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Effect of Soaking Step on Tempeh Quality: A Narrative Review

Rifqi Ahmad Riyanto*
Department of Food Technology, Universitas Sultan Ageng Tirtayasa, Banten, Indonesia

*Corresponding Author : rifqi.ar@untirta.ac.id

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ABSTRACT
Tempeh is a traditional Indonesian fermented food in which soaking plays an important role in determining fermentation performance and final product quality. Previous studies have reported varied effects of soaking duration and soaking media on the chemical, microbiological, physical, and sensory characteristics of tempeh. This study aimed to review and synthesize existing research on the effects of soaking conditions on tempeh quality. A narrative literature review was conducted using scientific articles published in national and international journals between 2015 and 2026. Relevant studies were identified through searches of Google Scholar, Scopus, ScienceDirect, and Garuda, and the findings were synthesized descriptively according to raw materials, soaking duration, soaking media, and reported tempeh quality parameters. The reviewed literature indicates that appropriate soaking conditions can improve hydration, lower pH, promote the development of amino acids, and reduce antinutritional compounds, thereby supporting *Rhizopus* spp. growth during fermentation. Soaking media containing organic acids, including pineapple peel extract, bilimbi juice, vinegar, and lactic acid bacteria, have also been reported to influence fermentation performance and tempeh quality. However, the effects vary depending on the raw materials, soaking conditions, and experimental methods used. Overall, optimizing soaking duration and soaking media represents a promising strategy for improving tempeh quality and promoting more consistent production. Further research is needed to establish optimal soaking conditions for different raw materials and production systems.

INTRODUCTION

Tempeh is a traditional Indonesian fermented food that has long been consumed and is recognized as a high-quality plant protein source. It is produced by fermenting soybean kernels with *Rhizopus* spp., resulting in physical, chemical, and biological changes that improve nutritional value, protein digestibility, and sensory characteristics compared with the raw material (Romulo & Surya, 2021). In addition to protein, tempeh contains bioactive compounds such as peptides, isoflavones, vitamins, and antioxidants that contribute to its potential health benefits (Prativi et al., 2023).

Tempeh production involves several important stages, including sorting, washing, boiling, soaking, dehulling, starter inoculation, wrapping, and fermentation. Among these stages, soaking plays a critical role because it is an initial step that influences subsequent fermentation. During soaking, soybean hydration and softening occur, some antinutritional compounds are released, and naturally occurring lactic acid bacteria may proliferate, resulting in a decrease in pH (Tamang et al., 2022). This acidic condition can inhibit contaminating microorganisms while creating an environment conducive to *Rhizopus* growth during fermentation.

Various studies have shown that differences in soaking duration and soaking media produce different effects on tempeh quality. Conventional water soaking for 8–24 h is still widely used in household-scale production, whereas other studies have explored soaking media containing pineapple peel extract, bilimbi juice, vinegar, Glucono Delta Lactone (GDL), or added lactic acid bacteria to accelerate acidification and improve product quality. These innovations aim to shorten processing time, enhance fermentation activity, improve chemical characteristics, and obtain better sensory quality.

Nevertheless, published findings remain considerably variable. Some studies report that 8 h of soaking is sufficient to produce optimal tempeh quality, whereas others indicate better results at 10, 12, or even 24 h. Differences in soaking media also produce diverse responses in chemical and sensory characteristics. Such variation may be influenced by raw material type, acidulant concentration, fermentation conditions, ambient temperature, and processing methods used in individual studies.

The diversity of findings highlights the importance of reviewing and synthesizing existing research to develop a broader understanding of the effects of soaking on tempeh characteristics. Therefore, this study aimed to review published research on the effects of soaking conditions on tempeh quality, with particular emphasis on soaking duration, soaking media, and their influence on the chemical, microbiological, physical, and sensory characteristics of tempeh. By examining findings across studies involving soybeans and alternative raw materials, this review seeks to identify common trends, variations in reported outcomes, and areas requiring further investigation. The findings are expected to provide a scientific basis for understanding the role of soaking in tempeh production and to inform future research aimed at optimizing soaking conditions and improving the consistency of tempeh quality.

MATERIALS AND METHODS

Materials

This review was based on secondary data obtained from scientific research articles published in national and international peer-reviewed journals. The articles examined soaking processes in tempeh production using soybeans and alternative raw materials, including lablab bean, jack bean, pigeon pea, rubber seed, faba bean, sorghum, and other legumes processed according to tempeh fermentation principles. The selected literature provided information on soaking conditions and their effects on the chemical, microbiological, physical, and sensory characteristics of tempeh.

Methods

This study employed a narrative literature review approach to examine and synthesize existing research on the role of soaking in tempeh production. Relevant literature was identified through searches of Google Scholar, Scopus, ScienceDirect, and Garuda. The search strategy combined keywords related to tempeh, soaking, and product quality, using the following Boolean expression: *(tempe OR tempeh) AND (soaking OR soaking time OR soaking duration) AND (quality OR protein OR fermentation)*. Additional relevant articles were considered when they provided information directly related to the research objectives.

The literature search focused on articles published between 2015 and 2026. Articles were considered relevant when they reported original experimental research on soaking treatments in tempeh production using soybeans or alternative raw materials. Particular attention was given to studies investigating variations in soaking duration, soaking media, the use of acidulants, and combinations of pretreatment conditions. Articles reporting quantitative results for at least one tempeh quality parameter and providing accessible full-text information were prioritized. Review articles, conference proceedings, unpublished undergraduate theses, master's theses, and doctoral dissertations were not included in the main analysis. Articles that did not adequately describe their experimental methods or examined fermentation without addressing a soaking treatment were also excluded from the synthesis.

The selection of literature was guided by its relevance to the research objectives, the adequacy of the reported methodology, the variables examined, and the availability of relevant data. The initial literature search identified 20 relevant articles, which were subsequently examined for their suitability for inclusion in the review. The final synthesis focused on studies providing information relevant to understanding the relationship between soaking conditions and tempeh quality.

Data from the selected articles were extracted and organized according to four main aspects: (1) raw material used in tempeh production; (2) soaking duration; (3) soaking medium and the use of acidulants; and (4) reported quality parameters, including chemical composition, microbiological characteristics, physical properties, and sensory attributes. The findings were analyzed descriptively and compared across studies to identify similarities, differences, and emerging patterns in the effects of soaking treatments on tempeh quality. Differences in raw materials, experimental conditions, and analytical methods were considered when interpreting the findings. The results were then synthesized narratively to provide an overview of current knowledge, highlight research gaps, and identify potential directions for further research on soaking optimization in tempeh production.

RESULTS AND DISCUSSION

Article Selection and Study Characteristics

Literature identification across several scientific databases yielded studies addressing the effects of soaking on tempeh quality. After screening for topic relevance, study type, data availability, and compliance with the inclusion and exclusion criteria, 20 articles were included in the synthesis. The studies comprised national and international research published during 2015–2026 and addressed variation in soaking duration, soaking media, and pretreatment conditions affecting tempeh quality.

The analyzed studies used diverse raw materials, including soybean (*Glycine max*), lablab bean (*Lablab purpureus*), jack bean (*Canavalia ensiformis*), pigeon pea (*Cajanus cajan*), rubber seed (*Hevea brasiliensis*), faba bean (*Vicia faba*), sorghum, and other legumes. This diversity indicates that soaking is an important processing step across tempeh-like fermentation systems based on legumes.

The studies also applied diverse soaking treatments. Some used water as a conventional medium, whereas others used natural acidulants such as pineapple peel extract, bilimbi juice, vinegar, Glucono Delta Lactone (GDL), or lactic acid bacterial inocula to accelerate acidification during soaking. These treatments were intended to accelerate fermentation, improve chemical quality, enhance sensory characteristics, and reduce antinutritional compounds.

The quality parameters evaluated included protein, moisture, fat, ash, pH, amino acids, antioxidant activity, lactic acid bacteria counts, mold counts, texture, color, aroma, mycelial compactness, and panelist acceptance. Collectively, these parameters represent chemical, physical, microbiological, and sensory aspects of tempeh quality.

Table 1. Characteristics of the analyzed studies

No.	Authors	Raw material	Soaking treatment	Main parameters	Main finding
1	Suparno et al. (2020)	Soybean	4, 8, 12, 16 h	pH, amino N, LAB, sensory	8 h produced the best quality.
2	Yusuf & Amaro (2021)	Soybean	Bilimbi juice, 0–62.5%	Chemical, microbiological, sensory	25% produced the best quality.
3	Putri et al. (2022)	Soybean	Pineapple core	Protein	Soaking increased protein content under the optimum treatment.
4	Dahlan et al. (2022)	Soybean	Vinegar	Metabolome, sensory	Vinegar soaking altered the metabolite profile and improved sensory acceptance.
5	Yarlina et al. (2023)	Jack bean	12, 24, 36, 48 h	Protein, amino acids	12 h produced the highest protein and amino acid contents.
6	Isnaini et al. (2024)	Local soybean	Pineapple peel extract + 6–10 h	Chemical	The fastest fermentation occurred with 30% pineapple peel extract and 10 h soaking.
7	Werdiningsih et al. (2024)	Lablab bean	LAB during soaking	pH, antinutritional compounds	LAB accelerated acidification and shortened soaking time.
8	Fernandez Castaneda et al. (2024)	Faba bean	Water, vinegar, $NaHCO_3$	Texture, boiling time	Soaking medium affected physical properties and processing time.
9	Siska et al. (2026)	Jack bean	Different soaking durations	Physical, chemical, sensory	Soaking significantly affected moisture, pH, fat, aroma, and taste.
10	Habibie et al. (2025)	Rubber seed	0–36 h	HCN, proximate composition	24 h effectively reduced HCN without major nutrient loss.

Table 1 indicates a consistent tendency that soaking is an important stage in determining the final quality of tempeh. Nearly all studies reported effects on at least one quality parameter, including protein content, pH, microbial activity, physical characteristics, or sensory quality. Although soaking durations and media varied, the overall direction suggests that optimizing the soaking stage can improve fermentation performance and produce better-quality tempeh.

Effect of Soaking Duration on the Chemical Characteristics of Tempeh

Soaking duration is an important factor determining the success of tempeh fermentation. During soaking, water absorption, tissue softening, activation of naturally occurring microorganisms, and pH reduction due to lactic acid bacterial activity occur (Drulyte & Orlien, 2019; Zhang et al., 2021). These changes create conditions that support Rhizopus spp. growth during subsequent fermentation. The synthesis indicates that soaking duration affects nearly all major chemical parameters of tempeh, particularly protein, moisture, pH, fat, amino acids, and antinutritional compounds.

Across the studies, an optimal soaking duration was associated with improved protein quality. During fermentation, *Rhizopus* produces proteolytic enzymes that hydrolyze complex proteins into peptides and amino acids, thereby improving protein digestibility. However, improved protein quality does not necessarily correspond to a quantitative increase in total protein. Excessively long soaking may cause soluble nitrogenous compounds to leach into the soaking water, resulting in lower protein content. In the study by Yarlina et al. (2023), 12 h of soaking produced the highest protein and amino acid contents in jack bean tempeh compared with 24, 36, and 48 h. The study also indicated that proteolytic bacteria developed during soaking and contributed to free amino acid formation before fermentation (Kieliszek et al., 2021).

Soaking duration also significantly affects pH. The decrease in pH during soaking is associated with lactic acid bacteria utilizing simple sugars as substrates and producing organic acids (Punia et al., 2022). This acidic condition is important because it inhibits contaminating microorganisms while supporting Rhizopus spp. during subsequent fermentation. Suparno et al. (2020) reported that soaking duration significantly affected pH, moisture content, amino nitrogen, lactic acid bacteria counts, and sensory quality of soybean tempeh, with 8 h producing the best overall quality.

Changes in moisture content during soaking are another factor affecting fermentation success. Water uptake softens the bean tissue and facilitates fungal mycelial penetration (Nout & Kiers, 2005). However, excessive moisture can increase the risk of undesirable microbial growth if fermentation is not initiated promptly. Therefore, soaking duration should be adjusted to provide sufficient moisture for fermentation without increasing deterioration risk. Research on jack bean tempeh showed that longer soaking increased moisture content and decreased pH, which subsequently affected physical characteristics and panelist acceptance (Siska et al., 2026).

Soaking also affects antinutritional compounds. During soaking, inhibitory compounds such as tannins, phytate, and hydrogen cyanide may diffuse into the soaking medium, reducing their concentrations in the raw material. Habibie et al. (2025) reported that 24 h soaking of rubber seeds effectively reduced HCN to a safe level after subsequent heating without substantial nutrient loss. This finding indicates that soaking serves not only as a fermentation preparation step but also as an important food-safety intervention.

Overall, the findings indicates a consistent influence of soaking duration on tempeh chemical characteristics, although the magnitude of the effect differs among studies. Such differences may be related to raw material type, soaking medium, process temperature, fermentation duration, and processing methods. Nevertheless, the studies generally agree that soaking is an initial process step that influences fermentation success by improving hydration, lowering pH, supporting protein and amino acid availability, and reducing antinutritional compounds. Optimization of soaking duration is therefore important for obtaining favorable chemical quality.

Effect of Soaking Media on Fermentation and Tempeh Quality

In addition to soaking duration, the soaking medium influences fermentation performance and the resulting quality of tempeh. Based on the synthesized studies, soaking media can be grouped into conventional water, media containing organic acids such as vinegar, pineapple peel extract, and bilimbi juice, and media enriched with lactic acid bacteria. Each medium produces different chemical and microbiological conditions that influence *Rhizopus* spp. growth and the final characteristics of tempeh.

Water soaking remains the most common method, particularly in household-scale production. During this process, naturally occurring lactic acid bacteria gradually proliferate and produce organic acids that lower the pH of the material. The pH reduction is necessary to suppress spoilage microorganisms while creating conditions suitable for the fermentation mold. However, natural acidification generally requires more time, and

its effectiveness may be affected by water quality, ambient temperature, and the microbial population associated with the raw material (Nout & Kiers, 2005).

Table 2. Comparison of soaking media

Soaking medium	Effect on pH	Effect on protein	Sensory effect	Conclusion
Water	Low	Moderate	Good	Conventional method
Pineapple peel extract	Lower	Moderate	Good	Promising
Bilimbi juice	Lower	Moderate	Good	Promising
Vinegar	Lower	Moderate	Better	Improves flavor
LAB (*L. plantarum*)	Lowest	Moderate	Good	Fastest fermentation

Several studies have explored natural acidulants as soaking media to accelerate acidification. Pineapple peel extract is one such medium because it contains organic acids and bromelain. Isnaini et al. (2024) reported that pineapple peel extract at an appropriate concentration accelerated fermentation and produced better chemical characteristics than water soaking. In addition to promoting a faster pH decrease, pineapple peel extract may enhance enzymatic activity during the early stage of fermentation, thereby supporting faster mold growth.

Bilimbi juice produced similar effects. Its citric acid and other organic acids can accelerate acidification and promote more stable fermentation. The reported optimum bilimbi concentration produced better chemical, microbiological, and sensory characteristics than treatments without an acidulant. Improvements included faster pH reduction, more uniform mold growth, and increased panelist acceptance of tempeh color, aroma, and texture (Yusuf & Amaro, 2021).

Vinegar has also been reported to accelerate acidification. Dahlan et al. (2022) found that tempeh produced using vinegar soaking exhibited a different metabolite profile from the conventional process. Metabolomic analysis indicated changes in fermentation-derived compounds associated with improved flavor and aroma. Sensory evaluation also showed higher acceptance for tempeh subjected to vinegar soaking than for the control.

Another approach is the addition of lactic acid bacteria during soaking. Werdiningsih et al. (2024) showed that inoculation with Lactiplantibacillus plantarum accelerated pH reduction, reduced tannin content, and improved the sensory characteristics of tempeh made from lablab bean. The presence of lactic acid bacteria also accelerated the establishment of a selective fermentation environment, potentially reducing the risk of spoilage microorganism contamination. This approach may provide an alternative for shortening production time while improving product-quality consistency.

Although various soaking media show positive effects on fermentation, their effectiveness depends on raw material type, acidulant concentration, soaking duration, and fermentation conditions. A medium that is excessively acidic may inhibit mold growth if pH decreases too far (Massa et al., 2024), whereas insufficient acidification may fail to suppress contaminating microorganisms. Therefore, soaking-medium selection should balance acidification, fermentation-microorganism activity, and final product quality.

CONCLUSIONS

Based on the review of published studies, soaking is an important processing stage that influences fermentation success and tempeh quality. Variation in soaking duration and soaking medium affects the chemical, microbiological, physical, and sensory characteristics of the product. An optimal soaking duration improves bean hydration, lowers pH, supports lactic acid bacterial growth, promotes amino acid formation, and reduces antinutritional compounds, thereby creating more favorable conditions for *Rhizopus* spp. growth during fermentation. In contrast, insufficient soaking may fail to establish optimal conditions, whereas excessive soaking may cause nutrient losses and increase the risk of microbial contamination. This also indicates that soaking media containing organic acids, including pineapple peel extract, bilimbi juice, vinegar, and lactic acid bacteria, tend to accelerate pH reduction, improve fermentation efficiency, and produce better-

quality tempeh than water soaking. Thus, optimization of both soaking duration and soaking medium is a promising strategy for improving tempeh quality and process consistency.

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