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Physical, Chemical, and Organoleptic Characteristics of Tempeh Cookies

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Abstract: Cookies are widely consumed snack options made primarily from wheat flour. Alternative materials, such as tempeh flour, are needed to replace wheat flour in the production of cookies. Tempeh contains high levels of protein and crude fiber. This research aims to determine the effect of wheat flour and tempeh flour formulations on the physical, chemical, and organoleptic characteristics of cookies. This research employed a one-factor Completely Randomized Design (CRD), with variations in wheat flour and tempeh flour (F1, 100% wheat flour: 0% tempeh flour), (F2, 75%: 25%), (F3, 50%: 50%), and (F4, 25%: 75%). A total of 31 untrained panelists carried out organoleptic evaluations on the product. The analyzed physical and chemical properties include moisture content, ash, protein, fat, carbohydrates, crude fiber, and texture. Organoleptic tests include taste, color, aroma, hardness, crispness, aftertaste, and overall impression. Data analysis was performed using One-way ANOVA at a 5% significance level, followed by Duncan's post-hoc test. Results indicate that the different formulations of wheat flour and tempeh flour significantly affect the moisture content, fat, protein, carbohydrates, crude fiber, hardness, crispness, and organoleptic properties of cookies. The best cookie formulation was obtained at F4 (25% wheat flour: 75% tempeh flour), with the highest protein and crude fiber content of 16.10% and 12.36%, respectively. This finding had implications for reducing dependence on imported wheat flour and encouraged the diversification of local food products based on fermented soybeans with high protein and fiber content.

Keywords: cookies, organoleptic, tempeh flour, wheat flour

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INTRODUCTION

The dietary pattern of the Indonesian population demonstrates an imbalance, with the majority of people preferring foods high in sugar but low in protein. A national food consumption survey found that approximately 65% of respondents frequently consume sweet foods such as soft drinks, cakes, and sweet snacks, while only 25% regularly consume adequate amounts of animal or plant-based protein sources (Kencanaputri et al., 2024). This imbalance potentially increases the risk of various health problems, including obesity, diabetes, and macronutrient deficiencies.

Research by Safitri & Fitranti (2016) revealed that young adult males aged 18–24 years had an average protein intake below 80% of the recommended dietary allowance. Similarly, females under 20 years and those aged 20–35 years also demonstrated inadequate protein intake (<80%) of the 2013 RDA (Nunes et al., 2022). In Indonesia, tempeh is a notable protein source, competing with animal protein in terms of both quality and quantity. Animal protein generally offers higher nutritional quality compared to plant protein; however, combinations of several plant protein sources yield a more complete amino acid profile with relatively high quality (Diana, 2009).

Alongside the insufficient protein consumption, most Indonesians also consume inadequate dietary fiber. Approximately 80% of the population consumes only 15 g of fiber per day, while the recommended intake is approximately 25 g per day (Soerjodibroto, 2004). Tempeh is highly nutritious, as it contains protein, essential amino acids, essential fatty acids, B-complex vitamins, and dietary fiber (Teoh et al., 2024). Soy tempeh, in particular, is rich in crude fiber due to the proliferation of Rhizopus sp. mycelia during fermentation (Hasibuan et al., 2024). Soy tempeh contains 7.2 g/100 g of crude fiber, which is higher than unfermented soybeans with 3.7 g/100 g (Turana et al., 2025). High-fiber foods benefit health by preventing constipation, diluting toxic compounds in the colon, and reducing the absorption of carcinogenic substances in the digestive tract, which are subsequently excreted from the body. Through these mechanisms, dietary fiber helps prevent various degenerative diseases (Silalahi, 2006).

Cookies are classified as baked biscuits with a soft, crumbly texture that is less dense when sliced (BSN, 2011). They are commonly made from wheat flour with low protein content (8–9%). One approach to improve the protein level in cookies is by incorporating tempeh flour. Although tempeh is rich in plant-based protein, it has a short shelf life of about two days (2 × 24 h) (Yolanda & Hidayah, 2024). Beyond this period, proteolytic bacteria rapidly spoil the product (Marcelli et al., 2024). Consequently, processing tempeh into flour form is required to extend its shelf life (Bastian et al., 2013).

Previous research showed that the partial substitution of wheat flour with tempeh flour increased the protein and fiber content in processed products; however, the optimal formulation and its effects on the physical and organoleptic characteristics of cookies had not been widely explored. The novelty of this research lay in determining the best proportion between wheat flour and tempeh flour that not only increased the nutritional value but also maintained the texture quality and consumer acceptance of the cookies. Based on this, the purpose of this study was to determine the effects of variations in wheat flour and tempeh flour formulations on the physical, chemical, and organoleptic properties of tempeh cookies. The research hypothesis stated that a higher proportion of tempeh flour used would increase the protein and crude fiber contents of the cookies but might affect the texture and sensory acceptance of the product.

METHODS

Equipment and Materials

The equipment used in this study included a balance, mixer, blender, baking trays, an 80-mesh sieve, oven, spoons, bowls, plates, spatula, cabinet dryer, grinder, electric stove, stopwatch, and gloves. Instruments for chemical analysis included a muffle furnace (B-One), oven, desiccator, analytical balance, Soxhlet apparatus, fat flask, Erlenmeyer flask, spatula, beakers, funnels, weighing bottles, porcelain crucibles, mortar and pestle, 10 mL pipettes, burettes, Kjeldahl flasks, water bath, and a texture analyzer.

The ingredients used to prepare tempeh cookies included wheat flour (Pita Merah, low protein 8–9%), tempeh (Super Murni Do'a Ibu), carrot (Nantes), powdered sugar (Jago), margarine (Palmia), chicken eggs, baking powder (Koepoe-Koepoe), salt (Daun), and powdered milk (Dancow). The chemicals used for analysis included ethanol 95%, acetone, Na_2SO_4, H_2SO_4 0.3 N, $CuSO_4$, selenium, NaOH 1.5 N, H_3BO_3 4%, NaOH 30%, $Na_2S_2O_3$ 5%, HCl 0.1 N, HCl 0.2 N, Whatman filter paper No.1, bromcresol green indicator, methyl red indicator, and petroleum benzene.

Procedures

1. Preparation of tempeh flour

   Fresh soybean tempeh ("Super Murni Do'a Ibu") was thinly sliced and placed on trays. It was dried in a cabinet dryer at 60 °C for 24 hours. The dried tempeh was then ground using a blender and sieved through an 80-mesh sieve (Madani et al., 2023).
2. Preparation of carrot flour

   Carrots were peeled, washed with running water, and sliced thinly or grated; excess water was removed by squeezing. The carrot slices were then oven-dried at 60 °C until thoroughly dried, ground into fine powder, and sieved (Madani et al., 2023).
3. Preparation of tempeh cookies

   Margarine and powdered sugar were creamed for about 3 minutes, and eggs were added and mixed for roughly 2 minutes. Other ingredients (tempeh flour, wheat flour, powdered sugar, powdered milk, carrot flour, and baking powder) were then added and mixed until a dough formed. The dough was shaped using a 3.5 cm diameter mold with 0.5 cm thickness and baked at approximately 150°C for 20 minutes. (Gardjito et al., 2018).
4. Chemical Analysis of Tempeh Cookies
   a. Moisture content: Determined according to Madani et al. (2023) by drying 2 g of sample at 105 °C for 3–5 hours until reaching constant weight.
   b. Ash content: Measured by incinerating 2 g of sample in a muffle furnace at 550 °C for 3–5 hours until reaching constant weight.
   c. Protein content: Determined using the Kjeldahl method (Apriantono, 2023).

d. Fat content: Analyzed using Soxhlet extraction with petroleum benzene (AOAC, 2005).
e. Carbohydrate content: Calculated by difference (100% – [moisture + protein + fat + ash]).
f. Crude fiber content: Analyzed according to AOAC (2005) using sequential acid and alkaline digestion.

5. Physical Analysis of Tempeh Cookies

   The texture of the cookies was evaluated using a texture analyzer, measuring hardness and fracturability (Madani et al., 2023).

Organoleptic Evaluation

A sensory evaluation was conducted to determine panelists' preferences for tempeh cookies. Thirty-one untrained panelists (22 females and 9 males, aged 20–25 years, students of Universitas Ahmad Dahlan) participated. A hedonic test was carried out using a 5-point Likert scale (1 = strongly dislike, 2 = dislike, 3 = slightly like, 4 = like, 5 = strongly like). The attributes assessed included taste, color, aroma, hardness, crispness, aftertaste, and overall acceptability (Kumalasari, 2024).

Data Analysis

Data were analyzed using One-way Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD) with SPSS version 21.0. If significant differences ($p < 0.05$) were found, Duncan's Multiple Range Test (DMRT) was performed as a post hoc analysis.

RESULTS AND DISCUSSION

Chemical Properties

Table 1

Proximate Composition of Tempeh Cookies

Formulation*	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Crude Fiber (%)
F1 (100:0)	5.13±0.06^{a}	2.45±0.07^{a}	59.60±0.08^{d}	7.49±0.11^{a}	25.33±0.27^{b}	4.71±0.05^{a}
F2 (75:25)	6.60±0.05^{b}	2.52±0.05^{a}	47.31±0.04^{a}	9.90±0.54^{b}	33.67±0.19^{c}	5.22±0.01^{b}
F3 (50:50)	6.77±0.08^{c}	2.54±0.04^{a}	52.27±0.03^{b}	12.97±0.01^{c}	25.43±0.09^{b}	10.50±0.09^{c}
F4 (25:75)	7.52±0.04^{d}	2.60±0.04^{a}	56.47±0.03^{c}	16.10±0.08^{d}	17.30±0.09^{a}	12.36±0.05^{d}

*Formulation based on wheat flour: tempeh flour ratio.
a, b, c, d = Means with different superscripts within the same column differ significantly ($p < 0.05$)

Moisture Content

Based on Table 1, the One-way ANOVA test showed significant differences ($p < 0.001$). Duncan's post hoc test indicated that all tempeh cookie formulations significantly affected the average moisture content. The highest moisture content was observed in sample F4 (7.52%), while the lowest was in F1 (5.13%). According to SNI 2973:1992, the maximum permitted moisture content is 5%; thus, only cookies from formulation F1 complied with the SNI standard. Sipayung (2014) reported that increasing the proportion of tempeh flour results in higher cookie moisture content. Similarly, Salim (2017) noted that tempeh is a high-protein food, and Marcelli (2024) stated that higher protein content reduces water release at the same heating temperature.

Ash Content

As presented in Table 1, the One-way ANOVA revealed no significant differences ($p = 0.526$) among formulations for ash content. The highest ash content was found in F4 (2.60%), while the lowest was in F1 (2.45%). According to SNI 2973:1992, the maximum permitted ash content is 1.5%. Therefore, cookies from formulations F1 and F2 complied with the SNI standard (<1.5%), whereas those from F3 and F4 exceeded it (>1.5%). Tempeh flour contains approximately 2.33% ash content (Bastian et al., 2013). In comparison, wheat flour typically contains 14% moisture, 8–12% protein, 0.25–0.60% ash, and 24–36% wet gluten. The relatively high ash content in tempeh cookies indicates a higher mineral content (Kaushik et al., 2015). This aligns with Koni et al. (2023), who noted that fermented products generally have elevated ash levels.

Fat Content

Table 1 demonstrated significant differences ($p < 0.001$), with Duncan's test confirming that all formulations significantly influenced fat content. The highest fat level was in F1 (59.60%), and the lowest in F2 (47.31%). According to SNI 01-2973-2011, cookies must contain at least 9.5% fat. All formulations exceeded this minimum standard. Similar findings were reported by Yolanda & Hidayah (2024), where the fat content of control and F1 tempeh cookies was 29.72% and 29.62%, respectively, also meeting SNI requirements. The addition of margarine significantly increased fat levels, as margarine contains 65–75% fat (Kaushik et al., 2015). Cookie fat primarily originates from margarine and eggs. Higher fat content may be associated with microbial cell mass in the substrate. However, high levels of fat, despite being nutritionally significant, may negatively impact storage stability due to rancidity (Turana et al., 2025).

Protein Content

As presented in Table 1, the One-way ANOVA indicated significant differences ($p < 0.001$). Duncan's test confirmed that all formulations had a significant effect on protein content. The highest protein content was found in F4 (16.10%), while the lowest was in F2 (7.49%). According to SNI 2973:2011, cookies must contain at least 5% protein. All formulations exceeded this requirement. Soybeans are recognized as a high-quality plant-based protein source (Rismayanthi, 2015). Yolanda & Hidayah (2024) also reported that black soybean tempeh flour increased cookie protein content, with the highest levels observed in F5 (100:50 wheat: tempeh flour) compared to the control (F0, 100:0). Rismayanthi (2015) found similar results, where biscuits enriched with red bean flour (10%, 17.5%, and 25%) increased protein to 7.27 g, 8.51 g, and 8.94 g per 100 g, respectively. Nariah et al. (2024) further confirmed that higher protein and fat levels in cookies were due to the increased protein and fat content of tempeh flour.

Carbohydrate Content

Duncan's test showed that F2 had the highest carbohydrate content (33.67%), while F4 had the lowest (17.31%). Madani et al. (2023) reported that the carbohydrate content in the F3 tempeh cookie was 53.96%, below the SNI 2973:1992 minimum of 70%. Thus, none of the formulations in this study met the carbohydrate standard. Sipayung et al. (2014) noted that carbohydrate content (by difference) depends on other proximate components (protein, fat, ash, and moisture), where lower values of these components result in higher carbohydrate content. Supported by Malau (2022) who found that increasing the proportion of tempeh flour (low in carbohydrates) while reducing kepok banana flour (high in carbohydrates) led to lower carbohydrate levels. Raw material analysis revealed that kepok banana flour contained 88.55% carbohydrates, whereas tempeh flour contained only 23.46%.

Crude Fiber Content

The One-way ANOVA test showed significant differences in crude fiber content among formulations ($p < 0.001$). Duncan's test revealed that the highest crude fiber was in F4 (12.36%), while the lowest was in F1 (4.71%). According to SNI 2973:1992, the maximum permitted crude fiber level is 0.5%; thus, all formulations exceeded the standard. Madani et al. (2023) reported that crude fiber in selected tempeh cookies was only 0.05%, which is within the acceptable range. Tempeh flour contains approximately 18.50% crude fiber Malau et al. (2022), making it a rich dietary fiber source. The higher crude fiber content is associated with cellulose in tempeh flour, which has strong water-binding properties. Istiqomah et al. (2019) reported that Rhizopus sp. fermentation enhances nutritional value by increasing crude fiber content. The formation of dense tempeh mass from fungal mycelia also contributes to higher crude fiber. Mycelia are composed of hyphae with cellulose and chitin in their cell walls (Soerjodibroto, 2004), which increases crude fiber levels. Sutomo (2018) similarly reported that crude fiber in soybean tempeh (7.2 g/100 g) was higher than in unfermented soybeans (3.7 g/100 g).

Physical Properties

Table 2

Texture Properties of Tempeh Cookies

Formulation*	Hardness	Fracturability
F1 (100:0)	363.31 ± 0.19^{a}	10.61 ± 0.27^{d}
F2 (75:25)	484.45 ± 0.35^{b}	7.61 ± 0.39^{c}
F3 (50:50)	499.25 ± 0.61^{c}	6.83 ± 0.20^{b}
F4 (25:75)	606.74 ± 0.46^{d}	2.37 ± 0.23^{a}

*Formulation based on wheat flour: tempeh flour ratio.
a, b, c, d = Means with different superscripts within the same column differ significantly ($p < 0.05$)

Texture

Texture analysis was conducted using a texture analyzer to objectively evaluate hardness and fracturability. According to Safitri & Fitranti (2016), hardness and fracturability are two key parameters for cookies, as they are strongly correlated. Both are considered critical indicators in analyzing the texture of baked products such as bread and biscuits (Bastian et al., 2013).

Hardness

Based on Table 2, the results of the One-way ANOVA test on the hardness parameter show significantly different results ($p<0.001$). Duncan's post-hoc test indicates that all tempeh cookie formulations significantly affect the average hardness of the tempeh cookies' texture. Table 2 presents that the hardness value increases with the addition of more tempeh flour. The lowest hardness was found in the F1 sample with a value of 363.31 N, while the highest hardness was found in the F4 sample with a value of 606.74 N. According to Kristanti et al. (2020), the addition of tempeh flour increases the hardness of the cookies but reduces their fracturability. This occurs because the cookies have low expansion power, making the texture hard. Protein denaturation and the inhibition of water penetration, caused by the formation of a layer on the surface of starch granules by fat, prevent cookies from rising and result in a hard texture (Sari et al., 2019). The results align with Kristanti et al. (2020), which states that more tempeh flour increased the hardness of the cookies. According to Salim (2017), a higher proportion of tempeh flour causes a harder and more compact snack bar texture. Supported by Koni et al. (2023), stated that the texture of the snack bar becomes harder as the proportion of tempeh flour increases.

Fracturability

The One-way ANOVA test results for the fracture parameter show significant differences ($p<0.001$). Duncan's post-hoc test indicates that all tempeh cookie formulations significantly affect the average fracture value of the tempeh cookies' texture. Table 2 presents that the breaking strength decreased as the amount of tempeh flour increased. The highest breaking strength was found in sample F1 with a value of 10.6168 N, while the lowest breaking strength was found in sample F1 with a value of 2.379 N. According to Kristanti et al. (2020), the addition of tempeh flour reduces the breaking strength value, indicating less cookie expansion due to a higher tempeh flour ratio. Increasing the ratio of tempeh flour raises the protein and fat content of the cookies. The protein in the cookie dough will denature during the baking process, making the cookies difficult to rise. This is supported by Kristanti et al. (2020), which states that more tempeh flour results in a lower breaking strength value. The F0 cookies without the addition of tempeh flour had a breaking strength of 21.96 N, while the cookies with the most tempeh flour added (F5) had a breaking strength of 20.76 N.

Organoleptic Evaluations

Organoleptic tests were conducted with 31 untrained panelists aged 20–25 years to assess the acceptability of color, aroma, hardness, crispness, taste, aftertaste, and overall preference.

Color

Based on Table 3, the One-way ANOVA results for the hedonic evaluation of color preference showed significant differences ($p <0.001$). Duncan's post hoc test indicated that the color attribute of tempeh cookies in F1 was significantly different from F2, F3, and F4. Specifically, F2 and F3 were significantly different from F1 and F4, while F4 was significantly different from F2 and F3 but not from F1. The highest color preference score was for F1 (4.07, 'like'), whereas the lowest score was for

F4 (3.10, 'slightly like'). These findings demonstrate that the preference for cookie color decreased with increasing levels of tempeh flour substitution. This result is consistent with Lailatul & Anna (2019), who reported that higher levels of tempeh flour substitution resulted in a darker brown color in Kembang Goyang cookies, which reduced panelist acceptance.

Furthermore, the One-way ANOVA for the descriptive color evaluation (Table 4) also showed significant differences (p <0.001). Duncan's test revealed that F1 was significantly different from F2, F3, and F4, while F2 and F3 were significantly different from F1 and F4, and F4 was significantly different from F1, F2, and F3. The descriptive assessment indicated that increasing tempeh flour levels produced progressively darker cookies. According to Kristanti et al. (2020), higher proportions of tempeh flour result in darker (brownish) color changes. This is further explained by the production of brown pigments through the high protein content of the cookies, which promotes Maillard reactions. Malau et al. (2022) noted that the Maillard reaction occurs between reducing sugars and amino groups at high temperatures, leading to browning.

Table 3

Organoleptic Tests Results

Formulation*	Color	Aroma	Hardness	Crispness	Taste	Overall
F1 (100:0)	4.07 ± 0.94^{b}	3.83 ± 1.02^{a}	4.20 ± 0.66^{b}	4.17 ± 0.87^{b}	4.10 ± 0.80^{c}	4.17 ± 0.79^{c}
F2 (75:25)	3.90 ± 0.92^{b}	3.87 ± 0.86^{a}	4.03 ± 0.76^{b}	4.07 ± 0.83^{b}	3.93 ± 0.94^{bc}	4.07 ± 0.87^{c}
F3 (50:50)	3.77 ± 0.89^{b}	3.80 ± 0.85^{a}	3.53 ± 0.82^{a}	3.43 ± 0.73^{a}	3.53 ± 0.86^{b}	3.60 ± 0.89^{b}
F4 (25:75)	3.10 ± 0.99^{a}	3.37 ± 1.07^{a}	3.43 ± 0.90^{a}	3.37 ± 0.93^{a}	2.97 ± 0.96^{a}	3.13 ± 0.77^{a}

*Formulation based on wheat flour: tempeh flour ratio.
a, b, c, d = Means with different superscripts within the same column differ significantly ($p < 0.05$)

Aroma

As presented in Table 3, the One-way ANOVA results for aroma preference revealed no significant differences among formulations (p <0.001). The highest aroma score was in F2 (3.87, 'slightly like'), while the lowest was in F4 (3.37, 'slightly like'). These findings indicate that aroma preference decreased as the proportion of tempeh flour increased. Lailatul & Anna (2019) reported that a 30% substitution of tempeh flour added a sweet, savory taste, along with the distinctive aroma of tempeh, in *kembang goyang* cookies. In addition, the One-way ANOVA for descriptive aroma evaluation (Table 4) revealed significant differences (p <0.001). Duncan's test showed that F1 differed significantly from F3 and F4 but not from F2; F2 differed significantly from F4 but not from F1 and F3; F3 differed significantly from F1 but not from F2 and F4; and F4 differed significantly from F1 and F2 but not from F3. The descriptive evaluation indicated that tempeh cookies with carrot flour additions exhibited a characteristic tempeh aroma that became more prominent with higher levels of tempeh flour. Madani et al. (2023) noted that greater substitution levels intensified the characteristic aroma of the raw material. Similarly, Seveline et al. (2019) reported that increasing the amount of tempeh flour and roselle extract enhanced the intensity of the characteristic tempeh aroma.

Taste

According to Table 3, the One-way ANOVA for taste preference revealed significant differences (p <0.001). Duncan's post hoc analysis showed that F1 was significantly different from F3 and F4 but not from F2; F2 differed significantly from F3 and F4 but not from F1; F3 differed significantly from all other formulations; and F4 was significantly different from F1, F2, and F3. The highest taste preference score was for F1 (4.10, 'like'), while the lowest was for F4 (2.97, 'dislike'). These results indicate that taste acceptability decreased with increasing levels of tempeh flour substitution. The taste of cookies is strongly influenced by the ingredients used (Malau et al., 2022). Lailatul & Anna (2019) reported that adding higher levels of black soybean tempeh flour to cookies resulted in a more pronounced bitter taste. The bitterness arises from amino acid hydrolysis during Maillard reactions in tempeh flour and cookie processing. Lysine, in particular, produces a notably bitter taste compared to other amino acids (Istiqomah et al., 2019). Consequently, in this study, panelists generally disliked cookies with higher tempeh flour due to the bitter taste.

Table 4

Descriptive Organoleptic Evaluation Results

Formulation*	Color	Aroma	Texture Hardness	Texture Crispness	Taste	Aftertaste
F1 (100:0)	1.63 ± 0.964^{a}	1.97 ± 0.964^{a}	2.30 ± 0.750^{a}	2.80 ± 0.887^{b}	1.40 ± 0.968^{a}	1.70 ± 1.179^{a}
F2 (75:25)	2.13 ± 0.681^{b}	2.33 ± 0.994^{ab}	2.07 ± 0.868^{a}	2.77 ± 0.858^{b}	2.13 ± 1.252^{b}	2.13 ± 1.252^{ab}
F3 (50:50)	2.27 ± 0.691^{b}	2.67 ± 0.994^{bc}	2.50 ± 1.042^{a}	2.27 ± 0.868^{a}	2.30 ± 1.291^{b}	2.57 ± 1.165^{b}
F4 (25:75)	3.03 ± 0.890^{c}	3.13 ± 0.973^{c}	2.57 ± 1.194^{a}	2.27 ± 1.015^{a}	3.30 ± 0.988^{c}	3.20 ± 0.925^{c}

*Formulation based on wheat flour: tempeh flour ratio.
a, b, c, d = Means with different superscripts within the same column differ significantly ($p < 0.05$)

Descriptive Taste Evaluation

As presented in Table 4, the One-way ANOVA followed by Duncan's test indicated significant differences at a significance level of 0.000 ($p < 0.05$). Specifically, the taste attribute of tempeh cookies in F1 differed significantly from F3 and F4, but not from F2. F2 differed significantly from F4 but was not significantly different from F1 and F3. F3 differed significantly from F1 but not from F2 and F4, whereas F4 was significantly different from F1, F2, and F3. These findings demonstrate that increasing levels of tempeh flour significantly affected the taste of cookies, with higher substitution resulting in a more pronounced bitter taste. According to Madani et al. (2023), the taste of cookies is influenced by the ingredients used. Nariah et al. (2024) also reported that increasing the proportion of black soybean tempeh flour in cookies led to increased bitterness. The bitter taste is attributed to the hydrolysis of amino acids during Maillard reactions in both tempeh flour and cookie processing. Lysine is known to be the most bitter-tasting amino acid compared to others (Sari et al., 2019).

Overall Acceptability

Based on Table 3, the One-way ANOVA results for overall acceptability showed significant differences (p <0.001). Duncan's test indicated that F1 differed significantly from F3 and F4, but not from F2. F2 differed significantly from F3 and F4 but not from F1. F3 differed significantly from F1, F2, and F4, and F4 was significantly different from F1, F2, and F3. The highest overall acceptability score was F1 (4.17, 'like'), while the lowest was F4 (3.13, 'slightly like'). These results indicate that tempeh cookies were generally acceptable to panelists, with scores ranging from slightly like to like. Kristanti et al. (2020) also reported that the overall acceptability of cookie formulations, F0 (3.80), F1 (3.64), F2 (3.07), F3 (2.67), and F4 (2.66) fell within the slightly like to like categories, whereas F5 (2.42) was not accepted. Similarly, Lailatul & Anna (2019) found that kembang goyang made with tempeh flour substitution was moderately accepted by panelists. In terms of consumer acceptance, F1 was identified as the best formulation, with an overall acceptance score of 3.64 on a 5-point scale.

Texture Hardness

Based on Table 3, the One-way ANOVA results for hedonic evaluation of hardness showed significant differences ($p < 0.001$). However, Duncan's test revealed that there were no significant differences among formulations for hardness preference. The highest score for hardness preference was in F4 (2.57, 'dislike'), while the lowest was in F1 (2.07, 'dislike'). Overall, all samples received a score of around 2 (dislike) for hardness, which was attributed to the cookies being excessively hard. According to Kristanti et al. (2020), the addition of tempeh flour increased hardness by reducing cookie expansion, leading to a harder texture.

Similarly, the One-way ANOVA for descriptive hardness evaluation (Table 4) showed significant differences ($p = 0.025$). Duncan's test revealed that F1 differed significantly from F3 and F4, but not from F2. F2 differed significantly from F3 and F4, but not from F1. F3 differed significantly from F1 and F2, but not from F4, whereas F4 differed significantly from F1 and F2 but not from F3. These findings suggest that increasing tempeh flour levels did not consistently influence hardness perception, as panelists generally rated all samples as slightly hard. According to (2020), increased hardness was associated with lower expansion power in cookies made with modified cassava flour (MOCAF), resulting in a harder texture.

Texture Crispness

As presented in Table 3, the One-way ANOVA for hedonic crispness evaluation revealed significant differences ($p < 0.001$). However, Duncan's test indicated no significant differences among formulations. The highest crispness score was F1 (2.80, 'dislike'), while the lowest was F3 and F4 (2.27, 'dislike'). Overall, panelists rated crispness negatively across all samples, mainly due to the hard texture of the cookies, which reduced brittleness and crispness. According to Kristanti et al. (2020), increasing tempeh flour substitution reduced fracturability, indicating lower cookie expansion. The One-way ANOVA for descriptive crispness evaluation (Table 4) also showed significant differences ($p < 0.001$). Duncan's test revealed that F1 differed significantly from F3 and F4 but not from F2. F2 differed significantly from F3 and F4 but not from F1. F3 differed significantly from F1 and F2 but not from F4. F4 differed significantly from F1 and F2 but not from F3. These findings show that increasing tempeh flour levels did not yield consistent effects on crispness, with panelists generally describing all samples as slightly crispy. Crispness depends on how easily cookies break when bitten and is influenced by factors such as flour type, flour moisture content, fat, eggs, sugar, baking soda, and skim milk (Malau et al., 2022). According to Yolanda & Hidayah (2024), texture is also influenced by the ratio of amylose to amylopectin in wheat flour.

Aftertaste

As presented in Table 4, the One-way ANOVA followed by Duncan's test indicated significant differences at the 0.000 level ($p < 0.05$). F1 differed significantly from F2, F3, and F4. F2 differed significantly from F1 and F4 but not from F3. F3 differed significantly from F1 and F4 but not from F2, whereas F4 differed significantly from F1, F2, and F3. The descriptive evaluation showed that increasing tempeh flour levels resulted in a stronger bitter aftertaste, whereas cookies without tempeh flour exhibited a sweet aftertaste. Aftertaste is defined as the flavor or taste that lingers in the mouth (Turana et al., 2025).

CONCLUSION

The results of chemical analysis showed that the substitution of tempeh flour significantly affected the moisture, fat, protein, carbohydrate, and crude fiber contents of cookies. Physical analysis demonstrated significant effects on hardness and fracturability. Sensory evaluation revealed that the level of tempeh flour addition significantly influenced consumer acceptance in terms of color, hardness, crispness, taste, and overall preference in the hedonic test, as well as color, aroma, crispness, taste, and aftertaste in the descriptive test. This study comprehensively reported the physicochemical, physical, and sensory properties of cookies with varying wheat-to-tempeh flour ratios. However, this study did not examine cookies made entirely from 100% tempeh flour. Future research is therefore recommended to investigate the formulation, quality, and sensory characteristics of cookies made with full tempeh flour substitution.

The implications of this study suggested that tempeh flour had potential as an alternative raw material to increase the protein and fiber contents of cookie products, while simultaneously supporting the diversification of fermented soybean-based foods and reducing dependence on imported wheat flour. These findings could be utilized by the food industry and MSMEs to develop functional food products with higher nutritional and economic value. However, this study had certain limitations, as it only included chemical, physical, and organoleptic analyses without testing the product's storage stability and shelf life. Further research was needed to evaluate the effects of long-term storage, additional formulations (such as the use of natural binders), and broader consumer acceptance of tempeh flour-based cookies.

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Author Contribution Statement

Amelya Aprilliani Yolanda: Data Collection; Data Analysis; Writing Original Draft. **Nurul Hidayah:** Conceptualization; Validation; Writing, Review & Editing.

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