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Physicochemical and functional characteristics of protein co-precipitates from tempeh and egg white flours

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ABSTRACT

Recent developments in Indonesia have seen a growing interest in tempeh, with new activities emerging that combine animal and plant proteins through innovative processing technologies. This study examined the blending of egg white and tempeh flour (TF) using a co-precipitation method. The process involved solubilizing the proteins at pH 12, followed by precipitation at the isoelectric point around pH 4.4. Four types of precipitates were produced based on the protein source: co-precipitate of mixed tempeh flour (FMP), egg white protein co-precipitate (EMP), tempeh flour precipitate (TFP), and egg white precipitate (EFP). Among these, FMP demonstrated superior qualities, containing 79.68% protein and achieving 89.80% in vitro digestibility. Notably, the protein was enriched with essential amino acids such as lysine, leucine, and methionine compared to single-source proteins. Functionally, FMP exhibited excellent water and oil holding capacities, measuring 512.82% and 233.82%, respectively. Supporting analyses confirmed these findings: FTIR indicated minor shifts in amide bands, primarily secondary structures; SEM revealed porous, water-stable structures capable of absorption; SDS-PAGE showed minimal fragmentation, with protein bands characteristic of soybean and egg white proteins (15-75 kDa). The bioactive profile of FMP included antioxidant activity (9.45% RSA via DPPH), moderate antioxidant capacity, phenolic content of 4.44 mg gallic acid equivalents per gram, and detectable isoflavones such as daidzein (17.77 mg/100 g) and genistein (3.16 mg/100 g). These results support the potential of locally co-precipitated proteins to contribute to functional food innovation and development.

Contribution/Originality: This study introduces a novel plant-animal protein system based on combining tempeh and egg-white flours through co-precipitation, a process with no prior similar research. It investigates the composition, digestibility, functional properties, and bioactive potential of these co-precipitated mixtures, providing new insights into their applications.

1. INTRODUCTION

Proteins are essential macronutrients crucial for human health, affecting food structure and function. Recent scientific progress emphasizes sustainable, balanced diets, especially in the Global South, such as Indonesia. Although soy proteins are affordable and sustainable, they are relatively low in sulfur amino acids like methionine and cysteine

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(Guo et al., 2020). A fermented soybean product, tempeh, is more bioactive and digestible due to the action of Rhizopus oligosporus fungus, which enhances protein availability and removes anti-nutritional factors like trypsin inhibitors and phytic acid. However, methionine and cysteine remain the amino acids in shortest supply (Purwandari et al., 2025). On the other hand, egg white proteins are well-balanced in amino acid composition and have gelling and foaming strengths (Razi, Bagheri, Mohammadian, Mirarab-Razi, & Rashidinejad, 2024). Therefore, the two sources together have the potential to yield proteins of greater nutritional and functional value.

The phenomenon described here, known as protein co-precipitation, enables the capture of diverse proteins from different sources into a single entity. This technique begins with the solubilization of proteins under alkaline conditions, followed by the stepwise removal of proteins from the solution, resulting in a final protein precipitate near their respective isoelectric points. This process allows some proteins to undergo structural changes, such as partial unfolding, leading to molecular rearrangements. Such protein engineering is accompanied by synergistic interactions between proteins and the soluble polar components of the co-precipitate, forming complexes with improved solubility, digestibility, and stability. As these multi-protein complexes are engineered, numerous studies have quantified improvements in digestibility, solubilization, foaming stability, and the micro- and macro-structure of protein foams. However, there are very few studies on the co-precipitate of fermented soy protein, such as tempeh, and its interaction with egg whites, with limited experimental research available.

Other studies investigating plant-animal protein systems yield positive data on emulsification, structural stability, and hydration. Alu'datt et al. (2013) present examples of better emulsification in milk-soy systems, better structure stability in pea-whey proteins by He, Wang, Feng, Chen, and Wang (2020), and greater water-binding in soy-egg blends by Tian et al. (2022). Other recent work, Liu, Tan, Hong, Liu, and Zhou (2024), has also illustrated a soy-tilapia co-precipitate having high solubility (81.90%), better protein digestibility, and higher levels of amino acids than both soy and tilapia isolates. Proteomic analyses of that complex also revealed a considerable number of unique proteins (n=989), suggesting that molecular complexity resulted in significant interactions and improved functional properties. Notwithstanding these studies, the co-precipitation and fermentation-based protein potential of tempeh has been relatively unexplored. The protein interactions that are likely to occur when tempeh is combined with egg whites are hypothesized to improve digestibility, structure, and bioactivity due to the alterations occurring from fermentation.

This study evaluates the physicochemical properties of co-precipitates derived from tempeh and egg-white proteins. It examines composition, digestibility, amino acid profiles, functional properties, and structural analyses using FTIR, SEM, and SDS-PAGE. Additionally, antioxidant and phenolic activities are measured. The findings aim to support the development of protein-rich ingredients for functional foods and enhance bioactivity for specific applications, providing a scientific basis for future food formulation and nutritional improvements.

2. MATERIALS AND METHODS

The co-precipitation technique was adapted to extract proteins from egg-white flour and tempeh flour. The method was modified from standard alkaline extraction and isoelectric precipitation to better suit fermented soybean proteins. Protein solubilization occurred at pH 12.0, with precipitation at pH 4.4. These conditions were selected based on preliminary solubility tests, which showed maximum protein recovery. Unlike most studies focusing on unfermented plant proteins, this research emphasizes fermented sources, making its optimized parameters distinct. The adjustments improve protein extraction efficiency from fermented soybean products, highlighting the importance of tailored methods for different protein sources.

2.1. Materials

The procedures described in previous studies, Herawati, Febrisiantosa, Ningrum, and Santoso (2024), and Herawati, Febrisiantosa, Ningrum, Hendrasty, et al. (2024), facilitated the preparation of tempeh flour and egg white

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flour. The process of making tempeh began at Rumah Tempe Gunungkidul (Yogyakarta, Indonesia) using Anjasmoro soybean variety. The beans were cleaned, soaked, boiled, dehusked, inoculated with Rhizopus oligosporus, and fermented for two days at 30 °C in plastic bags. After fermentation, the tempeh was sliced, blanched in hot water for 10 minutes, then freeze-dried in a Lyovapor L-200 (Buchi, Switzerland) at −50 °C for 30 hours. The dried slices were ground, sieved through a 60-mesh screen, and stored in a freezer at -20 °C. For egg white flour, local chicken eggs were collected, and the yolks separated. The whites were homogenized using an Ultra-Turrax T-25 (22,000 rpm for 1 minute). The homogenized egg whites were then freeze-dried under the same conditions as the tempeh, ground, sieved through a 60-mesh screen, and stored at -20 °C for further analysis.

2.2. Preparation of Protein Co-Precipitates Using Alkaline–Acid Treatment

Protein coprecipitates from tempeh and egg-white using an alkaline solubilization and acid-induced precipitation method, citing (Alu'datt et al., 2020). Two preparation methods were employed: flour-based and extract-based. In the flour-based method, 10 g of tempeh and egg-white flour were dispersed in 200 mL of 1.0 M NaOH (pH 12). The mixture was stirred for 1 hour at 25°C, then centrifuged at 2500 × g for 20 minutes at 4°C. The clear supernatant was filtered and acidified stepwise with 1.0 M HCl to pH 4.4. The precipitate obtained from this acidified supernatant was centrifuged again, freeze-dried, and designated as the mixed flour coprecipitate (FMP). For the extract-based method, tempeh and egg-white extracts were prepared separately under the same alkaline conditions, then combined in a 1:1 ratio. This mixture was adjusted to pH 12, stirred for 1 hour, and centrifuged. Acidification with HCl to pH 4.4 induced precipitation, and the resulting solids were freeze-dried to produce the co-precipitate of the extract (EMP). Additionally, individual procedures were applied to each flour to obtain separate precipitates: tempeh flour precipitate (TFP) and egg-white flour precipitate (EFP).

2.3. Physicochemical Characteristics of Protein Co-Precipitates

2.3.1. Protein Content, Protein Digestibility

The protein concentration in the co-precipitates was measured using the Kjeldahl method, which calculates protein content based on nitrogen measurement (Association of Official Analytical Chemists (AOAC), 2004). According to the method described by Priyatnasari et al. (2024), in vitro protein digestibility was assessed. A 200 mg sample was placed into the Walpole buffer, with the pH adjusted to 2.0. Then, 2% pepsin was added, and the mixture was incubated at 37°C for 1.5 hours. After digestion, the sample was centrifuged at 1000 × g for 20 minutes. The supernatant was mixed with 20% TCA, incubated again, and filtered. The resulting filtrate was analyzed using the micro-Kjeldahl method to quantify the digestible protein content.

The formula below was used to determine the percentage of protein digestibility.

$$\text{Digestibility (\%)} = \frac{Pd}{Pt} x\ 100$$

Where Pd and Pt refer to the protein content (g/100 g) of the digested and undigested samples.

2.3.2. Amino Acid Profile

The amino acid composition was analyzed using an HPLC system (Thermo Dionex UltiMate 3000, USA) equipped with a fluorescence detector. Approximately 60 mg of dried sample was hydrolyzed in 6 N HCl at 110°C for 24 hours, then neutralized with 6 N NaOH, diluted to 10 mL with deionized water, and filtered through a 0.2 µm Whatman membrane. A 50 µL aliquot was derivatized with 300 µL of OPA reagent, re-filtered, and 10 µL was injected into the HPLC. Separation was performed on a LiChrospher 100 RP-18 column (5 µm) using solvent A (methanol, 50 mM sodium acetate, tetrahydrofuran in a 2:96:2 v/v/v ratio, pH 6.8) and solvent B (65% methanol) with a 35-minute gradient at 1.5 mL/min. Fluorescence detection was set at 300 nm excitation and 500 nm emission.

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2.3.3. Functional Properties

The evaluated functional parameters included water-holding capacity, oil-holding capacity, swelling power, and solubility.

2.3.3.1. WHC

Water-holding capacity (WHC) was analyzed as described by Nguyen, Mounir, Allaf, and Allaf (2015). A 1-gram sample was mixed with 10 mL of distilled water, vortexed for 2 minutes, allowed to stand for 30 minutes, and centrifuged at 1000 × g for 30 minutes. The residue weight was recorded to calculate WHC (%).

$$\text{WHC (\%)} = \frac{W2 - W1}{Wo} x\ 100$$

Where W_0, W_1, and W_2 are the weights of dry sample, empty tube, and residue are measured accurately.

2.3.3.2. OHC

Oil-holding capacity (OHC) was determined according to Nguyen et al. (2015). A 1-gram sample was mixed with 10 mL of vegetable oil, vortexed, and left to stand for 30 minutes. It was then centrifuged at 1000 × g for 30 minutes. The oil retained was measured by the weight difference before and after centrifugation.

$$\text{OHC (\%)} = \frac{W2 - W1}{Wo} x\ 100$$

Where W_0, W_1, and W_2 are the weights of the dry sample, empty tube, and residue after centrifugation.

2.3.3.3. Swelling Power

Swelling power (SP) was determined following Kusumayanti, Handayani, and Santosa (2015). A sample of approximately 0.1 g was dispersed in 10 mL of distilled water, heated at 60 °C for 30 minutes, then centrifuged at 300 × g for 15 minutes. The sediment was weighed after decanting the supernatant to determine the SP.

$$\text{Swelling power (g/g)} = \frac{W2 - W1}{Wo}$$

Where W_0, W_1, and W_2 represent the weights of the dry sample, empty tube, and swollen sediment.

2.3.3.4. Solubility

Solubility was determined based on the method of Kusumayanti et al. (2015). A 0.5 g sample was dispersed in 10 mL of distilled water, heated at 60°C for 30 minutes, then centrifuged at 300 × g for 10 minutes. A 5 mL aliquot of the supernatant was dried at 105°C to a constant weight.

$$\text{Solubility (\%)} = \frac{W1}{Wo} x\ 100$$

Where W_0 is the dry sample weight (g) and W_1 is the weight of dried soluble solids (g).

2.3.4. Surface Morphology Analysis

Surface morphology was examined using a scanning electron microscope (SEM; Hitachi SU-3500, Japan) operated at 4.0 kV and 100× magnification.

2.3.5. FTIR Analysis

Functional groups were analyzed using a Fourier transform infrared spectrometer (FTIR; Shimadzu 8201 PC, Japan). Dried samples were placed directly on the ATR crystal, and spectra were obtained from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

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2.3.6. Total Phenolic Content and Antioxidant Activity

2.3.6.1. Total Phenolic Content

The total phenolic content (TPC) of the extracts was measured using the Folin–Ciocalteu colorimetric method, following the procedure of Yu et al. (2002) with minor modifications. A reaction mixture was prepared by combining 10 µL of extract, 50 µL of Folin–Ciocalteu reagent, and 150 µL of 20% sodium carbonate solution, then adjusted to a final volume of 1 mL with distilled water. The mixture was allowed to react for 2 hours at room temperature. Its absorbance was then measured at 765 nm using a Multiskan GO microplate reader (Thermo Scientific, Finland). The phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g) and calculated using the appropriate equation.

$$\text{TPC (mg GAE/g)} = \frac{C \times V}{m} x\ 100$$

Where: C = Gallic acid concentration from the calibration curve (mg/mL); V = Extract volume (mL); m = Sample mass (g).

2.3.6.2. Antioxidant Activity by DPPH Radical Scavenging Assay

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to Indrianingsih et al. (2021). Samples were dissolved in methanol and mixed with 1.01 mM DPPH solution (1:1, v/v). The mixtures were kept for 30 minutes at room temperature in the dark, and absorbance was measured at 517 nm using an Epoch microplate reader (BioTek, Tokyo, Japan).

$$\text{DPPH Scavenging Activity (\%)} = \frac{Ao - A1}{Ao} x\ 100$$

Where A_0 = Absorbance of the control; A_1 = Absorbance of the sample mixture.

2.3.7. Isoflavone Contents

Isoflavones were determined according to Sulistyowati, Martono, Riyanto, and Lukitaningsih (2019) with slight adjustments. Samples (2.5 g) were extracted in 50% methanol, filtered (0.45 µm), and analyzed using UHPLC (Thermo Scientific™ Vanquish™, UV–Vis detector) on a Hypersil GOLD™ C18 column (250 × 4.6 mm, 5 µm). Isocratic elution with methanol water (40:60, v/v; 0.1% acetic acid) at 0.8 mL min^{-1} was used, with detection at 258 nm. Isoflavone levels were quantified using daidzein and genistein standards and expressed as mg/100 g dry weight.

2.3.8. Protein Profiles were Analyzed using SDS-PAGE

Protein profiles were determined by SDS–PAGE according to Luo et al. (2022) with minor modifications. Each 20 mg sample was dissolved in PBS (pH 7.0), combined with loading buffer, and heated at 95°C for 2 minutes. Ten microliters of the prepared solution were loaded with a molecular weight marker (10–180 kDa), and electrophoresis was performed at 100 V for 90 minutes on 12% resolving and 5% stacking gels. The gels were stained with Coomassie Brilliant Blue R-250, destained, and the band profiles were analyzed using ImageJ software.

2.4. Statistical Analysis

All measurements were expressed as mean ± standard error (SE) from three independent replicates. Statistical analysis was performed using SPSS (version 23). Differences among treatments were assessed by one-way ANOVA, with means compared via Duncan's test at a 95% confidence level ($p < 0.05$). Most analyses were in triplicate; single measurements were reported without SE due to limited samples.

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3. RESULTS AND DISCUSSION

3.1. Protein Content and Protein Digestibility

Table 1 shows that EFP has the highest protein content at 90.69%. This high protein content results from egg white proteins such as ovalbumin and ovotransferrin. FMP protein was also high at 79.68%, though lower than EFP. EMP was significantly lower at 44.30%. This indicates that the protein and non-protein fractions from the flour-based co-precipitation process were indeed protein fractions, demonstrating improved overall protein yields. Similar statements have been reported in the literature where co-precipitation in multiple systems resulted in the recovery of more protein than was previously possible, including whey-rapeseed (Thompson, 1977) soybean-cottonseed (Thompson, 1978), and flaxseed- soybean (Alu'datt et al., 2020) systems.

Table 1. Protein content, protein digestibility of precipitates, co-precipitates, and raw materials.

Sample	Protein content (%db)	Protein digestibility (%db)
FMP	79.68 ± 0.11^{e}	89.80 ± 0.03^{e}
EMP	44.30 ± 0.12^{a}	86.68 ± 0.03^{a}
TFP	70.09 ± 0.04^{c}	86.77 ± 0.03^{b}
EFP	90.69 ± 0.01^{f}	88.09 ± 0.00^{c}
TF	61.12 ± 0.00^{b}	88.42 ± 0.02^{d}
EF	77.62 ± 0.16^{d}	90.87 ± 0.02^{f}

Note: Values are expressed as mean ± SD. Different superscript letters (a–f) within the same column indicate significant differences ($p < 0.05$). FMP: co-precipitate of mixed flour proteins; EMP: Co-precipitate of protein extracts; TFP: Tempe flour precipitate; EFP: Egg white flour precipitate; TF: tempe flour; EF: Egg white flour.

The data are expressed as means and standard deviations. Letters above columns indicate statistically significant differences ($p < 0.05$). FMP, EMP, TFP, and EFP denote specific co-precipitates; TF and EF refer to flour types.

The FMP protein was 89.80% digestible, slightly lower than EF at 90.87% and higher than TF at 88.42%. This high digestibility likely results from Schiff and hydrophobic bonds that enhance enzymatic digestion and solubility. These observations align with existing literature, indicating the structural factors contributing to protein digestibility and solubility are consistent with previous research findings (Liu et al., 2024; Zhou et al., 2022). When TFP (86.77%) and TF (88.42%) digestibility rates are compared to FMP, the contribution of the egg white protein to the nutritional quality of the co-precipitates becomes apparent. This is contrary to the case of EMP, which had a lower digestibility (86.68%) and a lower protein percentage (44.30%). This could be explained by differences in the methods of protein extraction: flour-based systems tend to retain a greater variety of proteins in a non-disrupted state, while systems based on extracts may suffer loss of proteins and handle denaturation due to the pH and solvent conditions. According to Verkempinck, Duijsens, Mukherjee, and Wilde (2024), the alkaline extraction of pea proteins unfolds and aggregates, resulting in reduced solubility and slow digestion in flour-based systems. The lower-than-expected digestibility of EFP may result from protein structure loss during enzyme precipitation, which restricts enzyme access to peptide bonds.

3.2. Amino Acid Profile

The distribution of amino acids in FMP was more balanced between essential and non-essential amino acids than in its individual components, TF and EF. Tempeh, a fermented soybean food, is typically low in sulfur-containing amino acids like methionine, affecting its nutritional profile (Syida, Noriham, Normah, & Yusuf, 2018). As shown in Table 2, FMP was better than EMP, in particular methionine and lysine. The analysis indicates that flour-based co-precipitation was more effective in retaining and concentrating amino acids, likely due to better preservation of soluble material and proteins, with less denaturation. The FMP contained 6.43% lysine, an amino acid typically low in plant proteins but abundant in egg white protein. This suggests co-precipitation of both plant and egg proteins, leading to effective nutritional complementation. Significant amino acids such as leucine (6.05%) and methionine (1.78%) were also present in high concentrations, indicating a synergistic effect between egg and soybean proteins.

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These findings imply that the FMP could be nutritionally more complete, as it incorporates essential amino acids from both sources, enhancing its overall amino acid profile and potential nutritional value.

FMP clearly identified glutamic acid (13.05%) and aspartic acid (8.65%) as among the amino acids with the highest concentrations. These amino acids are likely responsible for the savory flavor and umami taste, potentially enhancing flavor in food systems. The absence of histidine in FMP, EMP, TFP, and EFP may result from detection limits or inherently low levels; however, its presence in TF and EF suggests possible partial degradation during processing. The methionine content in FMP (1.78%) confirms that co-precipitation with egg white effectively supplies sulfur-containing amino acids, which are limited in tempeh. Egg-white proteins are well known as rich sources of sulfur amino acids, particularly methionine and cysteine, both associated with health-promoting effects (Matsuoka & Sugano, 2022).

Table 2. Amino acid profile of precipitates, co-precipitates, and raw materials.

Amino acid	FMP (%w/w)	EMP (%w/w)	TFP (%w/w)	EFP (%w/w)	TF (%w/w)	EF (%w/w)
Aspartic acid	8.65	8.41	5.69	6.73	4.09	7.08
Glutamic acid	13.05	12.97	8.77	10.87	6.62	9.07
Serine	4.60	3.87	2.15	5.32	2.04	5.00
Histidine	n.d.	n.d.	n.d.	n.d.	1.28	2.19
Glycine	2.67	2.34	1.38	2.99	1.70	2.79
Threonine	2.92	2.47	1.30	2.82	1.58	3.46
Arginine	5.13	4.72	3.35	4.88	3.44	5.11
Alanine	4.33	3.46	1.75	4.71	1.91	4.73
Tyrosine	3.28	3.07	1.86	3.52	2.96	6.47
Methionine	1.78	0.63	0.26	2.96	0.30	2.76
Valine	4.32	3.86	2.15	4.33	2.00	4.98
Phenylalanine	4.24	4.10	2.37	4.46	2.35	4.66
Isoleucine	3.89	3.65	2.27	3.54	2.12	4.33
Leucine	6.05	5.94	3.43	6.30	5.55	11.07
Lysine	6.43	6.06	3.30	10.48	3.34	6.10
Glutamine	2.34	2.08	1.08	2.64	n.d.	n.d.

Note: FMP: Co-precipitate of mixed flour; EMP: Co-precipitate of protein extract; TFP: Tempe flour precipitate; EFP: Egg white flour precipitate; TF: Tempe flour; EF: Egg white flour. n.d: Not detected

3.3. Functional Properties

The functional properties of the samples, including water holding capacity, oil holding capacity, swelling power, and solubility, are detailed in Table 3. The highest water-holding capacity (WHC) was observed in EFP (558.99%) and FMP (512.82%), indicating greater exposure of hydrophilic groups within the co-precipitated proteins. Conversely, EF exhibited the lowest WHC (167.20%), consistent with the compact structural conformation typical of egg-white proteins. Oil-holding capacity (OHC) was highest in EMP (271.32%) and TF (290.87%), likely due to the exposure of hydrophobic sites during extraction. EFP (219.55%) and EF (221.05%) showed lower OHC values. Swelling capacity followed a similar trend, with EFP (13.16%) and FMP (9.64%) demonstrating greater flexibility of the protein network. Solubility tests revealed EF (81.71%) as the most soluble, aligning with its intrinsic protein nature, while EMP and EFP had lower values. FMP, TFP, and EFP showed significantly reduced solubility (10–12%), probably due to aggregation near their isoelectric points. Similar results have been reported for other plant-based proteins. Verkempinck et al. (2024) observed that alkaline-extracted pea proteins had much lower solubility (27 %) than salt-extracted ones (77 %), owing to hydrophobic interactions that cause partial denaturation. Although EMP was prepared under highly alkaline conditions (pH 12), its solubility of 48.36% remained moderate, indicating that a semi-native protein conformation was retained, preventing excessive aggregation. Jiang, Xiong, and Chen (2010) observed that soy-protein isolates treated at pH 12 experienced molecular unfolding but regained solubility after neutralization due to partially refolded molten-globule-like structures.

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Table 3. Functional properties of precipitates, co-precipitates, and raw materials analyzed and corrected.

Sample	Water holding capacity (%)	Oil holding capacity (%)	Swelling power (%)	Solubility (%)
FMP	512.82 ± 10.59^{e}	233.82 ± 1.40^{b}	9.64 ± 0.11^{c}	10.93 ± 2.02^{a}
EMP	240.39 ± 2.40^{b}	271.32 ± 3.44^{c}	2.69 ± 0.00^{a}	48.36 ± 0.10^{c}
TFP	416.23 ± 3.95^{c}	222.23 ± 3.39^{a}	5.28 ± 0.10^{b}	11.32 ± 1.23^{a}
EFP	558.99 ± 0.52^{f}	219.55 ± 1.88^{a}	13.16 ± 0.51^{d}	12.04 ± 1.66^{a}
TF	470.85 ± 1.75^{d}	290.87 ± 1.20^{d}	4.93 ± 0.02^{b}	18.46 ± 0.49^{b}
EF	167.20 ± 4.74^{a}	221.05 ± 6.31^{a}	2.86 ± 0.12^{a}	81.71 ± 3.70^{d}

Note: Values are given as mean ± SD. Different superscript letters (a–f) within the same column indicate significant differences ($p < 0.05$). FMP: co-precipitate of mixed flour; EMP: co-precipitate of protein extract; TFP: tempe flour precipitate; EFP: egg white flour precipitate; TF: tempe flour; EF: egg white flour.

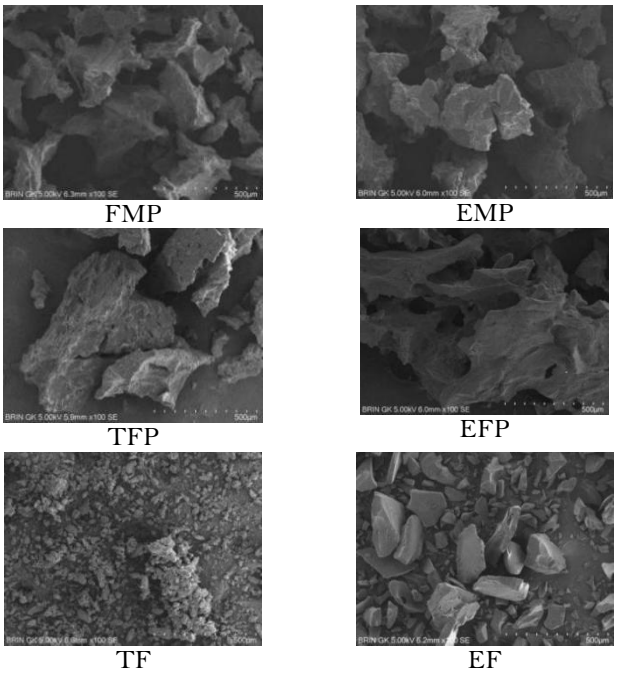

Figure 1. Morphological characteristics of precipitates, co-precipitates, and raw materials.

Note: FMP: Co-precipitate of mixed flour; EMP: Co-precipitate of protein extract; TFP: Tempe flour precipitate; EFP: Egg white flour precipitate; TF: Tempe flour; EF: Egg white flour.

3.4. Morphological Characteristics

As shown in Figure 1, the FMP appeared as a porous, sponge-like matrix composed of loosely connected particles. The open spaces within this structure facilitated water entrapment, explaining its high water-holding capacity (WHC). Conversely, EMP samples appeared denser with fewer pores, correlating with their lower WHC and reduced swelling behavior. Their relatively high solubility may be due to smaller protein aggregates that disperse more evenly in water. Tempe Flour (TF) and its precipitate (TFP) exhibited granular, irregular morphologies consistent with their intermediate WHC and swelling values. The EFP displayed rough surfaces with uneven cavities and had the highest WHC and swelling among all samples. Loosely organized protein networks with irregular voids tend to absorb more water, enhancing hydration. In comparison, EF showed a smooth, compact surface with limited porosity. This dense structure restricted water uptake and swelling, although the natural solubility of ovalbumin still supported high solubility.

3.5. FTIR Analysis

FTIR spectra of the samples (Figure 2) exhibited characteristic amide I (≈1650 cm^{-1}) and amide II (≈1540 cm^{-1}) bands, indicating that the protein backbone structure was preserved following co-precipitation. These bands correspond to C=O stretching and N–H bending vibrations, representing α-helix and β-sheet conformations of

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proteins. Similar patterns have been reported for egg albumin (Kuligowski, Pawłowska, Jasińska-Kuligowska, & Nowak, 2017) and soy–whey co-precipitates, where intermolecular interactions caused slight band shifts and an increased α-helix proportion. Tian et al. (2022) observed comparable FTIR characteristics in soy–whey co-precipitates, representing a combination of the distinctive spectra of each protein component. The spectra indicate that proteins in the tempeh–egg-white co-precipitates are partly unfolded and reorganized, yet they still maintain their primary structural form.

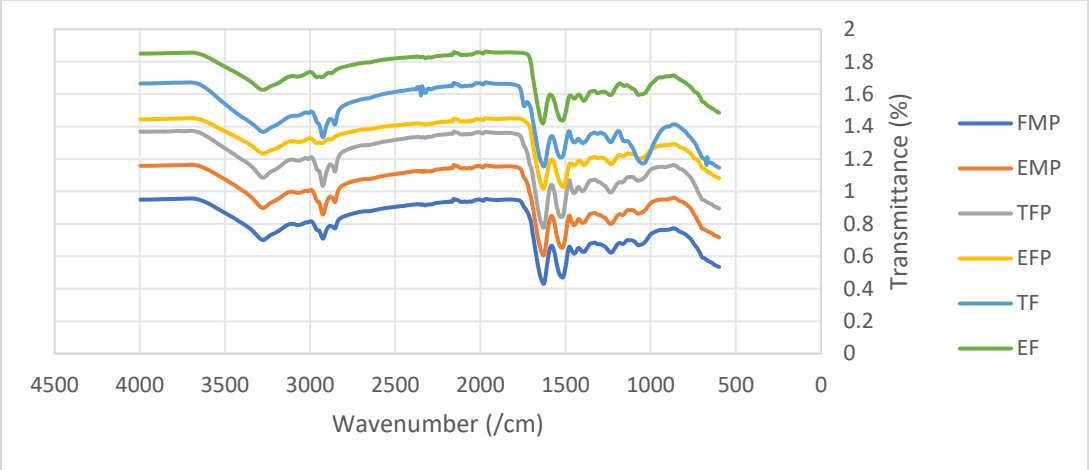

Figure 2. FTIR spectra of precipitates, co-precipitates, and raw materials.

Note: FMP: Co-precipitate of mixed flour; EMP: Co-precipitate of protein extract; TFP: Tempe flour precipitate; EFP: Egg white flour precipitate; TF: Tempe flour; EF: Egg white flour.

3.6. Total Phenolic Content and Antioxidant Activity

The total phenolic content (TPC) and antioxidant activity (%RSA) are summarized in Table 4. The mixed-flour co-precipitate (FMP) contained a moderate amount of phenolics (4.44 mg GAE/g) and exhibited 9.45% RSA, indicating that some bioactivity was retained after processing. Tempeh flour (TF) showed the highest TPC value and demonstrated strong antioxidant capacity, consistent with the enhancement often seen in fermented products. Conversely, egg-white flour (EF) had low phenolic content but relatively high antioxidant activity (22.22% RSA), likely due to bioactive peptides formed during processing that contribute to its antioxidant effects (Rao et al., 2020).

Table 4. Total phenolic and antioxidant activity of precipitates, co-precipitates, and raw materials.

Sample	Total phenolic content (mg GAE/g db)	Antioxidant activity (%RSA)
FMP	4.44 ± 0.59^{a}	9.45 ± 0.73^{b}
EMP	7.61 ± 2.66^{a}	11.93 ± 0.52^{b}
TFP	6.33 ± 0.44^{a}	11.45 ± 0.55^{b}
EFP	1.22 ± 0.10^{a}	5.91 ± 0.67^{a}
TF	426.67 ± 15.89^{c}	20.93 ± 0.77^{c}
EF	243.03 ± 5.55^{b}	22.22 ± 3.31^{c}

Note: Values are expressed as mean ± SD. Different superscript letters (a–c) within the same column indicate significant differences ($p < 0.05$). FMP: Co-precipitate of mixed flour; EMP: Co-precipitate of protein extract; TFP: Tempe flour precipitate; EFP: Egg white flour precipitate; TF: Tempe flour; EF: Egg white flour.

3.7. Isoflavone Content

Isoflavone profiles are presented in Figure 3. Daidzein and genistein were detected in all tempe samples (FMP, EMP, TFP, TF). Tempe flour (TF) exhibited the highest levels, with 65.69 mg/100 g daidzein and 6.83 mg/100 g genistein, indicating abundant aglycone forms released during fermentation. TFP and EMP had intermediate levels (27.52 and 21.00 mg/100 g daidzein), while FMP showed the lowest at 17.77 mg/100 g.

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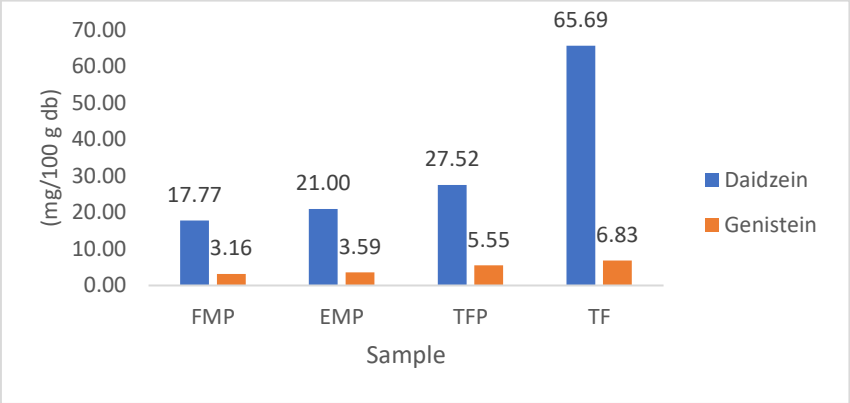

Figure 3. Isoflavone content of precipitates, co-precipitates, and raw materials.

Note: FMP: Co-precipitate of mixed flour; EMP: Co-precipitate of protein extract; TFP: Tempe flour precipitate; TF: Tempe flour.

Isoflavone levels in FMP, EMP, and TFP were lower than in raw materials, likely due to structural changes during co-precipitation. Compared to TF (65.69 mg/100 g daidzein; 6.83 mg/100 g genistein), reductions were approximately 73% and 54% in FMP, 68% and 47% in EMP, and 58% and 19% in TFP, respectively. Isoflavones may bind to proteins via hydrophobic or hydrogen bonds, limiting their release. pH shifts during precipitation can also cause partial degradation. In mixed systems like FMP and EMP, protein–isoflavone competition may further decrease free aglycones. Conversely, TF showed the highest concentrations of daidzein and genistein, consistent with fermentation processes that naturally convert glycosides into aglycones (Lo et al., 2022; Wang, Wang, Feng, Wang, & Wang, 2021).

3.8. Protein Profile Analysis (SDS-PAGE)

The SDS-PAGE patterns are presented in Figure 4. In tempeh-based samples, protein bands between 33–70 kDa corresponded to β-conglycinin (7S) and glycinin (11S). EF and EFP showed strong bands near 45–70 kDa, typical of ovalbumin and ovotransferrin. The profile in FMP indicated successful co-precipitation of plant and animal proteins. Minor smearing above 100 kDa suggested limited aggregation, likely from hydrophobic or disulfide interactions. Similar associations between soy 11S/7S fractions and egg proteins are reported to improve interfacial stability and gel cohesiveness (Guo, Deng, Hu, Zhu, & Zhu, 2024; Liang et al., 2026). In agreement with previous reports on soy–whey co-precipitates (Kristensen et al., 2021; Kuligowski et al., 2017), the major protein bands remained evident across all samples, and variations in their intensity were likely influenced by the initial proportion of the two proteins. The comparable band patterns indicate that SDS treatment disrupted hydrogen-bonded associations, suggesting that the co-precipitated *proteins* were mainly associated through non-covalent interactions (Tian et al., 2022).

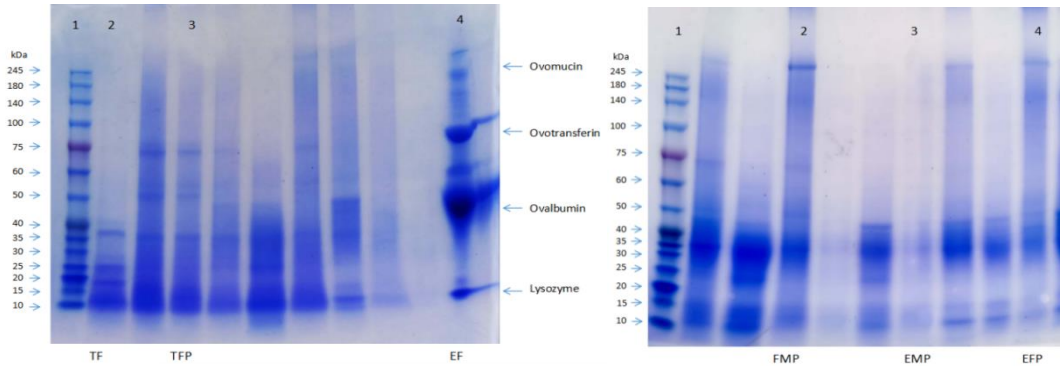

Figure 4. SDS-PAGE of precipitates, co-precipitates, and raw materials.

Note: (A) *Left gel* displays protein bands from individual materials: Lane 1, molecular weight marker; Lane 2, TF (Tempeh flour); Lane 3, TFP (Tempeh flour precipitate); Lane 4, EF (Egg-white flour). (B) *Right gel* shows protein profiles of combined systems: Lane 1, molecular weight marker; Lane 2, FMP (Mixed-flour co-precipitate); Lane 3, EMP (Protein-extract co-precipitate); Lane 4, EFP (Egg-white flour precipitate).

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4. CONCLUSION

4.1. Implications

This study confirmed that integrating tempeh and egg-white flours through co-precipitation significantly enhanced nutritional and functional properties. The mixed-flour co-precipitate (FMP) exhibited high protein content (79.68%) and in vitro digestibility (89.80%), along with water- and oil-holding capacities (512.82% and 233.82%). Structural analyses (SEM, FTIR, SDS-PAGE) verified improved protein network integrity. The co-precipitate could serve as a protein-rich additive for gluten-free products, fortified snacks, or functional beverages, utilizing affordable, local materials. This aligns with Indonesia's food diversification and nutrition-sensitive value chain policies.

4.2. Limitations

Results of this study were conducted under laboratory conditions, which means that conclusions may not be applicable to industrial-scale applications or the product's stability after long-term storage.

4.3. Future Research Directions

To enhance the practical use of the tempeh-egg-white co-precipitates in functional food systems and the sustainability of such systems, additional research should be directed to the stability of the product over time, the adaptability and acceptance of the product by consumers, and the scalability of the production methods.

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