medium.

In conclusion, the addition of different types of carbon sources, both tapioca and wheat flour, with different concentrations in tempeh fermentation significantly affects the growth of yeast, fungi, and β-glucan content. Adding 10% tapioca (w/w) in tempeh fermentation resulted in the highest amount of yeast with 9.505 log CFU/g and the highest content of β-glucan with 0.707% (w/w), whereas the addition of 10 wheat flour only produced 0.676% (w/w) β-glucan content.

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