

The Addition Effect of Cheese to Tempeh Against Flavor, Function, and Product Attractiveness

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ABSTRACT

Tempeh is a type of soybean-based food made by a fermentation process. Processing soybeans into tempeh can involve adding natural additives to increase nutritional value and to make it more fulfilled. Cheese is a food derived from animal milk with high protein content. Adding cheese to soybean tempeh fermentation can increase tempeh protein so that the protein in tempeh consists of vegetable and animal protein. The study's objective was to measure protein content, moisture content, and organoleptic tests (taste, smell, color, and mycelial appearance) on tempeh when given cheese powder. The method used is firstly drying cheese into powder, then making tempeh with cheese powder added to various concentrations (0%, 0.5%, 1%, 1.5%, and 2%). The Lowry method tested the protein content, while the water content was tested using gravimetry. An organoleptic test was carried out based on several parameters: odor, taste, color, and mycelium density. Results from this study, the results of the increase in protein levels from each given concentration. The water content did not provide a significantly different effect from any given concentration of cheese. And based on the organoleptic test, the addition of cheese to tempeh did not affect the mycelium's color, taste, smell, and density. Adding cheese to soybean tempeh can be an alternative in increasing protein content; besides, adding cheese does not significantly affect the product's taste, smell, color, and Mycelium distribution density.

Keywords: *Tempeh; Cheese; Nutritional; Protein*

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ABSTRAK

Tempe merupakan jenis makanan berbasis kedelai yang pembuatannya dengan proses fermentasi. Pengolahan kedelai menjadi tempe dapat melibatkan penambahan zat aditif guna menambah nilai gizi agar lebih terpenuhi. Keju merupakan jenis pangan yang berasal dari susu hewani dengan kandungan protein hewani tinggi. Penambahan keju dalam fermentasi tempe kedelai dapat menambah protein tempe, sehingga protein dalam tempe terdiri dari protein nabati dan hewani. Penelitian ini bertujuan untuk mengukur kadar protein, kadar air, dan uji organoleptik (rasa, bau, warna dan kenampakan misellium) pada tempe ketika diberi serbuk keju. Metode yang digunakan yaitu pertama pengeringan keju menjadi serbuk, kemudian pembuatan tempe yang ditambah serbuk keju dengan berbagai konsentrasi (0%, 0,5%, 1%, 1,5%, dan 2%). pengujian kadar protein dengan metode lowry, kadar air dengan gravimetri dan uji organoleptik dengan parameter bau, rasa, warna dan kepadatan misellium. Hasil dari penelitian ini diperoleh hasil peningkatan kadar protein dari setiap konsentrasi yang diberikan. Untuk kadar air tidak memberikan pengaruh yang berbeda nyata dari setiap konsentrasi keju yang diberikan. Dan berdasarkan uji organoleptik, pemberian keju pada tempe tidak mempengaruhi warna, rasa, bau maupun kepadatan misellium. Penambahan keju pada tempe kedelai dapat menjadi alternatif dalam peningkatan kadar protein selain itu penambahan keju juga tidak memberikan pengaruh secara signifikan terhadap rasa, bau, warna, dan kepadatan misellium dari hasil fermentasinya.

Kata kunci: Tempe; Keju; Nutrisi; Protein.

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INTRODUCTION

Food is essential for humans to survive and even supports individual growth and development to be more optimal. Because it is a need, nutritional adequacy must be a concern, especially

for developing countries, to ensure adequate amounts of nutritious food production. The lack of balance in producing healthy food causes the fulfillment of nutrition for the entire population in Indonesia, especially being a challenge to process food ingredients with adequate nutritional sources.

Foodstuffs derived from soybeans can be the main food product that is more economical because they can be used as food sourced from protein.

Tempeh is a food that comes from the main ingredient, soybeans, and has a moderately high protein content of about 35%, so it is widely consumed by many people (Roni, 2013). The nutritional content found in tempeh includes fatty acids, vitamins, minerals, and antioxidants. Tempeh can be categorized as a food with high protein and a relatively low price compared to eggs or meat. The manufacturing undergoes a fermentation process with the help of the *Rhizopus fungus*, which will benefit the manufacturing process. This fungus will make tempeh dense and compact textures (Raswanti *et al.*, 2019).

Soy-based tempeh also has antibacterial compounds that can inhibit the growth of *Escherichia coli* bacteria in the human body, which can become pathogens in the body (Barus *et al.*, 2019). The fungus *Rhizopus* sp. in tempeh will experience growth with the hyphae formation. *Rhizopus* sp hyphae are found on the entire surface of soybean seeds. It is usually white with a smooth texture. The collection of hyphae found in tempeh is called mycelium. In addition, the decomposition protein in tempeh is a fungal activity through producing protease enzymes so that it can break down into free amino acids. Soy food variations can be in the form of adding additional ingredients, such as developing food sourced from vegetable protein at relatively affordable prices in the community (Barus *et al.*, 2019).

Cheese is a fermented food generally made with the main ingredient, animal milk. Animal milk is also a consumption material for the entire world community. Because it is a source of protein with a reasonably high level, this cheese can also be used as an additional ingredient in tempeh, making it possible to increase the nutritional content such as protein in it. In addition, adding cheese to tempeh is also innovative as a flavour enhancer. Cheese can be placed as a solid substrate as a place for *Rhizopus* sp. activity and is expected to affect the growth of mold mycelium better as a fermentation product. The combination of cheese in tempeh with a reasonably high protein content can be an alternative to healthy food because cheese is also very popular with the community, although not as much as tempeh. Cheese is also a source of protein and calcium and contains high saturated fatty acids (Usmiati & Damayanthi, 2013). The critical thing to note in adding cheese

to tempeh is the concentration and quality of the cheese. At the given concentration level, further testing can be carried out to determine the limit of administration as an additional ingredient to increase the protein and taste of tempeh, as well as organoleptic tests to assess the texture, taste, and aroma of fermented tempeh on the growth of *Rhizopus fungus* (Komar *et al.*, 2009).

It is made from soybeans through a fermentation process, plus yeast as a fungus to help the fermentation process. Soybeans added with yeast will make tempeh with the growth of fungi from the inoculum. It can break down soybeans into easily digested ingredients with a distinctive tempeh taste. The breakdown occurred through the enzymatic degradation of macromolecular compounds as substrates by *Rhizopus* sp. (Murtini *et al.*, 2011). The potential for adding cheese as an additive to fermented soybeans can also add a distinctive taste. But, it can also be used as an ingredient to increase protein content during the fermentation process (Suknia & Ahmani, 2020). The addition of this cheese goes through a drying process first so that the bacteria in the cheese do not interfere with the growth of fungi on tempeh when the soybean fermentation process occurs. This addition goes through a drying process first so that the bacteria in the cheese do not interfere with the growth of fungi in Tempeh during the soybean fermentation process. In addition, the drying process reduces the activity of secondary metabolites contained in cheese and could affect unwanted microorganisms (Amir, 2017).

MATERIALS AND METHODS

This study was experimental research conducted at the Microbiology and Biochemistry Laboratory, Faculty of Biology, Satya Wacana Christian University, Salatiga.

Materials and equipment

The materials used in this study were boiled soybeans, dry cheese, tempeh yeast (RAPRIMA Indonesia).

Sample preparation

The cheese was cut into small pieces and dried using a dehydrator (Memmert, Germany) at a temperature of 60°C for 2 hours; depending on whether the cheese dry, if it was dry, the cheese was mashed with a blender until smooth and then

ready to be mixed in the tempeh fermentation. In the process of making tempeh, 2 g of inoculum tempeh was added to 100 g of soybeans. Then added cheese powder concentration of 0%; 0.5%; 1%; 1.5%; and 2%. For the weight of cheese powder at a concentration of 0%, which was 0 g, 0.5% was 0.5 g, 1% was 1 g, 1.5% was 1.5 g, and 2% was 2 g of cheese powder. Each concentration was wrapped in ziplock plastic, and the plastic was perforated using a toothpick to allow oxygen to enter to facilitate the tempeh fermentation process. Then the tempeh was incubated at room temperature for 48 hours. After the tempeh finish, it was analyzed for protein content, moisture content, organoleptic tests, and observations of the mycelium density of molds.

Water Content Analysis

Measuring water content in tempeh that has added cheese using the gravimetric method is also known as the drying method. The process serves to evaporate the water or remove the water contained in the sample using heating. Previously, when the sample was still wet, it was weighed first to determine the initial weight. Furthermore, when it was dried, it continued by weighing to decide the final weight of the sample until it was constant. The water content in a sample will affect the shelf life and quality of a product. Therefore, the water condition in a product becomes an essential component during the processing (Ningrumsari & Herlinawati, 2020).

It tested the water content using the gravimetric method; firstly, the porcelain cup was dried in an oven (Memmert, German) at a temperature of 100°C for 1 hour. Then cooled in a desiccator for 15 minutes. If it is cold, it is weighed, and the initial weight of the porcelain cup will obtain. Furthermore, the tempeh samples were weighed as much as 2 g using an analytical balance. The sample was put into a porcelain dish that had been weighed. Then it was weighed again to determine the initial weight of the cup that already contained the selection. After heating in the oven at a temperature of 105°C for 5 hours, the cup and sample were cooled using a desiccator for 15 minutes and re-weighed using an analytical balance.

Protein Content Analysis

Soluble protein in tempeh, which add to cheese with various concentrations, obtained the following data. This study used the analysis

method of protein content, namely the Lowry method. This research method begins by using a standard curve defined as a form of protein qualification for an unknown initial sample concentration using Bovine Serum Albumin (BSA) solution (Merck Germany). The Lowry method is used to measure dissolved protein levels simply by the reaction of a Cu (II) protein complex formed in an alkaline atmosphere. Cu (II) will be reduced to Cu (I). Then ions from Cu⁺ will reduce Folin's reagent. The phosphomolybdo tungstate complex will produce hetero-polymolydenum blue. Hetero-polymolydenum blue in a solution can be formed due to the oxidation reaction of the Cu aromatic group, which produces a blue color and can be detected using coulometry. The level of strength of this blue color depends on the content of tyrosine and tryptophan residues (Puteri *et al.*, 2020). First, Lowry A reagent was made by adding Folin Ciocalteu solution. Furthermore, to manufacture Lowry B reagent, namely mixing a solution of 98 mL of 2% Na₂CO₃ (Merck Germany) in 0.1 N NaOH (Merck Germany) with 1 mL of 1% C₆SO₄.5H₂O (Merck Germany) and 1 mL of 2% Na-K-tartrate (Merck Germany). Then, make a stock solution using BSA (Bovin Serum Albumin) as much as 0.015 g and dissolve it in 50 mL of distilled water in a volumetric flask. So that the results obtained a stock solution concentration of 300 mg/L. Then proceeded with making BSA standard solutions with concentrations of 0 mg/L, 25 mg/L, 75 mg/L, 150 mg/L, 250 mg/L, and 300 mg/L. Each standard solution concentration was taken as much as 0.5 mL and then treated with Lowry B reagent as 4 ml, then incubated for 5 minutes. After incubation, 0.5 ml of Lowry A reagent add and vortexed (Thermolyn, 37600 Mixer, America). Furthermore, each concentration was incubated for 3 minutes, and the absorbance value was measured using a spectrophotometer at 600 nm.

After the standard solution was complete, it was continued to measure the protein content of tempeh by weighing 1 g of the tempeh sample and then dissolving it in 9 mL of distilled water and mashing using a mortar until smooth. When it was soft, the solution was allowed to stand for 10 minutes in cold conditions so that the protein could settle. After 10 minutes, it was continued by centrifugation (Merck Hettich) at 5000 rpm for 10 minutes. Then the supernatant was taken and frozen. In the sample solution, 0.5 mL was taken, 4 mL of Lowry B reagent was added and then incubated for 5 minutes. After incubation, 0.5 mL

of Lowry A reagent add and vortexed. It was then set for 3 minutes. The absorbance value was measured using a spectrophotometer UV-Vis (Shimadzu UV mini 1240, Japan) at 600 nm. If the absorbance value is high, the protein content is also high (Puteri *et al.*, 2020).

Organoleptic Test

The organoleptic test on tempeh used sensory tests such as color, taste, smell, and mycelium density conducted by panelists. The panelists needed about 25 people randomly to carry out organoleptic tests by responding to the results of taste, smell, and mycelium density in tempeh on the condition that the panelists were not hungry or sick. The panelists' level of preference for the given results will transform on a numerical scale with the following parameters: For color, taste, and smell tests, namely, 1 (Do not like it), 2 (Dislike), 3 (Neutral), 4 (Liking), 5 (Like). While for the observation of mycelium density, namely 1 (Not growing), 2 (Grow a little), and 3 (Grow a lot).

Statistical Analysis

The data from the nutritional analysis of tempeh were calculated from 3 replicates and presented as the mean value \pm standard deviation (SD). The Completely Randomised Design was designed to compare the chemical analysis of the five treatments. The results of moisture and protein contents were tested on variance. Tukey HSD was used to compare the differences between the four levels of cheese with a control. The statistical analysis was performed using the statistic program, Statistix 10, USA.

RESULTS AND DISCUSSION

The Water Content of Tempeh

Based on the statistical analysis in Table 1, the moisture content of tempeh with cheese added is no significant difference in the water content of various types of given cheese concentrations. Even though dried cheese was added to the product, the activity of molds during a fermentation process, where microbes could use the substrate and water for their metabolic processes. This follows the explanation from Laksono *et al.* (2019) that the high and low water content could be influenced by

differences in water penetration and mold growth during the fermentation process. The water content of all treatments is under the Indonesian National Standard (SNI) regarding tempeh, which is around a maximum of 65%. If the water content in tempeh is more than 65%, it will be highly vulnerable to being overgrown with other microorganisms (Salim, 2017). While the decrease in water content in cheese tempeh can be caused by the higher concentration of cheese given, the more water molecules contained in tempeh were used for respiration or metabolism by the fungus *Rhizopus* sp. The higher the concentration of cheese, the more fat content was higher so that it could inhibit the binding of water by soybeans which causes the use of water by molds during the high respiration process (Priadi *et al.*, 2018). This phenomenon follows the opinion (Astawan *et al.*, 2013) that the mold synthesized the lipase enzyme during the fermentation process, then hydrolyzed triacylglycerol into free fatty acids

Table 1. Water content in tempeh with various concentrations of cheese

Treatments	Water Content (%) ns
Concentration 0% (Control)	54.00 \pm 2.00
Concentration 0.5%	57.00 \pm 1.73
Concentration 1%	60.33 \pm 1.15
Concentration 1.5%	58.33 \pm 2.08
Concentration 2%	56.33 \pm 4.04

Note: ns means no significant difference.

In addition, when viewed from the lowest to the highest concentration, the water content in tempeh increased but only reached a concentration of 1%. The cheese concentration of 1% to 2% decreased in moisture, probably due to water utilization during the growth of *Rhizopus* sp. Adding cheese to dry cheese did not inhibit mold growth because *Lactobacillus* spp. Bacteria acted as anti-pathogenic agents. The working process was by inhibiting the growth of pathogens or contaminants during the tempeh fermentation process, for example, *Salmonella infant*, *Enterobacter aerogenes*, and *Escherichia coli* bacteria. The development of contaminant bacteria did not inhibit the fermentation process by *Rhizopus* sp. because this mold had a synergistic role with lactic acid bacteria during the tempeh fermentation process (Hamzah *et al.*, 2014).

The Protein Content of Tempeh

Soluble protein is an oligopeptide or amino acid easily absorbed by the digestive system. The increase in dissolved protein levels can be further increased based on the high concentration of protein given. Cheese has a reasonably high protein content. When added to tempeh, the protein content in tempeh will increase (Setyawan & Rahayu, 2015). Based on the results of the Robust Test statistical test. It can obtain the value of dissolved protein content with a sig value ($P < 0.05$). This result shows the effect of giving cheese with different concentrations on the dissolved protein content in tempeh. The concentration of protein content corresponds to the level of dissolved protein, which means that the level of dissolved protein produced was also higher, along with the increase in engagement (Noviyanti & Soepriyanto, 2010).

Table 2. Dissolved protein levels in cheese tempeh with various concentrations

Treatments	Dissolved Protein Content (%)
Concentration 0% (Control)	46.7±0.01 c
Concentration 0.5%	51.3±0.05 c
Concentration 1%	58.0±0.13 b
Concentration 1.5%	58.7 0.14 b
Concentration 2%	65.40±0.45 a

Note: a-c following the mean value (n=3) of dissolved protein content suggested a significant difference between treatments at $p < 0.05$.

Based on the results obtained from the dissolved protein levels tested using the Lowry method on tempeh given cheese, the results in Table 2 show that the higher the concentration given, the higher the dissolved protein content produced. Concentration of 0% to a concentration of 0.5%, the results started from 46.7% to an increase of 51.3%. They obtained the highest dissolved protein content at the greatest concentration of 2%, with a value of 65.4 % dissolved protein content. The high soluble protein levels can be caused by adding cheese. Cheese has a relatively high protein content of about 22.8 g. According to Abubakar (2016), cheese that previously had a protein content when added to tempeh and had a protein content could increase

the dissolved protein content in the cheese tempeh fermentation process.

It plays a role in its metabolic process by using protein in cheese to produce protease enzymes. This enzyme will break down proteins into simpler components in peptides formed into soluble proteins. In addition, the protease enzyme produced by the fungus *Rhizopus* sp. can remodel complex compounds into simple compounds, which plays an essential role in determining tempeh quality, which functions as a source of vegetable protein with a high digestibility value (Affandi *et al.*, 2010).

The amount of cheese concentration given increased soluble protein content in tempeh. During the incubation, there was an enzymatic protein hydrolysis process between cheese and soy protein. Researchers found that the protein content in cheese was hydrolyzed with protein in soybeans at optimum temperature or room temperature with the help of protease enzymes in *Rhizopus* sp. There was no systematic breakdown or protein denaturation to be produced amino acids. The process of protein hydrolysis in cheese could run well and increase the levels of dissolved protein in tempeh. This protein breakdown made the final product in the form of dissolved protein in food products; This hydrolysis also did not inhibit the growth of the fungus *Rhizopus* sp. because it was seen through the mycelium formed (Khanifah, 2018). So, it can be said that there was a relationship between hydrolysis activity and the growth of mycelium formation. With objective observation, the growth of the mycelium was arranged compactly as seen in Figure 1.

Organoleptic Test on Tempeh

Organoleptic tests were carried out to analyze tempeh added with cheese; there were various parameters, namely taste, smell, and color. These three parameters test with ripe tempeh without using additional flavouring ingredients. Meanwhile, the mycelium density parameter used tempeh samples that had not been fried. This treatment aimed to see how mycelium growth from tempeh was produced by adding various concentrations of given cheese. Based on the overall results, it can be seen that the addition of cheese did not affect the different parameters tested, such as taste, smell, or color.

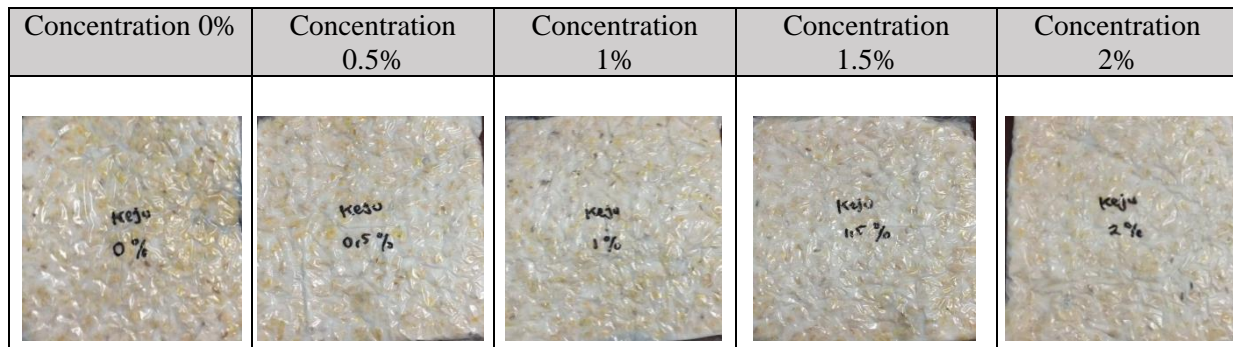


Figure 1. The growth of the mycelium on tempeh with different concentrations of cheese

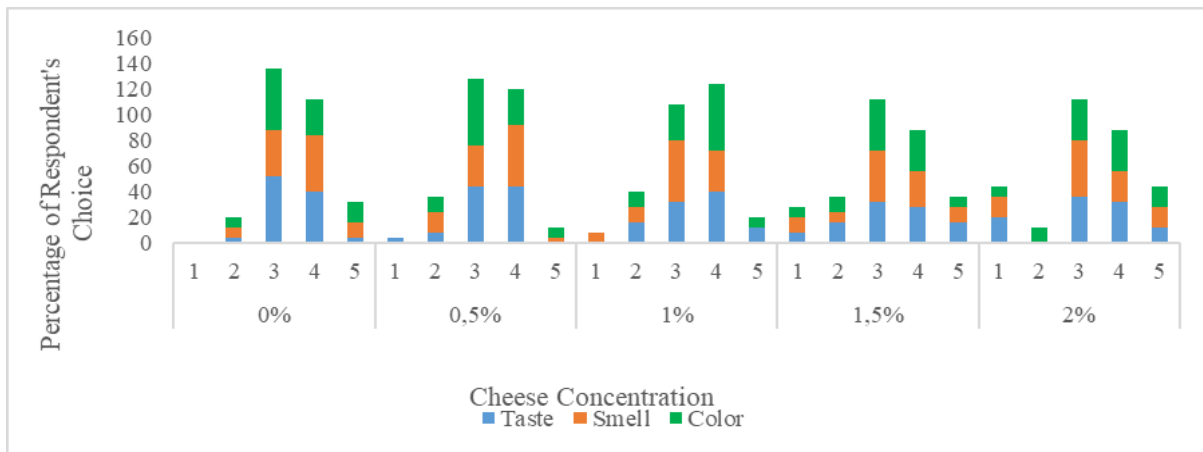


Figure 2. Graph of percentage of organoleptic test parameters (1: Don't like; 2: Don't like it much; 3: Neutral; 4: Like; 5: Really like)

Organoleptic tests on various concentrations of cheese were carried out using the parameters of taste, smell, color, and mycelium density using samples cooked without adding spices. The taste parameter was viewed from various concentrations, and the most preferred by the panelists was a concentration of 0.5%, as shown in Figure 2. However, the higher the concentration of cheese given, the lower the level of preference of the panelists. This condition was because the addition of cheese could affect the taste produced by tempeh. The flavour produced at specific concentrations of cheese gave it a bitter taste.

In comparison, respondents in the organoleptic test stated that cheese with a 0% content had a savorier taste and did not have a bitter taste. The bitter taste produced can come from the activity of lactic acid bacteria from tempeh fermentation, causing a sour or bitter taste in tempeh (Hamzah *et al.*, 2014). Cheese raw materials could also cause this in the form of milk, affecting the preference for tempeh.

The sour taste of cheese—could also taste tempeh when used as an additional ingredient for making tempeh. The cheese used in this

fermentation process was cheddar cheese that had gone through a drying process first so as not to cause spoilage during the fermentation process in tempeh. The drying process aimed to prevent the bacteria in the cheese from interfering with the growth of fungi on tempeh when the soybean fermentation process occurs. In addition, it reduces the activity of secondary metabolites contained in cheese and could affect the growth of unwanted microorganisms (Amir, 2017). The addition of cheese did not significantly affect the taste of tempeh (Radiati, 2016).

The odor parameter with the highest preference level was at a concentration of 0.5%. It can be seen that the higher the concentration, the lower the level of liking for the odor. This impact can cause because the addition of cheese could also affect the smell of the tempeh. The addition of cheese could give the panelists an unpleasant odor. This smell could have been from the aroma of cheese, as for the highest assessment score from various concentrations, namely the assessment of N (neutral) or number three. So it can be concluded that adding cheese was still acceptable to the community (Rati *et al.*, 2017).

The color parameter with the highest preference level was at a concentration of 1%. But for the assessment of the panelists with a high average percentage, there is an N (neutral) or number three assessment, so from these results, it can be explained that the addition of cheese did not affect the color of tempeh (see Figure 1.). Although given the proliferation of yellow cheese, the final result when frying tempeh still produced a standard color and did not affect the shape or characteristics of the original tempeh without adding cheese. Before frying, the color of tempeh was still like ordinary tempeh, which is white because it comes from the growth of mycelium on the *Rhizopus* sp. mold that grows around the surface of soybean seeds (Laksono *et al.*, 2019).

The mycelium density produced from the five concentrations had no different growth rate starting from the smallest to the largest concentration. It was found that the mycelium density remained similar. Adding cheese to tempeh did not affect the growth of mycelium produced by the mold *Rhizopus* sp. The mycelium produced at various concentrations of cheese given with control also had regular mycelium growth, which was white and grew on the surface of soybean seeds. The resulting mycelium texture was also compact because the assessment from the panelists showed that the mycelium growth rate was very high. The use of cheese as an additive in tempeh in our study was congruent with Pebrianti *et al.* (2020), who found that tempeh in the presence of cheese did not inhibit mycelium growth in tempeh.

CONCLUSION

Based on the research, it can be concluded that giving cheese to the tempeh fermentation process could increase the protein content by 2%. Meanwhile, the water content did not have a significant effect, and the addition of cheese did not inhibit the growth of *Rhizopus* sp. For the organoleptic test. The addition of cheese did not affect the taste, color, and smell. The highest value obtained from various parameters was neutral, so the community could still accept the provision of cheese on tempeh. The mycelium density of the five concentrations did not make a significant difference. It can be seen that the growth rate and mycelium density were still relatively similar, so the addition of cheese in the tempeh fermentation process did not inhibit the growth of mycelium.

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