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The Effect of Tofu Liquid Waste Acidity as a Soaking Agent for Soybeans in Expediting the Tempeh Fermentation Process

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Abstract

The tofu industry is a crucial food industry in Indonesia, providing highly nutritious food as an alternative to animal protein. In its production process, besides yielding tofu, it also generates liquid tofu waste. Despite its negative environmental impact, liquid tofu waste has a beneficial aspect—it serves as a soaking agent for soybeans, expediting the tempe fermentation process. Tempe, an authentic Indonesian food, undergoes a critical fermentation process, where any malfunction can significantly affect its quality. This study involved the creation of tempe, utilizing soybeans soaked in liquid tofu waste for 6, 9, and 12 hours, along with soybeans solely immersed in well water as a control. The subsequent steps included draining, peeling, steaming the soybeans for 15 minutes, pouring them into trays, letting them cool, sprinkling 3 grams of tempe yeast, packaging them in plastic with 100 grams of soybeans each, and then fermenting. The observation of tempe involved assessing the mold growth rate and the whitest color of tempe across various treatments. A comparison of the tempe preference test with soybeans soaked in liquid tofu waste, featuring different pH levels, was conducted through a physical examination. Twenty panelists were asked to compare tempe variants (A, B, C, and control) based on color, aroma, texture, and taste. The results are tabulated, and a taste test was performed by cutting each tempe into cubes, seasoning with salt, frying, and obtaining evaluations from 20 panelists. The research findings indicate that the optimal soaking time for soybeans in liquid tofu waste, to achieve the ideal pH for tempe fermentation, is 9 hours, resulting in a pH of 4.2. The optimal fermentation time for soybeans is 8 hours, coinciding with a pH of 4.2. Soybeans soaked in liquid tofu waste with varying pH levels produce tempe with nearly identical preference scores.

Keywords: Tofu Liquid, Tempeh, Soybeans

1. Introduction

Tempe and tofu are distinctive Indonesian foods widely consumed across various societal strata [1, 2]. Among households, tempe is favored as a culinary ingredient due to its savory taste and high protein content [3]. Roni, A. (2013) [4, 3] revealed that tempe contains protein equivalent to that of beef. Additionally, tempe is rich in various B vitamins, including B1, B2, pantothenic acid, niacin, B6, and B12 (PUSIDO Team of National Standardization Body [5]. It also contains minerals such as calcium, phosphorus, and iron. Furthermore, tempe exhibits antioxidant properties that contribute to its anti-cancer attributes [6, 1].The production of tempe involves the fermentation process of soybeans with the assistance of microorganisms, specifically Rhizopus oryzae and Rhizopus oligosporus [7, 2]. These fungi produce enzymes capable of breaking down soy protein into simpler forms, facilitating absorption by the human digestive system across all age groups [8]. The fermentation process also yields antioxidants, which prevent cancer and premature aging. This antioxidant formation is attributed to the activities of Micrococcus luteus and Corynebacterium bacteria during fermentation (PUSIDO Team of National Standardization Body [9]. These bacteria are obtained during the soybean soaking process. Despite being contaminants, these bacteria have the potential to produce antioxidant factor-II [10], which, according to Aryanta, W. (2020) [11], holds beneficial properties for human health, including inhibiting aging processes and preventing various diseases such as diarrhea, coronary heart disease, diabetes mellitus, cancer, osteoporosis, among others. Fermentation process significantly influences the quality of tempe products. Bintari *et al*. (2008) [10] noted that fermentation is influenced by various factors, including pH, with the optimal pH range for tempe fermentation being 4.3 - 4.5. Deviation from this range can disrupt the activity of Rhizopus sp. fungi, leading to

prolonged fermentation. Prolonged fermentation, as highlighted by Rahayu *et al*. (2021) [13], notably affects the chemical and sensory characteristics of tempe. Optimal fermentation results in tempe with a white color and thick mold [4]. Factors affecting the fermentation process, if unmet, hinder the process and prolong its duration. Prolonged fermentation results in tempe with thin mycelium, lacking longevity. Tofu liquid waste, with pH level below 5 [14], influences soybean fermentation for tempe production. The observed pH range of tofu liquid waste is 2.0 to 4.5. pH is a crucial factor determining microorganism growth [15]. Tempe fungi, specifically Rhizopus oryzae and Rhizopus oligosporus, thrive in a low pH environment. Low pH in tofu liquid waste induces an acidic atmosphere in soybeans during tempe soaking. The typical fermentation time for soybeans into tempe at room temperature is approximately 36-48 hours [16] or at least overnight. The pivotal stage in tempe production is the fermentation process, transforming organic compounds in soybeans with the assistance of microorganisms [17]., particularly Rhizopus oryzae and Rhizopus oligosporus [18]. These fungi actively produce white mycelium, binding soybeans together, resulting in a solid, white tempe. During fermentation, several events occur: (a) soybeans soften, and proteins break down into simpler forms for easy digestion; (b) mycelium grows from Rhizopus sp., entering soybeans and compacting them [19]; (c) an increase in unsaturated fatty acids occurs, reducing cholesterol; and (d) the formation of vitamins B1, B2, pantothenic acid, niacin, B6, and B12 (PUSIDO Team of National Standardization Body, 2012) [9]. Successful fermentation yields high-quality tempe with a clean white color and thick mycelium [8]. The thickness of tempe is dependent on the optimal growth of Rhizopus sp. during fermentation, significantly influencing its nutritional content. Thus, the fermentation process plays a crucial role in determining the quality of tempe. One of the important factors determining the success of fermentation is pH, or acidity level. According to Bintari *et al*. (2008) [10], the optimal pH for tempe fermentation is 4.3 - 4.5. Rhizopus sp. fungi thrive in acidic conditions. In less acidic conditions, the growth of fungi is hindered, consequently impeding the fermentation process. Tofu liquid waste, obtained during the tofu production process by separating protein and water, is known to contain various microorganisms due to exposure in open environments. It is highly acidic with an average temperature ranging from 40°C to 60°C, higher than the surrounding environmental temperature [20]. Such conditions contribute to a decrease in oxygen levels in water, resulting in significantly high pollution levels. Besides the highly acidic pH, the discharge of hot tofu liquid waste can lead to the mortality of aquatic organisms, accompanied by the emergence of foul odors, disrupting the environment [21]. Given this background, the authors aim to investigate the effect of the pH of tofu liquid waste used as a soybean soaking agent to expedite the fermentation process.

2. Method

2.1 Time and Place

The research was conducted in May 2023 at the Environmental Laboratory, Universitas Kristen Teknologi Solo.

2.2 Data Source, Tools, and Materials

Data were obtained from observations of pH during soybean soaking in tofu liquid waste, observations of mold growth on tempe treated with different soaking times for soybeans, and preference test.

Tools: Bucket, strainer, tray, stove, pot, tablespoon, rice spoon, scale, pH meter.

Materials: 2 kg of soybeans, RAPRIMA tempe yeast package, 20 liters of tofu liquid waste, and plastic wrap.

2.3 Data Collection Method

2.3.1 Analyzing the soaking time for soybeans to achieve the optimum pH for tempe fermentation

Activities involved in obtaining this data included measuring the pH of tofu liquid waste used for soybean soaking and soaking soybeans in tofu liquid waste:. Soybeans A, B, and C were soaked for 6, 9, and 12 hours, respectively. Meanwihle, Soybean K (Control) was soaked in well water for 9 hours.

2.3.2 Analyzing the optimum fermentation time for soybeans with different pH levels

Activities involved in this analysis included: 1. The soybeans used in this study were soaked in tofu liquid waste for 6, 9, and 12 hours, and soybeans soaked in well water were used as a control. The steps for making tempe were as follows: a) draining each soybean, peeling its skin, and steaming it for 15 minutes. b) Pouring it into a tray and letting it cool. c) Sprinkling 3 grams of tempe yeast on the cooled soybeans. D) Packing them in individual plastic wraps, each filled with 100 grams of soybeans, and fermenting. 2) For all differently treated tempe, mold growth and the whitest color were observed.

2.3.3 Comparing the preference test for tempe with soybeans soaked in tofu liquid waste with different pH levels

1**.** During the physical test, 20 panelists were asked to compare Tempe A, B, C, and the control regarding color, aroma, texture, and taste. The results were tabulated. Meanwhile, during the taste test, each tempe was diced, seasoned with salt, fried, and evaluated by 20 panelists.

3. Result and Discussion

3.1 Analysis of Soybean Soaking Time to Achieve Optimal pH for Tempe Fermentation

The results of pH observations during soybean soaking at different durations are presented in Table 1.

Table 1: Soybean soaking pH

Duration (Hours) / pH	0	6	9	12	15
Well Water	6.5	6.5	6.6	6.6	6.7
Tofu Liquid Waste	2.7	-	-	-	-
Soybean Soaking	2.9	3.5	4.2	5.4	6.3

3.1.1 Analysis of Optimal Fermentation Time for Soybeans with Different pH

To analyze the optimum fermentation time for soybeans to become tempe, observations were made regarding the color, aroma, texture (hardness), and mold growth. This treatment was conducted on Tempe A (soaked for 6 hours in tofu liquid waste), Tempe B (soaked for 9 hours in tofu liquid waste), Tempe C (soaked for 12 hours in tofu liquid waste), and a control group. The analysis of the optimal fermentation time for tempe is presented in Table 2.

Table 2: Optimal Fermentation Duration

Fermentation Duration	Condition	Tempe A	Tempe B	Tempe C	Control
0 Hour	Color	Soybean Color	Soybean Color	Soybean Color	Soybean Color
	Aroma	Soybean Aroma	Soybean Aroma	Soybean Aroma	Soybean Aroma
	Texture	Not Solid	Not Solid	Not Solid	Not Solid
	Mold	Not grown	Not grown	Not grown	Not grown
					
8 Hour	Color	Soybean Color	Thin White	Soybean Color	Soybean Color
	Aroma	Soybean Aroma	Tempe Aroma	Soybean Aroma	Soybean Aroma
	Texture	Not Solid	Slightly Solid	Not Solid	Not Solid
	Mold	Not grown	Thin growth	Not grown	Not grown
					
12 Hour	Color	Thin White	Thick White	Thin White	Soybean Color
	Aroma	Tempe Aroma	Tempe Aroma	Tempe Aroma	Soybean Aroma
	Texture	Slightly Solid	Solid	Slightly Solid	Not Solid
	Mold	Thin growth	Thick growth	Thin growth	Not grown
					
24 Hour	Color	Thick White	Thick White	Thick White	Thin White
	Aroma	Tempe Aroma	Tempe Aroma	Tempe Aroma	Tempe Aroma
	Texture	Solid	Solid	Solid	Slightly Solid
	Mold	Thick growth	Thick growth	Thick growth	Thick growth
					
36 Hour	Color	Thick White	Thick White	Thick White	Thick White
	Aroma	Tempe Aroma	Tempe Aroma	Tempe Aroma	Tempe Aroma
	Texture	Solid	Solid	Solid	Solid
	Mold	Thick growth	Thick growth	Thick growth	Thick growth

3.1.2 Preference Test for Tempe with Soybeans Soaked in Tofu Liquid Waste with Different pH

A preference test was conducted on Tempe A, B, C, and the control group, with the participation of 20 panelists. The number of panelists who liked each parameter is presented in Table 3.

Table 3

Parameter \ Tempe	A	B	C	Control
Color	18	20	19	17
Aroma	19	19	19	19
Texture	20	20	20	18
Taste	18	19	19	19
Mean Score	18.75	19.5	19.25	18.25

3.2 Analysis of Soybean Soaking Time to Achieve Optimal pH for Tempe Fermentation

From Table 1 concerning soybean soaking pH, an increase in pH is evident.

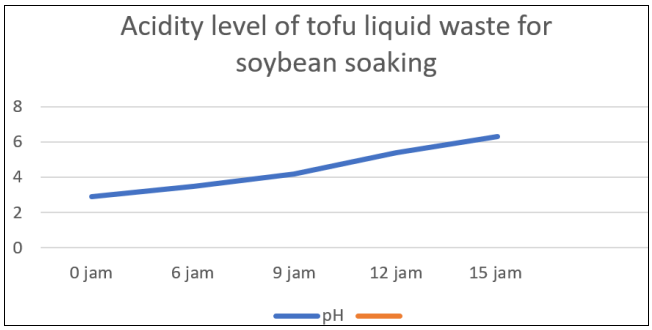

Fig 1: pH of tofu liquid waste for soybean soaking

The Figure shows that as the soaking time of soybean increases, the pH also rises. According to Suparno *et al.* (2020) [22], during soaking, the soybean water content increases. This increase in water content leads to the degradation of carbohydrates into carbon dioxide and water, resulting in a rise in pH.

A low pH is favorable for the growth of tempe mold. According to Bintari *et al.* (2008) [10], the optimal pH for tempe fermentation is 4.3 - 4.5, at which Rhizopus mold grows optimally. This pH range is achieved after 9 hours of soybean soaking. Suknia and Rahmawati (2020) [23] state that in general tempe production, soybean soaking is conducted for 12 – 24 hours. In this study, soybean soaking was done using well water.

After soaking for more than 12 hours, the pH reached 6.3, which is almost the same as the pH of well water. The pH increase occurs due to the growth of mold on the surface of the soaking liquid. The mold decomposes nitrogen residues in tofu liquid waste, producing ammonia, which causes an increase in pH [8].

3.3 Analysis of Optimal Fermentation Time for Soybeans with Different pH

From the observations presented in Table 2, Tempe B (soaked for 9 hours in tofu liquid waste) undergoes the fastest tempe fermentation, requiring 8 hours. Tempe A and C require 12 hours of fermentation, while the control group requires 24 hours. This aligns with the findings of Suknia and Rahmawati (2020) [23], suggesting that the typical fermentation time for tempe production is 24 hours. However, Rahayu *et al.* (2015) [13] note that the fermentation time for tempe production is 12-15 hours. The fermentation durations appear as shown in Fig 2.

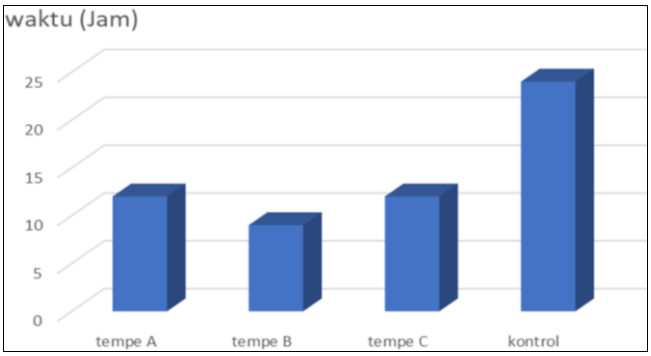

Fig 2: Tempe Fermentation Duration

Tempe B, with a soaking pH of 4.2, exhibits the optimum pH for the growth of Rhizopus mold. In contrast, Tempe A has a soaking pH of 3.5, which is too acidic, hindering the growth of Rhizopus mold. The same applies to Tempe C, which has a soaking pH of 5.4, not suitable for Rhizopus mold growth. This aligns with Mahadi's theory (2016) [12], stating that at excessively acidic pH levels, Rhizopus mold growth is inhibited, consequently impeding fermentation.

3.4 Comparison of Tempe Preference Test with Soybeans Soaked in Tofu Liquid Waste with Different pH

The preference test was conducted when the tempe was 36 hours old, and all tempe samples were in a ready-to-consume condition. The results of the preference test indicated nearly identical values for Tempe A, B, C, and the control group, with only insignificant differences. The highest score was obtained for Tempe B, which had the shortest fermentation time. From the sensory evaluation results, it can be inferred that all tempe variations were well-received by the participants.

4. Conclusion

This study concludes that the optimal soaking time for soybeans in tofu liquid waste to achieve the optimum pH for tempe fermentation is 9 hours, resulting in a pH of 4.2. The optimum fermentation time for soybeans is 8 hours, coinciding with the pH of 4.2. Soybeans soaked in tofu liquid waste with varying pH levels produce tempe with nearly identical preference scores.

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