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Fermentation Improves Protein Content and Physicochemical Characteristics of Tempe Protein Concentrate Compared to Soy Protein

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***Abstract*— The growing demand for sustainable plant-based proteins has increased interest in fermented soy products due to their improved nutritional and functional properties. This study investigated the effect of fermentation on the physicochemical and functional properties of tempe protein concentrate (TPC) compared to conventional soy protein products. Soybean flour (SF), soybean protein concentrate (SPC), tempe flour (TF), and TPC were produced and analyzed for proximate composition, antioxidant activity, physicochemical and functional properties. Soybeans and tempe were processed into flours, and protein concentrates were obtained by hexane defatting. Fermentation significantly increased protein content and bioactivity, as TF had higher protein content (48.27%) and antioxidant activity (58.54 mg AEAC/100 g) than SF. The concentration process further increased protein levels while reducing fat content, with TPC showing the highest protein content (68.80%), exceeding SPC (57.10%). TPC also exhibited improved physicochemical characteristics, including lower water activity (0.38), higher bulk density (0.51 g/mL), and better flowability (angle of repose 20.60°), suggesting improved stability and processability. Functional properties such as water absorption, oil absorption, and foaming capacity were significantly higher in both protein concentrates compared to their respective flours, although no significant differences were observed between TPC and SPC. These improvements are attributed to structural modifications during defatting, which increase protein solubility and expose hydrophilic and hydrophobic groups. These findings indicate that fermentation can improve the application and nutritional quality of soy protein products, supporting the development of functional foods and sustainable plant-based protein formulations.**

***Keywords*— Fermentation; physicochemical characteristics; plant-based protein; protein concentrate; soybean tempe**

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I. INTRODUCTION

In Indonesia, soybeans are the third most important agricultural commodity after rice and maize [1]. Soybeans are highly popular as a relatively affordable source of plant-based protein compared to animal-based protein. In addition, as a protein source, soybeans are also rich in isoflavones, dietary fiber, and other bioactive components, classifying them as functional foods with the potential to prevent various degenerative diseases. Based on their chemical composition, soybeans have strong potential to be used as raw materials in the food, pharmaceutical, beverage, and animal feed industries. Processed soybean-based food products can generally be categorized as either fermented or non-fermented. Fermented soybean products include tempe, soy sauce, and tauco, while non-fermented products include tofu, soy milk, and yuba (tofu skin). In Indonesia, soybean utilization is primarily focused on the production of tempe and tofu [2].

Tempe is a fermented soybean product that offers several advantages, such as the inactivation of anti-nutritional factors and off-flavor compounds, improved digestibility, and a high isoflavone content. The fermentation process in tempe production occurs in two stages. The first stage involves soaking the cooked soybeans at room temperature for 12 hours, during which lactic acid bacteria carry out the initial fermentation. The second stage requires inoculation with *Rhizopus* spp. molds, which are incubated for 48 hours at room temperature (28–30°C). The growing mold forms hyphae (white threads covering the surface of the soybeans), which develop into a network of mycelium that binds the soybeans together, creating a compact structure with a firm texture [3]. The mycelium produces several enzymes, such as protease, lipase, and amylase [4]. These enzymes hydrolyze complex nutrients into simpler forms, making them easier to digest and absorb [5].

A common issue with fresh tempe is its short shelf life, mainly due to its high moisture content (approximately 65%). Prolonged fermentation can also lead to protein degradation and ammonia production, resulting in an unpleasant odor [6]. Processing tempe into intermediate food products is one strategy to extend its shelf life and improve its utility. These intermediate products can take the form of flour, protein concentrates, or protein isolates, each with varying protein contents. Tempe flour has low moisture content, which inhibits microbial growth and chemical reactions [7]. Protein concentrate is a refined product derived from flour, where a portion of carbohydrates and fats is removed to increase the protein fraction. Protein concentrates typically contain 65–90% protein [8]. Non-protein components can be removed through extraction using alcohol or acid solutions [9,10].

Tempe flour and protein concentrates differ in chemical composition, particularly protein content. Therefore, it is necessary to evaluate the potential of tempe as a raw material for flour and protein concentrate production, and to compare their physicochemical and functional properties with those of soybean-based flour and protein concentrates. This study aimed to optimize the utilization of soybeans and tempe as raw materials for flour and protein concentrates to support the availability of alternative protein sources in the food industry.

II. MATERIAL AND METHODS

A. Materials

The primary raw material used in this study was soybean, obtained from the Tempe and Tofu Producers Cooperative in Bogor, West Java, Indonesia. Additional materials for tempe production included a commercial tempe inoculum (Raprima®) and polypropylene plastic packaging. The raw material for soybean flour production was dehulled soybeans, while tempe flour was made from fresh tempe produced by Rumah Tempe Indonesia in Bogor, West Java, Indonesia. The main ingredients for the preparation of soybean protein concentrate and tempe protein concentrate were soybean flour and tempe flour, respectively. Other materials included Whatman filter paper no. 42 and n-hexane solvent, both purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals used for analysis were also purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

B. Methods

The research was conducted in three stages: (1) preparation of raw materials, including the production of dehulled soybeans and tempe; (2) product development, including the production of soybean flour (SF), soybean protein concentrate (SPC), tempe flour (TF), and tempe protein concentrate (TPC); and (3) product analysis, including chemical, physical, and functional properties of the proteins.

1. Preparation of dehulled soybeans

The preparation of dehulled soybeans followed the standard operating procedure for tempe production at Rumah Tempe Indonesia [11]. The soybeans were sorted, washed to remove impurities, and soaked for 2 hours. The first boiling was conducted for 30 minutes at 100°C, followed by overnight soaking in the same boiled water. After soaking, the soybeans were dehulled and split using a dehulling machine. The resulting clean, dehulled soybeans were washed to obtain clean, hull-free soybeans.

2. Tempe production

Tempe was produced following the procedures applied at Rumah Tempe Indonesia, similar to the preparation of dehulled soybeans [12]. The clean, dehulled soybeans underwent a second boiling for 30 minutes at 100°C, then were drained and cooled. The soybeans were inoculated with 0.2% (w/w) of *Rhizopus* spp.-containing tempe inoculum and packed in perforated plastic bags (holes 2 × 2 cm apart). The soybean-inoculum mixture was fermented at room temperature (28–30°C) for 48 hours.

3. Soybean flour production

Soybean flour production was based on Astawan et al., with slight modification [11]. Dehulled soybeans were dried using a fluidized bed dryer at 50°C for 8 hours. The dried soybeans were ground using a pin disc mill and sieved through an 80-mesh screen.

4. Tempe flour production

Tempe flour was produced by slicing fresh tempe, blanching the slices with steam for 10 minutes, and then drying them using a fluidized bed dryer at 50°C for 8 hours. The dried tempe slices were ground using a pin disc mill and sieved through an 80-mesh screen [11].

5. *Soybean and tempe protein concentrate production*

The production of soybean protein concentrate (SPC) and tempe protein concentrate (TPC) was based on the extraction method by Prayudani et al. with slight modification [13]. Soybean flour and tempe flour (80 mesh) were extracted with n-hexane using an ultrasonic sonicator, in two steps. The first extraction was done with a flour-to-solvent ratio of 1:3 (b/v) for 20 minutes, followed by filtration using a vacuum pump to separate the filtrate and solid residue. The residue was extracted again using the same solvent at a 1:4 (b/v) ratio with ultrasonic assistance for 20 minutes and then filtered again. The final residue was left in a fume hood for 12 hours and then dried in a drying oven at 50°C for 2 hours.

6. *Chemical, physical, and functional properties analysis*

Soybean flour (SF), soybean protein concentrate (SPC), tempe flour (TF), and tempe protein concentrate (TPC) were analyzed for chemical, physical, and functional protein properties. Chemical analysis included proximate composition and crude fiber [14], as well as antioxidant capacity using the DPPH method [15]. Physical analysis included yield, water activity (a_w), bulk density [16], angle of repose [13], color, and whiteness index [13]. Functional protein properties were analyzed for water absorption capacity [17], oil absorption capacity [18], foaming capacity [19], and emulsifying capacity [20].

7. *Data processing and analysis*

Data on the chemical, physical, and functional protein characteristics of the samples (SF, SPC, TF, and TPC) were analyzed using a Completely Randomized Design (CRD) with two replicates, each in duplicate. Results were presented descriptively and analyzed using Analysis of Variance (ANOVA). Data processing was performed using IBM SPSS Statistics 26 to compare significance at the 5% level. Treatments showing significant differences ($p < 0.05$) were further analyzed using Duncan's Multiple Range Test.

III. RESULT AND DISCUSSION

A. *Yield*

Yield was calculated based on the ratio between the weight of the final product (flour or protein concentrate) and the weight of the raw material used (soybeans or tempe). Results showed that soybean flour (SF) and tempe flour (TF) had no significant difference in yield (p>0.05). Similarly, soybean protein concentrate (SPC) and tempe protein concentrate (TPC) showed no significant difference in yield. However, both had significantly lower yields (p<0.05) compared to SF and TF (**Figure 1**). The production of protein concentrates led to the extraction of most of the fat content from SF and TF by the hexane solvent. Fat loss during the hexane extraction process contributed to the significantly lower SPC and TPC yields than SF and TF.

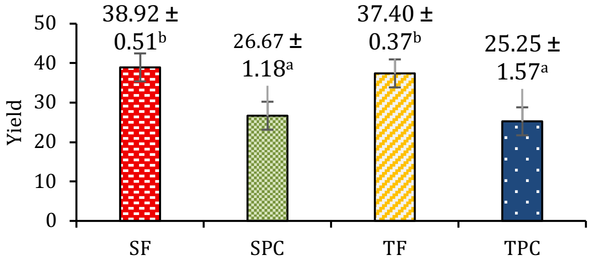

Fig 1. Flour and protein concentrate yield (SF: soybean flour, SPC: soybean protein concentrate, TF: tempe flour, TPC: tempe protein concentrate).

*Numbers followed by different letters indicate significant differences (p<0.05) based on the DMRT test. Results were obtained from the average of two replications (duplo)

B. *Chemical characteristics*

The proximate composition and antioxidant capacity of soybean flour (SF), tempe flour (TF), soybean protein concentrate (SPC), and tempe protein concentrate (TPC) is summarized in **Table 1**. Fermentation significantly improved protein quality and antioxidant capacity, as indicated by higher protein and antioxidant capacity of TF compared to SF (p<0.05).

TABLE 1. CHEMICAL COMPOSITION OF SOYBEAN FLOUR, SOYBEAN PROTEIN CONCENTRATE, TEMPE FLOUR, AND TEMPE PROTEIN CONCENTRATE

Parameters	**Product**			
	SF	**SPC**	**TF**	**TPC**
Moisture (% wb)	6.90 ± 1.53^{ab}	7.40 ± 0.66^{b}	5.46 ± 0.55^{a}	7.12 ± 0.90^{b}
Ash (% db)	3.76 ± 0.61^{b}	4.88 ±0.74^{c}	2.06 ± 0.16^{a}	2.62 ± 0.14^{a}
Fat (% db)	21.14 ± 1.11^{b}	1.33 ± 0.24^{a}	24.52 ± 1.74^{c}	2.26 ± 0.52^{a}
Protein (% db)	43.46 ± 1.03^{a}	57.10 ± 1.14^{c}	48.27 ± 1.19^{b}	68.80 ± 0.80^{d}
Carbohydrate (% db)	24.74 ± 2.06^{b}	29.28 ± 0.11^{c}	19.69 ± 0.97^{a}	19.19 ± 0.53^{a}
Crude fiber (% db)	3.20 ± 0.61^{b}	1.99 ± 0.53^{a}	2.43 ± 0.25^{a}	3.17 ± 0.33^{b}
Antioxidant capacity (mg AEAC/100 g db)	35.18 ± 0.73^{c}	16.83 ± 1.07^{a}	58.54 ± 0.87^{d}	28.42 ± 1.10^{b}

Note: SF: soybean flour; SPC: soybean protein concentrate; TF: tempe flour; TPC: tempe protein concentrate.

*Value followed by different letters in the same row indicate significant differences (p<0.05) based on the DMRT test. Results were obtained from the average of two replications (duplo)

Fermentation improved the nutritional profile, particularly protein content due to enzymatic activity by *Rhizopus* spp., which breaks down complex proteins into more digestible peptides and amino acids. This fermentation process also reduces carbohydrate content, resulting in a relative increase in protein concentration [4]. The production of protein concentrates further increased protein content while reducing fat due to hexane defatting. Hexane is the preferred solvent for oil extraction due to its efficiency in removing lipids while preserving protein quality. Defatted protein fractions typically exhibit better amino acid composition, solubility, and functional properties compared to those extracted using polar solvents such as methanol or ethanol [10]. TPC had the highest protein level (68.80%), exceeding SPC (57.10%), and retained higher antioxidant activity than SPC despite a reduction during defatting.

Fat content decreased significantly in concentrates compared to their flour forms. Fermentation also reduced ash content significantly due to the mineral loss during various stages of tempe production, such as soaking and boiling the soybeans. Carbohydrate and crude fiber levels decreased slightly after fermentation but remained within Codex standards. According to Codex Standard 175-1989, soybean protein concentrates should contain 65–90% protein [8]. In this study, only the tempe protein concentrate (TPC) met this requirement, while the soybean protein concentrate (SPC) fell slightly below, indicating the positive impact of fermentation on protein enrichment. Both SPC and TPC complied with Codex and Indonesian National Standard (INS 3751:2009) requirements for moisture (≤10% for concentrates, ≤14% for flour), ash (≤8%), and crude fiber (≤6%), confirming their quality, safety, and storage stability.

Microorganism activity during the fermentation process can secrete both primary and secondary metabolites that significantly improve the antioxidant activity of tempe [21]. Isoflavones in soybeans are typically conjugated with sugar molecules or other compounds. The mold involved in fermentation secretes the enzyme β-glucosidase, which breaks these conjugated bonds, releasing free isoflavones [22]. However, antioxidant capacity decreased in protein concentrates, likely due to the extraction of non-polar antioxidant compounds such as isoflavones during the defatting process [13]. Nonetheless, polar phenolic compounds may remain, contributing to the residual antioxidant activity observed.

C. Physical characteristics

Physical characteristics of soybean flour (SF), tempe flour (TF), soybean protein concentrate (SPC), and tempe protein concentrate (TPC) is summarized in **Table 2**. Fermentation slightly affected color which can be seen in **Figure 2**, as TF had darker color than SF due to Maillard reactions and pigment formation during microbial metabolism [11]. In contrast, the defatting step significantly brightened SPC and TPC, as hexane removed fat-soluble pigments such as carotenoids, resulting in higher whiteness indices [13]. Light-colored protein concentrates are preferred in formulated foods, especially bakery and beverage products, where appearance is critical [23].

Fig 2. The photograph of soybean flour/SF (top left), tempe flour/TF (top right), soybean protein concentrate/SPC (bottom left), tempe protein concentrate TPC (bottom right)

TABLE 2. PHYSICAL CHARACTERISTICS OF SOYBEAN FLOUR, SOYBEAN PROTEIN CONCENTRATE, TEMPE FLOUR, AND TEMPE PROTEIN CONCENTRATE

Parameters	Product			
	SF	SPC	TF	TPC
L	90.18 ± 0.53^{b}	92.77 ± 0.93^{c}	87.90 ± 0.56^{a}	92.49 ± 0.22^{c}
a	-2.01 ± 0.62^{a}	-1.47 ± 0.13^{b}	-0.94 ± 0.08^{c}	-0.16 ± 0.12^{d}
b	27.75 ± 1.17^{d}	15.28 ± 1.33^{b}	23.63 ± 0.48^{c}	12.35 ± 0.29^{a}
Whiteness index	70.41 ± 1.06^{a}	82.99 ± 1.01^{c}	73.43 ± 0.68^{b}	85.54 ± 0.35^{d}
Water activity (a_w)	0.54 ± 0.08^{b}	0.40 ± 0.06^{a}	0.55 ± 0.01^{b}	0.38 ± 0.12^{a}
Bulk density (g/ml)	0.43 ± 0.01^{a}	0.49 ± 0.01^{b}	0.44 ± 0.00^{a}	0.51 ± 0.01^{c}
Angle of repose	43.49 ± 1.60^{d}	25.65 ± 0.98^{b}	40.89 ± 0.76^{c}	20.60 ± 1.32^{a}

Note: SF: soybean flour; SPC: soybean protein concentrate; TF: tempe flour; TPC: tempe protein concentrate.
*Value followed by different letters in the same row indicate significant differences ($p<0.05$) based on the DMRT test. Results were obtained from the average of two replications (duplo)

Water activity (a_w) values were low in all products (<0.6), indicating good microbial stability at ambient temperature. The decrease of a_w in both protein concentrates compared to flours demonstrated that fat extraction also affects flour and protein concentrates' water activity (a_w). Hexane removes the natural lipid layer that normally traps free water within the capillary structure of flour. Once defatted, the surfaces of protein and starch particles become cleaner, allowing water molecules to bind more tightly to polar groups such as –OH and –NH. This reduces the amount of free water, which directly contributes to a_w, while increasing bound water fractions, ultimately lowering the overall a_w value [24]. A lower water activity indicates greater product stability and safety. An a_w below 0.6 creates an unfavorable environment for the growth of pathogenic and spoilage microorganisms, effectively halting microbial activity [25]. Furthermore, an a_w range of 0.2–0.4 can significantly slow down chemical and biochemical reactions that may compromise food quality, such as enzymatic activity, Maillard browning, and hydrolysis, due to reduced water mobility as a reaction medium [26]. TPC showed the lowest a_w (0.38), which combined with its reduced moisture content (**Table 1**) will improve its shelf life and storage stability.

Bulk density is a key parameter used to evaluate flour-based products. It is calculated by dividing the mass of the flour particles by the total volume they occupy, which includes the volume of the particles themselves, internal pores, and spaces between particles. A higher bulk density indicates the particles are more tightly packed, resulting in fewer voids and more solid matter per unit volume [27]. Bulk density increased significantly in both protein concentrates compared to both flours, demonstrating denser particle packing after fat removal. This characteristic is beneficial for industrial applications, as it improves packaging efficiency and mixing behavior in powder-based formulations. In contrast, flours with lower bulk density are more suitable for weaning foods due to their lighter texture and easier digestibility [28]. Several factors, including moisture content, starch content, and the size and shape of the flour particles influence bulk density. Generally, lower moisture levels lead to higher bulk density, as less free water allows particles to pack more tightly together [29].

Flowability was measured by the angle of repose, which improved significantly after concentration, with TPC showing the best flow properties (20.60°), indicating free-flowing characteristics which suitable for large-scale handling and processing. High fat content in flour can make protein particles more cohesive, reducing their ability to move freely. This increased cohesiveness raises the static friction between particles, resulting in a higher angle of repose. A higher angle of repose indicates greater caking and agglomeration in powdered products, negatively affecting flowability. Materials with a low angle of repose (less than 40°) are typically considered free flowing [13]. Low water activity (a_W) also supports good flowability, as tightly bound water in monolayer form around biomolecules helps prevent powder clumping and ensures smoother flow [30].

D. Functional properties

Functional properties of soybean flour (SF), tempe flour (TF), soybean protein concentrate (SPC), and tempe protein concentrate (TPC) is summarized in **Table 3**. SF and TF showed no significant differences in water absorption, oil absorption, and foam capacity parameters, suggesting that fermentation did not affect these properties. However, TF demonstrated higher emulsion capacity compared to SF significantly, which likely due to structural changes in proteins during fermentation that improve their surface activity. In contrast, converting SF and TF into protein concentrates significantly increased water absorption, oil absorption, and foam capacity properties, but decreased emulsion capacity. No significant differences found between SPC and TPC for these functional properties.

TABLE 3. PROTEIN FUNCTIONAL PROPERTIES OF SOYBEAN FLOUR, SOYBEAN PROTEIN CONCENTRATE, TEMPE FLOUR, AND TEMPE PROTEIN CONCENTRATE

Parameter	**Product**			
	SF	**SPC**	**TF**	**TPC**
Water absorption (g/g)	2.54 ± 0.09^a	3.47 ± 0.40^b	2.32 ± 0.16^a	3.42 ± 0.04^b
Oil absorption (ml/g)	1.78 ± 0.18^a	2.56 ± 0.16^b	1.58 ± 0.08^a	2.61 ± 0.08^b
Foam capacity (%)	15.55 ± 1.31^a	48.57 ± 2.14^b	14.21 ± 1.03^a	46.36 ± 2.03^b
Emulsion capacity (%)	5.57 ± 0.12^b	2.46 ± 0.27^a	9.32 ± 0.57^c	2.15 ± 0.16^a

Note: SF: soybean flour; SPC: soybean protein concentrate; TF: tempe flour; TPC: tempe protein concentrate.
*Value followed by different letters in the same row indicate significant differences ($p<0.05$) based on the DMRT test. Results were obtained from the average of two replications (duplo)

Water and oil absorption capacities were significantly improved in both protein concentrates, likely due to increased surface polarity from defatting. During the production of protein concentrates, the defatting process, which often involves solvents under specific temperature and pH conditions, can induce structural changes in soy proteins. These alterations affect protein-protein interactions, such as hydrogen bonding and hydrophobic effects, ultimately influencing the protein's functionality [31]. These properties are crucial for determining texture, mouthfeel, and moisture retention in food formulations. Hydrophilic amino acid residues facilitate water binding, while hydrophobic surface characteristics affect oil absorption [19].

Foaming capacity and stability are key functional parameters, particularly in the formulation of bakery products [32]. In legumes like soy, the predominant proteins such as glycinin and β-conglycinin play a major role in several functional properties, including water holding capacity (WHC), emulsification, foaming, gelatinization, and solubility [33]. Structural changes in proteins caused by heat treatment or solvent use during protein concentrate production can alter the quantity and type of proteins and their secondary structures. These changes contribute to the differences in functional properties and exhibit improved foaming capacity compared to their flour counterparts, likely due to these structural modifications [31]. The ability of protein to incorporate and stabilize air bubbles, forming stable foam structures within food matrices is beneficial in improving texture, volume, and creaminess [34]. Therefore, tempe protein concentrate is a promising candidate for use in beverages, whipped toppings, and ice cream.

Emulsifying capacity refers to a material's ability to form and stabilize emulsions. A good emulsifier can absorb and retain water and oil effectively, creating a stable mixture. In soybean flour, emulsifying capacity is primarily influenced by its protein content. The emulsions formed in soy-based tempe are typically oil-in-water systems [17]. However, in this study, both flour and protein concentrate samples exhibited low emulsifying capacities, which suggests limited effectiveness as emulsifying agents under the tested conditions. Further optimization or functional enhancement may be needed to improve their emulsifying potential for food applications.

IV. CONCLUSION

Fermentation prior to protein concentration significantly improved the protein content and physicochemical characteristics of tempe protein concentrate (TPC) compared to conventional soy products. TPC exhibited the highest protein content and better physicochemical stability, as indicated by lower water activity, higher bulk density, and improved flowability, which are advantageous for storage and industrial processing. Functional properties, including water and oil absorption capacities and foaming ability, were significantly higher in both protein concentrates compared to their respective flours. Despite similar functional properties to SPC, TPC provides an additional advantage of fermentation-improved protein quality and antioxidant activity, offering potential health benefits. These findings suggest that TPC is a promising fermented soy-based protein ingredient suitable for bakery products, plant-based meat analogs, and other functional foods. However, this study is limited by its small number of replicates and the absence of sensory and digestibility evaluation, which may affect application interpretation. Therefore, future studies should explore sensory properties, digestibility, and formulation trials to optimize its industrial application.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

During the preparation of this manuscript, generative AI tools (Grammarly, Quillbot, MsOfficeAI, OpenAI) were used to improve the clarity, grammar, and overall readability of the text. The use of these tools was conducted under full human oversight, and all intellectual and scientific content, data interpretation, and conclusions are entirely the responsibility of the authors. No generative AI tool was used to generate or analyze scientific data, and AI tools are not listed as authors or co-authors.

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