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The effect of soybean substitution with pigeon peas (*Cajanus cajan*) on physical characteristics, antioxidant properties, nutrient content, and sensory characteristics of tempe

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ABSTRAK

Latar Belakang: *Kacang gude (Cajanus cajan) memiliki kandungan komponen bioaktif yang tinggi, namun pemanfaatannya dalam produk fermentasi seperti tempe masih terbatas..*

Tujuan: *Penelitian ini bertujuan untuk mengevaluasi pengaruh substitusi kedelai dengan kacang gude terhadap sifat fisik, komponen bioaktif, kandungan gizi, bioaksesibilitas mineral, dan sifat sensori tempe.*

Metode: *Tempe dibuat dengan variasi rasio kacang gude dan kedelai (F1: 60:40, F2: 50:50, F3: 40:60) menggunakan rancangan acak lengkap (RAL) dengan tiga ulangan. Sifat fisik yang dianalisis meliputi pertumbuhan miselium, daya iris, analisis warna dan rendemen. Komponen bioaktif meliputi analisis total kandungan flavonoid, fenolik, serta dengan metode penghambatan DPPH. Kandungan gizi dianalisis yakni kandungan serat total dan kandungan zat besi serta bio aksesibilitasnya. Sifat sensoris tempe dianalisis dengan uji hedonik menggunakan 35 panelis semi terlatih untuk memperoleh formula yang paling banyak disukai. Selanjutnya dilakukan uji deskriptif dengan metode konsensus pada formula terpilih.*

Hasil: *Semua formula tempe berhasil dikembangkan yang ditunjukkan dengan sifat fisik yang baik, termasuk pertumbuhan miselium, warna putih, dan tekstur yang kompak. Tempe substitusi kacang gude menunjukkan penurunan kecerahan dan peningkatan daya iris seiring bertambahnya proporsi kacang gude. Selain itu terdapat peningkatan kandungan total flavonoid, fenolik serta antioksidan seiring peningkatan kandungan kacang gude. Analisis kandungan gizi menunjukkan terjadi penurunan signifikan kandungan serat total pada F3 (40:60). Kadar zat besi (Fe) antar formula tidak menunjukkan perbedaan yang signifikan, namun bioaksesibilitas Fe menurun signifikan seiring peningkatan rasio kacang gude. Uji kesukaan menunjukkan penurunan signifikan pada F3 (40:60), sehingga formula F2 (50:50) dipilih untuk uji deskripsi. Tempe substitusi kacang gude segar memiliki aroma fermentasi yang dominan, namun proses penggorengan dapat mengurangi intensitasnya.*

Kesimpulan: *Penelitian ini menunjukkan bahwa substitusi kedelai dengan kacang gude berpotensi meningkatkan nilai fungsional tempe, khususnya potensi antioksidan dengan tetap mempertahankan penerimaan sensori, sehingga berpotensi sebagai bahan alternatif yang dapat dikembangkan pada pembuatan tempe.*

KATA KUNCI*:* *antioksidan; fermentasi; kacang gude; kedelai; tempe*

ABSTRACT

Background: *Pigeon pea (Cajanus cajan) recognized for its high content of bioactive compounds; however, its application in fermented products, such as tempeh, remains underexplored.*

Objectives: *This study aimed to evaluate the impact of substituting soybeans with pigeon peas on the bioactive components, mineral bioaccessibility, and sensory properties of tempeh.*

Methods: *A completely randomized design (CRD) with three replications was employed, using different soybean-to-pigeon pea ratios: F1(60:40), F2(50:50), F3 (40:60).. The physical properties analyzed included mycelial growth, cutting force, color analysis, and yield. Bioactive components evaluated through total phenolic and flavonoid content, and antioxidant activity using the DPPH radical scavenging assay. Nutritional analysis covered total dietary fiber, iron content, and their bioaccessibility. Sensory evaluation involved a hedonic test with 35 semi-trained panelists to determine the most preferred formulation, followed by a descriptive sensory analysis using the consensus method.*

Results: *All tempeh formulas were successfully developed, exhibiting good physical properties, including mycelium growth, white color, and compact texture. The tempeh samples demonstrated decreased lightness and increased cutting force with the addition of pigeon peas. The results indicated that increasing the proportion of pigeon peas significantly enhanced total antioxidant activity, total phenolic content, and flavonoid levels ($p < 0.05$). Nutrient analysis showed total fiber content significantly decreased in F3 (40:60). Iron (Fe) levels remained consistent across formulations (8.55 to 8.92 mg/100 g); however, Fe bioaccessibility significantly declined with higher pigeon pea ratios. The acceptance test revealed a notable decrease in acceptability for F3 (40:60), leading to the selection of F2 (50:50) for descriptive analysis. Fresh tempe substituted pigeon peas exhibited a dominant fermentation aroma, which was subsequently mitigated by the frying process.*

Conclusions: *These findings suggest that soybean substitution with pigeon peas can enhance the functional value of tempeh, especially its antioxidant potential, without compromising sensory quality, presenting a promising alternative for further tempeh development.*

KEYWORDS*: antioxidants, fermentation, pigeon pea, soybean, tempeh*

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INTRODUCTION

Asian countries are the highest consumers of plant-based foods globally. In Indonesia, plant-based foods occupy the primary position in consumption compared to animal-based foods. Recent data from Indonesia show a continued increase in plant-based food consumption, from 63.7% in 2022 to 64.1% in 2023 (1). Legumes are the second-most consumed plant-based food after rice, the national staple. Despite this, legume consumption remains heavily focused on soybeans, highlighting a significant opportunity for diversification. The highest consumption rates of soybean-based products come from tofu (7.53 kg/capita/year) and tempeh (7.47 kg/capita/year). When adjusted for fresh soybean equivalents, tempeh consumption surpasses that of tofu due to their respective conversion values of 35% and 50% (2). Despite the high demand, domestic soybean production remains inadequate to meet national needs (3). Consequently, the government relies on imports to meet national demand (4). From 2017 to 2022, Indonesia's soybean imports fluctuated but averaged 2.5 million tons per year (5). Despite this dependency, alternative locally available legumes suitable for tempe production remain underutilized.

Pigeon pea (Cajanus cajan) is a legume native to Asia and Africa, thrives in tropical to subtropical climates and exhibits high drought tolerance (6) (7). In Indonesia, pigeon pea is widely distributed in Java, Bali, West Nusa Tenggara, East Nusa Tenggara, and South

Sulawesi. However, pigeon pea has not yet achieved its potential as a commercial crop, and its utilization remains limited to basic culinary applications such as stir-frying, steaming, boiling, and frying (8). The nutritional content of pigeon pea is comparable to that of soybeans, making it a promising substitute in soybean-based processed products. In terms of mineral content, pigeon pea exhibits a superior iron (Fe) level of 25.04 mg/100g (9) compared to soybeans, which contain 10 mg/100g (10). Considering the high prevalence of iron deficiency in populations consuming predominantly plant-based diets, incorporating iron-rich foods such as pigeon pea represents an effective and relevant nutritional strategy. In addition to its high energy, carbohydrate, protein, and fat content, pigeon pea also contains bioactive compounds with therapeutic potential (11).

Overall, the phytochemicals in pigeon pea demonstrate antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities (12). A study by Jayanti (2019) reported that pigeon pea possesses physical characteristics similar to soybeans, thus potentially supporting mycelial growth of mold during tempe fermentation (13). Despite this recognized potential, a comprehensive investigation into the direct impact of utilizing pigeon peas as a substitution material in tempeh production remains largely unexplored. Based on these considerations, considering its nutritional potential and availability, this study aims to evaluate the substitution of soybeans with pigeon peas in tempeh production, with focus on sensory, nutritional, and antioxidant properties, as well as panelist acceptance.

MATERIALS AND METHODS

A completely randomized design with three replicates was used for each treatment. The treatments consisted of variations in the combination of soybeans and pigeon peas (%) in each tempeh formula: F1 (60:40), F2 (50:50), and F3 (40:60). The production of pigeon pea-substituted tempeh modified based on the tempeh production process by Rumah Tempe Indonesia (RTI)(14). Pigeon peas (purchased from a local store in Denpasar, Bali) and soybeans (obtained from Rumah Tempe Indonesia (RTI), Bogor, Indonesia) were weighed and manually sorted. Subsequently, both pigeon peas and soybeans were washed under running water, followed by a 2 h soaking period. The raw materials were then boiled at 100°C for 30 minutes. After draining and air-cooling, the pigeon peas and soybeans underwent a 12 h soaking period with a water-to-raw material ratio of 2:1 (w/v). Hull removal was performed using a bean peeling machine (custom-made) at RTI, followed by separation of the raw materials from the hulls. The peeled raw materials were rinsed with boiling water (±100°C, 15 minutes), drained, cooled, and air-dried with the aid of a fan. The pigeon peas and soybeans were weighed and combined according to the predetermined formulas. Subsequently, starter culture (Raprima®, LIPI, Bandung, Indonesia) was added at 0.1% (w/w), and the mixture was packaged in perforated polypropylene (PP) plastic bags. The fermentation process of pigeon pea-substituted tempeh was carried out for 44 h at 30°C.

Physical evaluation included mycelial growth, color, cutting force, and yield. Mycelial growth was observed through subjective observation and measurement of compactness. Measurement of tempeh compactness refers to SNI 3144: 2015, which states that tempeh can be declared compact if it is not destroyed when pressed or sliced (15). Color measurement was conducted following the method outlined by (16) assessing six different sides of the tempeh, using a portable colorimeter (AMT511, AMTAST, USA) employing CIELAB coordinates (L*, a*, b*). The L* coordinate indicates brightness ranging from black (0) to white (100), the a* coordinate indicates reddish (+) and greenish (-), and the b* coordinate indicates yellowish (+) and bluish (-). Cutting force was analyzed using a texture analyzer (TA1Plus, Ametek, USA). The probe used was a Warner-Bratzler blade (speed setting 1.5 mm/sec, distance 35 mm), and the force was evaluated from the curve area obtained from calculating the force and time required to slice tempe samples, expressed in units of gf/s (17). Yield analysis was calculated based on the weight of pigeon pea tempe before and after fermentation and the results are expressed as percentages (18).

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, following (19). Gallic acid standards (0–100 mg/L) and sample extracts (1000 mg/L) were prepared. A 0.5 mL aliquot of each was mixed with 2.5 mL of 1:10 diluted Folin–Ciocalteu reagent and incubated for 5 minutes at room temperature. Subsequently, 2 mL of 75 g/L sodium carbonate (Na_2CO_3) solution was added, followed by incubation for 30 minutes. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Total Phenolic Content (TPC) was quantified and expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method based on (20) with modifications. A quercetin standard curve (0–30 mg/L) and sample extracts (1000 mg/L) were prepared. Each 0.5 mL aliquot was mixed with 0.15 mL of 15% $NaNO_2$, allowed to stand for 6 minutes, followed by 0.15 mL of 10% $AlCl_3$ and incubated for 60 minutes. Then, 2 mL of 4% NaOH and 2 mL of ethanol were added, and the mixture to stand for 15 minutes. The absorbance was measured at 420 nm using a spectrophotometer.

The analysis of antioxidant capacity referred to the method by (20) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Sample extracts were dissolved and diluted in absolute ethanol to obtain concentration ranges of 2.5, 5, 10, 12.5, and 12 × 10^3 mg/L. A volume of 1 mL from each concentration was mixed with 3 mL of DPPH solution (60 mg/L). The mixture was incubated for 30 minutes at 37°C in the dark. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Total dietary fiber analysis was conducted semi-automatically using an enzymatic-gravimetric method with a commercially available analysis kit (K-TDFR, Megazyme, Cork, Ireland), following AACC 32-05.01 and AOAC 985.29 modified by megazyme (21).

Fe content analysis in this study was conducted according to AOAC 999.11 (18). Samples were mineralized using wet ashing. Then, Fe content was measured using AAS at a wavelength of 515 nm. The standard curve equation was calculated, and the sample absorbance was plotted onto the standard curve equation. In vitro digestion simulation for measuring Fe bioaccessibility was conducted according to Cabero et al (2020) (22). A 10 mL aliquot of the filtered digestion solution from the tempe sample was taken, and the Fe analysis procedure was performed.

Table 1. Aroma descriptors of fresh and fried pigeon pea-substituted tempeh

Sensory Attributes and Descriptors	Sample Standard (Intensity Score)	References
Fresh pigeon pea-substituted tempeh		
Beany	4 g boiled soybeans for 30 minutes	(26)
Pigeon pea like	4 g boiled pigeon peas for 30 minutes	(26)
Tempeh	3 x 5 x 1 cm fresh tempeh (Tempe Kita, Rumah Tempe Indonesia, Bogor, Indonesia)	(15)
Fermented	10 g tapai	(27)
	Fried pigeon pea-substituted tempeh	
Beany	4 g boiled soybeans for 30 minutes	(26)
Pigeon pea like	4 g boiled pigeon peas for 30 minutes	(26)
Tempeh	3 x 5 x 1 cm soybean tempe (Tempe Kita, Rumah Tempe Indonesia, Bogor, Indonesia) fried in 140°C oil for 3 minutes	(15)
Fermented	10 mL liquid from tapai solution (1:1 tapai and mineral water)	(27)
Rancid	5 mL oil oven-heated at 200°C for 6 h	

Thirty-five semi-trained panelists were selected from students at the Department of Public Nutrition, IPB University, Indonesia. The panelists were asked to rate their degree of liking for the following attributes of cooked tempeh: appearance, aroma, texture, taste, aftertaste, and overall acceptability. A 9-cm line scale was used (0 cm = strongly dislike; 9 cm = strongly like). The fried tempeh was prepared by slicing it to a thickness of approximately 1 cm and a weight of around 30 g, followed by frying in cooking oil at a temperature of 140°C (23). The acceptance test was conducted to select the best formula for further descriptive testing. Descriptive sensory analysis followed the consensus method Chambers (2018) (24) modified by Azra et al (2021) (25) with twelve trained panelists. The descriptive test was conducted for 3 h per day over 4 days. The sensory attributes evaluated in the descriptive test were aroma and taste. Panelists were recruited and screened through a triangle test, evaluating basic tastes (sweet, sour, salty, and bitter) for taste parameters and aroma parameters. Initially, panelists smelled and tasted pigeon pea-substituted tempeh samples, describing the perceived aromas and tastes. Attributes were recorded and refined through consensus, with reference standards adapted from literature. The aroma and descriptors of fresh and fried pigeon pea-substituted tempeh are detailed in **Table 1**.

The taste and descriptors of fried pigeon pea-substituted tempeh are shown in **Table 2**. Panelists evaluated aroma and taste attributes using a 0 to 15 intensity scale. Samples (10 g) were served in coded containers, with white bread and water as palate cleansers. Evaluations were discussed to establish agreed-upon sensory descriptions, which were later visualized in scatter plots. All procedures in this study were approved by the Ethics Committee of IPB University (Approval No. 1472/IT3.KEPMSM-IPB/SK/2024). Data were analyzed using IBM SPSS Statistics 25.0 (SPSS Inc, Chicago). Statistical significance between groups was determined using One way ANOVA followed by Duncan's test with p value < 0.05. All data are presented as mean values ± standard deviations

Table 2. Taste descriptors of fried pigeon pea-substituted tempeh

Sensory Attributes and Descriptors	Sample Standard (Intensity Score)	Reference
Beany	4 g boiled soybeans for 30 minutes	(26)
Pigeon pea like	4 g boiled pigeon peas for 30 minutes	(26)
Tempeh	3 x 5 x 1 cm soybean tempeh (Tempe Kita, Rumah Tempe Indonesia, Bogor, Indonesia) fried in 140°C oil for 3 minutes	(15)
Fermented	5 g tapai dissolved in 10 mL water	(27)
Astringency	10 mL 2% brewed dried green tea (100°C hot water, steeped for 5 minutes, filtered)	(28)
Sour	10 mL of 50% Greek yoghurt	
Salty	10 mL of 0,5% table salt	
Umami	10 mL of 0,2% MSG	

RESULTS AND DISCUSSIONS

Physical characteristics of pigeon pea-substituted tempeh

The physical characteristics analyzed in this study included mycelial growth, cutting force, color, and yield. Tempeh's surface was covered with a white mycelium layer due to the activity of Rhizopus mold used during fermentation. Mycelial growth was assessed through subjective observation and compactness (15). In this study, all tempeh samples were found to undergo the fermentation process, as evidenced by the inoculated beans intertwining to form a compact white mycelium cake that was not slimy on the surface. The texture of the tempeh was developed by the action of fungi that break down the intercellular matrix between plant cells. The appearance of each treatment was rated as good, characterized by a white mycelium color without greyish or black spots from spore growth on the surface of the tempeh. The mycelium contributed to the characteristic appearance of tempeh and helped bind the beans together, resulting in a firm and compact texture.

This structure made the tempeh dense yet slightly spongy when pressed, allowing it to be cut easily without crumbling, which indicates successful fermentation and adequate binding of the mycelium (15) (29).These results are consistent with previous research on the development of tempeh from different types of beans (cowpea, pigeon peas, velvet beans), which produced a proper physical appearance similar to soybean tempeh (30)(31). The physical properties of the tempeh samples produced in this study are presented in **Table 3**.

Color is one of the most important attributes determining food quality, as it could affect consumer acceptance (32). Increasing the pigeon peas ratio significantly decreased the samples' lightness value (L*). Meanwhile, the redness values (a*) increased following the increased use of pigeon peas, although these increases were not statistically significant ($p > 0.05$). On the other hand, the yellowish values (b*) decreased, with sample F3 (50:50) found to be significantly different from the others. This finding is consistent with the study of Sharma et al. (2019), which reported similar results on the chroma color of pigeon pea flour with soaking variations (32). The decrease in lightness (L*) and yellowishness (b) observed in tempeh with a higher proportion of pigeon pea is attributed to the natural pigmentation of soybeans and pigeon peas. Soybeans naturally contribute to the yellow hue and overall lightness of the tempeh due to their carotenoid content, which is responsible for the bright colors in plants (33). In contrast, pigeon peas possess an inherently darker natural pigmentation than soybeans. Yuniasti (2020) states that pigeon pea can have dark colors, ranging from dark purple to black and brownish (8). These colors are primarily due to polyphenolic substances such as tannins or anthocyanins, which are concentrated in the pigeon pea's seed coat (34). Peeled pigeon peas had a fairly bright color, but the soaking process in the production of tempeh resulted in a darker color change in the peas. The boiling and soaking processes facilitate the transfer of soluble pigments from the pigeon pea seed coat to the cotyledons, visually resulting in a darkening of the seeds (35)(35).

Table 3. Physical characteristics of pigeon pea-substituted tempeh

Parameter	Treatment (soybean:pigeon peas(%))		
	F1 (60:40)	**F2 (50:50)**	**F3 (40:60)**
Mycelium growth	good	good	good
Surface color	white	white	white
Compactness	compact	compact	compact
Yield (%)	96.57±1.10[a]	95.67±0.50[a]	95.47±0.72[a]
Color			
L*	58.09±3.54[c]	56.14±3.71[b]	51.07±4.59[a]
a*	4.22±1.33[a]	4.37±0.64[a]	4.45±0.87[a]
b*	10.76±1.98[b]	10.51±2.45[b]	8.21±1.81[a]
Cutting force (gf/s)	19.12±2468[a]	21.79±1854[a]	23.41±871[a]

[a-c] Different superscript letters in the same row indicate significant differences (*p*-value<0.05) ± standard deviation.

Optically, the darker a material, the greater its ability to absorb light and the lesser its ability to reflect light. As the proportion of pigeon pea in the tempeh formula increases, the total content of dark pigments within the tempeh mass also rises. This causes the tempeh to absorb more visible light and reflect less, resulting in a darker appearance. In color measurements using the CIE L*a*b* system, this phenomenon is reflected by a decrease in the L* value (lightness), where a lower L* value indicates a darker color (36). Thus, the darkening of the color of pigeon peas after the preliminary process in tempeh production resulted in color differences between each sample, as the increasing proportion of pigeon peas in the tempeh also affected the color of the tempeh produced. The cutting force values of tempeh samples increased with the higher amount of pigeon peas used, this supported by the increasing cutting force values obtained from texture analysis. The data indicated that the cutting force required for slicing tempeh samples increased, suggesting a higher hardness level. The increase in cutting force observed in this study was attributed to the higher carbohydrate content of pigeon pea (58.0 g %db) compared to soybean (24.9 g %db) (10). These findings align with the research conducted by Astawan et al 2024 on tempeh made from velvet beans, which also exhibited a high carbohydrate content (37). The research concluded that as the proportion of velvet beans increased, the hardness of the resulting tempeh likewise increased. According to Amanah et al (2019), elevated carbohydrate levels contribute to the increased hardness observed in the samples (38). Starch is the dominant type of carbohydrate found in pigeon pea, with a content ranging from 41 to 53% (39). The research before found the dominant starch component is amylose (54.74 to 58.51%) (40). This high amylose content tends to result in a harder texture due to its ability to undergo retrogradation. This occurs because amylose has a linear chain structure, which facilitates the re-alignment of amylose chains that were disrupted during gelatinization (cooking), thus causing the product to become firm again after cooling (41).

Total phenolic, flavonoid compounds, and antioxidant activity

Plant-based food sources are rich in bioactive compounds that function as antioxidants. Legumes and grains are recognized as foods abundant in phenolic bioactive compounds, which can act as antioxidants. The main phenolic compounds found in legumes include phenolic acids, flavonoids, and condensed tannins, with the majority located in the seed coats of legumes (42). Food processing can either enhance or diminish antioxidant activity. The results of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity analyses of pigeon pea-substituted tempeh for each formula are presented in **Table 4**.

This study found an increase in phenolic and flavonoid compound levels in pigeon pea-substituted tempeh with increasing pigeon pea ratios in the tempeh formula. Total phenolic content (TPC) increased from 13.26 mg GAE/g in F1 to 14.95 mg GAE/g in F2, and further to 15.92 mg GAE/g in F3. Similarly, Total Flavonoid Content (TFC) increased from 5.17 mg QE/g in F1 to 6.17 mg QE/g in F2, and reached 6.78 mg QE/g in F3. Yang et al (2022) confirms the phenolic and flavonoid content in the ethanol extract of pigeon pea seeds, which were 23.15 mg GAE/g dw and 15.13 mg QE/g dw, respectively (43).

Meanwhile, the phenolic and flavonoid compounds in soybean extract were 16.94 mg GAE/g dw and 8.77 mg CTE/g dw, respectively. It is crucial to acknowledge that there are differences in the standards used for flavonoid quantification from the citations in this study; literature regarding soybeans uses catechin as a standard, while research on pigeon peas and the samples in this study employs quercetin. Nevertheless, both catechin and quercetin are classified as flavonoid compounds. Therefore, results obtained using these respective standards can still indicate the presence and general range of flavonoids within each ingredient. Another study by Arinanti (2018) showed no significant difference in flavonoid levels between pigeon peas and soybeans, with contents of 4.13 and 4.60 mg QE/g in powder form (44).

Table 4. Total phenolic content, total flavonoid content, and antioxidant activity of pigeon pea-substituted tempeh

Antioxidant Properties	Unit	Treatment (soybean:pigeon peas(%))		
		F1 (60:40)	F2 (50:50)	F3 (40:60)
Total Phenolic	mg GAE/g	13.26± 0.38 [a]	14.95± 0.09 [b]	15.92± 0.54 [b]
Total Flavonoid Content	mg QE/g	5.17± 0.51 [a]	6.17± 0.55 [ab]	6.78± 0.52 [b]
Antioxidant activity	% inhibition	53.44± 3.01 [a]	66.07± 1.88 [b]	77.99± 1.16 [b]

[a-c] Mean values followed by different letters within the same row indicate significant differences ($p < 0.05$) ± standard deviation (mg QE/g), Antioxidant activity based on 10 g/L concentration of pigeon pea-substituted tempeh extract.

Despite the overall increase in both TFC and TPC with higher pigeon pea ratios, it is important to consider the impact of the entire tempeh production process on the initial concentrations. Quantitatively, when compared to the phenolic and flavonoid content in all treatments of pigeon pea-substituted tempeh, a decrease in both compounds is apparent. This reduction can be attributed to compound loss during processing steps such as washing, hulling, soaking, and boiling. Thepthanee et al, 2024 (45) revealed that soaking striped beans in water for 2 to 12 h impacts the reduction of TFC levels. Similarly, Meital et al (2023) (46) stated that faba beans experienced a decrease in TPC (13–18%) and TFC (19–36%) after the soaking process, with subsequent reductions in TPC and TFC of up to 77% and 73% respectively, after boiling. That study found most of this compound loss was due to leaching into the soaking water (39–77% TPC; 28–42% TFC) and boiling water (57–62% TPC; 71–92% TFC); however, there is also a possibility that a small fraction underwent degradation during these processes. Despite these losses, the total phenolic content observed in our study is consistent with previous research that reported TPC in tempeh ranging from 10 to 18 mg GAE/g tempeh extract (47).

Although a decrease occurred during the initial stages of tempeh production, the fermentation process itself can enhance the availability and bioactivity of phenolic and flavonoid compounds. The increase in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) during tempeh fermentation can be attributed to the activity of microorganisms capable of producing enzymes. These enzymes break down macromolecular polyphenols into smaller, more efficient parts, which then contribute to the increased measured TPC and TFC values, thereby potentially enhancing their availability and bioactivity for the body (43). The increase in flavonoid content observed in pigeon pea-substituted tempeh in this study is consistent with previous research, which reported that fermentation during tempeh production can enhance flavonoid levels by up to 20 times compared to the raw materials (48).

The antioxidant activity of pigeon pea-substituted tempeh showed a significant increase with pigeon pea, ratios with DPPH radical inhibition increased from 53.44% for F1, 66.07% for F2, and 77.99% for F3. Table 4 shows a linear increase relationship between flavonoid, phenolic compound levels, and antioxidant activity in pigeon pea-substituted tempeh. These results are consistent with research indicating a positive correlation between total phenolic and flavonoid compounds and antioxidant activity. Correlation analyses of antioxidant activity in pigeon peas with TFC and TPC levels showed positive correlations and antioxidant activity (43). Based on the antioxidant activity in pigeon pea-substituted tempeh extract, increasing the pigeon pea ratio in tempeh enhances its ability to inhibit oxidative stress. The research observed antioxidant activity in tempeh with natural color modifications in the formula, and also found similar results, with an increase corresponding to the increase in coloring compounds in tempeh, although the antioxidant activity remained relatively low (49). Elevated TPC values directly correspond to increased antioxidant activity, confirming that phenolic compounds are the primary contributors to the antioxidant capacity.

While observed an increase in antioxidant activity across the different formulations (F1 to F3) relative to each other, the overall absolute remained comparatively low. The decrease in antioxidant activity can be attributed to oxidation or degradation of antioxidant compounds during tempeh processing. This means that antioxidant compounds may be damaged, lost, or converted into less active forms (50). The low antioxidant activity in tempeh may also be due to the absence of the skin during tempeh production, whereas the highest antioxidant activity in legumes is found in the skin (51). Additionally, tempeh is not primarily intended as an antioxidant product. The lengthy production process can also reduce several compounds with antioxidant potential, such as certain antinutrients and water-soluble vitamins, which can affect antioxidant activity. Overall, this study demonstrates that the substitution of pigeon peas in the tempeh production process effectively enhances antioxidant properties and presents potential as a high-antioxidant plant-based food.

Dietary Fiber in Pigeon Pea-Substituted Tempeh

Dietary fiber primarily consists of carbohydrate polymers, which are resistant to hydrolysis by digestive enzymes in the mammalian small intestine but can be fermented by large intestinal bacteria. One of the main benefits of dietary fiber is related to its fermentability, which influences the diversity and function of microbes in the gastrointestinal tract, as well as the byproducts of the fermentation process (52). The Indonesian Ministry of Health recommends a daily fiber intake ranging from 25 to 30 g/day to provide health benefits (53). Plant-based foods generally have a high fiber content and can be consumed to help meet daily fiber intake. Table 5 showed the results of the dietary fiber content analysis in this study.

Table 5. Dietary fiber of pigeon pea-substituted tempeh (% db)

Nutrient (%db)	Unit	Treatment (soybean:pigeon peas(%))		
		F1 (60:40)	F2 (50:50)	F3 (40:60)
Dietary fiber	%	7.40± 0.20[b]	7.67± 0.35[b]	6.78± 0.16[a]

[a-b] Mean values followed by different letters within the same row indicate significant differences ($p<0.05$) ± standard deviation.

Legumes are among the food materials with high dietary fiber content, providing numerous health benefits. Dietary fiber is a type of carbohydrate that cannot be digested or absorbed in the intestine (54). The study revealed that while fiber content remained statistically unchanged between formulations F1(60:40) and F2 (50:50), with values of 7.40 % and 7.67% respectively, a notable reduction was observed in F3 (60:40), which had 6.78%, suggesting that higher substitution levels may negatively impact dietary fiber content. This finding contradicts the assumption that tempeh would experience an increase in fiber content with the addition of pigeon peas, which have a higher fiber content than soybeans. Legumes typically contain a higher proportion of soluble dietary fiber, including pectin, cellulose, and xyloglucan (34)(48). However, the composition of fiber types can vary depending on the legume species. In soybeans, insoluble fiber is the predominant fiber fraction (55). Meanwhile, pigeon peas are dominated by soluble fiber and polysaccharides from the raffinose family, which are easily fermented (54). The decrease in fiber content in F3 is likely due to the fermentation of soluble fiber, which is the predominant fiber type in pigeon pea cotyledons. Increasing the pigeon pea ratio in tempeh may alter the composition of available fiber types, with an increase in soluble fiber, making it more easily fermentable.

The pigeon pea-substituted tempeh in this study exhibited a higher total fiber content compared to soybean tempeh, which typically ranges from 1.4 to 1.6 g (56). Therefore, the utilization of pigeon peas as a substitution material in tempeh production can yield a high-fiber tempeh product, effectively contributing to the fulfillment of daily fiber intake requirements.

Iron (Fe) Content and Bioaccessibility

Iron deficiency is one of the most prevalent micronutrient deficiencies worldwide, causing anemia, fatigue, and impaired mental growth, and can lead to risks during child birth. The Indonesian Ministry of Health recommends a daily Fe intake of 9-11 mg/day for men and 15-18 mg/day for women (53). Legumes are inherently rich in minerals, but their bioaccessibility is low due to the presence of non-heme iron and mineral absorption inhibitors (57). Bioaccessibility measures the fraction of a consumed compound released from the food matrix and available for absorption through the small intestinal mucosa or altered by gut microbiota (58). The iron content and bioaccessibility of pigeon pea-substituted tempeh are presented in **Table 6**.

Table 6. Fe content and fe bioaccessibility

Nutrients (%wb)	Unit	Treatment (soybean:pigeon peas(%))		
		F1 (60:40)	F2 (50:50)	F3 (40:60)
Fe Content	mg/100 g	8.55 ± 0.40[a]	8.53 ±0.11[a]	8.92 ±0.132 [a]
Fe Bioaccessibility	%	9.99 ± 0.24[b]	9.66 ± 0.33[ab]	9.05± 0.08[a]

[a-b] Mean values followed by different letters within the same row indicate significant differences ($p<0.05$) ± standard deviation.

There were no significant differences in Fe content among the samples; however, increasing the pigeon pea ratio while reducing soybeans tended to raise Fe levels. Pigeon peas and soybeans have comparable Fe content, with pigeon peas ranging from 19.70 to 35.18 mg/100g (9) and soybeans at 10 mg/100g (3). The soaking and fermentation processes increase moisture, reducing the apparent Fe concentration in tempe. Sine et al (2018) reported over 50% Fe loss after pigeon peas were processed into tempeh (59).

Although fermentation may lead to a reduction in total iron content, it has been shown to significantly enhance iron bioaccessibility by improving mineral solubility and antinutritional factors. Previous research noted a 2.2 fold increase in Fe bioaccessibility through fermentation. However, in this study, increasing the pigeon pea ratio led to reduced Fe bioaccessibility (9.05 to 9.99%) (60) This is consistent with previous studies indicating that non-heme iron in plant-based foods can be absorbed at rates of 5–15% (61). The observed decrease in iron bioaccessibility with increasing pigeon pea ratio in tempeh is likely due to the presence of inhibitory compounds such as phytic acid, phenolics, and dietary fiber (57). Although fiber content decreased in F3, Fe bioaccessibility also declined from 9.99% (F1) to (9.05%), possibly due to stronger inhibitory compounds such as phytic acid and phenolics which are more abundant in darker legumes like pigeon peas (62).

Nonetheless, the Fe bioaccessibility of pigeon pea-substituted tempeh remained higher than in unfermented legumes (63). Furthermore, the enhancement of Fe bioaccessibility can be facilitated through combination with other food sources that promote Fe absorption, such as vitamin C, which is abundant in plant-based foods, thereby offering a beneficial option for vegan populations.

Sensory characteristics of tempeh substitution with pigeon peas

Sensory characteristics were evaluated in this study through acceptance testing of fried pigeon pea-substituted tempeh to determine the preferred formulation, followed by descriptive analysis. Table 7 presents the acceptance values of pigeon pea-substituted tempeh.

Table 7. Acceptance values of fried pigeon pea-substituted tempeh

Attribute	**Treatment (soybean:pigeon peas(%))**		
	F1 (60:40)	**F2 (50:50)**	**F3 (40:60)**
Appearance	6.21±1.98^{b}	5.63±1.84^{b}	4.13±2.02^{a}
Aroma	6.64±1.55^{b}	6.75±1.59^{b}	5.71±1.44^{a}
Taste	6.18±1.90^{b}	5.99±2.13^{b}	4.04±1.80^{a}
Texture	6.01±2.01^{b}	5.59±2.06^{b}	4.53±2.20^{a}
Aftertaste	6.02±1.78^{b}	5.53±1.99^{b}	3.86±1.72^{a}
Overall acceptability	6.51±1.47^{b}	6.18±1.47^{b}	5.78±1.64^{a}

$^{a-b}$ Mean values followed by different letters within the same row indicate significant differences (p<0.05) ± standard deviation

There were significant differences in panelist liking for all attributes between samples F1 and F2 compared to F3, with a trend of decreasing liking as the pigeon pea ratio increased and the soybean ratio decreased ($p < 0.05$). Formula F2 was selected as optimal formulation for descriptive sensory analysis. This choice was based on its balanced sensory acceptance and a favorable nutritional profile. F2 exhibited acceptability levels comparable to F1 across all attributes, particularly in overall acceptability (F1: 6.51;. F2: 6.18, p-value < 0.05). Furthermore, F2 contained a higher proportion of pigeon peas (soybean:pigeon peas 50:50) than F1 (soybean:pigeon peas 60:40), which positively correlated with increased levels of bioactive compounds (such as phenolics and flavonoids) and enhanced antioxidant activity, while maintaining other essential nutritional components. This optimal balance makes F2 a superior choice, offering improved functional benefits without compromising desirable sensory characteristics.

This descriptive analysis was conducted on the selected formula of pigeon pea-substituted tempeh (F2), focusing on aroma attributes for fresh and fried tempeh, and taste attributes for fried tempeh. The aroma descriptors identified by the panelists for fresh pigeon-pea-substituted tempeh (**Figure 1a**), included beany, pigeon pea-like, tempeh, and fermented aromas. Fried pigeon-pea-substituted tempeh (**Figure 1b**) descriptors included beany, pigeon pea-like, fried tempeh, fermented, and rancid aromas. The taste descriptors for fried pigeon pea-substituted tempeh (**Figure 1c**), comprised beany, pigeon pea-like, fried tempeh, fermented, astringent, sour, salty, and umami tastes.

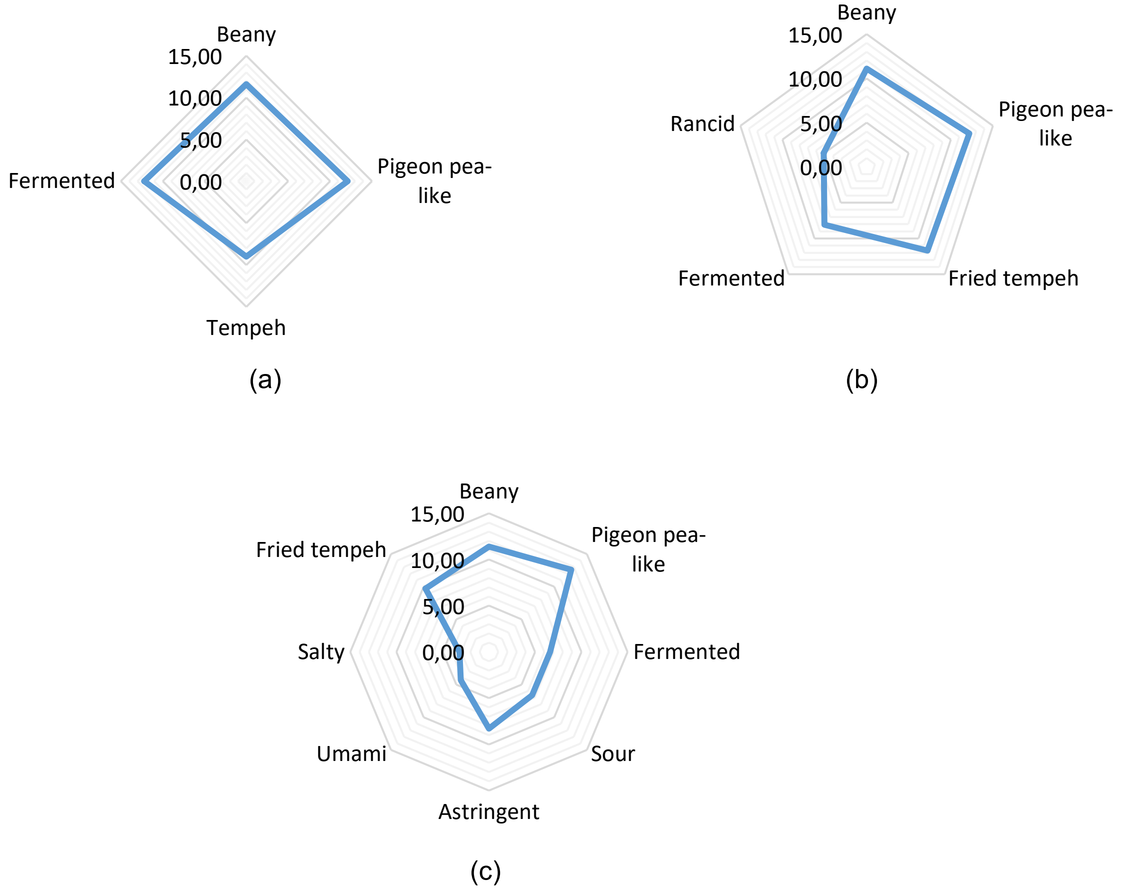

Figure 1. Descriptors identified by the panelists for fresh pigeon-pea-substituted tempeh

Fermentation plays a crucial role in developing the aroma and flavor profiles of various food products. The identified characteristics are similar to those in previous studies that identified raw soybean tempeh with the addition of S. cerevisiae yeast as having a characteristic tempeh aroma, beany notes, and tapai/alcohol aroma, while fried modified tempeh had oily food, alcoholic, bitter, astringent, salty, sour, and umami aromas (27). The dominant aroma and flavor identified were fermentation aromas, considered nearly similar to tapai aroma. These results align with Astuti et al (2022) which analyzed volatile components in bran fermentation products (64). The fermentation process can enhance volatile aromas in products as carbohydrate content in the substrate increases. During the fermentation process, yeast enzymes metabolize carbohydrates in the form of simple sugars or hydrolyzed starches, which are subsequently converted into ethanol (65). Increased carbohydrates substrate availability enhances fermentation activity, leading to greater production of ethanol and other volatile compounds, thus enhancing the fermentation aroma. The volatile aroma components of tempe are predominantly alcohols, esters, and aromatic groups (27). Meanwhile, another study by Astuti et al (2022) reported that volatile components in carbohydrate-rich bran fermentation products were dominated by aldehydes, followed by alcohols, esters, and acids (64). Aldehyde components are formed through lipid oxidation, which increases during fermentation and becomes easily identifiable by human olfactory senses due to their low odor thresholds. Alcohol aromas also contribute to the volatile components of fermentation products, originating from glucose catabolism into pyruvate through the glycolysis pathway.

Beany aromas and flavors are frequently found in various soybean-based products. The research before indicated that high moisture content (40-80%) in legume products results in an increased beany aroma and flavor in the resulting products (66). Simultaneously, astringent, savory, and sour tastes were also identified. Astringent and bitter tastes in pigeon pea-substituted tempe products may be associated with high phenolic compounds in legumes (67). Additionally, the amino acid profile components in food products are related to tempeh aroma and flavor. This study found that the highest contents were glutamic acid and aspartic acid, which contribute to the umami taste of tempeh (68).

CONCLUSION AND RECOMMENDATION

This study shows that pigeon pea can be a viable alternative in tempeh production. Substitution of soybean with pigeon peas in tempeh production significantly improved antioxidant properties and increased bioactive compound levels, while maintaining key nutritional components. However, higher substitution ratios, particularly in the F3 formulation, led to decreased Fe bio accessibility and reduced sensory acceptance due to intensified fermentation aroma and taste. Notably, the 50:50 formulation (F2) presented a balanced and optimal approach, this formula successfully maintaining satisfactory sensory acceptance (an overall acceptability score of 6.18) while also significantly improving functional properties, such as antioxidant activity (66.07% inhibition). This suggests pigeon peas are a viable alternative for tempeh diversification. Processing of pigeon pea-substituted tempeh is recommended for wet seasoning dishes due to its tendency towards a harder texture. Alternatively, processing it as a dry snack can also yield a crispier texture. Further research is warranted to refine the fermentation process to mitigate the intense fermentation aroma and taste, thereby enhancing the acceptability of tempeh with higher pigeon pea proportions. This includes optimizing fermentation time and temperature, exploring various starter cultures and their quantities.

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