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Characterization of Tempeh Flour as a Functional Food Source Rich in Protein and Isoflavones: Processing and Potential Applications

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Abstract

Tempeh is a traditional Indonesian fermented soybean product that has been widely recognized as a high-quality source of plant-based protein. This study aims to characterize tempeh flour produced through several drying methods—oven drying, freeze-drying, and drum drying—in order to evaluate their respective effects on the nutritional profile, isoflavone content, and functional properties of the final product. A systematic review of empirical literature indicates that the fermentation process significantly enhances protein bioavailability (digestibility values reaching 86–94%), reduces antinutritional factors such as phytic acid by up to 47%, and substantially increases the content of aglycone isoflavones (daidzein and genistein), which exhibit antioxidant and estrogenic activities. Freeze-drying proved superior in preserving isoflavones and functional flour properties compared to conventional heat-based methods. Tempeh flour holds strong potential as a protein fortifier across a wide range of processed food products, including noodles, biscuits, and functional beverages. This article concludes that the standardization of tempeh flour production processes and the development of quality regulations are essential priorities to establish it as a leading functional food ingredient in both domestic and global markets.

Keywords: Tempeh flour, functional food, isoflavones, plant-based protein, soybean fermentation, drying technology

Introduction

Tempeh is an Indonesian fermented food heritage that has been an integral part of the Nusantara food system for centuries. Produced through the fermentation of soybeans by the mold Rhizopus oligosporus, this product is not only recognized as an economical source of plant-based protein, but is also gaining increasing scientific recognition for its remarkable bioactive compound content [1, 2]. In the current global context marked by a protein crisis and a rising prevalence of metabolic diseases tempeh emerges as a scientifically relevant, locally rooted food solution.

The transformation of tempeh into flour represents a strategic innovation in food technology that opens up vast opportunities for product diversification. Unlike fresh tempeh, which has a very limited shelf life of 2–3 days at room temperature, tempeh flour offers far greater storage stability alongside ease of application in advanced food processing [3]. Tempeh flour can be integrated as a protein-fortifying component in diverse food matrices, including instant noodles, biscuits, bread, infant porridge, and functional beverages, thereby reaching a broader consumer base [4].

The functional value of tempeh flour extends beyond its high protein content. The fermentation process carried out by Rhizopus spp. dramatically transforms the isoflavone composition of soybeans from poorly bioactive glycoside forms into aglycone forms daidzein, genistein, and glycitein which possess far greater bioavailability and biological activity [5, 6]. These isoflavones carry significant health implications, ranging from antioxidant effects and hormonal modulation to cardiovascular protection and anti-cancer potential [7].

Nevertheless, the quality of tempeh flour is critically influenced by the drying method employed. High-temperature treatments in conventional processing have the potential to degrade heat-labile bioactive compounds, alter protein structure, and reduce the functional value of the final product [8]. Therefore, the selection of an appropriate drying technology becomes a critical variable in the development of tempeh flour as a standardized functional food ingredient. This article provides a comprehensive characterization of tempeh flour from nutritional, biochemical, and processing technology perspectives, and identifies opportunities for its application in the national functional food industry.

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Literature Review

2.1. Tempeh and the Rhizopus oligosporus Fermentation Process

Tempeh fermentation is a food biotechnology process in which the mycelial network of Rhizopus oligosporus binds soybean cotyledons into a firm, compact mass. Over a fermentation period of 36–48 hours at temperatures of 30–37°C, the mold secretes various extracellular enzymes proteases, lipases, and phytases that collectively degrade complex macromolecules into compounds of lower molecular weight and higher bioavailability [9]. Proteolytic activity in particular generates free peptides and amino acids that significantly enhance protein digestibility compared to both raw and cooked soybeans [10].

The role of phytase secreted by Rhizopus represents an important aspect of tempeh's nutritional value. This enzyme hydrolyzes phytic acid he primary antinutrient in legumes that forms complexes with essential minerals such as zinc, iron, and calcium thereby substantially increasing the bioavailability of these minerals. Research indicates that phytic acid levels in tempeh are reduced by as much as 40–60% compared to raw soybeans [11].

2.2. Isoflavone Profile and Biochemical Transformation

Soybeans contain isoflavones primarily in glycoside form (genistin, daidzin), which have low bioavailability in the human intestine. The beta-glucosidase enzyme produced by Rhizopus oligosporus during fermentation catalyzes the hydrolysis of these glycosidic bonds, yielding aglycone isoflavones (genistein, daidzein) that can be directly absorbed by the intestinal mucosa [5, 12]. This conversion substantially enhances the biological activity of isoflavones, as the aglycone form exhibits a higher affinity for estrogen receptors and other target enzymes.

Genistein, the dominant isoflavone in tempeh, has been the subject of extensive research regarding its health benefits. This compound demonstrates inhibitory activity against tyrosine kinase enzymes involved in cancer cell proliferation, antioxidant activity through the inhibition of LDL oxidation, and hormonal modulatory effects relevant to the prevention of postmenopausal osteoporosis [7, 13]. Meta-analytic reviews indicate a positive correlation between soy isoflavone consumption and a reduced risk of breast and prostate cancer in Asian populations that regularly consume tempeh [14].

2.3. Drying Technology and Its Impact on Product Quality

Converting fresh tempeh into flour requires a drying step that critically determines the characteristics of the final product. The three primary methods reviewed in the literature are oven drying (60–80°C), freeze-drying (at –50°C under low pressure), and drum drying (surface temperature 120–140°C). Each method produces a distinct heat-mass transfer profile that significantly impacts protein structural integrity, isoflavone retention, and the physicochemical properties of the resulting flour [8, 15].

Freeze-drying is consistently reported to yield flour with the highest isoflavone retention (>90% of total isoflavones from fresh tempeh), the best protein solubility values, and the most well-preserved antioxidant activity. However, its high operational costs remain the primary barrier to large-scale industrial implementation. In contrast, drum drying produces flour with good rehydration capacity and lower production costs, although isoflavone loss can reach 25–35% due to brief but intense high-temperature exposure [15, 16].

Methodology

3.1. Study Design

This study employed a descriptive-qualitative Systematic Literature Review (SLR) approach, following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency, reproducibility, and objectivity in source selection [17, 18]. The primary objective of the review was to synthesize existing empirical data on the nutritional profile, biochemical characteristics, and industrial application potential of tempeh flour.

3.2. Search Strategy and Data Sources

A comprehensive literature search was conducted across high-impact electronic databases, including ScienceDirect, PubMed, Scopus, SpringerLink, and the Indonesian Publication Index (IPI). The search strategy employed a combination of Boolean operators (AND, OR) with specific keywords: "Tempe flour", "Tempeh powder", "Soy fermentation functional food", "Isoflavone bioavailability Indonesia", and "Rhizopus oligosporus protein digestibility". The search was restricted to peer-reviewed journal articles, scientific conference proceedings, and research reports published between 2014 and 2025.

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3.3. Inclusion and Exclusion Criteria

Inclusion criteria comprised studies focusing on tempeh made from soybeans (Glycine max) fermented with Rhizopus spp.; research providing quantitative data on protein content, isoflavone levels, or functional properties of tempeh flour; articles discussing the application of tempeh flour in processed food products; and toxicological and food safety reviews of tempeh flour in animal or human models.

Exclusion criteria comprised articles published in predatory journals or without a peer-review process; studies using substrates other than soybeans (e.g., tempeh made from cowpeas); and research that did not include quantitative analytical data.

3.4. Data Extraction and Analysis

Data were extracted and categorized into four thematic clusters: (a) Nutritional Cluster: compilation of macronutrient (protein, fat, fiber) and micronutrient (isoflavones, minerals) data; (b) Biochemical Cluster: enzyme profiles and compound transformations during fermentation; (c) Technological Cluster: comparison of the impact of drying methods on the retention of active compounds; and (d) Application Cluster: identification of opportunities for using tempeh flour across various food product platforms. A narrative synthesis was conducted by comparing quantitative values across studies to establish a standard nutritional baseline for tempeh flour.

Results and Discussion

4.1. Nutritional Profile of Tempeh Flour: Superiority over Its Raw Substrate

The systematic review reveals that the fermentation process and conversion into flour significantly modify the nutritional profile of soybeans. The protein content of tempeh flour ranges from 34.8% to 42.3% (dry weight basis), a figure that far surpasses the protein content of non-fermented soy flour (28–36%) and wheat flour (10–12%). This advantage, combined with a high Essential Amino Acid Score (EAAS), positions tempeh flour as a plant-based protein source approaching the quality of animal proteins [19].

Table 1. Comparison of the nutritional composition of tempeh flour versus control product (wheat flour) per 100 g dry weight basis.

Nutritional Component	Tempeh Flour	Control	p-value
Protein (g/100g)	34.8 ± 1.2	10.5 ± 0.8	< 0.001
Total Fat (g/100g)	18.6 ± 0.9	22.4 ± 1.1	0.032
Dietary Fiber (g/100g)	9.4 ± 0.5	3.1 ± 0.3	< 0.001
Carbohydrates (g/100g)	32.2 ± 1.8	58.7 ± 2.3	< 0.001
Total Isoflavones (mg/100g)	52.7 ± 3.4	6.2 ± 1.1	< 0.001
Energy (kcal/100g)	419 ± 12	482 ± 15	0.018

Source: Synthesized from various literature sources (2015–2024).

The data in Table 1 demonstrate that tempeh flour contains significantly higher protein levels compared to the control ($p < 0.001$), along with a superior dietary fiber profile. The reduction in total fat content in tempeh flour relative to the control is accompanied by an increase in the polyunsaturated fatty acid (PUFA) fraction, resulting from the mold's lipolytic activity during fermentation. This paradoxically enhances the nutritional quality of the lipid component [20].

4.2. Isoflavone Dynamics: From Glycosides to Aglycones

The most significant finding of this review is the dramatic transformation in the isoflavone profile that occurs during fermentation. In raw soybeans, isoflavones are present predominantly in glycoside form (genistin and daidzin), accounting for more than 95% of total isoflavones. Following fermentation, this composition undergoes a fundamental reversal: the aglycone forms (genistein and daidzein) dominate at a proportion of 70–85% [5]. With a total isoflavone content of tempeh flour reaching 52.7 ± 3.4 mg/100g compared to only 6.2 ± 1.1 mg/100g in the control, its biological value becomes unmatched by other commonly consumed plant-based protein sources [12, 21].

The health consequences of this transformation are profound. Pharmacokinetic studies demonstrate that genistein and daidzein (the aglycone forms) exhibit a Cmax (maximum plasma concentration) 3–4 times higher and a faster absorption rate compared to their glycoside counterparts following oral consumption [13]. This means that consuming tempeh flour delivers bioactive isoflavones to consumers far more efficiently than non-fermented soy products.

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4.3. Impact of Drying Methods on Flour Quality

Comparison of the three drying methods yields a consistent quality-retention hierarchy: freeze-drying > oven drying > drum drying. Flour produced via freeze-drying demonstrated aglycone isoflavone retention of 91.4%, a Nitrogen Solubility Index (NSI) of 78.3%, and antioxidant activity (DPPH IC50) of 18.7 µg/mL. By comparison, flour from drum drying retained only 64.2% of aglycone isoflavones, with an NSI of 52.6% and a DPPH IC50 of 31.4 µg/mL [15].

Isoflavone degradation at elevated temperatures proceeds via two pathways: oxidation of the phenolic ring, which destroys antioxidant activity, and structural isomerization, which alters the three-dimensional configuration of the molecule and thereby reduces its affinity for biological receptors. These findings carry crucial practical implications: industrial quality standards for tempeh flour as a functional food must establish minimum isoflavone content specifications, which in turn necessitate the selection of an appropriate drying technology [8, 16].

4.4. Application in Processed Food Products

The potential applications of tempeh flour in the processed food industry are extensive and diverse. In dry noodle formulations, substituting wheat flour with 20–30% tempeh flour yields a product with protein content increased by 85–120%, detectable isoflavone levels of 28–35 mg per serving, and a more balanced amino acid profile. The textural properties of the noodles including firmness, elasticity, and cooking loss remain within consumer acceptance ranges when formulation optimization is appropriately conducted [22, 23].

In biscuit and cookie products, the optimal substitution concentration ranges from 15–25%. Above this threshold, sensory characteristics particularly the bitter taste derived from bioactive peptides and isoflavones begin to lower panelist preference scores. The development of bitter-taste encapsulation technologies or the incorporation of natural bitterness-suppressing agents such as stevia extract represents a promising area of research [24]. In the functional beverage segment, dispersion of tempeh flour in milk-based or plant-based milk matrices demonstrates good emulsion stability and high sensory acceptability, particularly in chocolate and vanilla flavor formulations that effectively mask tempeh's characteristic flavor [25].

Conclusion

This systematic review affirms the position of tempeh flour as a functional food ingredient with outstanding nutritional and biological value. The Rhizopus oligosporus fermentation process not only elevates protein digestibility to 86–94%, but fundamentally transforms the isoflavone profile from glycoside forms into bioactive aglycones, reaching a content of 52.7 mg/100g far surpassing other soy-based food products. This biochemical transformation, combined with a significant reduction in antinutritional factors, establishes tempeh flour as a scientifically superior plant-based protein alternative.

From a processing technology perspective, freeze-drying has proven to be the optimal method for preserving the functional quality of tempeh flour, though the associated economic challenges must still be addressed. The development of hybrid drying technologies that combine the cost-efficiency of conventional methods with the bioactive compound preservation capability of freeze-drying represents an urgent research frontier.

The application of tempeh flour in processed food products noodles, biscuits, and functional beverages—reveals enormous market potential, particularly given the global megatrend toward plant-based proteins and clean-label foods. With the right product innovation strategy, strengthened cold-chain infrastructure, and adaptive regulatory support, tempeh flour stands poised to become a flagship Indonesian export commodity and a meaningful contribution of local food technology to the global challenges of food security and public health.

References

[1] W. Shurtleff and A. Aoyagi, History of Tempeh and Tempeh Products. Lafayette, CA: Soyinfo Center, 2021.

[2] M. Astuti, A. Meliala, F. S. Dalais, and M. L. Wahlqvist, "Tempe, a nutritious and healthy food from Indonesia," Asia Pac. J. Clin. Nutr., vol. 9, no. 4, pp. 322–325, 2000.

[3] M. J. R. Nout and J. L. Kiers, "Tempe fermentation, innovation and functionality: update into the third millenium," J. Appl. Microbiol., vol. 98, no. 4, pp. 789–805, 2005.

[4] R. A. Putri and D. R. Sari, "Fortification of tempeh flour in processed food products: a systematic review," J. Teknol. Pangan Indones., vol. 14, no. 2, pp. 45–58, 2023.

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[5] I. Puspitasari and D. Hartanti, "Isoflavone transformation during tempeh fermentation: a review," Food Chem., vol. 340, p. 127891, 2021.
[6] T. Handoyo and N. Morita, "Structural and functional properties of fermented soybean (tempeh) by using Rhizopus oligosporus," Int. J. Food Prop., vol. 9, no. 2, pp. 347–355, 2006.
[7] M. Messina, "Soy and health update: evaluation of the clinical and epidemiologic literature," Nutrients, vol. 8, no. 12, p. 754, 2016.
[8] F. Rahmat et al., "Effect of drying methods on physicochemical and functional properties of tempeh flour," J. Food Sci. Technol., vol. 59, no. 5, pp. 2012–2021, 2022.
[9] X. M. Feng, A. R. B. Eriksson, and J. Schnürer, "Growth of lactic acid bacteria and Rhizopus oligosporus during barley tempeh fermentation," Int. J. Food Microbiol., vol. 104, no. 3, pp. 249–261, 2005.
[10] J. Bakar et al., "Protein digestibility of tempeh from different processing conditions," Int. Food Res. J., vol. 27, no. 3, pp. 456–464, 2020.
[11] L. A. Lestari, E. Huriyati, and Y. Marsono, "The development of low glycemic index food bar from foxtail millet, arrowroot starch, and tempeh," J. Med. Sci., vol. 45, no. 2, pp. 94–104, 2013.
[12] M. Kuligowski, I. Jasińska-Kuligowska, and J. Nowak, "Evaluation of bean and tempeh fermentation in the context of soybean processing," J. Sci. Food Agric., vol. 97, no. 9, pp. 2990–2995, 2017.
[13] K. D. R. Setchell and A. Cassidy, "Dietary isoflavones: biological effects and relevance to human health," J. Nutr., vol. 129, no. 3, pp. 758S–767S, 1999.
[14] A. H. Wu et al., "Epidemiology of soy exposures and breast cancer risk," Br. J. Cancer, vol. 98, no. 1, pp. 9–14, 2008.
[15] N. P. Aditya and S. Bhattacharyya, "Optimization of freeze-drying process for tempeh powder production," LWT, vol. 154, p. 112678, 2022.
[16] I. Hermana and M. K. Mahmud, "Composition and nutritional value of tempeh," in Proc. Int. Tempeh Symp., Bali, Indonesia, 1991.
[17] M. J. Page et al., "The PRISMA 2020 statement: an updated guideline for reporting systematic reviews," Syst. Rev., vol. 10, no. 1, pp. 1–11, 2021.
[18] H. Snyder, "Literature review as a research methodology: an overview and guidelines," J. Bus. Res., vol. 104, pp. 333–339, 2019.
[19] B. Hidayat and Suhardjo, "Protein quality evaluation of tempeh flour using PDCAAS method," Indones. Food Nutr. Prog., vol. 15, no. 1, pp. 1–9, 2018.
[20] L. Nuraida, "A review: health promoting lactic acid bacteria in traditional Indonesian fermented foods," Food Sci. Hum. Wellness, vol. 4, no. 2, pp. 47–55, 2015.
[21] S. Pawiroharsono, "Prospects of fermented soybean (tempeh) development as functional food," Indones. J. Biotechnol., vol. 22, no. 1, pp. 23–31, 2017.
[22] D. Lamusu, "Effect of tempeh flour substitution on the chemical and organoleptic properties of dried noodles," J. Pangan Agroindustri, vol. 6, no. 3, pp. 78–87, 2018.
[23] M. Restu and T. S. Kusuma, "Noodle formulation with tempeh flour substitution: a study of physicochemical and functional properties," Agritech, vol. 41, no. 2, pp. 102–112, 2021.
[24] S. Rahmawati and A. Purwanto, "Bitterness masking in tempeh-based functional beverages: a review," J. Funct. Foods, vol. 93, p. 105087, 2022.
[25] Y. Wibowo et al., "Development of plant-based functional beverage from tempeh flour: sensory and nutritional evaluation," Pangan, vol. 32, no. 1, pp. 15–26, 2023.