



## Optimization of Tempe Fermentation With the Addition of a Combination of Yeast and Starch Flour: Effect on Process Speed and Product Freshness Period

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### ABSTRACT

This research explores the potential for adding a combination of tempeh yeast and starch to the tempeh fermentation process to speed up fermentation and extend the product's freshness period. Through an experimental approach, we investigated the effect of the proportion of yeast and starch on the fermentation speed and shelf life of tempeh. We found that a yeast-to-starch ratio of 1:2 significantly accelerated the fermentation process, while a ratio of 1:1 was effective in extending the shelf life of tempeh up to 80 hours. Organoleptic tests show positive consumer acceptance of the tempeh variations produced. This research makes a significant contribution to tempeh processing technology, offering practical solutions for the fermented food industry to improve the quality and attractiveness of tempeh products on the market.

## **INTRODUCTION**

Tempeh, a traditional fermented soybean food that is popular in Southeast Asia, especially in Indonesia, is made by fermenting soybeans that have been peeled and cooked using the *Rhizopus* fungus (Chen et al., 2021). Tempe is a favorite choice for housewives throughout Indonesia because of its economical price and ease of processing into a variety of delicious dishes (Astuti et al., 2023; Sudaryantiningsih et al., 2022). From a health perspective, tempeh is rich in nutrients, including vitamins B1, B2, pantothenic acid, nicotinic acid, vitamins B6, and B12, as well as minerals such as calcium and phosphorus, and also contains antioxidants (PUSIDO Team, National Standardization Agency, 2012).

The process of making tempeh, a traditional fermented food, involves the fungus *Rhizopus* sp. which plays a key role in producing the enzyme phytase. This enzyme turns hard soybeans into soft ones, making digestion easier for humans, so tempeh is very suitable for consumption by babies and the elderly (Roni Ahmad, 2013). In addition, tempeh is known to contain antioxidants that are formed during fermentation, thanks to the activity of bacteria such as *Micrococcus luteus* and *Corynebacterium*. These antioxidants have an important role in preventing cancer and premature aging (PUSIDO Team, National Standardization Agency, 2012). According to Aryaanta, W. (2020), tempeh is also effective in preventing degenerative diseases such as diabetes, coronary heart disease and atherosclerosis.

Quality tempeh, according to Roni Ahmad (2013), is characterized by good taste, perfect softness of the soybeans, and long shelf life. However, tempeh craftsmen often face challenges in achieving efficient fermentation.

Razie et al. (2018) found that the fungus *Rhizopus* sp. responsible for tempeh fermentation will die after 48 hours, replaced by bacteria that cause spoilage. As a solution, the addition of starch in the fermentation process has been proposed. Starch, with its ability to bind water and high carbohydrate content, has the potential to support better mold growth, thereby extending the freshness of tempeh (Rahayu, S., 2020). This research aims to explore the potential of mixing starch with tempeh yeast to extend the freshness of tempeh.

The formulation of this research problem includes three main questions: First, what is the potential of a mixture of tempeh yeast and starch in speeding up the fermentation process and extending the freshness of tempeh; secondly, determine the appropriate composition of the ratio of

tempeh yeast and starch to achieve this goal; and third, evaluating consumer acceptance of tempeh enriched with this mixture.

The objectives of this research include analyzing the potential of tempeh yeast and starch in accelerating fermentation and extending the freshness of tempeh, as well as describing the correct composition ratio to achieve optimal results. In addition, this research aims to test consumer acceptance of tempeh produced from this process.

The benefits of this research are quite extensive, including providing important information about the health benefits of tempeh, how to speed up the fermentation process, and how to extend the freshness of tempeh. In addition, this research provides valuable insight into the benefits of a mixture of tempeh yeast and starch in extending the freshness of tempeh and determining the right mixture composition to get the best results.

## LITERATURE REVIEW

### 2.1 Tempe

Tempeh is a processed soybean food that is processed by fermentation using mushrooms. The mushrooms used are from the genus *Rhizopus*, species *Rhizopus oryzae* and *Rhizopus oligosporus* (Hernawati, D. and Vita, M., 2019)

The process of making tempeh is not difficult. Tangkabiringan, et al., (2019), wrote about the process of making tempeh as shown in the following picture:

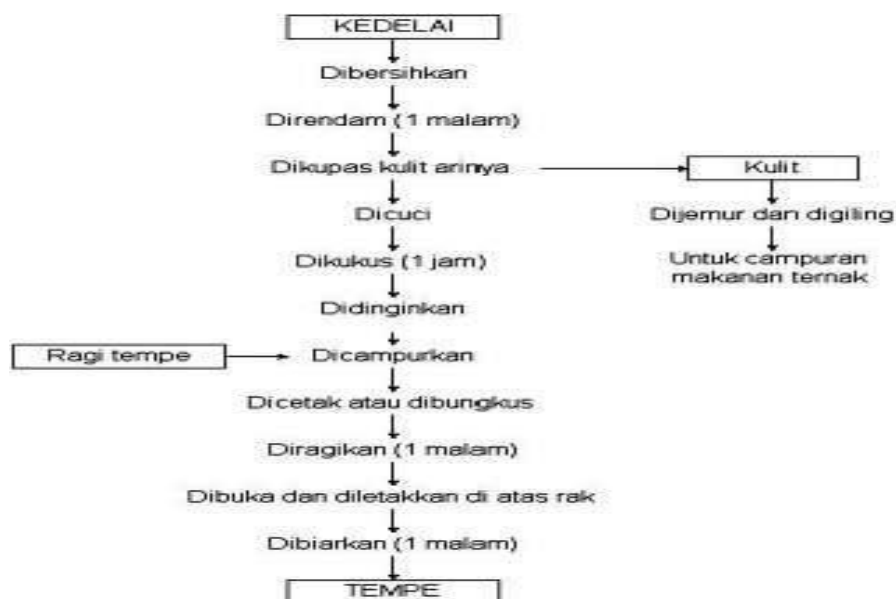


Figure 1. Process of Making Soybean Tempe

During the tempeh fermentation process by *Rhizopus* sp., various physical and chemical changes occur in the soybeans. Some of the important events that occur during this fermentation include:

1. Soybeans soften, and the proteins in them break down into simpler forms, facilitating the human digestion process.
2. Mycelium from *Rhizopus* sp. grows and penetrates the soybean, making it more compact and compact, as described by Razie et al. (2018).
3. There is an increase in levels of unsaturated fatty acids which play a role in lowering cholesterol.
4. Formation of various vitamins such as B1, B2, pantothenic acid, nicotinic acid, B6, and B12, as noted by the PUSIDO Team of the National Standardization Agency (2012).

Apart from that, tempeh also contains short peptides, free amino acids, fatty acids, and carbohydrates in simpler forms, which are easily absorbed by the body (Pinasti, L., et al., 2020). The protein content in tempeh is higher compared to other processed soybean products, including extracted products such as soy sauce or soybean juice (Rahayu, P., et al., 2015).

However, tempeh has limitations in terms of freshness, which only lasts less than 48 hours. Razie et al. (2018) noted that after this period, the fungus *Rhizopus* sp. will die, replaced by other bacteria which causes the tempeh to emit a foul smell and become unfit for consumption.

## **2.1.Fermentation**

Fermentation is a chemical transformation of organic substrates by microorganism enzymes, essential in making tempeh using the fungus *Rhizopus* sp. (Suryani, et al., 2017; Kusuma, et al., 2020). The duration of fermentation is important, according to Fauziah, P., et al. (2022), because it affects mold growth and tempeh quality. Fast fermentation produces quality tempeh with thick and dense mold, while the slow process increases the water content and reduces mold density (PUSIDO Team, National Standardization Agency, 2012). The length of fermentation also has an impact on tempeh protein, with the fermentation of more than 36 hours reducing protein levels of lysine and methionine and hydrolyzing important fatty acids such as linoleic acid and oleic acid (Rahayu, W., et al., 2015; Fauziah, P., et al., 2022; Sudaryantingsih, Cicik, and Supyani, 2010). The addition of carbohydrates such as starch or rice can speed up fermentation, support mold growth, and improve the quality of tempeh (Suryani, et al., 2017; Surbakti, et al., 2022).

## **2.2. Tempeh Yeast**

Tempeh yeast, also known as tempeh starter, plays a key role in turning soybeans into tempeh through fermentation (Hernawati, D. & Vita, M., 2019). The quality of the yeast greatly influences the tempeh produced, measured by the concentration of active spores (Amaliyah, F., et al., 2017). Quality tempeh is characterized by white, clean and thick mold (Roni Ahmad, 2013). Using yeast evenly is important to avoid the blackening of tempeh (Alvina & Hamdani, 2019). Mixing yeast with flour, such as starch, helps evenly distribute the soybeans, producing quality tempeh after overnight fermentation.

## **2.3. Starch**

Starch, also known as tapioca flour or aci flour, is a processed cassava product that is widely used in making various foods because of its ability to bind water and make food more flexible (Ministry of Agriculture of the Republic of Indonesia, 2021). This flour, low in protein but rich in carbohydrates, is often used as a thickener in foods (Jayanti, U., et al., 2017). In tempeh fermentation, the addition of starch can function as a source of nutrition for the growth of the fungus *Rhizopus* sp., which is essential in making high-quality tempeh (Roni Ahmad, 2013).

## **METHODOLOGY**

### **1.1. Data Sources, Tools and Materials**

This research uses data obtained from observing the growth of mold on tempeh, which is processed by adding a mixture of yeast to tempeh and starch in various ratios. The tools used include plastic buckets, trays, stoves, pans, large rice buckets or spoons, tablespoons and digital scales. The ingredients consist of 2 kg of soybeans, 1 packet of tempeh yeast, 500 grams of starch, and plastic wrap.

### **1.2. Data Acquisition Method**

#### **A. Analyze the potential of tempeh yeast and starch as ingredients to extend the freshness of tempeh.**

1. Make a ratio of tempeh yeast mixture and starch

The ratio of the mixture of tempeh yeast and starch made is as follows:

Formula-A (2 grams of tempeh yeast : 2 grams of starch, or 1 : 1);

Formula-B (2 grams of tempeh yeast : 4 grams of starch, or 1 : 2);

Formula-C (2 grams of tempeh yeast : 6 grams of starch, or 1 : 3);

Control (K) is fermented soybeans without the addition of starch.

## 2. Making tempe

The stages of making tempeh are as follows

- 1) 2 kg of soybeans washed, then soaked overnight;
- 2) Drain, peel, then steam for 1 hour;
- 3) Poured into a tray, left to cool;
- 4) Next, 500 grams of cold soybeans are taken and sprinkled with formula A, then made again for formula-B, formula-C, and control;
- 5) Next, it is packaged in plastic which has been cut with small holes;
- 6) Each plastic is filled with 100 grams of soybeans and fermented.

## 3. Observation of tempeh

Observed the growth of mold and weight of tempeh

- 1) To observe the speed of the fermentation process, observe the mold growth every 10 hours until all the tempeh is ready to be consumed;
- 2) To observe the shelf life of tempeh, further observations were carried out until it was seen that the tempeh was no longer good, and compared with the control.

### **B. Describe the correct ratio of tempeh yeast and starch to speed up the fermentation process and extend the freshness of tempeh.**

From the data obtained above, observe which ratio of tempeh yeast mixture and starch can produce tempeh with the longest shelf life and the greatest weight.

### **C. Analyze the potential of tempeh yeast and starch as ingredients to extend the freshness of tempeh, whether they can be accepted by consumers**

At this stage data is obtained from organoleptic tests.

- 1) Tempeh with the best ratio of yeast and starch is diced, seasoned with salt and then fried;
- 2) The same thing is also done for tempe control;
- 3) Asked 20 panelists or respondents to provide assessments.

## RESULTS AND DISCUSSION

### 1.1.RESULTS

- A. Analyze the potential of tempeh yeast and starch as ingredients to speed up the fermentation process and extend the freshness of tempeh.



**Figure 2. Tempeh Fermentation for 0 Hours** All tempeh is still in the form of soybeans.



**Figure 3. Tempeh Fermentation for 10 Hours**

The condition of the control tempeh did not grow mold

**Caption:**

Tempe 1:1 has not yet grown soybeans, but there is visible dew on the wrapper and the temperature is warm

Tempeh 1:2 has started to grow mold, thin, white-like cotton

Tempe 1:3 has not grown mold, it still looks like soybeans.



**Figure 4. Tempeh Fermentation for 20 Hours**

**Caption:**

Condition 1:2 tempeh has a thicker mold. Tempeh 1:1 has mold growth, but it is thinner than tempeh 1:2

Control tempeh and tempeh 1:3 started to grow thin mold



**Figure 5. Tempeh Fermentation for 30 Hours**

**Caption:**

When the tempeh is 30 hours old or on day 2 (two), you can see that all the tempeh is in good condition, white, dense, with thick mold. This condition is a condition where all tempeh is good for consumption.



**Figure 6. Tempeh Fermentation for 40 Hours**

**Caption:**

When the tempeh was 40 hours old, you could see that all the tempeh was in good condition and white, getting denser, marked by wrinkles on the plastic, and the mold was starting to thin out. This condition is a condition where all tempeh is still good for consumption.



**Figure 7. Tempe Fermentation for 50 Hours**

**Caption:**

All tempeh is still in good condition, white and firm.



**Figure 8. Tempe Fermentation for 60 Hours**

**Caption**

In the control tempeh, brown spots formed, while in the other tempeh, there were no brown spots.



**Figure 9. Tempeh Fermentation for 70 Hours**

**Caption**

The brown spots on the control tempeh looked increasingly clear, slimy and smelly. In tempeh 1:3, brown spots also form, but there is no strong smell.



**Figure 10. Tempe Fermentation for 80 Hours**

**Caption:**

The brown spots on the control tempeh became more widespread, with an increasingly pungent odor. At tempeh 1:3 the brown spots become more widespread and start to smell

At 1:2 tempeh, brown spots start to appear

In 1:1 tempeh there are no brown spots.



**Figure 11. Tempe Fermentation for 90 Hours**

**Caption:**

Brown spots with a pungent odor were visible covering all of the control tempeh. The smell is very strong. At 1:3 tempeh, brown spots also form which spread and smell. In 1:2 tempeh the brown spots also spread even though they don't have a strong odor. Tempeh 1:1 is starting to form brown spots, even though it doesn't smell yet.



**Figure 12. Tempe Fermentation for 100 Hours**

**Caption:**

At this time all the tempeh has become rotten and smells strong, slimy, and not solid (soft). This tempeh is no longer suitable for consumption.

**B. Describe the correct ratio of tempeh yeast and starch to speed up the fermentation process and extend the freshness of tempeh.**

From the observation data above, tempeh with a ratio of 1:2 tempeh yeast and starch has the fastest fermentation time, namely 10 hours.

Meanwhile, 1:1 tempeh has the longest freshness period, namely up to 70 hours

**C. Analyze the potential of tempeh yeast and starch as ingredients to extend the freshness of tempeh, whether they can be accepted by consumers**

At this stage, a Tempe taste test was carried out on 20 panelists or respondents, who had various professions. A summary of the results of this test can be seen in Table 2:

**Table 2. Tempeh Organoleptic Test Results**

	TEMPE FLAVOR VALUE															
	CONTROL				1:1				1:2				13			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<b>APPRAISAL QTY (person)</b>	3	5	7	5	2	3	7	8	1	4	7	8	3	3	7	7
<b>TOTAL VALUE</b>	3	10	21	20	2	6	21	32	1	8	21	32	3	6	21	28

**1.2.DISCUSSION**

**A. Analyze the potential of tempeh yeast and starch as ingredients to speed up the fermentation process and extend the freshness of tempeh.**

This discussion analyzes the potential of tempeh yeast and starch in accelerating fermentation and extending the freshness of tempeh. In 10 hours of fermentation, control tempeh and tempeh in a 1:1 ratio did not show mold growth, but the presence of dew and warm temperatures indicated yeast spore

activity (Fauziah, et al., 2022; Rahayu, W.P., et al., 2015). Meanwhile, in Tempeh 1:2, there was light mold growth, indicating earlier fermentation, by research by Alvina and Hamdani (2019) and Suryani, et al. (2017). Rahayu, P., et al. (2015) linked the growth of mold spores to the uptake of glucose, which is necessary for the growth of dense, white mold.

At 30 hours of fermentation, all tempeh showed a thick white mold, with a dense texture, by the findings of Surbakti, et al. (2022). Roni, Ahmad (2013) emphasized that good quality tempeh is characterized by clean and thick white mold. However, at 60 hours of fermentation, the control tempeh began to show brown spots and thinning of mold, which became more obvious at 70 hours. Kristiadi, O.H., and Arina (2022) describe this as the death phase of *Rhizopus*, where many molds die, causing the color to change to brown and the appearance of a pungent odor due to protein degradation and the activity of contaminating bacteria (Roni, A., 2013).

At 80 hours of fermentation, control tempeh, tempeh 1:2, and 1:3 experienced spoilage, but tempeh 1:1 remained fresh for up to 90 hours. This shows that 1:1 tempeh can last longer. However, after 100 hours of fermentation, all the tempeh spoiled

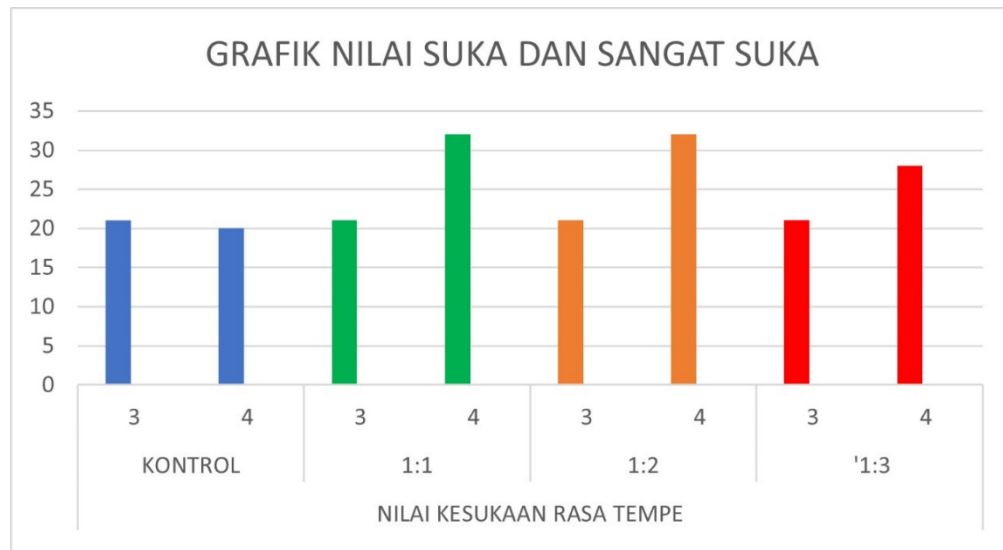
**B. Describe the correct ratio of tempeh yeast and starch to speed up the fermentation process and extend the freshness of tempeh.**

From the observations above, it can be seen that tempeh with a yeast: starch ratio of 1:2 has a fast fermentation time, namely 10 hours. This shows that the addition of starch during tempeh fermentation can accelerate mold growth (Suryani., et al. 2017).

Meanwhile, when observing the freshness of tempeh, the yeast: starch ratio of 1:1 has a long freshness period, namely up to day 4. This is because the nutritional power of the starch is sufficient for the mold growth process (Surbakti, et al., 2022). However, if you give too much nutrition, it will inhibit the growth of mold.

**C. Analyze the potential of tempeh yeast and starch as ingredients to extend the freshness of tempeh, whether they can be accepted by consumers**

From Table 2, it can be seen that control and treated tempeh have almost the same liking values. A rating of 1 indicates a dislike, a score of 2 is a dislike, a score of 3 is alike, and a score of 4 is very like. If you make a comparison graph of preferences, it will look as follows:



**Figure 13. Likeability value for tempeh**

**Caption:**

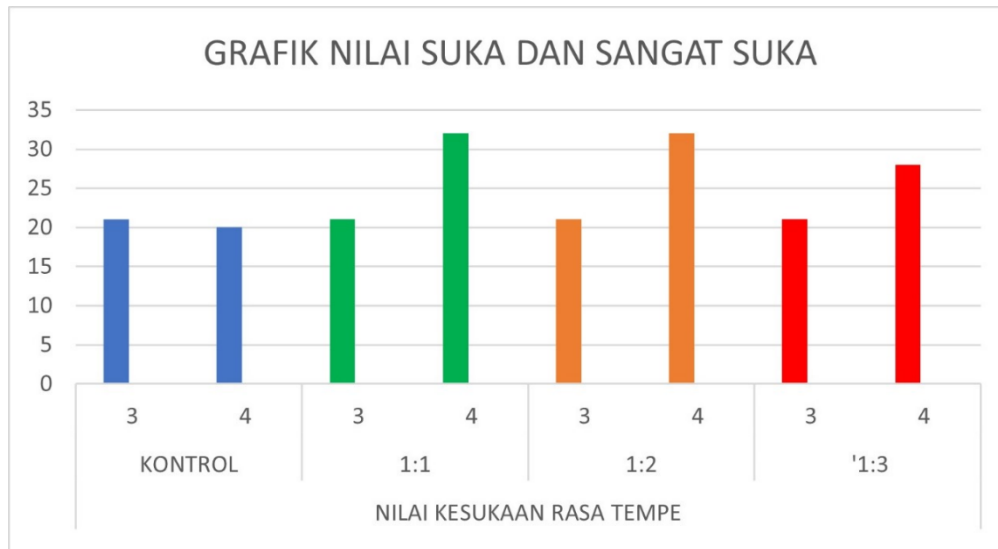
value 3: "Like"

value 4: "Very Like"

From Table 2, it can be seen that the panelists or respondents had the same liking score (21) for the control tempeh and all treated tempeh. This shows that there is no difference between control tempeh (without the addition of starch) and tempeh with the addition of starch.

While the value is 4 (like very much), tempeh 1:1 and 1:2 have a high value, namely above 30. According to Surbakti, et al., (2022) adding flour to the tempeh fermentation process is useful for providing nutrients for mold growth. Meanwhile, according to Roni Ahmad, (2013), the more fertile the mushrooms, the thicker the mold will be and the better the quality of tempeh. Adding starch can improve the taste of tempeh, making it more palatable.

This means that the addition of starch can be accepted by the panelists or respondents of this study.



**Figure 13. Likeability value for tempeh**

**Caption:**

value 3: "Like"

value 4: "Very Like"

From Table 2, it can be seen that the panelists or respondents had the same liking score (21) for the control tempeh and all treated tempeh. This shows that there is no difference between control tempeh (without the addition of starch) and tempeh with the addition of starch.

While the value is 4 (like very much), tempeh 1:1 and 1:2 have a high value, namely above 30. According to Surbakti, et al., (2022) adding flour to the tempeh fermentation process is useful for providing nutrients for mold growth. Meanwhile, according to Roni Ahmad, (2013), the more fertile the mushrooms, the thicker the mold will be and the better the quality of tempeh. Adding starch can improve the taste of tempeh, making it more palatable.

This means that the addition of starch can be accepted by the panelists or respondents of this study.

## CONCLUSION AND RECOMMENDATIONS

This research produces several important conclusions. First, the addition of starch during tempeh fermentation has proven to be effective in speeding up the fermentation process and extending the freshness of tempeh. Second, it was found that a 1:2 ratio of yeast to starch was very efficient in speeding up the fermentation process, with fermentation occurring in just 10 hours. Meanwhile, a 1:1 ratio shows the ability to maintain tempeh freshness for up to 80 hours, or

around three days. Lastly, tempeh enriched with starch during fermentation had a similar taste to control tempeh, indicating good acceptance by panelists or respondents.

Based on these results, this study also puts forward several suggestions for future research. First, it is recommended to carry out further research regarding the effect of adding starch on the protein content and other nutrients in tempeh. Second, additional studies need to be conducted to explore whether there are differences in the taste, aroma and texture of tempeh with the addition of starch when using different packaging, such as leaves, to better understand the potential and application of these findings.

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