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**PRELIMINARY OBSERVATION OF FUNGAL
CONTAMINATION IN PEANUT TEMPEH
FERMENTATION:
CHALLENGES IN SMALL-SCALE PRODUCTION**

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Abstract:

Tempeh is a traditional fermented cuisine, generally produced from soybeans and *Rhizopus* spp., with great nutritional content and functional health advantages. In recent years, the increasing demand for locally available, sustainable and cost-effective protein sources has led to significant interest in developing non-soy tempeh using alternative substrates such as peanuts. Although peanut-based tempeh has been suggested as a substitute for soybean tempeh, there is insufficient empirical investigation of the fermentation behaviour, failure mechanisms and process stability of peanut substrates under controlled small-scale production circumstances. This study was conducted to investigate the fermentation behaviour of peanut tempeh and to determine the possible reasons for fermentation failure, with the practical relevance in small-scale and community-based production. Peanut substrates were inoculated with commercial *Rhizopus* starter and fermented under ambient conditions. The fermentation process was tracked over a period of three days using visual observation, temperature profiling and pH testing. The results showed that the fermentation did not occur. On Day 1 there was little mycelial development seen, peanut kernels were loose, and surface wetness was

present. By Day 2 it showed greyish mycelial development, uneven colonisation and increasing condensation. By the third day, there was heavy contamination with grey-black mycelium and green-yellow patches of fungus, and this was a complete failure of fermentation. The temperature profile was non-linear with an increase from 26.18°C to 31.01°C on Day 1, decrease to 29.29°C on Day 2 and increase again to 31.22°C on Day 3. In addition, pH increased from 5.46 to 6.62, indicating alkaline conditions consistent with microbial deterioration. The observed failure in fermentation might have been caused by insufficient compacting of the substrate, uneven moisture distribution and inappropriate fermentation conditions. Nevertheless, further controlled experiments are necessary to confirm these contributing factors. Results from the point of view of small-scale production show practical difficulties in adapting peanut tempeh for small-scale and community production where control over the process is limited. These results provide a basis for the optimisation of fermentation techniques and improved practicality of peanut-based tempeh as a sustainable alternative protein product for community-scale production.

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Keyword:

Contamination, Food Fermentation, Peanut Fermentation, *Rhizopus*, Tempeh



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Introduction

Tempeh is a traditional fermented cuisine that is renowned for its nutritional and functional properties, and it is made from soybeans and *Rhizopus* spp. (Rizzo, 2024; Teoh *et al.*, 2024). In recent years, the diversification of plant-based protein sources has gained increasing attention due to sustainability concerns, dietary preferences, and the need to reduce reliance on imported soybean supplies. As a result, there has been growing interest in adapting traditional fermentation processes to alternative substrates that are locally available and economically viable. Interest in non-soy alternatives is expanding, leading to research on alternative substrates such as legumes, grains, and seeds (Sulasfiana Alfi Raida *et al.*, 2025; Górska *et al.*, 2025; Quinny, 2023). But the successful application of tempeh fermentation to non-soy substrates is not always straightforward since differences in chemical composition, especially lipid, protein and moisture content, may have a substantial effect on microbial growth and fermentation dynamics. For example, peanuts have a larger lipid content as compared to soybeans which may alter oxygen diffusion, water activity and overall microbial ecology in the fermentation system. Nevertheless, the stability of fermentation of peanut tempeh is an area that has not been studied extensively, particularly at the small scale.

Additionally, many small-scale fermentation systems, such those employed in laboratory or educational settings, could not have accurate environmental control and are more prone to process variability and contamination. Tempeh fermentation needs the controlled growth of

microbes and environmental variables such temperature, aeration, and moisture content (Rizal *et al.*, 2021). Peanuts are different from soybeans in terms of having a larger lipid content and various physical properties which could alter the fermentation process and fungal colonisation (Commeey *et al.*, 2021; Indiarito & Rezaharsamto, 2020).

Despite the increasing interest in non-soy tempeh, there is a scarcity of documentation regarding the fermentation challenges associated with peanut substrates, particularly in small-scale laboratory settings. Consequently, the objective of this investigation is to record initial observations of contamination during the fermentation of peanut tempeh and to identify potential process-related factors that may contribute to the failure of the fermentation process.

Literature Review

Tempeh is a traditional fermented cuisine from Indonesia, renowned for its high protein content, digestibility, and health benefits. It is generally manufactured from soybeans fermented by *Rhizopus* spp., especially *Rhizopus oligosporus* (Dwiatmaka *et al.*, 2022) which creates a dense white mycelial network that consolidates the substrate into a compact cake. The fermentation process boosts the nutritional content of soybeans, improves nutrient bioavailability, and diminishes antinutritional components (Azri *et al.*, 2026).

Recently, there has been an increasing interest in creating non-soy tempeh utilising various substrates, including legumes, grains, and nuts (Rahmawati *et al.*, 2021; Tan *et al.*, 2024). The movement is driven by the increased demand for sustainable protein sources, diversification of raw resources (Sulasfiana Alfi Raida *et al.*, 2025), and concerns surrounding the availability of soybeans and allergens (Alejandra *et al.*, 2025; Khosyati *et al.*, 2024). Of them, peanuts (*Arachis hypogaea*) have emerged as a potential substrate, due to their high-protein and fat content, widespread availability and distinctive flavour profile. However, the adaptation of tempeh fermentation in peanut substrates provides unique challenges due to differences in chemical composition and physical structure compared to soybeans.

The successful fermentation of tempeh is largely dependent on the growth and dominance of *Rhizopus* spp. and hence requires optimal environmental factors such as temperature, moisture content, aeration and substrate composition. The fungal mycelium must rapidly colonise the substrate to outcompete other bacteria and generate a uniform product (Samsul Rizal *et al.*, 2023). Insufficient early fungal dominance can lead to contamination with unwanted bacteria, spoilage, and ultimately rejection of the product. This is particularly important in small size fermentation systems because the ability to control the environment is sometimes limited.

The moisture content is a very important aspect for successful fermentation. Too much moisture increases the water activity, which provides an environment for bad bacteria and moulds to grow. On the other hand, lack of moisture may limit the growth of fungi and delay colonisation. Proper drying of substrates after boiling is critical for enhancing *Rhizopus* growth and minimising contamination risks (Moustapha Soungalo Drabo *et al.*, 2023) The high lipid content of the peanut substrates can influence the movement and retention of water and thus influence control of moisture during fermentation.

Another important factor is aeration for the aerobic metabolism of *Rhizopus* spp. Usually, tempeh is fermented in packaging with holes that permits oxygen exchange and maintains structure. If aeration is poor, localised anaerobic conditions might develop which encourage

the growth of undesirable bacteria. Excessive exposure, on the other hand, might cause dehydration and abnormal growth of fungi. Thus, the design of the packaging, including hole size, distribution, and substrate thickness, has a considerable impact on fermentation results.

The structure of the substrate and its packing density significantly affect the efficacy of fungal colonisation. Proximity among substrate particles promotes the development of a continuous mycelial network, allowing the substrate to join into a cohesive mass. Inadequate or irregular packing may result in voids that hinder mycelial development and cause uneven fermentation. Prior research has highlighted the necessity of sustaining uniform thickness and density to guarantee even heat distribution and oxygen accessibility (Nussbaum *et al.*, 2025).

Temperature is a crucial factor in tempeh fermentation, as it indicates microbial metabolic activity. In successful fermentation, temperature generally rises due to heat produced by fungal activity and thereafter stabilises (Castaneda *et al.*, 2026). Deviations from this trend, including abrupt declines or erratic oscillations, may signify disturbances in microbial activity or contamination. Temperature monitoring has been proposed as a straightforward and efficient method for evaluating fermentation progress in small-scale systems.

Accompanying physical and environmental considerations, pH variations offer insight into the metabolic processes that happen during fermentation. In regulated tempeh fermentation, pH variations are typically moderate and indicate the equilibrium between acid generation and protein breakdown. A substantial rise in pH may signify the formation of alkaline substances like ammonia, frequently linked to microbial degradation and the proteolytic actions of contaminating organisms (Ahnan-Winarno *et al.*, 2021; Wang D *et al.*, 2022). Consequently, pH monitoring can act as a gauge of fermentation quality and stability.

While these factors are well established, most of the research on tempeh fermentation has been conducted on systems utilising soybeans, whilst non-soy substrates, such as peanuts, have been relatively underexplored. The high oil content and the peculiar physical features of peanuts might influence the diffusion of oxygen, microbial activity and heat transfer in the substrate. The differences imply that the process parameters optimised for soybeans may not be directly applicable for peanut tempeh and the need for substrate-specific research.

Socio-technical variables are influencing the development of peanut tempeh. As the demand for sustainable and locally derived foods increases, peanut tempeh could be an alternative source of protein in locations where soybeans are not easily available or more expensive. Such innovation must be not only technically possible but also adaptable to small-scale production systems, especially in community-based and educational environments. Limited access to controlled fermentation facilities may increase the risk of contamination and process failure as it would not be possible to closely monitor environmental conditions.

The present study emphasises the complex nature of tempeh fermentation and the need for the optimisation of several, intersecting parameters to succeed. However, the knowledge of specific concerns connected to peanut-based tempeh is still missing, particularly at small-scale. Therefore, further research is needed to investigate the interaction between substrate characteristics, environmental variables and microbial behaviour to develop reliable and robust protocols for non-soy tempeh production.

Methodology

The fermentation technique was adapted to the preferability environment of the area as described by Adhianata *et al.* (2022).

Preparation of Peanut Substrate

The raw peanuts were sorted, rinsed and steamed for around 40 min till they were soft but still intact. The peanuts were cooked; the skins were manually peeled and completely drained. The substrates were chilled to <35 °C before to inoculation.

Inoculation and Packaging

The cooled peanuts were inoculated with commercial tempeh starter (*Rhizopus* spp.) at 0.3% (w/w) and mixed uniformly. The inoculated substrate was packed into perforated plastic bags with standardized thickness (1.7 cm) and surface area (15.5 cm X 10.0 cm) to ensure consistent fermentation geometry.

Fermentation Conditions

Fermentation was conducted at ambient temperature (28–32°C) for a maximum of approximately 96 hours. In order to facilitate sufficient ventilation, the samples were positioned on a wire rack. The experiment was conducted in nine replicates (N=9).

Fermentation Monitoring

Fermentation progress was monitored at 24-hour intervals. Observations included:

- Presence of contamination
- pH readings before and after
- Visual assessment of mycelial growth
- Temperature measurement using a probe thermometer

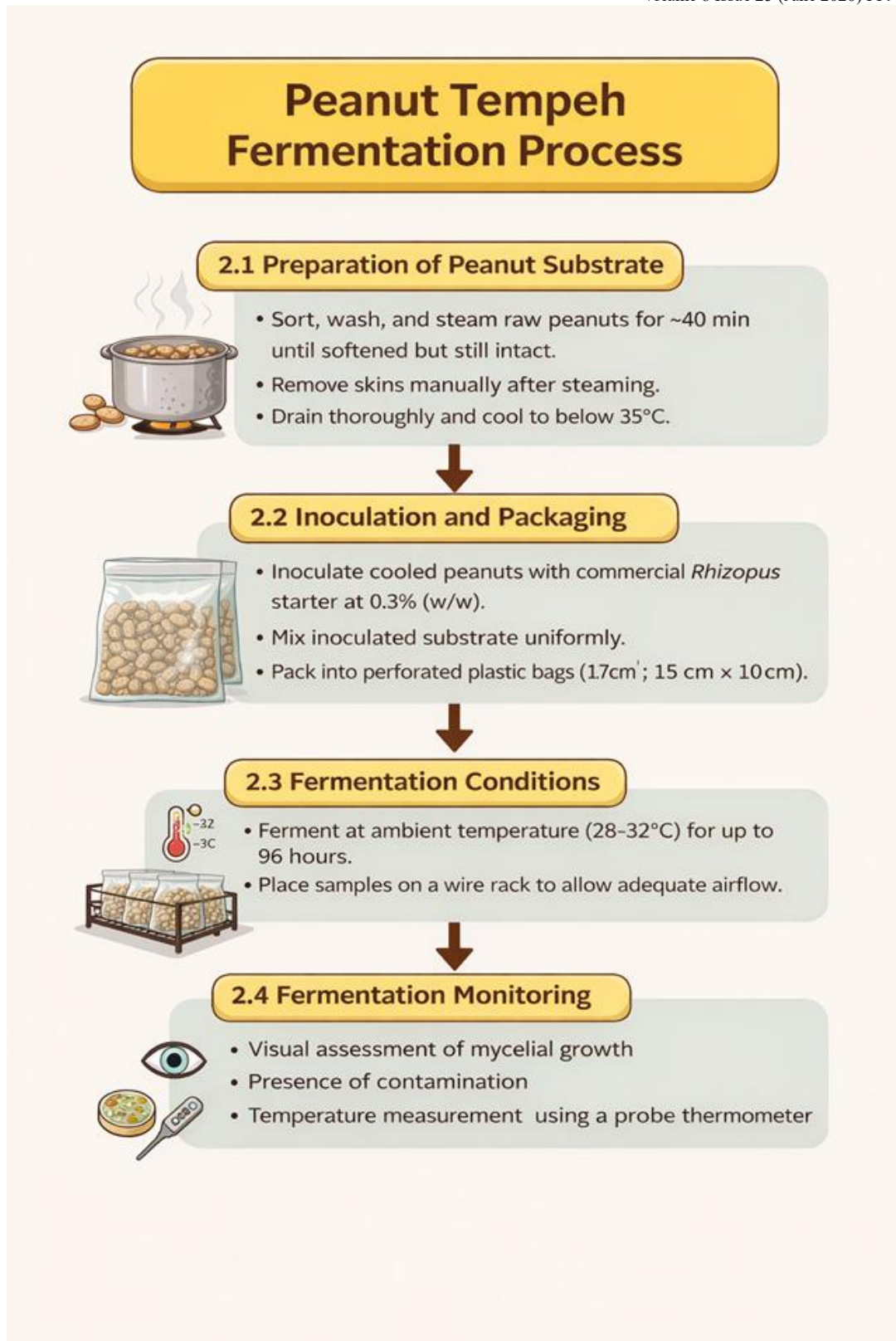


Figure 1: The Procedures of Peanut Tempeh Fermentation.

Results

Mycelium Growth Profile

A gradual decline in fermentation quality was observed from Day 0 to Day 3 (Figure 2). The peanut substrate showed minimal mycelial development on Day 1. The kernels remaining unbound and only minor surface moisture apparent, suggesting the early-stage colonisation. Day 2 revealed greyish mycelial growth and uneven colonisation, as well as increased condensation within the packaging, which might imply an unstable fermentation environment. Severe contamination was observed on Day 3, as evidenced by the presence of green-yellow fungal regions and grey-black mycelial overgrowth. Confirmation of complete fermentation failure was obtained when the substrate failed to establish a compact structure. *Rhizopus* spp.'s inability to establish dominance was likely influenced by early-stage conditions, such as moisture and substrate compactness, as per this progression.

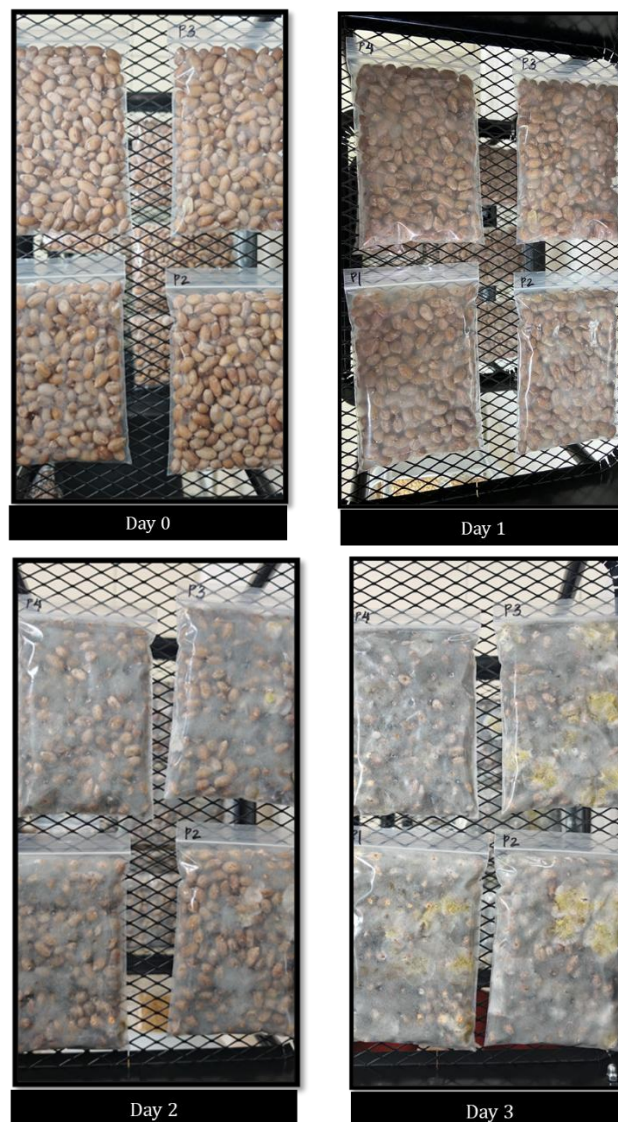


Figure 2: Temporal Progression of Peanut Tempeh Fermentation From Day 0 To Day 3.

Temperature Profile

Figure 3 illustrates the temperature profile of peanut tempeh fermentation from Day 0 to Day 3. The onset of microbial metabolic activity was suggested by the increase in mean temperature from 26.18°C on Day 0 to 31.01°C on Day 1. Nevertheless, a decrease in temperature was observed on Day 2 (29.29°C), which implies a decrease in fermentation activity. The temperature subsequently rose to 31.22°C on Day 3. The anticipated trend of stable or progressively increasing temperature in successful tempeh fermentation is not reflected in this fluctuating temperature pattern. The subsequent increase on Day 3 is likely to be associated with the metabolic activity of contaminating microorganisms rather than controlled fermentation, while the decrease in temperature on Day 2 may suggest that *Rhizopus* spp. growth has been disrupted. Supporting the existence of fermentation instability, the temperature trend is consistent with the visual progression of contamination that is observed throughout the fermentation period.

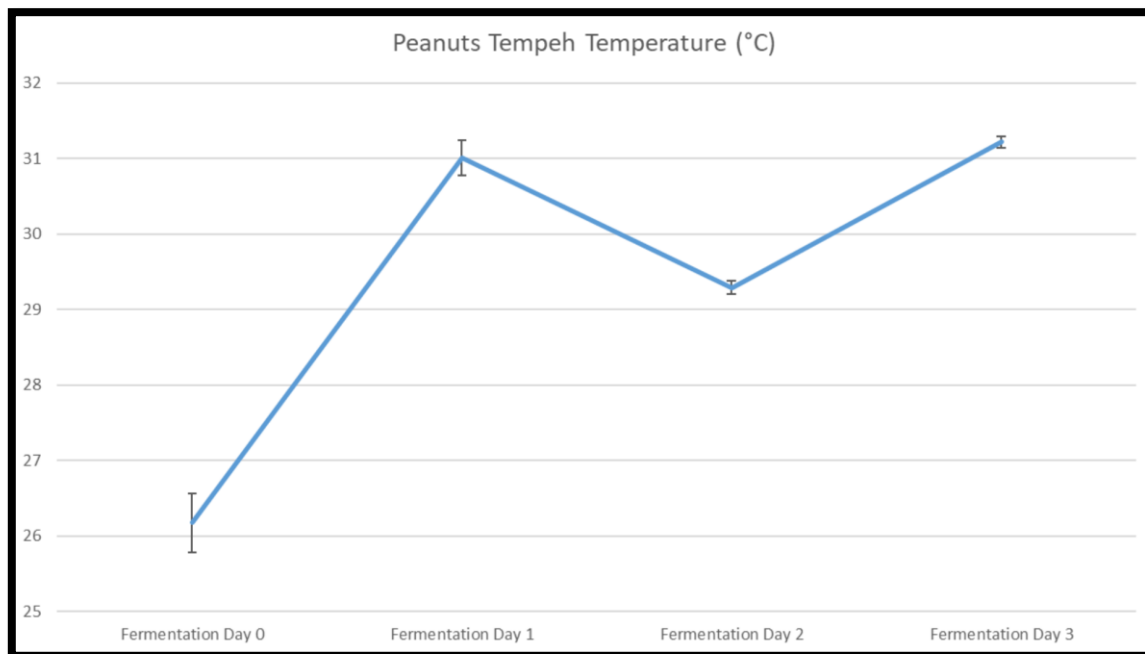


Figure 3: Temperature Profile of Peanut Tempeh Fermentation from Day 0 to Day 3.
(N = 9)

pH Profile

Results in Table 1 shows that the pH of peanut tempeh increased from 5.46 at Day 0 to 6.62 at Day 3. The rise in pH indicates a shift towards alkaline conditions during the fermentation process. This observation suggested the probability of undesirable microbial activity.

**Table 1: pH Values of Peanut Tempeh at Different Fermentation Stages.
Values Are Expressed as Mean (N = 9).**

PERIOD	AVERAGE PEANUTS TEMPEH (PH)
Fermentation Day 0	5.46
Fermentation Day 3	6.62

Discussion

Results reveal that contamination may be a reason for early fermentation failure. *Rhizopus* did not colonise successfully as there is no white mycelium present. It could be a result of poor aeration and high wetness which might have increased the competition between the bacteria. The temperature trend is strongly correlated with the visual trend of contamination from Day 0 to Day 3 and confirms the failure of controlled fermentation.

The first contamination was due to a fermentation environment not dominated by *Rhizopus* spp., as evidenced by the presence of greyish-black mycelium and green-yellow fungal colonies. *Rhizopus* produces a dense white mycelial network binding the substrate into a dense structure during tempeh fermentation (Tomás Vellozo-Echevarría *et al.*, 2024). The absence of this distinguishing feature and the presence of the fungal growth with varied colours indicate contamination by competing microorganisms.

Condensation in the packaging shows that there was an excess of retained moisture that may have allowed the growth of pollutants and impeded the correct colonisation of *Rhizopus*. The moisture content is an important factor influencing the success of fermentation, since the excessive water activity encourages the development of unwanted bacteria (Tomás Vellozo-Echevarría *et al.*, 2024).

Peanuts have a higher lipid content and different surface characteristics than soybeans which may influence microbial competition and aeration. The efficiency of the fermentation that impacts the growth of the fungal organisms and the creation of the metabolites (Irvan *et al.*, 2021) is significantly affected by the composition of the substrate.

The temperature profile during fermentation of peanut tempeh (Figure 3) was irregular and unstable, indicating the failure of the fermentation process. Temperature profiling has been shown to be an effective indication of fermentation progress and active tempeh fermentation is often associated with heat output due to fungal metabolism (Górska *et al.*, 2025). The initial rise in temperature from approx. 26.18°C on Day 0 to 31.01°C on Day 1 would indicate the beginning of microbial metabolic activity, possibly coinciding with the first colonisation by *Rhizopus* spp. The rise in temperature is attributed to the heat produced by the rapid growth of fungus (Ahnán-Winarno *et al.*, 2021; Wu *et al.*, 2025).

The temperature showed a significant dip on the second day (about 29.29°C). The decline is not according to the predicted pattern in successful tempeh fermentation where the temperature usually increases or remains the same due to the continued microbial activity (Ahnán-Winarno *et al.*, 2021; Wu *et al.*, 2025). The observed decline reflects a likely drop in the metabolic

activity of the selected starter culture, implying that *Rhizopus* spp. may not have retained its dominance in the substrate.

Interestingly, the temperature was again increased to around 31.22°C on Day 3. But the rise is not likely to be successful fermentation. This behaviour is explained by the metabolic activity of contaminating microorganisms, which is confirmed by visual evidence with grey-black mycelium and green-yellow fungal patches detected during this period. This suggests the fermentation system has progressed from regulated fungal fermentation to uncontrolled development of microorganisms.

The pattern of variable temperature shows the fragility of the fermentation process. It proves that the early stages of the process were disturbed probably because of high moisture, low density of the substrate and uneven aeration, which caused the failure of *Rhizopus* colonisation.

The rise in pH from 5.46 to 6.62 reported (Table 1) is not indicative of effective tempeh fermentation but is a reflection of the shift to alkaline circumstances. The *Rhizopus* spp. used in the traditional tempeh brewing process allows the controlled biochemical changes such as small amount of protein hydrolysis but not too much alkalinity (Devagopalan & Krishnaswamy, 2023). However, the considerable pH increase in this study is indicative of alkaline metabolite formation, perhaps as a consequence of contaminating bacteria activity.

This conclusion is also visually confirmed by the appearance of grey-black mycelium and green-yellow fungal patches indicating that mixed microbial populations rather than the starter culture dominated the fermentation system contrary to the intention. These bacteria might have decomposed protein to ammonia and other nitrogenous chemicals, which led to the observed pH increase (Ahn-an-Winarno *et al.*, 2021; Wang X *et al.*, 2022).

In addition, the alkaline shift is compatible with the unstable temperature profile observed, further confirming the disruption of the controlled fermentation. Increase of pH, temperature fluctuation and apparent contamination confirmed the failure of fermentation process of peanut tempeh under the conditions.

This study has used a packing geometry of 15.5 cm X 10 cm with a thickness of 1.7 cm which may have also contributed to the unsatisfactory fermentation outcome. The peanut substrate ziplock bags were not firmly packed, but the thickness was quite moderate. Therefore, the packaging structure was loose and the spaces between kernels could be observed. In tempeh fermentation, the substrate particles need to be in close proximity because the growing *Rhizopus* mycelium has to join the neighbouring kernels and bond them gradually into a compact cake (Irvan *et al.*, 2021; Ellent *et al.*, 2022). The substrate may be weakly structured resulting in discontinuous and uneven fungal colonisation and hence reduce the creation of a stable mycelial network. This inadequate structural contact may prevent or delay the proper binding of the substrate, especially at the early stage of fermentation, when the starter culture is still gaining the dominance.

Loose substrate arrangements can also lead to an unstable interior microclimate within the container. Excess empty space can cause to uneven moisture distribution, localised condensation and irregular heat transfer. The uneven growth of fungi is due to the fact that certain regions can have too much airflow, and others can be too damp. Under such conditions, the predicted *Rhizopus* culture may not colonise the substrate homogeneously, thus increasing

the possibility for competing microbes to settle. This could explain the visible looseness of the peanut kernels on Day 0 and contamination by Day 2. Therefore, the failure of this investigation might not be fully due to the peanut substrate per se but the interaction between the qualities of the substrate and the packing arrangement specifically the lack of compactness inside the fermentation sack.

In addition to the technical issues, this study brings out certain socio-technical issues in the development of peanut-based tempeh. Growing interest in non-soy alternatives makes customer acceptance, raw material availability and small-scale production practicality critical factors. Peanut tempeh has the potential to be a regionally adaptable, sustainable alternative to regular soybean tempeh, particularly where the availability or cost of soybean is limited.

However, the fermentation instability found in this work indicates that further process optimisation is necessary if such products are to be made reliably at community level. This study revealed that the danger of contamination could be higher in small-scale or home-based production systems where the environmental variables are more difficult to control. Thus, simple and realistic process controls such as improved substrate drying, compact packing and basic monitoring of temperature and pH are needed to improve the dependability of fermentation.

Furthermore, the results are of instructional relevance to expose real life challenges in fermentation processes. Incorporation of such case-based observations into the laboratory instruction may strengthen the student comprehension of microbial dynamics, process control and food safety. In general, the development of peanut tempeh should be done not only from the technical side but also from the practical implementation in actual life.

Practical Implication

The present work has various practical implications for the improvement of peanut tempeh fermentation in small scale settings. Given the reported failure of fermentation, it is advised that the substrate of peanuts be sufficiently dried after boiling to keep the surface moisture to a minimum. And the substrate also needs to be packed more densely, so that a sufficient contact between the kernels is provided for an effective binding of the mycelium during fermentation.

The packaging design should also be optimised to allow better air movement and to minimise condensation in the fermentation bag. An increase in the number or distribution of perforations and a constant and moderate thickness may aid to generate a more stable fermentation environment. The daily recording of temperature and pH levels as basic monitoring tools also helps in early detection of aberrant fermentation patterns and prompt action.

These practical adaptations are especially valuable for student laboratories, community-based producers and small-scale food entrepreneurs who may not have access to regulated fermentation facilities. These low-cost process changes may increase fermentation dependability and reduce contamination risk in the development of peanut-based tempeh products.

Conclusion

This study demonstrates that peanut tempeh fermentation under the tested small-scale conditions was unsuccessful, as evidenced by visual contamination, unstable temperature patterns, and an increase in pH. The fermentation process failed to establish effective *Rhizopus* colonization, resulting in incomplete substrate binding and the development of mixed microbial contamination.

The temperature profile showed a non-linear trend, with an initial increase followed by a decline and a subsequent rise, indicating disruption of normal fermentation activity. In addition, the increase in pH from 5.46 to 6.62 suggests the production of alkaline metabolites associated with microbial spoilage rather than controlled fermentation. These findings are consistent with the observed progression from minimal mycelial growth at early stages to severe contamination at later stages.

The failure of fermentation was likely influenced by multiple interacting factors, including excessive moisture retention, insufficient substrate compactness within the packaging, and uneven aeration. The loose arrangement of peanut kernels may have limited effective mycelial bridging, delayed the establishment of *Rhizopus* dominance and allowed competing microorganisms to proliferate.

Overall, this study highlights the critical importance of early-stage process control in non-soy tempeh fermentation. Parameters such as substrate moisture, packing density, and aeration must be carefully optimized to ensure successful fermentation. The findings provide practical insights for improving peanut tempeh production and support future optimization studies aimed at developing reliable non-soy tempeh products. Future studies should focus on optimizing substrate preparation and packaging conditions to enhance fermentation stability and product quality.

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