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Physicochemical properties of meat analogs from cowpea (*Vigna unguiculata*) tempeh substituted with soy protein isolate

Aan Sofyan[1]*, Eni Purwani[1], Sudrajah Warajati Kisnawaty[1], Setyaningrum Rahmawaty[1], Sihti Widya Rizkawati[1], Satiy Annisa Fakita[1], Fathia Azzahra Shafa Sabiella[1]

[1]Universitas Muhammadiyah Surakarta, Nutrition Science, Surakarta - Indonesia

***Corresponding Author:** Aan Sofyan, Universitas Muhammadiyah Surakarta, Nutrition Science, Jl. Ahmad Yani, Tromol Pos1, Pabelan Kartasura, Central Java, CEP: 57162, Surakarta – Indonesia, e-mail: aa122@ums.ac.id

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Abstract

Legumes are valuable food sources that can serve as an alternative supply of plant-based protein. Among them, cowpea has been explored, although its utilization in food processing remains limited. This legume holds potential to be developed into meat analog products, particularly when subjected to prior fermentation. This study aimed to evaluate the substitution of soy protein isolate (SPI) on the physicochemical properties of meat analogs from cowpea (*Vigna unguiculata* (L.) Walp) tempeh. The parameters, SPI substitution (0% to 15%) and cowpea tempeh (85% to 100%), were varied in the manufacture of meat analogs, then analyzed for moisture, texture, ash, and nutrient contents (carbohydrate, protein, fat, and crude fibre). The substitution of SPI decreased the moisture (57.7% to 52.6%) and hardness (92.3 N to 77.3 N); increased ash (2.9% to 3.4%), carbohydrate (26.6% to 29.8%), protein (12.04% to 13.02%), fat (0.7% to 1.1%), and crude fibre (5.6% to 9.3%). This work contributes to the alternative use of cowpea and SPI as the main ingredients in the manufacture of meat analogs.

Keywords: Cowpea tempeh; Meat analog; Physicochemical characteristics; Soy protein isolate.

Highlights

- Soy protein isolate - SPI levels enhanced the protein fiber, ash, and carbsin tempeh-based meat analogues
- Texture properties improved with higher levels of SPI incorporation
- Cowpea tempeh with SPI showed technological potential for meat analogs

1 Introduction

Meat analogs are imitation meats developed as alternatives to conventional animal-based meat that are carefully designed to mimic animal-based meat products by modifying appearance, texture, taste, digestibility, and the bioavailability of nutrients (Toh et al., 2024). They are considered healthier due to their potential health benefits (Ishaq et al., 2022) and are considered a substitute for processed meat, which is reported to have negative health impact. Research suggests that consuming approximately 0.6-57 g/day of processed meat is associated with an 11% and 7% increased risk of type 2 diabetes and colorectal cancer, respectively (Haile et al., 2025). Plant-based meat analogs can also be a viable option for individuals who do not consume animal products (Joshi & Kumar, 2015), such as vegetarians (Singh et al., 2021).

In general, the primary ingredient of meat analogues is plant-based proteins (Purnawijayanti et al., 2024) such as legumes. Their high protein content and ability to retain texture, bind and stabilize water and fat components during processing (Shen et al., 2022), make legumes as a good vehicle for making meat analogs. Hence, it can improve the nutritional quality, texture, and shelf life of meat analogs (Baune et al., 2021). Fermented legumes (called tempeh) provide better nutritional value due to they improve the bioavailability of nutrients in the human body (Adebo et al., 2022). It can enhance the nutritional profile of foods through enzymatic activity released by microorganisms, which degrade macromolecules in legumes, resulting in various biochemical changes (Law et al., 2024). In addition, it can inhibit the growth of pathogenic microorganisms (Adebo et al., 2022) and lead to nutritional improvements, such as increased levels of vitamins, essential amino acids, and fatty acids, as well as detoxification and the reduction of anti-nutritional factors (ANFs) due to yeast enzyme activity (Mani & Ming, 2017).

Tempeh has been increasingly recognized as a plant-based meat analog due to its high protein content, unique fermentation-derived characteristics, and favorable texture (Sun & Ruiz-Carrascal, 2023). Traditionally, soybeans are the primary raw material for tempeh production, and they remain the most common ingredient used in the development of tempeh-based meat analogs (Ishaq et al., 2022). However, the reliance on soybeans has raised concerns regarding allergenicity, sustainability, and diversification of raw materials (Górska et al., 2025).

Cowpea (*Vigna unguiculata* (L.) Walp) is a legume with promising potential as a raw material for meat analogs. Although it has been studied for this purpose (e.g., Wibowo et al., 2023), research remains limited compared to soybeans, indicating the need for further exploration of its functionality and applications. Various legumes have been widely utilized in the development of meat analogs, with soybeans being the most common due to their high protein content and established functional properties. Other legumes such as peas, chickpeas, and lentils have also been explored as alternative protein sources in plant-based meat formulations (Górska et al., 2025). Compared to the common meat analogs made from legumes, cowpea contains low fat (1%), high total protein (23% to 32%) and carbohydrate (50–60%), also rich amino acids including 17 types of amino acids, the majority of which are essential, including valine, leucine, phenylalanine, and lysine (Jayathilake et al., 2018). Cowpea (*V. unguiculata*), an underutilized legume, offers promising potential as an alternative substrate for tempeh fermentation (Górska et al., 2025).

To support the mimicry of the physical and chemical characteristics of animal meat, soy protein isolate (SPI) is added in making the cowpea meat analog. SPI contains 90% protein on a dry weight basis and is widely applied in food products (Bu et al., 2022), especially in various protein-based food formulations due to its excellent nutritional and functional properties, such as emulsifying ability and film-forming capacity (Zhang et al., 2015). In meat analog applications, SPI serves not only as a binding agent that enhances firmness and water-holding capacity but also contributes to the physical structure by producing fibrous textures that closely resemble muscle fibers of animal meat (Chiang et al., 2019; Purnawijayanti et al., 2024). When the moisture content in meat analogs is low, the texture tends to become harder (Yadav et al., 2015). In addition to influencing texture, protein isolates also affect the color of meat analogs. A higher proportion of protein isolate in a food product typically results in a darker color (Kang et al., 2022).

To date, there is a lack of research on the development of meat analogs using cowpea tempeh. Therefore, this study aimed to evaluate the effect of SPI substitution on the physicochemical properties of meat analogs produced from cowpea (*V. unguiculata*) tempeh.

2 Materials and Methods

2.1 Raw material

The materials used in this study included cowpeas, which were purchased from Sidodadi Market in Surakarta, Central Java Province, Indonesia and SPI used in this study was obtained from Para Agribusiness, containing 90.08% protein and 5.4% ash on a dry weight basis, as reported by the manufacturer. Information on other proximate components (lipids, carbohydrates, and moisture) was not provided by the supplier. Other ingredients used were Raprima-brand yeast, Rumah Mocaf-brand mocaf flour, Top-Ho-brand angkak (red yeast rice), KRI-02-brand carrageenan, Baker's Bonus A-brand emulsifier, sodium tripolyphosphate (STPP) from Weihai Sing Lih Chemicals Co., Ltd., R&W-brand baking powder, and cocoa butter from Tri Tunggal Supplier. The equipment used included an oven laboratory BINDER ED 56, a Samsung refrigerator, Hakka Commercial 12 Tray Food Dehydrator Electric, Grinder Flour Machine FCT-Z, thermometer, Minolta chroma meter, AMETEK TA1 LLOYD Texture Analyzer, Kjeldahl flasks, fume hood, and muffle furnace.

2.2 Research design

This study was conducted as a laboratory experiment using a completely randomized design (CRD). Three treatment groups consisted of varying formulas of SPI (0%, 10%, and 15%) substitution in the meat analog formulation were applied, with two experimental replications and two analytical replications for each treatment.

2.3 Research procedure

2.3.1 Fermentation of cowpea

The cowpea fermentation procedure was based on the method described by Tan et al. (2024), with modifications. The fermentation process began with sorting and weighing the cowpeas, followed by washing and boiling them at 100 °C for 30 minutes using a water-to-cowpea ratio of 4:1 (v/w). The cowpeas were then soaked for 12 hours and dehulled to remove the seed coats. A second boiling step was performed at 100 °C for 20 minutes using a water-to-cowpea ratio of 3:1 (v/w), followed by draining until the surface of the cowpeas was dry. The next step involved inoculation with tempeh starter culture at a ratio of 2 g of starter per 1 kg of cowpeas. The inoculated cowpeas were then packed in perforated plastic bags to allow air circulation. Finally, the fermentation was carried out by incubating the cowpeas at 32 °C for 48 hours until tempeh was formed.

2.3.2 Cowpea tempeh flour preparation

The procedure for producing cowpea tempeh flour was based on the method by Jonesti et al. (2023), with modifications. The preparation of cowpea tempeh flour followed the processing steps illustrated in Figure 1. The process began with weighing and slicing the fermented cowpea tempeh into pieces approximately 1 cm thick, followed by steaming at 100 °C for 10 minutes. Steaming was carried out using a stainless-steel household steamer pot (diameter 24 cm, Maxim, Indonesia) placed on a Modena gas stove (Modena BH 1725, Indonesia) until the temperature reached 100 °C. The steamed tempeh was then dried using a Hakka Commercial 12 Tray Food Dehydrator Electric at 60 °C for 6 hours. The dried tempeh was ground using a Grinder Flour Machine FCT-Z for 3 minutes and subsequently sieved through a 60-mesh screen to obtain fermented cowpea tempeh flour.

2.3.3 Meat analog production

The production of meat analogs began with preparing ingredients (Table 1), then mixing the dry ingredients—cowpea tempeh flour, SPI, mocaf flour, angkak (red yeast rice), sodium tripolyphosphate, emulsifier, baking powder, and carrageenan—in a container until homogeneous. The dry mixture was then combined with melted cocoa butter and mixed thoroughly. The resulting mixture was transferred to a Chopper Mitochiba CH 200, and 160 mL of crushed ice was gradually added during the grinding process until a smooth and elastic dough was formed. The dough was then refrigerated at 4 °C for 10 minutes, molded into the desired shape, and steamed at 100 °C for 45 minutes to produce the meat analog. The steaming process was conducted using the same household steamer setup described in Section 2.3.2, but with a steaming duration of 45 min at 100 °C.

Table 1. Formulation of meat analog.

Composition	F1	F2	F3
Fermented cowpea flour (g)	100	90	85
Soy protein isolate (g)	0	10	15
Mocaf flour (g)	15	15	15
STPP (g)	1	1	1
Angkak (red yeast rice) (g)	1	1	1
Emulsifier (g)	1	1	1
Baking Powder (g)	1	1	1
Carrageenan (g)	1	1	1
Cocoa Butter (g)	1	1	1
Ice (g)	160	160	160

Note: Formula 1 (F1): 0% soy protein isolate substitution; Formula 2 (F2): 10% soy protein isolate substitution; Formula 3 (F3): 15% soy protein isolate substitution.
STPP = sodium tripolyphosphate

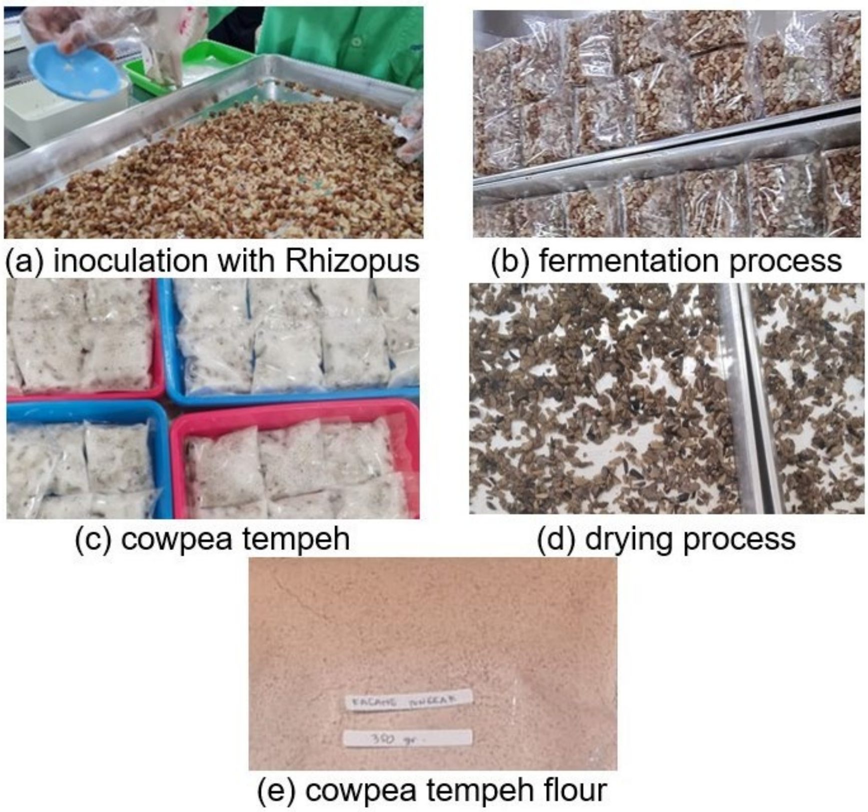

Figure 1. Processing steps of cowpea tempeh flour production.

2.3.4 Proximate analysis

Proximate analysis was conducted according to the official methods of the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 2005). Moisture content was determined using the oven-drying method (AOAC 925.10), ash content by the dry ashing method (AOAC 923.03), protein content by the Kjeldahl method (AOAC 2001.11), and fat content by the Soxhlet extraction method (AOAC 920.39). Carbohydrate content was calculated by difference.

2.3.5 Color analysis

Color analysis was carried out using a Minolta Chroma meter CR. The instrument measures color based on the reflection of light emitted by the device onto the surface of the sample. The results of the color measurements were expressed in terms of L*, a*, and b* values (Saldanha do Carmo et al., 2021).

2.3.6 Texture analysis

Texture testing was performed using the Texture Profile Analysis (TPA) method with a Texture Analyzer (AMETEK TA1 LLOYD). The instrument operates by measuring the resistance of the sample to compression force. The texture parameters obtained from the analysis included hardness, gumminess, cohesiveness, and chewiness.

2.3.7 Data analysis

The experimental data were analyzed using SPSS version 20. One-way analysis of variance (ANOVA) was performed, and when a significant effect was found ($p < 0.05$), the differences among means were further evaluated using Duncan's Multiple Range Test (DMRT).

3 Results and discussion

3.1 Proximate value

The moisture values of the meat analogs tended to decrease (from 57.76% to 52.56%) with an increase in SPI substitution, in contrast, for nutrition values, including carbohydrates, protein, fat, fiber, and ash content were increased (Table 2).

Table 2. Proximate value of Meat Analogs.

Proximate parameters	F1	F2	F3
Moisture	**57.76 ± 0.72^{c}**	**55.12 ± 0.98^{b}**	**52.56 ± 0.80^{a}**
Carbohydrates	26.62 ± 0.93^{a}	28.05 ± 0.21^{b}	29.77 ± 0.64^{c}
Protein	12.04 ± 0.15^{a}	12.85 ± 0.57^{b}	13.02 ± 0.05^{b}
Fat	0.66 ± 0.11^{a}	0.88 ± 0.06^{a}	1.12 ± 0.87^{b}
Crude fiber	5.65 ± 0.32^{a}	6.20 ± 0.66^{a}	9.27 ± 2.23^{b}
Ash	2.90 ± 0.07^{a}	3.06 ± 0.20^{a}	3.37 ± 0.07^{b}

Note: Different superscript letters in the same row indicate significant differences ($p < 0.05$).

This reduction of moisture of the meat analog may be attributed to the ability of soy protein isolate to form a gel network that can retain water, thereby influencing water retention in the meat analog (Shih et al., 2016). In addition, SPI has hygroscopic properties in both its native and denatured forms. Denatured proteins possess more polar sites, which enhance their water absorption capacity through liquid imbibition or vapor adsorption (Jovanovich et al., 2003).

In this study, the carbohydrate content of the analog meat formulations showed a slight increase with the addition of SPI, ranging from 26.62% in the control to 29.77% at 15% SPI substitution. Several possible factors could explain this result. First, the SPI used in this study may not have been of maximum purity (≥ 90% protein), as the actual composition of commercial SPI can vary depending on the manufacturer and processing method, sometimes containing 3–8% carbohydrates in addition to protein. Although SPI typically contains more than 90% protein, certain SPI products may still retain carbohydrate content of up to 22% (Alarape et al., 2024). Second, the method of proximate analysis (by difference) could influence the carbohydrate value, especially since carbohydrates were calculated as the remaining fraction after moisture, ash, protein, and fat determinations. Consequently, any minor variations in the other components would affect the calculated carbohydrate content. Third, the substitution of SPI may alter the food matrix and water-binding properties, potentially influencing the apparent carbohydrate content due to interactions among macronutrients (Hu et al., 2024).

The protein contents of the meat analogs for F1, F2, and F3 treatments were 12.04%, 12.85%, and 13.0%, respectively. The protein content of the meat analogs increased with higher levels of SPI substitution. However, no significant difference ($p > 0.05$) was observed between F2 and F3, despite F3 containing a higher proportion of SPI. This could be attributed to the influence of moisture content, as F3 had lower moisture content compared to F2, which may have masked the difference in protein values when expressed on a wet basis. This finding is in line with a previous study on soy protein-substituted sausages, which also showed an increasing trend in protein content (Kang et al., 2022). The increase in protein content in the meat analogs may be attributed to the high protein concentration of the soybean protein isolate used, which can reach over 90% (Qin et al., 2022).

The fat content of the meat analogs showed an increase in line with the higher percentage of SPI substitution, with values of 0.66% (F1), 0.88% (F2), and 1.12% (F3). This increase may be related to the emulsifying properties of SPI (Galani et al., 2023). Soy protein isolate is known to have a high emulsifying capacity, as it can form and stabilize emulsions between water and fat by reducing surface tension (Castro-Criado et al., 2023). This allows more fat to be retained within the product matrix during the meat analog production process. The higher the SPI level, the greater its fat-binding capacity, resulting in a higher measured fat content in the final product.

The fiber content of the meat analogs increased with the higher percentage of SPI substitution. This increase may not only be attributed to the fiber content of the ingredients themselves, but also to the interaction between proteins and polysaccharides during the meat analog production process. Emulsion stability can be enhanced through the appropriate combination of proteins and polysaccharides (Xu et al., 2023). The heating process during meat analog formation causes protein unfolding, improving the protein's ability to stabilize emulsions. The presence of SPI enhances hydrophobic interactions and facilitates the formation of more stable protein–polysaccharide networks (Zhang et al., 2015). Protein and polysaccharides can form electrostatic interactions to produce complex molecules, which may contribute to the increase in fiber content.

The ash content of the meat analogs increased along with the higher percentage of SPI substitution, with values of 2.90%, 3.06%, and 3.37%, respectively. Although the numerical increase appears relatively small, statistical analysis indicated a significant difference. This increase in ash content may be attributed to the contribution of minerals present in the soybean protein isolate used as a substitution ingredient (Schmid et al., 2024).

3.2 Texture profile

The variation in the percentage of SPI substitution had a significant effect on the hardness of the meat analog. The hardness values in treatments F2 and F3 were lower than those of F1 (Table 3).

Table 3. Texture Profile of Meat Analogs

Texture parameters	F1	F2	F3
Hardness (N)	92.29 ± 8.65[b]	65.32 ± 10.17[a]	77.28 ± 13.78[a]
Cohesiveness	0.45 ± 0.04[a]	0.53 ± 0.04[a]	0.51 ± 0.04[a]
Chewiness (N)	47.18 ± 4.99[a]	35.47 ± 5.34[a]	43.10 ± 8.06[a]
Gumminess (N)	54.55 ± 6.90[a]	40.92 ± 6.52[a]	49.49 ± 9.04[a]
Springiness	0.86 ± 0.021[a]	0.86 ± 0.004[a]	0.87 ± 0.006[a]

Note: Superscript letters that differ indicate significant differences at the 0.05 level of significance

Carbohydrate, fat, and moisture contents are among the most influential factors affecting food texture (Ata et al., 2023). In addition, lower water-holding capacity is positively correlated with increased hardness, resulting in a firmer and more elastic texture (Flory et al., 2023). The hardness value may also be affected by the presence of air pockets and fat globules that disrupt the structural integrity, thereby decreasing the firmness of the product (Godschalk-Broers et al., 2022).

The hardness of the meat analog without SPI substitution was higher compared to those with SPI substitution. This may be attributed to the ability of certain proteins, such as SPI, to form stable gels during heating, thereby contributing to a softer texture in the meat analog (Flory et al., 2023). Textured soy protein is considered one of the most suitable proteins for improving the texture of meat analog products (Yadav et al., 2015). Similarly, processing parameters such as moisture content and temperature have been shown to significantly influence the hardness of extruded cowpea-based products (Silva et al., 2023). These findings support the present study, in which both protein composition and moisture content played a key role in determining the final texture.

The hardness of the control formulation (F1) was the highest (92.29 N), whereas formulations containing SPI (F2 and F3) showed significantly lower values (65.32 and 77.28 N, respectively). This reduction in hardness is desirable, since excessive hardness is usually associated with toughness and lower consumer acceptance. The hardness of cowpea tempeh-based meat analogs in this study (65–92 N) was comparable to some commercial plant-based meat analogs, such as Beyond Meat (88 N) and Greenway Burger (92 N), as reported by Godschalk-Broers et al. (2022). However, the values were lower than real beef (210 N) or certain other analogs (e.g., Gardein Ultimate Beefless Burger, 254 N), indicating that cowpea-based formulations may produce a softer texture.

Statistical analysis showed that varying the substitution percentage of SPI had no significant effect on the cohesiveness, chewiness, gumminess, or springiness of the meat analog. However, according to Ishaq et al. (2022), a combination of SPI and rice protein isolate can enhance water absorption and porosity, thereby improving hardness and chewiness in meat analogs. Soy protein isolate is also widely used in meat products due to its ability to maintain texture and retain water (Nishinari et al., 2014). The hydration properties of SPI are strongly influenced by particle size, which in turn affects its gelling behavior and consequently the texture of the final product (Schmid et al., 2024).

3.3 Color attributes

The brightness of the meat analog decreased significantly with the increasing percentage of SPI (Table 4). The L* value in the color analysis of the meat analog indicates its brightness level. Soaking and cooking processes can increase lightness (L* value), whereas fermentation tends to darken cowpea tempeh (Cuevas-Rodríguez et al., 2006). This may be attributed to the incorporation of plant-based protein isolates, which can lead to a darker product color (Yeater et al., 2017). SPI increases the availability of amino compounds to react with aldehydes in the Maillard reaction, forming melanoidin pigments that contribute to the darker coloration (Akesowan, 2009).

Table 4. Color Attributes of Meat Analogs

Color Parameter	F1	F2	F3
L*	47.97 ± 0.63[b]	44.07 ± 2.32[a]	41.45 ± 2.49[a]
a*	12.95 ± 0.26[a]	15.92 ± 2.48[a]	14.05 ± 0.61[a]
b*	13.22 ± 0.33[a]	16.97 ± 3.07[a]	14.42 ± 1.95[a]

Note: Superscript letters that differ indicate statistically significant differences at the 0.05 significance level.

Color is one of the key attributes influencing consumer perception and evaluation of product quality (Hoppu et al., 2018). The lightness (L) of the meat analog decreased, the yellowness (b) increased, while the redness (a*) remained relatively unchanged (Figure 2) which it is in line with previous study (Majzoobi et al., 2018).

The color analysis of meat analogs revealed significant variations in lightness (L*), redness (a*), and yellowness (b*) among the three formulations (Table 4). The control sample (F1) exhibited the highest L* value (47.97), indicating a lighter appearance compared to F2 (44.07) and F3 (41.45). This reduction in lightness with higher SPI substitution suggests a darker color development, which could be attributed to the inherent color of soy protein isolate and its interaction with tempeh flour during processing. The a* values indicated that redness was slightly higher in F2 (15.92) compared to F1 (12.95) and F3 (14.05), while b* values showed a tendency toward higher yellowness in F2 (16.97) compared to F1 (13.22) and F3 (14.42). These variations reflect the contribution of SPI to the Maillard reaction and pigment interactions that affect the overall color profile.

To better quantify these changes, the total color difference (ΔE) was calculated using F1 as the reference. The results showed that F2 and F3 had ΔE values of 6.17 and 6.72, respectively, when compared to F1. According to the general interpretation of ΔE, values greater than 5 indicate color differences that are easily perceptible to the human eye (Ly et al., 2020). This finding demonstrates that the substitution of tempeh flour with SPI at levels of 10% (F2) and 15% (F3) resulted in a visually noticeable color shift compared to the control.

Figure 2. Color of Meat Analog.

The addition of red mold rice (angkak) was intended to enhance the redness (a*) of the meat analogs. However, the results showed that the redness values among F1, F2, and F3 did not differ significantly (Table 4). This indicates that the contribution of angkak to the overall redness was consistent across formulations, regardless of the proportion of soy protein isolate (SPI) substitution. Since all samples received the same amount of angkak, the pigment input from this ingredient was constant, and therefore, it did not create measurable differences in redness among treatments.

Red mold rice, a fermentation product of *Monascus* spp., supports health when used in appropriate quantities and is widely applied as a natural colorant in food processing (Yanli & Xiang, 2020). The red color of meat analogs can also be achieved using leghemoglobin, annatto extract, beetroot juice extract, or lycopene. On the other hand, color formation in meat analogs depends on the combination of reducing sugars and colorants that are thermally unstable (Ishaq et al., 2022).

Based on the overall findings, both F2 (10% SPI substitution) and F3 (15% SPI substitution) demonstrated improvements in certain quality attributes compared to the control (F1). F2 showed a significant increase in protein content while maintaining desirable textural characteristics, with lower hardness values that may contribute to better chewability. In addition, its color attributes were closer to those expected for meat analogs, particularly in terms of brightness (L*). Meanwhile, F3 also provided a higher protein content and slightly better crude fiber levels, but its hardness increased compared to F2, which might negatively influence consumer perception of texture.

Considering the balance between nutritional composition, texture, and color, F2 appears to be the most suitable formulation. The 10% SPI substitution level provided a notable protein improvement without compromising texture quality, and the color remained acceptable. Therefore, increasing the SPI substitution to 15% does not seem strictly necessary, as F2 already offers favorable characteristics that may support good sensory acceptance. However, further sensory evaluation would be essential to confirm consumer preferences and acceptance of these formulations.

4 Conclusion

This study demonstrated that the incorporation of SPI into cowpea tempeh-based meat analogs influenced their nutritional composition, texture, and color attributes. Substitution with 10% SPI (F2) significantly increased protein content while maintaining favorable textural properties and acceptable color, suggesting that this formulation offers a balanced improvement in both nutritional and physical quality. Increasing the substitution to 15% SPI (F3) provided a further increase in protein content but was associated with a darker appearance and higher hardness, which may reduce consumer acceptance.

The findings highlight the potential of cowpea tempeh as a promising raw material for developing plant-based meat analogs, particularly when combined with SPI. This study provides new insight into optimizing legume-based formulations that balance protein enrichment with desirable sensory-related properties. Future research should include sensory evaluation and scale-up trials to confirm consumer acceptance and explore the industrial application of cowpea tempeh–SPI analogs as sustainable alternatives to animal-derived meat.

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Data Availability Statement

The data supporting this study are not publicly available, but can be requested from the corresponding author upon reasonable request.

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