

The Role of Rhizopus oligosporus in Enhancing Protein and Mineral Bioavailability in Tempeh: A Literature Review

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Abstract: Tempeh is a fermented soybean-based food that plays an important role in meeting the protein and mineral needs of the Indonesian population. The fermentation process of tempeh involves the mold Rhizopus oligosporus, which contributes to the transformation of soybean chemical components through enzymatic activity. This article aims to review the role of Rhizopus oligosporus in enhancing protein and mineral bioavailability in tempeh based on a literature review of Indonesian scientific journals and academic books published over the last five years. The method employed was a literature review focusing on accredited national publications related to tempeh fermentation, protein digestibility, and mineral bioavailability. The findings indicate that Rhizopus oligosporus produces proteolytic enzymes capable of hydrolyzing complex soybean proteins into peptides and free amino acids, thereby improving protein digestibility and biological quality. In addition, phytase enzyme activity during fermentation plays a significant role in reducing phytic acid content, an antinutritional compound, which subsequently enhances the availability of essential minerals such as iron and calcium. The increased bioavailability of protein and minerals positions tempeh as a potential functional food that can contribute to improving community nutritional status. Therefore, optimizing tempeh fermentation using Rhizopus oligosporus should continue to be developed to enhance nutritional quality and strengthen the competitiveness of tempeh as a superior local food product.

Keywords: tempeh, Rhizopus oligosporus, fermentation, protein, minerals

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INTRODUCTION

Tempeh is a fermented soybean-based food that has long been an important part of Indonesian dietary patterns. It is widely recognized as an affordable plant-based protein source with high nutritional value and a strategic role in meeting population-level protein needs. Compared with raw soybeans, tempeh has key advantages due to fermentation, which can improve nutritional quality, enhance digestibility, and increase the functional value of the food (Astuti, 2020).

Tempeh is produced through soybean fermentation. During fermentation, the white tissue that binds the beans is formed by mycelium from species within the genus Rhizopus. This mold functions to bind soybeans into compact cakes that take the shape of the container. Tempeh is generally prepared by boiling soybeans and fermenting them with Rhizopus spp. The fermentation process results in the production of proteolytic enzymes that break down proteins into peptides and free amino acids.

Several studies have discussed tempeh production. One study reported that tempeh processing consists of four stages: soaking, boiling, fermentation, and incubation at room temperature. Fermentation occurs after the beans are packaged, either in plastic or in leaf wrapping. The same study also noted that in Indonesia, innovations have expanded tempeh beyond soybeans to other legumes such as jack beans (koro), peas, and velvet beans. Another study identified additional raw materials besides soybeans, including corn, koro beans, lamtoro (Leucaena) beans, and mung beans.

Other research reported two general approaches to tempeh production: traditional and modern processing. Traditional processing includes boiling, dehulling, washing, reboiling, cooling, and packaging. Modern production begins with mechanical dehulling, followed by boiling, washing, reboiling, cooling, and inoculation with mold starter. According to this study, traditional processing may lead to uneven fungal growth, often appearing darker or blackish, whereas modern processing tends to produce more uniform white mycelial growth (Suknia S. L., & Rahmani, 2020).

Microorganisms are living organisms that cannot be seen with the naked eye and play critical roles in energy and nutrient cycles as decomposers. They can be found across diverse environmental conditions and spread through various mechanisms among living organisms or through the environment. Microbial growth is commonly measured by an increase in cell numbers, which can occur rapidly to yield large populations. Microorganisms include bacteria, protozoa, fungi, algae, and viruses. Microbes are also ideal systems for exploring fundamental biological questions such as specialization, origins, adaptation, cellular function, biochemistry, genetics, and other characteristics of living organisms. Microbial roles can be beneficial or harmful. Benefits to humans include microbial control to prevent the spread of disease or infection, inhibition of bacterial growth, prevention of material deterioration by microbes, and avoidance of unwanted bacterial contamination in growth media (Sari W. P., et al., 2024).

Fermentation is an important biochemical process that occurs naturally and has long been utilized in the food and beverage industries. One of the most commonly used microorganisms in fermentation is *Saccharomyces cerevisiae*, which can convert sugars into alcohol and carbon dioxide through anaerobic respiration. This process is not only commercially valuable but also provides an engaging scientific topic for learning contexts, particularly for elementary students who are beginning to explore science through practical activities. From a philosophical perspective, fermentation illustrates a harmonious relationship between humans and nature. Humans utilize natural microbial processes to meet daily needs, reflecting reciprocal interactions between people and the environment. Such understanding is important so that younger generations develop awareness of environmental stewardship and use scientific knowledge wisely (Laila I. N., et al., 2025).

Tempeh fermentation is carried out with molds from the genus *Rhizopus*, particularly *Rhizopus oligosporus*, which serves as the primary microorganism. This mold grows to form mycelium that binds soybean seeds and produces various extracellular enzymes, including protease, lipase, and phytase. These enzymatic activities are central to the chemical and biological transformations of soybeans during tempeh fermentation (Winarno, 2021).

Regarding protein, fermentation by *Rhizopus oligosporus* is known to increase protein availability and digestibility by breaking down complex proteins into peptides and free amino acids. This transformation makes tempeh protein easier to digest and absorb than protein in unfermented soybeans. Several Indonesian studies have reported that tempeh fermentation significantly improves protein digestibility and the biological quality of plant-based protein (Septiani & Nurhartadi, 2023).

In addition to protein, tempeh is a source of essential minerals such as iron (Fe), calcium (Ca), phosphorus (P), and magnesium (Mg). However, in raw soybeans, antinutritional compounds such as phytic acid can inhibit the absorption of these minerals. Fermentation by *Rhizopus oligosporus* reduces phytic acid levels through phytase enzyme activity, thereby improving mineral bioavailability in tempeh (Utami et al., 2021).

Improved protein and mineral availability through tempeh consumption has important implications for Indonesian public nutrition, particularly in preventing protein deficiency and micronutrient deficiencies such as iron-deficiency anemia. Given the high consumption of tempeh in Indonesia, a scientific understanding of the role of *Rhizopus oligosporus* in enhancing tempeh's nutritional value is highly relevant as a foundation for developing local fermented foods (Kementerian Kesehatan RI, 2020).

Based on the discussion above, this literature review aims to comprehensively examine the role of *Rhizopus oligosporus* in increasing protein and mineral bioavailability in tempeh. This understanding is expected to strengthen the scientific basis for tempeh as a local functional food and to encourage optimization of fermentation processes in developing tempeh products with higher nutritional value and competitiveness (Handayani & Marsono, 2022).

RESEARCHMETHOD

This study employed a literature review approach, which aims to collect, critically examine, and synthesize findings from previous studies relevant to the topic under investigation. A literature review was selected because it enables researchers to obtain a comprehensive understanding of the role of *Rhizopus oligosporus* in improving protein and mineral bioavailability in tempeh based on previously published empirical evidence (Sukmadinata, 2020).

The data sources consisted of accredited Indonesian national scientific journals, scholarly reference books, and official reports from Indonesian institutions published within the last five years (2020–2025). Literature was retrieved through searches on national journal portals such as Garuda (Garba Rujukan Digital), SINTA (Science and Technology Index), and Indonesian university repositories. The use of national sources was intended to ensure contextual relevance to Indonesian food systems, consumption practices, and tempeh-related research (Arikunto, 2021).

The inclusion criteria were as follows: (1) articles or books discussing tempeh fermentation, (2) studies examining the activity of *Rhizopus oligosporus* or tempeh-associated molds, (3) literature addressing the availability or bioavailability of protein and/or minerals, and (4) publications with clear methodology and academic credibility. Sources that were not relevant to the topic or lacked clear scientific validity were excluded from the review (Utami et al., 2021).

Data collection was conducted by searching Indonesian-language keywords, including “*Rhizopus oligosporus*,” “tempeh fermentation,” “tempeh protein digestibility,” and “tempeh mineral availability.” All retrieved articles were then read in full, key points were recorded, and the literature was classified according to the main focus: improvement of protein availability and improvement of mineral availability (Septiani & Nurhartadi, 2023).

Data analysis used a descriptive qualitative approach by comparing and synthesizing findings across sources to identify patterns, similarities, and differences regarding the role of *Rhizopus oligosporus* in tempeh fermentation. The results were then presented in a systematic and structured narrative to provide an integrated understanding of the mechanisms underlying nutritional enhancement in tempeh (Handayani & Marsono, 2022).

RESULTS AND DISCUSSION

Role of *Rhizopus oligosporus* in Enhancing Protein Availability in Tempeh

Tempeh is a fermented soybean-based food product with high nutritional value. It contains approximately 18–20 g of protein per 100 g, making it an excellent source of plant-based protein (Aryanta, 2020). In addition to protein, tempeh contains unsaturated fatty acids, dietary fiber, B-complex vitamins (B1, B2, B6, and B12), iron, calcium, magnesium, and isoflavones with antioxidant properties.

One of the main advantages of tempeh compared with raw soybeans lies in the fermentation process involving the mold *Rhizopus oligosporus*. This process not only enhances nutrient digestibility and availability but also reduces antinutritional compounds such as phytic acid and tannins, which can inhibit the absorption of essential minerals such as iron and calcium. Protein quality in tempeh also increases significantly because fermentation breaks down complex proteins into amino acids that are more easily digested by the human body (Azzahra et al., 2025). Previous studies reported that tempeh fermentation increases iron bioavailability by up to 60% compared with unfermented soybeans (Fujiana et al., 2021).

Fermentation by *Rhizopus oligosporus* plays a crucial role in improving protein availability through the activity of proteolytic enzymes produced during fermentation. Protease enzymes secreted by *R. oligosporus* hydrolyze complex soybean proteins into peptides and free amino acids. This transformation simplifies protein structures, making them more digestible and absorbable than proteins in raw soybeans (Septiani & Nurhartadi, 2023).

Improved protein digestibility in tempeh is also associated with structural modifications of the soybean matrix caused by fungal mycelial growth. The mycelial network penetrates soybean cell walls and softens seed texture, facilitating access for digestive enzymes. Several Indonesian studies have demonstrated that

protein digestibility in tempeh is higher than in boiled soybeans or non-fermented soybean products, confirming the essential role of fermentation in improving plant protein quality (Astuti, 2020).

In addition to digestibility, fermentation contributes to the enhancement of protein biological value. Protein hydrolysis increases the availability of essential amino acids, supporting tissue growth and maintenance. Consequently, tempeh serves as an effective plant-based protein source, particularly for populations with limited access to animal protein (Winarno, 2021).

Role of *Rhizopus oligosporus* in Enhancing Mineral Availability in Tempeh

Rhizopus oligosporus contributes significantly to increasing mineral availability in tempeh through the production of phytase enzymes during fermentation. Phytase hydrolyzes phytic acid naturally present in soybeans. Phytic acid is an antinutritional compound that chelates minerals such as iron, zinc, calcium, and magnesium, thereby reducing their absorption in the human body. Degradation of phytic acid by phytase releases bound minerals, making them more biologically available (Handoyo et al., 2021).

In addition, *R. oligosporus* enhances mineral solubility through changes in the fermentation environment. Metabolic activity during fermentation leads to a decrease in pH, causing minerals to remain in more soluble ionic forms. This increased solubility directly improves the bioavailability of both macro- and microminerals in tempeh compared with raw soybeans (Rahmawati & Nurhayati, 2022).

Another important function of *R. oligosporus* is the degradation of soybean cell wall structures through the production of cellulase and protease enzymes. This breakdown releases minerals previously trapped within cellular matrices and organic complexes, enabling greater accessibility for digestive enzymes and improving mineral absorption (Sari et al., 2023).

Furthermore, fermentation reduces mineral interactions with other inhibitory compounds such as polyphenols and crude fiber. Structural modification of these compounds during fermentation lowers their mineral-binding capacity, further enhancing mineral bioavailability in tempeh as a fermented food product (Pratama et al., 2024).

Collectively, the roles of *Rhizopus oligosporus* during tempeh fermentation include antinutrient degradation, increased mineral solubility, release of minerals from cellular matrices, and reduction of absorption inhibitors. These mechanisms contribute to the high nutritional value and improved mineral bioavailability of tempeh (Astawan & Wresdiyati, 2020).

Beyond protein enhancement, the role of *R. oligosporus* in mineral improvement is particularly significant. Raw soybeans naturally contain antinutritional compounds, especially phytic acid, which bind minerals such as iron (Fe), calcium (Ca), and magnesium (Mg), thereby limiting absorption. Tempeh fermentation markedly reduces phytic acid levels through phytase activity produced by *R. oligosporus* (Utami et al., 2021).

The reduction of phytic acid directly increases mineral bioavailability. National studies have shown that tempeh contains higher bioavailable iron and calcium levels compared with raw or boiled soybeans, demonstrating that fermentation not only preserves mineral content but also enhances mineral absorption efficiency (Handayani & Marsono, 2022).

Improved mineral availability in tempeh has important nutritional implications, particularly in preventing iron-deficiency anemia and bone health disorders. Regular tempeh consumption represents an effective local food strategy to help meet mineral requirements in Indonesia, considering its accessibility, affordability, and cultural acceptance (Ministry of Health of the Republic of Indonesia, 2020).

Implications of Tempeh Fermentation for Nutrition and Public Health

The combined improvement in protein and mineral availability positions tempeh as a functional food with strategic importance for enhancing public nutritional status. Fermentation by *Rhizopus oligosporus* enables tempeh to function not only as a source of energy and protein but also as a valuable provider of essential micronutrients. This role is highly relevant in addressing Indonesia's double burden of malnutrition, including protein deficiency and micronutrient insufficiency (Astuti, 2020).

From a sustainable food perspective, the utilization of *Rhizopus oligosporus* in tempeh production supports the development of high-nutritional-value fermented local foods with minimal environmental impact. Optimization of fermentation processes through appropriate starter selection and process control represents a strategic approach to improving tempeh nutritional quality without significantly increasing production costs (Winarno, 2021).

CONCLUSION

Based on the literature review, it can be concluded that the mold *Rhizopus oligosporus* plays a crucial role in enhancing the bioavailability of protein and minerals in tempeh through soybean fermentation. Enzymatic activities generated during fermentation—particularly protease and phytase—contribute to the hydrolysis of complex proteins into peptides and free amino acids that are more readily digested and absorbed by the human body.

Tempeh fermentation by *Rhizopus oligosporus* has also been shown to reduce antinutritional compounds, especially phytic acid, which is known to inhibit the absorption of essential minerals such as iron, calcium, and magnesium. This reduction in antinutrients directly increases mineral bioavailability, giving tempeh a nutritional advantage over non-fermented soybean products.

The combined improvement in protein and mineral bioavailability positions tempeh as a fermented functional food with strategic value for improving the nutritional status of the Indonesian population. Tempeh serves not only as an affordable source of plant-based protein but also as an important source of micronutrients that can help prevent nutritional problems such as protein deficiency and iron-deficiency anemia.

Therefore, continued efforts are needed to optimize tempeh fermentation through the appropriate and standardized use of *Rhizopus oligosporus*. Such optimization is expected to sustainably enhance the nutritional quality of tempeh and strengthen its position as a competitive, locally developed food product grounded in Indonesian traditional wisdom.

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