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The influence of inoculum types on the chemical characteristics and β-glucan content of tempe Gembus

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Abstract. Tempe Gembus is a type of tempe which is made by fermenting tofu dregs with *Rhizopus oligosporus*. Tempe Gembus has lower nutritional value than soybean tempe. The addition of *Saccharomyces cerevisiae* in the manufacture of tempe Gembus is expected to increase the added value of tempe Gembus.The aim of this study was to determine the effect of various types of inoculums on the chemical properties and β-glucan content of tempe Gembus. The study used a completely randomized block design (RBD) with 3 repetitions and 7 types of inoculums: commercial tempe inoculum (RAPRIMA) (T1), *Saccharomyces cerevisiae* (T2), *Rhizopus oligosporus* (T3), commercial tempe inoculum (RAPRIMA) + commercial yeast (Fermipan) (T4), commercial tempe inoculum (RAPRIMA) + *Saccharomyces cerevisiae* (T5), *R. oligosporus* + Fermipan (T6), and *R. oligosporus* + *Saccharomyces cerevisiae* (T7). Tempe Gembus produced was analyzed for the content of β-glucan, fat, protein, ash, water and carbohydrates. The data obtained was analyzed statistically using one way ANOVA and the Honest Significant Difference (HSD) test. The results showed that the type of tempe inoculum increased the levels of protein, ash, water and β-glucan but decreased the levels of fat and carbohydrates in tempe Gembus. A mixture of *R. oligosporus* and *S. cerevisiae* produced the best tempe Gembus containing 0.69% β-glucan, 6.98% protein, 0.48% ash, 83.98% water, 8.12% carbohydrates and 0.47% fat.

Key words: Tempe Gembus, *Saccharomyces cerevisiae*, *Rhizopus oligosporus*, β-glucan.

Abbreviations (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.

Running title: instant premium tempe starter

INTRODUCTION

Tempe Gembus is widely known in Java, especially in Malang, East Java, as a traditional food derived from by-products of tofu processing produced through fermentation. Tempe Gembus is a traditional fermented food made from tofu dregs fermented by *Rhizopus oligosporus*. Tofu dregs are a by-product of tofu processing which still contains nutrients so it has the potential to be further processed, one of which is to become tempe gembus. Secara umum, tempe gembus dibuat dengan cara yang sama dengan pembuatan tempe kedelai.

Tempe Gembus has a relatively low nutritional value as compared to soybean tempe. The protein ratio between tempe Gembus and soybean tempe is 1:10. The carbohydrate content (crude fiber) of tempe Gembus is only 11% of the total nutritional value of tempe Gembus (Murdiati et al. 2016). The low nutritional content of tempe Gembus makes the selling value low regarding health and the adequacy of nutrients for the body. The era of globalization and modernity makes consumers tend to consider choosing foodstuffs that affect health, not only seen of the nutritional content itself. It causes the foodstuffs consumed not only to meet basic needs (delicious and nutritious) but also to provide other needs for body health. Therefore, it is necessary to develop technology to increase the nutritional value of tempe Gembus.

The fermentation process of making tempe Gembus involves several organisms similar to the microorganisms found in tempe made from soybeans. The main microbe that plays a role in tempe fermentation is *Rhizopus oligosporus*. However, during the soybean tempe fermentation process, in addition to fungi, other microorganisms were also found that play an important role in the tempe fermentation process, namely lactic acid bacteria (LAB) and yeast (Efriwati et al. 2013). One type of yeast that plays a role in the tempe fermentation process is *Saccharomyces. cerevisiae* (Rizal and Kustyawati 2019). *Saccharomyces cerevisiae* is one of the potential types of yeast that can synthesize β-glucan in its cell wall (Rizal and Kustyawati 2019). The results of research by Rizal and Kustyawati (2019) revealed that tempe treated with the addition of *S. cerevisiae* resulted in a β-glucan content of 0.25%.

Tempe with the addition of *S. cerevisiae* in the form of fermipan (commercial instant baker's yeast) contains higher β-glucan than tempe without the addition of *S. cerevisiae* (Rizal et al., 2021). According to Rizal and Kustyawati (2019), tempe added to 1% *S. cerevisiae* has a β-glucan content of 0.181%, while the addition of 3% *S. cerevisiae* produced tempe with a higher β-glucan content of 0.25%. The addition of *S. cerevisiae* in the fermentation process of tempe Gembus is expected to produce tempe Gembus which contains high β-glucan due to the growth of yeast during fermentation.

The β-glucan is a type of polysaccharide with a D-gucose monomer attached to a β-(1,3)-glucoside bond. β-glucan has biological activity as an immunomodulator in enhancing the immune system (Di Domenico et al., 2017). According to Miro´nczuk-Chodakowska et al. (2021), β-glucan is a natural molecule that has great therapeutic potential due to its immunomodulatory, antioxidant, anti-allergic, antifungal, antibacterial, antineoplastic, anti-inflammatory and antiviral properties. Even in recent reports, the use of β-glucan from mushrooms shows great potential in the prevention and treatment of COVID-19. Therefore, it is necessary to conduct this research to determine the potential content of β-glucan in tempe Gembus which is added with *S. cerevisiae* in its manufacture.

Saccharomyces cerevisiae is estimated to become a potential source of protein, fat, carbohydrates and minerals. The role of *S. cerevisiae* which can grow and interact during the fermentation process, allows it to provide functional properties of Gembus tempe. Some of these functional properties are forming β-glucan components in tempe and improving nutritional quality such as protein, carbohydrate, fat, ash, and air content of Gembus tempe. Therefore, it is important to study how the influence of the type of inoculum and the addition of *S. cerevisiae* on the chemical and β-glucan content of tempe Gembus.

MATERIALS AND METHODS

Materials

The tofu dregs material was obtained from the Syafe'i tofu factory, Jl. Sukardi Hamdani Palapa, Langkapura, Bandar Lampung. *Saccharomyces cerevisiae* (trademark Fermipan), commercial tempe inoculum (trademark RAPRIMA), *Rhizopus oligosporus* FNCC 6010, *Saccharomyces cerevisiae* FNCC 3012 obtained from the Inter-University Center for Food and Nutrition UGM Yogyakarta, media Malt Extract Agar (MEA), Plate Count Agar (PCA), and other chemical analysis materials.

Methods

A completely randomized block design was used in this studyn with seven (7) types of inoculums commercial tempe inoculum (RAPRIMA) (T1), *Saccharomyces cerevisiae* (T2), *Rhizopus oligosporus* (T3), commercial tempe inoculum (RAPRIMA) + commercial yeast (Fermipan) (T4), commercial tempe inoculum (RAPRIMA) + *Saccharomyces cerevisiae* (T5), *R. oligosporus* + Fermipan (T6), and *R. oligosporus* + *Saccharomyces cerevisiae* (T7). Observations made in this study included water content using the gravimetric method (AOAC 2016), fat content using the Soxhlet extraction method (AOAC 2016), protein content using the Gunning method (Sudarmadji et al. 2010), ash content using the gravimetric method (AOAC 2016) and carbohydrate content using the by a different methods. Analysis of β-glucan levels in tempe Gembus used method of Rizal et al. (2020). The obtained data were analyzed statistically using the one way ANOVA test and then further tested with the Honest Significant Difference (HSD) Test at the 5% level.

Preparation of *Saccharomyces cerevisiae* culture

This step was performed following the procedure of Rizal et al. (2021). The *S. cerevisiae* from agar slant was cultured into MEA media using a sterilized ose needle. It was incubated for 24-48 hours at 30±2°C to obtain culture colonies of *S. cerevisiae*. The growing colonies were then collected by adding 10 mL of sterile distilled water and slowly taking it using a dry galski rod. The *S. cerevisiae* suspension was transferred into a tube of centrifuge and then centrifuged for 10 minutes at 3000 rpm to separate the pure culture from the supernatant. The supernatant in the centrifuge tube was removed to obtain pellets of pure *S. cerevisiae* culture. Cells *S. cerevisiae* was counted using a haemacytometer and adjusted to obtain *S. cerevisiae* amounted to 10^7 cells/mL.

Preparation of *Rhizopus oligosporus* culture

The pure cultures of *Rhizopus oligosporus* was prepared as per the procedure of Rizal et al. (2021). *Rhizopus oligosporus* was cultured on Potato Dextrose Agar media (PDA) in a petri dish using a sterilized ose needle, then was incubated for 5-7 days at a temperature of 30-35°C to obtain *R. oligosporus* in the form of colonies. Growing colonies were harvested by adding 10 mL of sterile distilled water mixed gently and slowly taking it using a drygalski rod. Furthermore, the spores of *R. oligosporus* were centrifuged for 10 minutes at 3000 rpm. The supernatant in the centrifuge tube was discarded and a pure culture pellet of *R. oligosporus* was obtained. The cells of *R. oligosporus* was calculated using *a haemacytometer* to get 10^7 spores/mL of *R. oligosporus*.

The procedure of making tempe Gembus

The process of manufacturing tempe Gembus was carried out following the procedure used Murdiati et al. (2016). The steps included: 500 g of tofu dregs was put into a filter cloth and squeezed by hand to remove most of the water contained therein. Furthermore, the tofu drinks were steamed for 45 minutes and cooled at 30 ± 2 °C (room temperature). The fermentation process of tempe Gembus is carried out by adding various types of tempe inoculum according to each treatment to 100 g of tofu dregs, namely commercial tempe inoculum (RAPRIMA) (T1), *S. cerevisiae* (T2), *R. oligosporus* (T3), commercial tempe inoculum (RAPRIMA) + *S. cerevisiae* (T4), *R. oligosporus* + Fermipan (T5), *R. oligosporus* + *S. cerevisiae* (T6) and commercial tempe inoculum (RAPRIMA) + Fermipan (T7). And after thoroughly mixed, packed in PE (polyethylene) plastic packaging with a thickness of 3 mm which was regularly perforated for aeration and incubated at a temperature of 31-32°C for 48 hours for fermentation condition.

RESULTS AND DISCUSSION

Chemical composition of steamed tofu dregs

Preliminary research was conducted to determine the chemical composition of steamed tofu dregs which was used as raw materials for making tempe Gembus. The results of testing the chemical composition of tofu dregs are presented in Table 1. Information on the nutritional composition of steamed tofu dregs was used as a comparison to determine whether there is an increase or decrease in the nutritional composition of tempe Gembus after the fermentation process.

Table 1. The nutritional composition of steamed tofu dregs

Component of nutrients	Content (%)
Water content	80.1
Fat	1.3
Carbohydrates	13
Ash	0.3
Protein	5.2

Water Content of Tempe Gembus

The results of the ANOVA test indicated that the type of inoculum treatment significantly affects the water content of tempe Gembus. Further investigation through the 5% HSD test (Table 2) indicated that the highest water content was found in the tempe Gembus sample produced by the T7 treatment (addition of *R. oligosporus* and *S. cerevisiae* inoculums), which was 83.98%, while, the lowest water content contained in tempe made with T4 treatment (Raprima and Fermipan inoculum mixture), which was 82.05%. The high water content in tempe Gembus was caused by the relatively high water content of tofu dregs, which is 80%. The addition of a combination of *R. oligosporus* and *S. cerevisiae* inoculums to tofu dregs fermentation effected the moisture content of tempe. This occurred because of the yeast respiration process during its growth and it is suspected that during the fermentation process, the respiration process contributes to the amount of water in tempe Gembus (Kustyawati and Pujiastuti, 2018).

Table 2. The water content of tempe Gembus with various types of inoculums

Type of inoculums	Water content (%)
T7 (*R. oligosporus* + *S. cerevisiae*)	83.98[a]
T2 (*S. cerevisiae*)	83.73[a]
T5 (Raprima + *S. cerevisiae*)	83.70[a]
T3 (*R. oligosporus*)	83.69[a]
T6 (*R. oligosporus* + Fermipan)	83.69[b]
T1 (Raprima)	82.17[b]
T4 (Raprima + Fermipan)	82.05[b]

Description: Water content marked with the same letter was not significantly different (HSD 5%= 1.346)

Table 1 shows the results of the proximate analysis of steamed tofu dregs. The moisture content of steamed tofu dregs was 80%, and after fermentation, it increased by ±4%. These results indicated that the fermentation process can increase the water content of tempe. Tofu dregs that were only inoculated with *S. cerevisiae* also showed an increase in water content even though tempe Gembus was not formed. During the fermentation of tempe Gembus, yeast needs oxygen for

the respiration process, which produce carbon dioxide and water (Kustyawati and Pujiastuti, 2018). The results of respiration in the form of water can increase the water content of tempe gembus.

Fat Content of Tempe Gembus

The highest fat content in tempe Gembus with commercial tempe inoculum and *R. oligosporus* of 0.51%, while the lowest fat content was in tempe Gembus with *R. oligosporus* and *S. cerevisiae*, namely 0.45%. However, the ANOVA test showed that the type of inoculum did not significantly affect the fat content of tempe Gembus (Table 3). Therefore, the BNT test was not carried out on the fat content of tempe Gembus.

Even though the ANOVA results were not significantly different, the addition of *R. oligosporus* and *S. cerevisiae* produced the lowest fat content in tempe Gembus. The low fat content is thought to be due to the interaction of *R. oligosporus* and *S. cerevisiae* during the breakdown of fat in tofu dregs. *Rhizopus oligosporus* has an important role in helping the growth of *S. cerevisiae* which can grow together during fermentation by utilizing carbon and nitrogen sources from tofu dregs and free fatty acids produced by *R. oligosporus* (Kustyawati and Pujiastuti, 2018). *Rhizopus oligosporus* uses free fatty acids as a nutrient for its growth (Asmoro, 2016), which is thought to cause the fat content of tempeh to decrease slightly.

Table 3. The fat content of tempe Gembus with various types of inoculums

Type of inoculums	Fat content (%)
T1 (Raprima)	0.51
T3 (*R. oligosporus*)	0.51
T2 (*S. cerevisiae*)	0.50
T6 (*R. oligosporus* + Fermipan)	0.50
T5 (Raprima + *S. cerevisiae*)	0.49
T4 (Raprima + Fermipan)	0.48
T7 (*R. oligosporus* + *S. cerevisiae*)	0.45

Protein Content of Tempe Gembus

Based on the results of the ANOVA test, the type of inoculum treatment significantly affected the protein content of tempe Gembus. A further test in the HSD level of 5% (Table 4) showed that the highest protein content of tempe Gembus was in the treatment of *R. oligosporus* and *S. cerevisiae* with a protein content of 6.98%, while the lowest protein content was in the commercial tempe inoculum treatment of 6,26%. Protein content of tempe Gembus increased after the fermentation process. Table 1 shows the results of the proximate analysis on steamed tofu dregs. The protein content of steamed tofu dregs was 5.25% and increased after the fermentation with an increase of up to 1%. These results proved that the fermentation process would increase the protein content of tempe Gembus. The high protein content is presumably due to the interaction between *R. oligosporus* and *S. cerevisiae*.

Based on Table 4, the protein content of Tempe Gembus increased with the addition of yeast s. cerevisiae in the fermentation process. The increase in the amount of protein in fermented tempeh using Raprima + *S. cerevisiae* and *R. oligosporus* + *S. cerevisiae* inoculums is thought to be caused by the higher number of *S. cerevisiae* cells in both tempeh compared to tempeh inoculated with other inoculums. *Saccharomyces cerevisiae* is a source of single cell protein, so the increase in the number of *S. cerevisiae* cells will increase protein levels in tempe. Research by Purwitasari et al (2004) showed that increasing *S. cerevisiae* cells in suitable media could increase single cell protein levels. In addition, in the inoculum mixture during fermentation, *S. cerevisiae* grows and develops, increasing the microbial mass rich in protein (*S. cerevisiae*) (Malianti et al. 2019). The genetic aspect of yeasts also plays an important role in producing a group of peptides and enzymes necessary for their work in the growth medium. According to Jach and Serefko (2018), yeast is known as a natural source of protein including enzymes, peptides, and amino acids.

Table 4. The protein levels of tempe Gembus with various types of inoculums

Type of inoculums	Protein content (%)
T7 (*R. oligosporus* + *S. cerevisiae*)	6.98[a]
T5 (Raprima + *S. cerevisiae*)	6.75[b]
T4 (Raprima + Fermipan)	6.59[c]
T6 (*R. oligosporus* + Fermipan)	6.51[d]
T3 (*R. oligosporus*)	6.28[e]
T2 (*S. cerevisiae*)	6.28[e]
T1 (Raprima)	6.26[e]

Description: Protein content marked with the same letter was not significantly different (HSD 5%= 0,070)

Carbohydrate Content of Tempe Gembus

Based on the results of the ANOVA test, the type of inoculum treatment significantly affected the carbohydrate content of tempe Gembus. A further test in the HSD level of 5% (Table 5) showed that the highest carbohydrate content was found in tempe gembus with the addition of commercial tempe inoculum type, which was 10.70%. In contrast, the lowest carbohydrate content was found in tempe gembus with the addition of *R. oligosporus* + *S. cerevisiae inoculum*, which was 8.12%. This study showed the same results as (Rizal et al. 2022), which stated that the low carbohydrate content in tempe gembus was presumably due to microbes using carbohydrates as a substrate for the cooling process. Apart from *R. oligosporus* which can break down carbohydrates, yeast *S. cerevisiae* can also convert polysaccharides into simple sugar because *S. cerevisiae* has the amylase enzyme (Dewi et al. 2014). Therefore, the tofu dregs that were only inoculated with *S. cerevisiae* experienced a decrease in the carbohydrate content although no tempe was formed.

Tabel 5. The carbohydrate content of tempe Gembus with various types of inoculum

Type of inoculums	Carbohydrate content (%)
T1 (Raprima)	10.70[a]
T4 (Raprima + Fermipan)	9.54[a]
T3 *(R. oligosporus)*	9.09[ab]
T2 (*S. cerevisiae*)	9.05[ab]
T6 (*R. oligosporus* + Fermipan)	8.89[ab]
T5 (Raprima + *S. cerevisiae*)	8.59[ab]
T7 (*R. oligosporus* + *S. cerevisiae*)	8.12[b]

Description:
Carbohydrate content marked with the same letter was not significantly different (HSD 5%= 1,870)

Table 5 shows the results of the proximate analysis on steamed tofu dregs. The carbohydrate content of steamed tofu dregs was 13%, and after the fermentation, it decreased by 3-5%. The decrease in carbohydrate levels occurs because during fermentation, microorganisms have widely used carbohydrates as a source of nutrition for their growth (Dewi et al. 2014). Carbohydrates are the primary energy source for microbial growth (Febriani et al. 2019). Mold can digest carbohydrates so there will be a significant change in the loss of hexoses and stakiose which undergo slow hydrolysis (Damanik et al. 2018).

Ash Content of Tempe Gembus

Based on the results of the ANOVA test, the type of inoculum treatment significantly affected the ash content of tempe Gembus. Further tests of HSD level 5% (Table 6) showed that the ash content of tempe Gembus among all treatments was not significantly different. The highest ash content of tempe Gembus was in the treatment of *R. oligosporus* + *S. cerevisiae inoculum* at 0.52%, while the lowest ash content was in the treatment of commercial tempe inoculum at 0.47%.

Table 6. The Ash content of tempe Gembus with various types of inoculums

Type of inoculum	Ash content (%)
T7 (*R. oligosporus* + *S. cerevisiae*)	0.52[a]
T5 (Raprima + *S. cerevisiae*)	0.48[a]
T4 (Raprima + Fermipan)	0.48[a]
T2 (*S. cerevisiae*)	0.48[a]
T6 (*R. oligosporus* + Fermipan)	0.47[a]
T3 *(R. oligosporus)*	0.47[a]
T1 (Raprima)	0.47[a]

Description: Ash content marked with the same letter was not significantly different (HSD 5%= 0,049).

Vitamin B12 in tempe is produced by *Klebsiella pneumoniae* and *Citrobacter freundii* bacteria during the fermentation process. According to Kustyawati and Pujiastuti (2018), Vitamin B12 cannot be produced by molds, but by contaminant

bacteria that grow in the fermentation process. *Klebsiella pneumonia* can synthesize sucrose contained in carbohydrates as a food source for its growth (Dewi et al. 2014). The formation of vitamin B12 causes an increase in the ash content during the combustion process due to the presence of cobalt (Co in vitamin B12) in the vitamin B complex (Fakruddin and Ahmed 2017). Tempe with relatively high ash content was found in tempe gembus with the addition of a mixture of inoculum *R. oligosporus* and *S. cerevisiae*. This treatment had an effect of 1% on the ash content of the tempe Gembus produced. That is because, in addition to vitamin B_{12}, it can naturally be formed during the tempe fermentation process. The presence of vitamin B_{12} produced by *S. cerevisiae* is thought to increase the content of vitamin B_{12} so the ash content also be higher due to the increase in cobalt (Co) molecules contained in vitamin B_{12}.

β-Glucan Content of Tempe Gembus

Based on the results of the ANOVA test, the inoculum type significantly affected the β-glucan content of tempe Gembus. Further test results with HSD at 5% (Table 7) showed that the highest β-glucan content was found in tempe Gembus made with a mixture of *R. oligosporus* and *S. cerevisiae* inoculums, which was 0.69%. In contrast, the lowest β-glucan content was found in tempe Gembus produced with commercial tempe inoculum, which was 0.2%. The results of this study are in line with research conducted by Rizal et al. (2021), which stated that the addition of a mixture of *R. oligosporus* and *S. cerevisiae* inoculum (pure culture) in soybean tempe fermentation had a higher effect on β-glucan content than the addition of commercial tempe inoculum, *S. cerevisiae*, and *R. oligosporus*.

Table 7. The β-glucan content of tempe Gembus with various types of inoculums

Types of inoculums	β-glucan content (%)
T7 (*R. oligosporus* + *S. cerevisiae*)	0.69[a]
T5 (Raprima + *S. cerevisia*)	0.62[b]
T6 (*R. oligosporus* + Fermipan)	0.59[c]
T4 (Raprima + Fermipan)	0.57[cd]
T3 *(R. oligosporus)*	0.27[d]
T2 (*S. cerevisiae*)	0.25[d]
T1 (Raprima)	0.20[e]

Description:
β-glucan content marked with the same letter was not siginicantly different (HSD 5%= 0,041)

Saccharomyces cerevisiae is one type of potential yeast that can synthesize β-glucan in its cell wall (Rizal and Kustyawati 2019). According to Kusmiati (2007), β-glucan production will increase as the number of *S. cerevisiae* cells increases. That means that the higher the amount of yeast, the greater the β-glucan content. According to Efriwati et al. (2013), during the tempe fermentation, in addition to *R. oligosporus*, there are other microorganisms, such as lactic acid bacteria (LAB) and yeast. Kustyawati (2009) reported that *S. boulardii, G. candidum*, and *Y. lipolytica* grew together with *R. oligosporus* with populations of 10^7, 10^8, and 10^9 CFU/g, respectively. Therefore, the addition of *R. oligosporus* and commercial yeast during the fermentation process also produced β-glucan content, although not as significant as other treatments.

Tempe gembus, with the addition of commercial tempe inoculum (Raprima), produced a different β-glucan content than tempe gembus with a mixture of Raprima and *S. cerevisiae*. That was because there was an increase in the amount of yeast added intentionally, which was 10^7 CFU/mL, causing the β-glucan content to increase or be more significant than the addition of only commercial tempe yeast.

The addition of a mixed inoculum of Raprima and *S. cerevisiae* resulted in different β-glucan content of tempe Gembus with the addition of mixed inoculum of *R. oligosporus* and *S. cerevisiae*. This might bebecause of the fact that *S. cerevisiae* utilizes compounds resulting from the breakdown of *R. oligosporus* during fermentation which are used for growth, so that yeast cells increase which causes the β-glucan content to increase (Rizal et al., 2020). The inoculum mixture produces a mutually beneficial symbiosis with synergistic growth.

Conclusions

The addition of *Saccharomyces cerevisiae* in the manufacture of tempe Gembus increased the water, ash, and protein content, but reduced the fat and carbohydrate content of tempe Gembus. The addition of *S. cerevisiae* increased the β-glucan content in gembus tempe. The highest yield of β-glucan was found in Tempe Gembus which was made with a mixture of *R. oligosporus* and *S. cerevisiae* inoculums with a β-glucan content of 0.69%. The existence of *S. cerevisiae* with *R. oligosporus* in fermentation of tempe increased the added value of tempe Gembus due to the β-glucan content in the tempe Gembus produced.

Conflict of interest

The authors declare no conflict of interest.

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