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Determination of optimum fermentation time through microscopic, sensory, and eating quality comparison of hyacinth beans, sword beans, and soybean tempeh

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ABSTRACT

Tempeh is a traditional Indonesian fermented food made from soybeans, which are rich in protein and essential nutrients. However, the increasing demand for soybeans, particularly in Indonesia, has led to a search for alternative legumes. This study aims to evaluate the potential of hyacinth beans (HB) and sword beans (SD) as alternative legumes for tempeh production in comparison to conventional soybean (SB) tempeh. The research was conducted in two phases. The first phase was determining the optimum fermentation duration for HB and SD tempeh compared to SB tempeh using sensory appearance, hardness, and microscopic observation. The second phase was to determine the eating quality of all tempeh, which were steamed and fried, by sensory evaluation supported by color and texture analyses. Protein content analysis was also conducted on raw and cooked tempeh. Results showed that optimal fermentation durations were determined as 36 hours for HB and SD and 48 hours for SB tempeh, which had optimum sensory appearance, hardness, and Rhizopus mycelial growth. SB tempeh was more favored in the second phase than HB and SD tempeh. The preference for SB tempeh compared to the other two was thought to be because the panelists were more familiar with the sensory characteristics of SB tempeh. Protein content was highest in fried SB tempeh (37.49%), significantly increasing due to moisture loss during frying. While the original tempeh made from SB remains the most favored, non-SB legumes, especially SD, also present viable alternatives for tempeh production. Consequently, non-SB tempeh has the potential to reduce dependence on soybeans and support food diversification.

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INTRODUCTION

Tempeh is a world-renowned Indonesian traditional fermented food that ferments soybeans (SB) using *Rhizopus sp.* The nutritional value of tempeh, including protein, B vitamins, and minerals, makes it a popular source of plant-based protein among the population (Alda et al. 2022). The average consumption of tempeh in Indonesia is estimated to reach 15.24 kg per capita per year and has been growing ever since (Astuti et al. 2023). However, the high consumption of tempeh is not supported by adequate local SB production capacity. Production decline has been reported since 2020, dropping from 632.2 tons to an estimated 558.3 tons in 2024 (Hulu 2023). This imbalance has resulted in more imports, and it is evident that only 32.72% of SB in Indonesia is produced locally (Setyawan and Huda 2022). The dependency of tempeh producers on SB is not ideal and indicates the need for measures and innovations to reduce soybean imports and support food sustainability in Indonesia (Krisbianto et al. 2023).

Over the years, the utilization of SB alternatives as replacements has been thoroughly researched. Widely available local legume species are the optimum alternatives for tempeh production (Ekafitri and Isworo 2014). Hyacinth beans (*Lablab purpureus (L.) Sweet*) and sword beans (*Canavalia gladiata*) are a few of the local legumes that meet these criteria. Hyacinth beans (HB) and sword beans (SD) are known for their higher production capacity (6-10 tons/ha and 3-5 tons/ha, respectively) compared to SB (2-3 tons/ha) (Darini and Kusdiarti 2017, Julisaniah et al. 2022). These two legumes are also resilient and capable of growing in various media, ranging from sand to clay.

In addition to being more affordable, these two legumes have nutritional values comparable to SB. Raw HB contains 24.63% protein, 0.9% fat, 65.85% carbohydrates, and 4.63% fiber (Mosisa and Tura 2017). Bioactive compounds are also abundant, predominantly phenolics, saponins, carotenoids, and fat-soluble vitamins (Zhou et al. 2024). Meanwhile, raw SD contains 27.4% protein, 66.1% carbohydrates, and 1.9% fat (Sari et al. 2022). SD is known to contain flavonoids, tannins, saponins, and terpenoids and exhibits antioxidants, anti-obesity, and anti-inflammatory properties (Hwang et al. 2023).

Various studies have explored the potential of non-soybean tempeh. Jayanti (2017) analyzed the protein content in various varieties of HB tempeh. The effect of different packaging materials on the final result of HB tempeh has also been observed (Werdiningsih et al. 2018). Susilo (2015) analyzed the prospects of SD as a substitute raw material for SB in the tempeh industry. However, comprehensive studies comparing the effect of fermentation time on the characteristics and eating quality of the resulting tempeh are lacking.

The first phase of this study determined the optimal fermentation duration for SB, HB, and SD tempeh based on microscopic and discriminative sensory analyses. In the second phase, the eating quality of the three types of tempeh was also compared using two commonly used cooking methods (steaming and frying). Aside from promoting the utilization of local commodities, the results of this research are expected to encourage the development of the tempeh industry with a focus on food sustainability.

METHOD

Tempeh Production and Maturity Analysis

Three types of legumes were used as raw materials for tempeh in this experiment: SB (Sidoarjo, East Java), SD (Ponorogo, East Java), and HB (Probolinggo, East Java). The samples were washed, soaked for 12 hours, boiled for 10 min for SD and 5 min for HB to help the seed coat peeling process, and further boiled at 90°C until tender, i.e., 45 min for SB, 50 min for SD, and 5 min for the soft HB. The boiled legumes were drained, cooled, and analyzed for moisture content before being aseptically mixed with 1% (w/w) *Rhizopus oligosporus* tempeh starter (Raprima, PT. Aneka Fermentasi Industri, Indonesia).

A total of 60 grams of the inoculated samples were placed into perforated polyethylene bags with 1 cm spacing between the holes. Fermentation was carried out in a controlled environment at 30-37°C, with a relative humidity of 70-80% for 24, 36, and 48 hours. Sampling was performed during 0, 24, 36, and 48 hours of fermentation duration for maturity analysis, macroscopically using sensory analysis and microscopically to observe sporangium formation. Protein and hardness analyses were also conducted to assess the impact of fermentation duration on the protein content and texture of tempeh.

Discriminative Sensory Analysis

The maturity standard was based on sensory analysis by a group of 30 semi-trained panelists who understood tempeh maturity standards based on color, aroma, and texture (compactness). The scoring used the Just About Right (JAR) scale, where number 1 means the tempeh is underfermented, number 5 means overfermented, and number 3 marks just about properly fully fermented. The sensory indicators were determined using focus group discussions and interviews with the panel group (Yonathan et al. 2021).

Microscopic Analysis

The microscopic structure of *Rhizopus* sp. was observed by placing tempeh mycelium on a glass slide with the addition of methylene blue. The preparation was covered with a cover slip and observed at 400x magnification using a light microscope (CX-23, Olympus, Japan).

Tempeh Eating Quality

All tempeh samples were further processed according to the method proposed by Setiarto (2021). Tempeh samples were cut into 2 cm cubic and divided into two parts. One part was fried at 160°C for 2 minutes, while the other was steamed at 100°C for 25 minutes. The samples were allowed to cool to room temperature before further testing using 31 non-trained panelists. The hedonic test assessed fried and steamed tempeh samples' appearance, aroma, texture (hardness), and taste parameters using a five-point Likert scale from immensely dislike to very like. Protein, hardness, and color analyses were also conducted to assess different types of raw materials and the cooking process on the protein content, texture, and color of tempeh.

Moisture Analysis

Moisture content was tested using the gravimetric method according to AOAC 930.15 (AOAC 1970). A two-gram sample was accurately weighed and heated at 105°C for 24 hours in an oven (UN30-230V, Memmert, USA). The sample's moisture content was calculated based on the ratio of water loss to the initial mass when equilibrium is reached. The moisture content of the sample can be calculated using the following Equation (1):

$$Moisture\ content\ (WB) = \frac{mass\ loss\ (g)}{initial\ mass\ (g)} \times 100\% \quad (1)$$

Protein Analysis

Protein content was measured using the Kjeldahl method. 0.2 g sample was mixed with 8 mL 98% H_2SO_4 and 4 g catalyst tablets (Buchi, Switzerland). The digestion process was done using a Speed Digester (K-425, Buchi, Switzerland) for 2 hours and then diluted with 38 mL of distilled water after reaching room temperature. The digested sample was mixed with 38 mL of 32% NaOH (Honeywell, USA) and distilled using a Kjel Line (K-365, Buchi, Switzerland) for 5 minutes. The distillate was captured by a 4% H_3BO_3 solution (Smart-Lab, Indonesia) in which three drops of bromocresol green-methyl red (BCG-MR) indicator in a 5:2 ratio had been added. The captured sample was titrated with a standardized 0.1N HCl solution (Sigma-Aldrich, USA) until it turned pink. The protein content of the sample was then determined using the following Equation (2):

$$\%Protein = 6.25 \times \frac{V_{(sample-blank)}(ml) \times N\ HCl \times 14.008}{m_{sample}(g)} \times 100\% \quad (2)$$

Physical Properties Analysis

Color parameters of the tempeh (CIE Lab) L* (lightness), a* (redness), and b* (yellowness) were tested using a color reader (CS-210, Njouka, China). Instrument calibration was performed before testing the samples. Browning Index (BI) of samples was calculated using the Equation (3) and (4)(Kasim and Kasim, 2015).

$$BI = \frac{X - 0.31}{0.172} \times 100\% \quad (3)$$

Where:

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (4)$$

The hardness of tempeh was tested according to the method by Erkan et al. (2020). Tempeh samples were cut into 2 cm cubes and tested using a texture analyzer (Imada, USA). A flat probe with a vertical speed of 5 mm/s was used.

Statistical Analysis

The significance of differences (α = 0.05) was conducted using analysis of variance (ANOVA), followed by the Tukey HSD test, using RStudio software (ver. 2024.04.2+764).

RESULT AND DISCUSSION

Optimum fermentation duration

The initial moisture content before tempeh inoculation plays an important role during tempeh fermentation, which is affected by the legume rehydration process after soaking and boiling. Boiled HB exhibited the softest texture, making it mushy and unsuitable for use as tempeh raw material if subjected to the same boiling time as SB and SD. That was why HB was only boiled for 5 minutes before being cooled and inoculated using the starter. The results of the moisture content analysis, presented in Table 1, show that HB had the highest moisture content, followed by SD and SB.

Table 1 Moisture content of boiled legumes.

Legumes	Moisture Content (%)
SB	57.82 ± 0.31^{c}
SD	60.98 ± 0.46^{b}
HB	63.44 ± 0.27^{a}

Values with different letters are significantly different ($P < 0.05$).

The hydration process during the soaking and cooking process is influenced by internal factors, including size, seed coat thickness, cotyledon chemical constituents, as well as hilum and micropyle size (Perera et al. 2023). Drying conditions, temperature, and ratio of water are also known to affect the amount of water molecules capable of penetrating the legumes (Wang et al. 2022).

Aside from softening the legumes, the moisture content also facilitates microbial activity, increases nutrient bioavailability, and reduces the amount of naturally occurring antimicrobial compounds (Mani and Ming 2017). *R. oligosporus*, the fungi species presented in tempeh fermentation in this study, has optimum growth at 35 °C and RH 95–97% (Han et al. 2003). On the other hand, high moisture content may lead to the unwanted, softer texture of legumes and increase the risk of bacterial contamination during tempeh fermentation. During fermentation, water vapor is released by respiration and increases the humidity inside the tempeh packaging. Thus, the higher humidity levels may inhibit the growth of the fungi and favor the growth of spoilage bacteria (Magdalena et al. 2024).

Rhizopus growth during the fermentation process is indicated by the formation of mycelium or hyphae, which binds the beans together into a compact tempeh. Underfermentation and the growth of contaminating bacteria will lead to poor mycelium structure, while overfermentation may lead to brown, pungent, and slimy tempeh caused by bacterial activity (Prameswari et al. 2021).

Macroscopic observation

The growth of *Rhizopus* during the fermentation process was indicated by the formation of mycelium or hyphae, which binds the beans together into a compact tempeh. Underfermentation will lead to poor mycelium structure, while overfermentation may lead to brown, pungent, and slimy tempeh caused by microbial activity (Prameswari et al. 2021).

Based on Figure 1, the best tempeh characteristics were observed in SB tempeh with great compactness and mycelium density. SB has a relatively small grain size (c. 8.56 mm), which allows for more excellent hyphae network formation, resulting in denser tempeh with fewer voids (Yuliani et al. 2022). Legumes with smaller particle sizes will increase the surface-to-volume ratio, creating more contact points on the surface and allowing them to reach the inner part of the legume faster (van Kuijk et al. 2016). In comparison, SB and HB with larger sizes (28.92 mm and 13 mm, respectively) inhibit the formation of mycelium networks (Hanapiah et al. 2022, Putri et al. 2023).

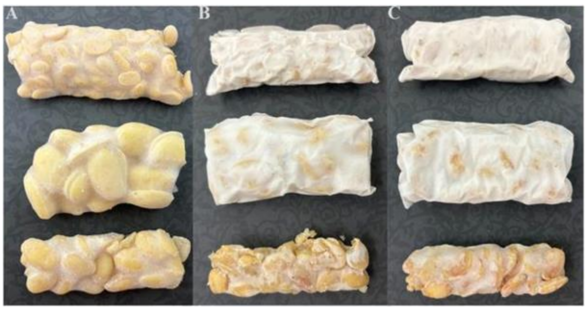


Figure 1 Tempeh fermented for 24 hours (A), 36 hours (B), and 48 hours (C) using different legumes, from top to bottom: SB, SD, and HB.

SD tempeh, with slightly higher moisture content than SB, exhibited significant fungal growth. The findings confirm a previous study that optimum tempeh fermentation occurs at 60% moisture content (Tahir et al. 2018). However, few hollow sections were found in SD tempeh due to its relatively large size. In contrast, HB tempeh shows the least desirable traits with soft texture and uneven mycelium growth. The high initial moisture content might inhibit growth and is known to rise over a prolonged fermentation period (Rizal et al. 2022). After 48 hours, slime can be observed on the surface of HB tempeh, which indicates the growth of spoilage bacteria.

Discriminative sensory evaluation

Based on the previous focus group discussion and interview with the semi-trained panel group, tempeh maturity parameters were determined as tempeh's appearance (color), aroma, and texture (compactness). When purchasing, consumers tend to focus more on sensory attributes that can be directly observed, such as color, aroma, and texture. Chirilli and Torri (2023) stated that appearance and smell are the primary sensory indicators that attract consumers' buying behavior. The discriminative sensory evaluation using the JAR method is shown in Table 2.

SB tempeh was considered underfermented by its appearance (color) after 24 hours of fermentation, which was about right after 36 and 48 hours. A similar trend was found in the aroma and texture parameters, where most panelists rated SB tempeh's aroma and texture (compactness) as insufficient during the 24 hours. Meanwhile, at 36 and 48 hours of fermentation, most panelists rated the aroma and texture as JAR, scoring close to 3 in the assessment.

Only 26.7% of panelists rated SD tempeh fermented for 24 hours with optimum color. At 36 and 48 hours of fermentation, most panelists rated the color of SD tempeh to match the well-fermented tempeh. Thus, the 36-hour fermentation period was selected as the optimum fermentation period for SD, considering better ratings than 48 hours of fermentation. Meanwhile, HB tempeh earns 53.3%, 60%, and 60% insufficient color, aroma, and texture ratings, respectively. Like SB and SD, most panelists rated it as JAR after 36 and 48 hours. However, a less favorable texture is observed at 48 hours due to decreased compactness.

Hardness of raw tempeh

The growth of *Rhizopus* will alter tempeh sensory characteristics. This process happens through enzymatic activity that breaks down proteins and carbohydrates in the beans, converting them into energy for fungal metabolism and producing mycelium, thus reducing the hardness of the beans. *Rhizopus oligosporus* is known to produce protease and lipase, which break down complex proteins and lipids into simpler compounds, generating volatile compounds such as aldehydes, ketones, and fatty acids that create the distinct aroma of tempeh (Sharma et al. 2020). The texture is affected by the breakdown of cell structure by cellulase enzymes and the formation of dense mycelium, resulting in a more compact tempeh.

Table 2 Evaluation of tempeh ripeness

Legumes	Fermentation Duration		
	24 hours	36 hours	48 hours
Color			
SB	2.33 ± 0.80^{cd}	2.93 ± 0.74^{abc}	3.03 ± 0.67^{ab}
SD	2.07 ± 0.94^{d}	3.00 ± 0.59^{abc}	3.33 ± 0.84^{ab}
HB	2.80 ± 1.13^{bc}	2.87 ± 0.68^{bc}	3.57 ± 1.14^{a}
Aroma			
SB	2.53 ± 1.04^{bc}	2.90 ± 0.71^{abc}	3.00 ± 0.64^{abc}
SD	2.20 ± 0.92^{c}	3.47 ± 0.90^{a}	3.10 ± 0.96^{ab}
HB	2.53 ± 1.33^{bc}	3.47 ± 1.04^{a}	3.70 ± 1.42^{a}
Texture			
SB	2.53 ± 0.73^{bcd}	3.03 ± 0.85^{ab}	3.13 ± 0.94^{ab}
SD	1.80 ± 0.81^{d}	2.57 ± 0.77^{abc}	3.30 ± 0.92^{a}
HB	2.47 ± 1.17^{bcd}	2.43 ± 0.97^{bcd}	2.20 ± 0.96^{cd}

Values in the same row with different letters are significantly different ($P < 0.05$).

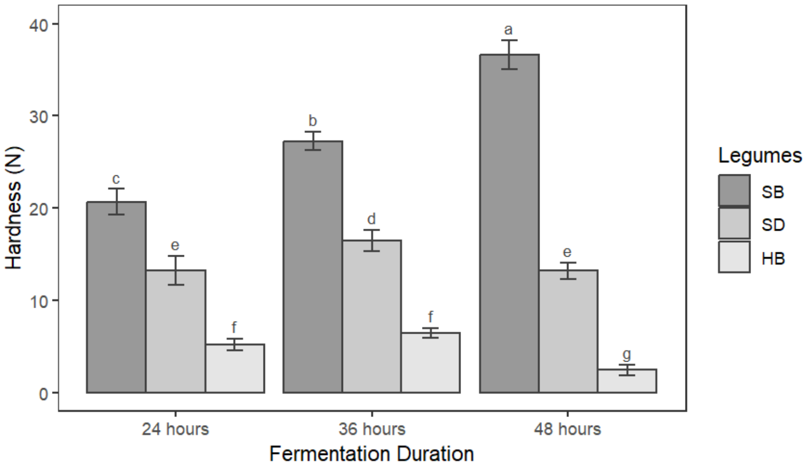
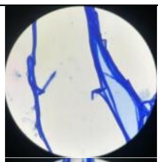
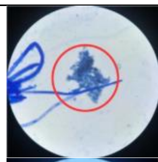
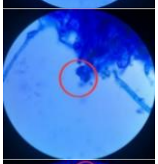
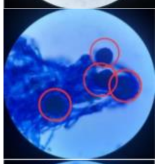
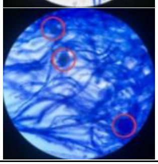
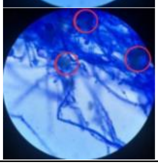
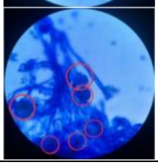


Figure 2 Hardness of SB, SD, and HB tempeh during fermentation.

Table 3 Microscopic appearance of tempeh mycelium observed at 400x magnification.

Legumes	Fermentation Duration		
	24 hours	36 hours	48 hours
SB			
SD			
HB			

Note: Sporangium and spores are marked with a red circle.

Figure 2 represents the hardness of tempeh made with different legumes in various fermentation durations. SB tempeh exhibited a significant increase in hardness, from 20.69 N at 24 hours to 36.62 N at 48 hours, due to the optimal growth of *Rhizopus spp.* mycelium, which forms a dense and strong structure. This aligns with findings by El-Zawawy et al. (2023) and Xu et al. (2021), suggesting that fungi release secondary metabolites like terpenoids can inhibit bacterial contamination, supporting better texture formation.

As for SD tempeh, an increase in hardness was initially observed, peaking at 16.51 N after 36 hours, followed by a decline to 13.21 N after 48 hours. This suggests that fungal overgrowth and tissue degradation occur beyond the optimal fermentation period, corroborating findings by Muthmainna et al. (2016). Similarly, HB tempeh showed an initial increase in hardness from 5.23 N to 6.46 N after 36 hours before decreasing significantly to 2.48 N at 48 hours. The high moisture content of HB (63.49%) accelerated fungal growth, but excessive fermentation led to structural degradation due to competition with bacteria for nutrients (Rizal et al. 2022).

These findings suggest that the longer fermentation period significantly decreases tempeh's hardness. The 36- and 48-hour periods were optimal for SB and SD tempeh, but they could potentially lead to overfermentation and reduced quality in HB tempeh.

Microscopic observation

The ripening process of tempeh is characterized by sporulation, or the dispersal of spores from their sporangium. This event marks the shift to the reproductive state of the fungi

(Rosidah et al. 2023). With the help of microscopic analysis, the spore formation of three tempeh samples can be observed (Table 3). HB exhibits the fastest growth, showing sporangium formation after 24 hours. This can be attributed to the higher initial moisture content discussed earlier. Meanwhile, SB and SD tempeh show sporangium formation at the 36-hour mark. Sporulation, or the release of spores from their sporangium, starts at 36 hours in SD and HB, followed by SB tempeh 12 hours later.

This microscopic data supports the macroscopic data. The high moisture content of boiled HB (Table 1) reduced the hardness (Figure 2), thus increasing the fermentation rate of HB tempeh (Table 3). Meanwhile, the appearance (Figure 1) and discriminative sensory evaluation (Table 2) also showed that SB tempeh had reached its full fermentation after 48 hours, while SD and HB tempeh had fermented for 36 hours. Upon considering these parameters, the optimum fermentation duration for SB tempeh was determined to be 48 hours. In comparison, SD and HB tempeh optimally need 36 hours of fermentation since prolonged fermentation leads to degradation.

Tempeh eating qualities

Eating quality refers to the perceived characteristics of a food product during consumption. For this testing, the optimum fermentation duration obtained earlier was used to make tempeh.

Color & texture

Among several characteristics, color is essential in determining customer perception of the flavor associated with the product. Substantial empirical research has consistently shown that the hue and saturation, or intensity, of color in food frequently affects how multisensory flavor is perceived (Spence, 2019). In the case of tempeh, sufficient browning is generally preferred.

Results in Table 4 show that fried tempeh had a significantly higher browning index than steamed tempeh. This browning was due to the Maillard reaction and caramelization during frying. The Maillard reaction between reducing sugars and amino acids forms melanoidin compounds, darkening the tempeh (Asokapandian et al. 2020). While caramelization, which requires temperatures above 120°C, also contributed to the browning of fried tempeh (Kocadağlı and Gökmen 2019). Among these samples, fried HB tempeh showed the darkest color, while all steamed tempeh exhibited lighter brownish hues reflecting the original color of the beans. It is suggested that the high moisture content of HB beans led to a higher gelatinization process, thus increasing the straight carbohydrate content compared to the other types of tempeh. In turn, it increased the Maillard and caramelization rate of HB tempeh, thus increasing its browning index.

Table 4 Browning index and hardness of cooked tempeh.

Tempeh	Browning Index (%)	Hardness (N)
Fried		
SB	35.50 ± 2.25^{b}	33.80 ± 0.55^{a}
SD	38.93 ± 15.93^{b}	29.24 ± 0.81^{b}
HB	66.96 ± 16.80^{a}	19.50 ± 0.72^{c}
Steamed		
SB	7.11 ± 0.61^{c}	12.40 ± 0.50^{d}
SD	0.75 ± 1.45^{c}	13.18 ± 0.86^{d}
HB	3.20 ± 2.38^{c}	8.34 ± 0.78^{e}

Values in the same column with different letters differ significantly ($P < 0.05$).

Aside from color, food texture is pivotal in shaping consumer preferences and final purchasing decisions (Baingana 2024). Table 4 shows that tempeh cooked by frying results in a significantly harder texture than steamed tempeh. In general, frying will produce harder tempeh due to the dehydration process, which forms a crust on the outer layer of the tempeh (Asokapandian et al. 2020). Ellent et al. (2022) also noted that frying leads to water evaporation, reducing the moisture content and coinciding with increased hardness.

In contrast, steaming did not promote crust formation while simultaneously promoting water absorption. This aligns with the findings of Tamsir et al. (2021), where fried foods typically have a higher hardness level than steamed ones. Differences in texture across tempeh samples may also be influenced by the initial moisture content of the beans before fermentation. HB tempeh with higher moisture content shows significantly lower hardness, and SB tempeh with the least amount of moisture yields harder tempeh in both treatments.

Hedonic sensory evaluation

The hedonics test was used to evaluate overall sensory acceptance of cooked tempeh. The results are shown in Table 5.

Table 5 Sensory evaluation of cooked tempeh

Legumes	Cooking method	
	Fried	Steamed
Appearance		
SB	4.06 ± 0.81[a]	3.84 ± 0.90[a]
SD	3.13 ± 1.02[b]	2.52 ± 1.09[b]
HB	4.00 ± 0.82[a]	3.00 ± 0.89[b]
Aroma		
SB	4.32 ± 0.75[a]	3.65 ± 1.05[b]
SD	3.39 ± 0.88[bc]	2.19 ± 0.87[d]
HB	2.77 ± 1.06[cd]	2.13 ± 0.81[d]
Texture		
SB	3.84 ± 0.69[a]	3.55 ± 0.96[ab]
SD	2.97 ± 1.11[bc]	2.45 ± 1.12[c]
HB	3.48 ± 0.96[ab]	2.45 ± 1.12[c]
Taste		
SB	3.23 ± 0.96[a]	3.19 ± 0.95[ab]
SD	2.55 ± 0.89[b]	1.65 ± 0.80[c]
HB	1.74 ± 0.96[c]	1.61 ± 0.80[c]

Values in the same sensory parameters with different letters differ significantly ($P < 0.05$).

Despite having significantly higher BI values, only HB tempeh shows a significant difference between the cooking methods used (Table 4). This may be due to fried HB tempeh being darker compared to fried SB and SD tempeh. On the other hand, the large bean size of SD might also contribute to the lower appearance score of SD tempeh, as some panelists stated that they were not used to seeing tempeh made of large beans.

The aroma of SB tempeh has a higher score compared to SD and HB tempeh due to the familiarity of panelists with the aroma of SB tempeh. Otherwise, fried tempeh earns a higher aroma score compared to its steamed counterparts, which is true for all types of tempeh. This finding was also observed by Syukri et al. (2023) while comparing soybean and corn tempeh. The maillard reaction produces a unique aroma closely associated with a savory smell (Gao et al. 2024). Higher temperature used while frying is also known to reduce beany aroma caused by volatile compounds, such as methyl-1-butanol, hexanal, 2,4-decadienal, and dimethyl disulfide (Purwandari et al. 2021). This explains the higher acceptance of panelists of fried tempeh than steamed tempeh.

Fried SB tempeh receives the highest texture rating, coinciding with the higher hardness evaluated previously. Panelists were more accustomed to fried soy tempeh's more compact and firmer texture. The texture score for SD and HB tempeh was more inconsistent due to partial fungal growth during fermentation, leading to the tempeh's less compact and dense texture. For SD tempeh, the relatively large size of SD beans had made some hollow sections inside the tempeh matrix, thus affecting its hardness as a whole. Meanwhile, the less optimum fungal growth of HB tempeh was affecting its texture.

The following sensory parameter is taste. For ready-to-eat food, taste is more relevant since it plays an essential role in influencing food choices, dietary behaviors, and overall intake (Kourouniotis et al. 2016). Taste evaluation proves that panelists might not be accustomed to the sensory characteristics of SD and HB tempeh. The use of seasoning such as salt might be able to increase the taste acceptance of all tempeh, as stated by some of the panelists.

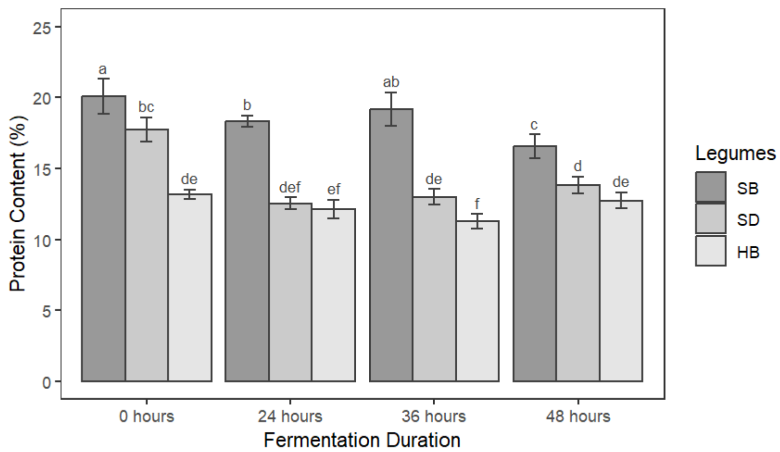


Figure 3 Effect of fermentation duration on tempeh protein content.

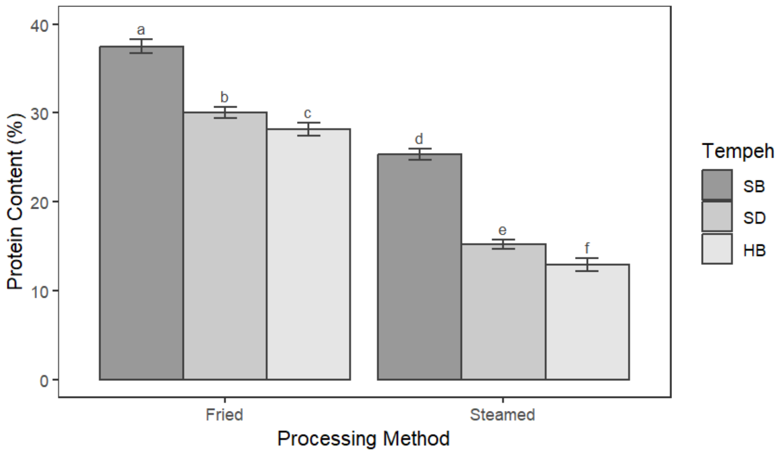


Figure 4 Effect of cooking method on tempeh protein content.

Tempeh nutrition quality

Being frequently used as a meat substitute in certain groups of the population, the protein content is one of the most crucial aspects of tempeh's nutritional quality (Aaslyng and Højer 2021). The duration of fermentation significantly impacts the protein content in tempeh, as the process naturally reduces protein levels through the breakdown into simpler peptides and amino acids (Acharya and Chaudhuri 2021). This is apparent across all samples used (Figure 3). Several factors contribute to variations in protein levels, such as biological differences in the beans, fluctuations in fermentation conditions, and microbial activity, which influence the protein breakdown during fermentation.

Observations from this study show that the protein content in tempeh ranged from 11.27% to 20.08%. The decline in protein levels becomes more pronounced as fermentation time increases, especially in SD tempeh, illustrating a direct relationship between fermentation duration and protein reduction. During fermentation, proteins degrade into simpler compounds through the action of protease enzymes (Nuraini et al. 2019).

As the fermentation progresses, the degradation of proteins into simpler compounds accelerates. Zhang et al. (2022) noted an increase in peptides from 20 to 200 mg/g after 36 hours of fermentation. This process might also lead to the formation of volatile ammonia. Some amino acids are also used by *Rhizopus sp.* for fungal growth, contributing to protein reduction after prolonged fermentation. Notably, increased protein content is observed in HB samples after 48 hours. This might be caused by the reduction of another component of tempeh, arguably the moisture content, which was not analyzed in this study.

The protein content of processed tempeh is shown in Figure 4. The highest protein content is found in fried SB tempeh (37.49%), while steamed HB tempeh has the lowest (12.90%). SB tempeh consistently has the highest protein content, followed by SD and HB. Processing leads to an increase in protein content due to evaporation. Frying leads to a more significant protein increase due to higher temperatures, causing more water to evaporate and concentrate the protein. This increase in protein content is relative, not an absolute gain in protein, as the water content decreases during cooking (Tan and Xie 2021).

Frying acts as a dehydration process, increasing protein concentration, but changes in other components, like fat, were not measured in this study. Steaming also raised protein content, though less than frying. Research by Syukri et al. (2023) found steaming reduces protein levels, while Wihandini et al. (2012) reports otherwise. Specific water-soluble proteins may leech out during steaming, explaining the lower protein content (Masson and Lushchekina 2022).

Further Simple Additive Weighting (SAW) calculations show that SB tempeh ranked the highest in both processing methods. Since SB tempeh has been a popular food item in Indonesia, especially in Java, for centuries, it may be more challenging for the general public to accept different characteristics associated with different legumes being used (Wijaya 2019). Although SD & HB tempeh are ranked lower in this SAW analysis, they still have a chance to be accepted by the public, especially the SD tempeh. The use of seasoning and cooking methods is expected to increase the public's familiarity and acceptance of non-soybean tempeh, thus promoting food diversification in Indonesia.

CONCLUSION

Based on macroscopic parameters such as appearance and discriminative sensory evaluation, as well as microscopic observations of sporangium presence, it was evident that different raw materials influenced the optimum fermentation period. SB tempeh achieved full fermentation after 48 hours, while SD and HB tempeh required only 36 hours to ferment fully. Among the three, SB tempeh had superior eating quality in fried and steamed forms. In contrast, HB tempeh was the least preferred due to its appearance, aroma, texture, and taste. Additionally, SB tempeh had the highest protein content. Despite these differences, non-soybean tempeh, particularly SD tempeh, has the potential to gain consumer acceptance depending on the cooking method and seasoning used. These findings highlight the viability of non-soybean legumes as alternative raw materials for tempeh production, supporting food diversification efforts and reducing reliance on soybean imports.

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