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Nutritional composition and sensory evaluation of tempeh from different combinations of beans

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Abstract

Tempeh is recognised as an excellent source of plant protein and a promising meat substitute in the diet. Recently, the development of tempeh by blending different legumes has gained more attention, but references are limited. This study aimed to develop tempeh combinations of soybean with chickpeas and red kidney beans. The physical characteristics of the tempeh samples were recorded, and their nutritional compositions were determined in accordance with the standard AOAC methods. A sensory evaluation was also conducted by using the hedonic 9-scale test to study the overall acceptance of the tempeh. Our results indicated that the soybean and chickpea tempeh combination contained 31.08±0.41% protein, 9.88±1.42% fat, 16.57±1.90% carbohydrates, 13.49±0.03% crude fibre, 4.40±0.02% dietary fibre, 39.91±0.23% moisture, and 2.57±0.21% ash content per 100 g. Meanwhile, the soybean and red kidney bean tempeh combination had 30.39±0.12% protein, 9.43±1.02% fat, 13.31±0.78% carbohydrates, 11.88±0.03% crude fibre, 2.50±0.02% dietary fibre, 44.17±0.03% moisture, and 2.70±0.26% ash content per 100 g. Both mixed bean tempeh samples demonstrated high nutritional value. However, the sensory evaluation showed that the soybean and red kidney bean tempeh combination was preferred by most panellists due to its appearance, texture, taste, and aroma, with the highest overall acceptance score (7.54±0.85). In conclusion, our study highlights the potential of blending soybean with chickpeas and red kidney beans to develop tempeh with a high nutritional value. These findings could have significant implications for developing plant-based meat substitutes with desirable nutritional and sensory properties.

1. Introduction

Tempeh is a well-known traditional fermented soy-based food that originated from Southeast Asia (Hashim *et al*., 2018). In addition, tempeh is a fermented food produced through natural culturing and controlled fermentation of cooked soybeans. Based on the Codex Alimentarius International Food Standards (2013), tempeh is defined as a white, compact cake-form food product that is made from dehulled and boiled soybeans through solid-state fermentation with Rhizopus moulds (Ahnan-Winarno *et al.,* 2021). Rhizopus mould is known as a tempeh starter that is responsible for binding the soybeans into a compact white cake form (Nursiwi *et al.,* 2021). After soaking, dehulling and cooking the soybeans, a solid substrate fermentation will be carried out by inoculating the soybeans with Rhizopus moulds (Puteri *et al.,* 2018). The fermentation of soybean with Rhizopus moulds enhances the nutritional values and digestibility of tempeh and provides vital living enzymes and beneficial microorganisms to the human body (Pangastuti *et al.,* 2019; Rizal *et al.,* 2022). Processing soybeans into tempeh involves the degradation of macromolecules into smaller units, making it easier for the body to digest and utilise (Dahlan *et al.,* 2022).

Legumes are used as a raw material in tempeh production due to their functional properties, such as sensory quality and nutritive value (Kustyawati *et al.,* 2020). The addition of legumes enhances the sensory quality of the tempeh, such as the texture, aroma, appearance and taste, and nutritional properties (Rahmawati *et al.,* 2021). The development of tempeh by different legumes has gained more attention in recent years, but existing references are outdated. Hence, this study investigated the development of tempeh containing

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soybean (*Glycine max*) and the mixtures between soybean with chickpea (*Cicer arietinum L.*) and red kidney bean (*Phaseolus vulgaris*), and the nutritional compositions and sensory evaluation of each tempeh are determined.

2. Materials and methods

2.1 Production of tempeh

A total of 200 g of bean was used in the soybean tempeh production, and 100 g of soybean and 100 g of chickpea or red kidney bean were used for the preparation of mixed bean tempeh. Firstly, the beans were washed and soaked in distilled water (1:10, w/v) at room temperature (25.0±1°C) for 12 hrs (Murdianto, 2022; Taib *et al.,* 2022). The soaked beans were rinsed three times, and the seed coats were manually removed by hand after rinsing. Thereafter, the peeled beans were cooked until they were tender but not mushy. The cooking times of each bean varied according to their characteristics (Wikandari *et al*., 2020). Accordingly, the soybeans were cooked for approximately 50 mins. Meanwhile, the red kidney beans and chickpeas were cooked for approximately 20 mins. The cooked beans were drained, surface dried and cooled at room temperature. Then, the cooled beans were inoculated with Rhizopus starter in a ratio of 200 g dried beans and 0.8 g tempeh starter (Erkan *et al*., 2020). After the inoculation, the beans were packed in the perforated zip-lock plastic bag for ventilation and incubated for 40 h at room temperature (Wikandari *et al.,* 2020).

2.2 Nutritional composition analysis

The fresh tempeh samples were oven-dried at 60°C and ground with a laboratory blender. The contents of protein, ash, crude fibre, and dietary fibre were analysed according to the Kjeldahl method (AOAC 200.11) (Horwitz, 2002), dry ashing method (AOAC 923.03) (Association of Official Analytical Chemists, 2000), ceramic fibre filter method (AOAC 962.09) (Helrich, 1990) and enzymatic–gravimetric method (AOAC 985.29) (Association of Official Analytical Chemists, 1985). The total carbohydrate content was determined by difference (Abu-Salem and Abou-Arab, 2011), and the fat content was analysed by the Soxhlet method (Egan *et al.,* 1981). The moisture content of the ground tempeh powder was measured with a rapid moisture analyser (Nielsen, 1998). All the analysis was conducted in triplicate.

2.3 Sensory evaluation

In this study, a nine-point hedonic test was used to evaluate the sensory attributes and overall acceptance of the tempeh from soybean, combinations of soybean with chickpea and soybean with red kidney bean (Meilgaard *et al.,* 2007). The type of scale used was a 1 to 9 scale, ranging from “dislike extremely” as “1” to “like extremely” as “9”. Approximately 35 untrained panellists participated in this sensory evaluation. The sensory assessment was based on the appearance, texture, taste, aroma, and overall acceptance of the tempeh samples. The fresh tempeh samples were cut into small pieces with a size of 2×2×2 cm^3 and fried using corn oil for 5 mins (Wikandari *et al.,* 2020). Food containers containing soybean tempeh, the combinations of soybean with chickpea and soybean with red kidney bean tempeh samples were coded as 321, 624 and 256, respectively, and served to the panellists.

2.4 Statistical analysis

The results of the study were expressed as mean±standard deviation. The significance of differences for the means of the formulations was determined by the Analysis of Variance (ANOVA) at a 95% confidence interval ($p<0.05$), followed by the Tukey HSD using IBM SPSS Statistics Version 23 (Wahid and Khattak, 2020).

3. Results and discussion

The physical characteristics of tempeh from different types of beans were investigated in this study. The researchers successfully produced three types of tempeh samples after 40 hrs of fermentation (Figure 1). The fermentation time was based on previous studies that suggested an optimal fermentation time of up to 48 hrs at room temperature (Tahir *et al.,* 2018). However, another study showed that tempeh started to rot after 42 hrs of fermentation at ambient temperature (*Dwiatmaka et al.,* 2021). This study found that a 40 hrs fermentation time was optimal and supported by the research of Kustyawati *et al*. (2020). The appearance of the tempeh samples was similar to that of soybean tempeh, with a white colour, and no grey or black patches of spores formed on the surface of the tempeh. The formation of the white colour was due to the mycelium growth of Rhizopus spp., which fully covered the whole surface of tempeh. The flavour of the tempeh samples was mushroom-like and nutty, without any ammonia smell. This result was consistent with the finding of Wang *et al*. (2022), who also found that fermentation with mushroom mycelium was able to change the aroma profile of highland barley. All the tempeh samples produced were considered successful because the inoculated beans were knitted together to form a firm, dense and chewy white mycelium cake, with no slimy texture on the surface. The texture of the tempeh was formed due to the action of the fungus, which breaks down the intercellular matrix between the plant cells (Syida *et al.,* 2018). This

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phenomenon resulted in a firm texture that allowed the tempeh to be sliced into thin pieces without easily disintegrating. Overall, the physical characteristics of tempeh in this study were consistent with those of previous research on tempeh fermentation. However, the optimal fermentation time found in this study was slightly shorter than that suggested in previous studies, which is an interesting finding. This result may be due to the differences in the beans used or in the fermentation conditions. Nevertheless, the finding that different types of beans can produce tempeh with similar physical characteristics is important because it suggests that tempeh can be made from a variety of sources, potentially expanding its availability and affordability.

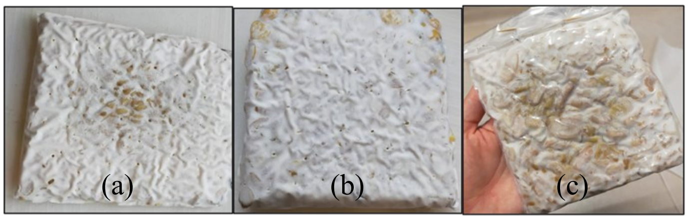

Figure 1. Physical characteristic of the tempeh samples: (a) tempeh produced from soybean, (b) tempeh combination of chickpea with soybean, (c) tempeh combination of red kidney beans with soybeans.

The nutritional composition of the tempeh samples, including the protein, fat, moisture content, ash, carbohydrate, crude fibre, and dietary fibre, was evaluated. The results are presented in Table 1. The findings indicated a significant difference ($p<0.05$) in protein content between the three types of tempeh samples. Soybean tempeh had the highest protein content (42.29±0.23%), whilst the tempeh combination of soybean and red kidney bean had the lowest protein content (30.39±0.12%). The high protein content of the tempeh samples was likely due to the main ingredients used in the study, which were soybean, chickpea, and red kidney beans. The protein contents of raw soybean and chickpea were approximately 34.3% and 23.33–30.95%, respectively (Gupta *et al.,* 2019; Kudełka *et al.,* 2021), whilst the protein content of red kidney bean ranged from 21.5% to 27.1% (Wang *et al.,* 2010). This study's findings suggested that adding soybean to chickpea and red kidney bean in the tempeh production process can significantly enhance the protein content of the tempeh. The protein content of both mixed bean tempeh samples was higher than that of red kidney bean tempeh (29.21% of protein) and chickpea tempeh (28.85% of protein) reported in previous research (Erkan *et al.,* 2020). Moreover, the protein content of the three tempeh samples was higher than those of eggs (13%), milk (3%–3.5%), duck (12.3%) and beef (22%) (Ahmad *et al.,* 2018; Goulding *et al.,* 2020; Revathy, 2020). The results of this study are consistent with previous research, suggesting that the Rhizopus mould that produces protease enzymes during fermentation can increase the protein content of tempeh. However, the protein content could vary depending on the fermentation conditions (Tahir *et al*., 2018). The high protein content and amino acid profile from different types of legumes (soybean with chickpea and red kidney bean) make tempeh a suitable meat substitute for vegetarians who are at risk of protein deficiency. The findings of this study demonstrate that mixed bean tempeh samples with high nutritional value can be successfully produced and adding soybean to chickpea and red kidney bean in the tempeh production process can significantly enhance the protein content of the tempeh, making it a suitable meat substitute for vegetarians due to its high protein content.

The results in Table 1 indicated that soybean tempeh had the highest fat content (12.79±0.26%) compared with the other two mixed bean tempeh, which was significantly different ($p<0.05$). The high-fat content could be attributed to the soybean, which had a higher fat content of approximately 20% compared with the raw chickpea and red kidney bean (approximately 6% and 3%, respectively) (Subroto, 2020; Madurapperumage *et al.,* 2021; Roy *et al*., 2021). The breakdown of lipids during the fermentation by lipase enzymes produced by *Rhizopus oligosporus* could have also contributed to the fatty acid content of the tempeh samples (Tahir *et al.,* 2018). The study by Erkan *et al.* (2020) reported a fat content of 6.31% for red kidney bean tempeh. Abu-

Table 1. The nutritional composition of soybean tempeh, combinations of soybean with chickpea tempeh and soybean with red kidney bean tempeh.

Nutritional composition (%)/100 g	Soybean tempeh	Combination of soybean and chickpea tempeh	Combination of soybean and red kidney bean tempeh
Protein	42.29±0.23^{a}	31.08±0.41^{b}	30.39±0.12^{c}
Fat	12.79±0.26^{a}	9.88±1.42^{b}	9.43±1.02^{b}
Carbohydrate	0.44±0.44^{c}	16.57±1.90^{a}	13.31±0.78^{b}
Ash	2.82±0.16^{a}	2.57±0.21^{a}	2.70±0.26^{a}
Moisture	41.66±0.61^{b}	39.91±0.23^{c}	44.17±0.03^{a}
Crude Fiber	10.69±0.04^{c}	13.49±0.03^{a}	11.88±0.03^{b}
Total Dietary Fiber	5.39±0.03^{a}	4.40±0.02^{b}	2.50±0.02^{c}

Values are presented as mean±SD ($n = 3$). Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

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Salem and Abou-Arab (2011) reported a fat content of 2.84% for chickpea tempeh. This finding suggested that the combination of soybean with other legumes in tempeh production can significantly decrease the fat content compared with soybean tempeh. The low unsaturated fat and presence of both essential fatty acids in soybean (Jeon *et al.,* 2019) could be responsible for the high fat content in soybean tempeh. The findings of this study indicate that tempeh production using a combination of soybeans with other legumes can significantly reduce the fat content of the final product compared with soybean tempeh. This situation could be of particular interest to individuals who are health-conscious and are looking for low-fat protein sources.

The results of this study showed a significant difference ($p<0.05$) in the moisture content between the three types of tempeh samples, with the soybean–red kidney bean combination having the highest percentage (44.17±0.03%), followed by soybean tempeh (41.66±0.61%) and the soybean–chickpea combination (39.91±0.23%). These findings are not comparable to the nutritional data reported by US Department of Agriculture (USDA) (2019), which shows a higher range of moisture content (60% to 66%). However, the moisture content reported in this study is consistent with the moisture loss as a result of the grinding during sample preparation (Oyinloye and Yoon, 2020). The study of Wikandari *et al.* (2020) demonstrated a higher moisture content in yellow soybean tempeh and red kidney bean tempeh, whilst Erkan *et al.* (2020) reported a moisture content of 44.88% in chickpea tempeh. These results suggest that the moisture content in tempeh may vary depending on certain factors, such as the type of legumes used, the processing method and the sample preparation process. Therefore, further studies are needed to explore the generality of these findings and better understand the factors that contribute to the moisture content in tempeh.

The results of the ash content showed no significant difference between the soybean tempeh and mixed bean tempeh samples. This result was expected because the ash content of these legumes was not expected to significantly differ. The ash content of the three types of tempeh samples produced in this study was within the range of 2.57% to 2.82%, in which soybean tempeh had the highest percentage of ash content. The ash content of soybean tempeh found in this study was in agreement with the data reported by Syamsuri *et al.,* (2020), which was approximately 2.0%. Moreover, the ash content of the chickpea tempeh and red kidney bean tempeh reported in the literature was 2.10% and 1.50%, respectively (Erkan *et al.,* 2020). By contrast, the tempeh combination of chickpea and red kidney bean with soybean produced a higher ash content compared with the chickpea or red kidney bean tempeh in previous studies. This situation could be due to the effect of the combination of legumes on the ash content. The findings of this study suggest that the ash content may vary depending on the type of legume used and the combination of legumes in tempeh production.

The results of this study showed that the soybean and mixed bean tempeh samples had varying carbohydrate contents. The soybean tempeh had the lowest percentage of carbohydrate content (0.44±0.44%). Meanwhile, the tempeh combination of soybean and chickpea had the highest percentage of carbohydrate content (16.57±1.90%). These results are consistent with previous studies that reported that chickpeas and red kidney beans were the main contributors to the carbohydrate content in mixed bean tempeh (USDA, 2019). In comparison with the results of this study to previous work, the carbohydrate content in the studied tempeh samples was significantly lower than that reported in other studies. Wikandari *et al.* (2020) reported that soybean tempeh and red kidney bean tempeh contained 21.83% and 30.64% carbohydrates, respectively. Abu-Salem and Abou-Arab (2011) reported that chickpea tempeh contained 64.53% carbohydrates. The lower carbohydrate content in the studied tempeh samples could be due to the consumption of carbohydrates by the moulds during fermentation (Kim *et al.,* 2022) and the loss of carbohydrates in the water during soaking, dehulling, washing and cooking (Xin *et al.,* 2022). Mixing soybeans with chickpeas and red kidney beans significantly decreased the carbohydrate content in the tempeh samples. This finding suggests that these three types of tempeh samples are suitable to be used in diabetic diets. However, these results may not be generalised to other types of tempeh or other food products.

The results of the study showed that the crude fibre content of the tempeh combination of soybean and chickpea was significantly higher (13.49±0.03%) than those of soybean tempeh (10.69±0.04%) and the tempeh combination of soybean and red kidney bean (11.88±0.03%). Crude fibre is categorised as a non-soluble carbohydrate. According to previous studies, the soybean tempeh and red kidney bean tempeh consisted of 2.64% and 3.76% crude fibre, respectively (Wikandari *et al.,* 2020), whilst the chickpea tempeh consisted of 1.68% crude fibre (Abu-Salem and Abou-Arab, 2011). The content of crude fibre in the studied tempeh samples was found to be higher than the previous data. The high crude fibre content in legumes can control the absorption of carbohydrates and reduce the intake of glucose, as stated by Powthong *et al.* (2021). This finding supports the claim that mixed bean tempeh samples can be added to a diabetic diet because they have beneficial effects on the regulation of blood sugar levels. The results of this study were somewhat expected, given that legumes are known to be good sources of fibre. However, the extent of the difference between the different tempeh samples was somewhat surprising. Therefore, these factors must be considered when interpreting the results. In terms of the generality of these results, other combinations of legumes could produce similar results. However, further research would be needed to confirm this. Overall, the results of this study suggest that mixed bean tempeh could be a valuable addition to a diabetic diet due to its high fibre content and beneficial effects on blood sugar regulation.

The total dietary fibre content of the three types of tempeh samples studied ranged from 2.50% to 5.39% ($p<0.05$), with soybean tempeh having the highest percentage of dietary fibre (5.39±0.03%) and the tempeh combination of soybean with red kidney bean having the lowest percentage (2.50±0.02%). These results are consistent with previous studies that reported legumes, especially soybeans, as a good source of dietary fibre (Saha and Mandal, 2019). However, the dietary fibre content of the studied tempeh samples was lower than that reported by Wu and Hasnah (2018) in their study of tempeh samples (average of 8.05%). The lower dietary fibre content in this study could be attributed to the decrease in the hemicellulose content of beans during fermentation (Bai *et al.,* 2020). Furthermore, the removal of bean husk during dehulling decreased the overall dietary fibre content of tempeh. Bean husk is rich in several non-starch polysaccharides and lignin, which contribute to the overall dietary fibre content of tempeh (Han *et al.,* 2021). Hutkins (2008) also reported that fibre is one of the degraded compounds during tempeh processing, such as soaking and fermentation. These results are consistent with the hypothesis that the dietary fibre content of tempeh is influenced by various factors, including the type of legume used, the dehulling and the fermentation. Furthermore, the lower dietary fibre content in this study compared with previous studies could be explained by the differences in the fermentation, which affects the degradation of compounds, such as fibre. Overall, these findings suggest that tempeh can still be considered a good source of dietary fibre, and future studies should investigate the effect of different processing methods on the dietary fibre content of tempeh.

The sensory analysis results of the fried tempeh samples displayed in Figure 2 and Table 2 suggest that the panellists preferred the sensory attributes of the soybean tempeh and the tempeh combination of soybean with red kidney beans (coded as 256). In terms of appearance, the sample coded 321 obtained the highest score (7.37), and the sample coded 624 acquired the lowest score (6.71). No significant difference in colour was observed amongst the tested samples because all samples appeared in golden-brown colour after frying due to the Maillard reaction. However, the shape of the beans in the tempeh samples might have influenced the panellist preference, with the shape of the chickpea being quite different from the soybean. The texture acceptance scores showed that the panellists preferred a harder and more compact texture, which was found in the soybean tempeh and the tempeh combination of soybean with red kidney beans. The tempeh combination of chickpeas and soybean had a softer and creamier texture, which was less favoured by the panellists. The taste and aroma acceptance scores for the samples coded 321 and 256 were equally high, with the panellists being more familiar with the beany flavour and nutty aroma of soybean tempeh. The sample code 256 was the most favourable amongst all sensory attributes, obtaining the highest score (7.54) in overall acceptance.

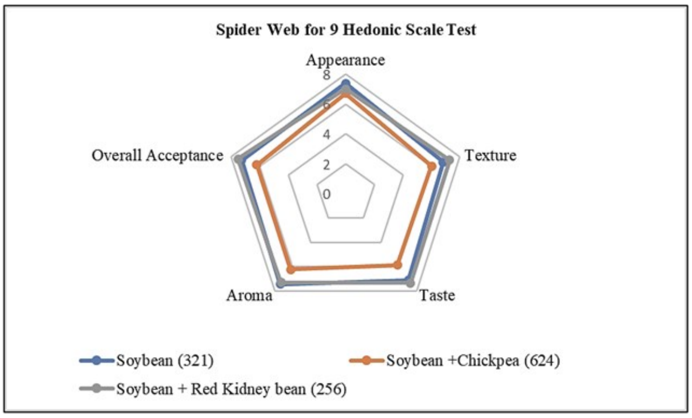

Figure 2. Sensory analysis of the tempeh samples.

Table 2. Sensory attributes of tempeh samples.

Attributes	Samples		
	321	624	256
Appearance	7.37±1.14^{a}	6.71±1.20^{b}	7.00±0.94^{ab}
Texture	6.83±1.25^{a}	6.03±1.29^{b}	7.26±0.78^{a}
Taste	7.11±1.23^{a}	5.86±1.54^{b}	7.34±0.73^{a}
Aroma	7.43±1.04^{a}	6.23±1.29^{b}	7.31±0.87^{a}
Overall Acceptance	7.29±0.96^{a}	6.23±1.22^{b}	7.54±0.85^{a}

The tempeh samples of soybean, combinations of soybean with chickpea and soybean with red kidney bean are coded as 321, 624 and 256, respectively. Values are presented as mean±SD (n = 3). Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

The results of this study are not entirely unexpected, as previous studies have also shown that soybean tempeh is widely accepted and preferred by consumers. However, this study provides new information on the acceptability of alternative tempeh made from a combination of soybean with red kidney beans and

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chickpeas. The finding that the tempeh combination of soybean and red kidney beans is highly accepted by panellists suggests that it could be produced as an alternative tempeh. The results of this study can be interpreted by comparing them with previous work. Sastry *et al.* (2014) reported that the shape of the beans in the tempeh samples might influence the panellist preference, which is consistent with the findings of this study. Starowicz and Zieliński (2019) reported that the development of food attributes, such as taste and aroma, is highly contributed by the Maillard reaction during frying, which is also consistent with the findings of this study. The findings of this study have implications for the production of alternative tempeh. Producers can offer a product that is similar in sensory attributes to the widely accepted soybean tempeh by producing a tempeh combination of soybean and red kidney beans, but it also provides an alternative option for consumers. Future research could investigate the sensory attributes of tempeh made from other combinations of beans and assess their acceptability amongst consumers. Accordingly, the sensory analysis results of the fried tempeh samples showed that the panellists preferred the sensory attributes of the soybean tempeh and the tempeh combination of soybean with red kidney beans. These results are consistent with previous studies and suggest that the tempeh combination of soybean and red kidney beans could be produced as an alternative tempeh. The results from the physical characteristics, nutritional composition and sensory analysis of the tempeh samples provide valuable insights into their potential as alternative sources of protein. The physical analysis showed that the moisture content of all the tempeh samples was below 70%, indicating that they are relatively stable products. The nutritional analysis revealed that all the tested samples had a high protein content, with soybean tempeh having the highest value. The sensory analysis demonstrated that the appearance, taste, aroma, and texture of the tempeh combination of soybean and red kidney beans (coded as 256) was similar to soybean tempeh, and it was highly accepted by the majority of the panellists. By contrast, the tempeh combination of chickpeas and soybean had a milder taste, yeasty odour, and creamy, soft and less chewy texture, which was less preferred by the panellists. These results are consistent with previous studies that have reported the nutritional value and sensory attributes of different tempeh types. Overall, the findings suggest that the tempeh combination of soybean and red kidney beans (coded as 256) has great potential as an alternative protein source and can be produced as an alternative tempeh.

4. Conclusion

In conclusion, the findings suggest that the combination of soybean and red kidney beans could be a promising alternative for tempeh production, offering a desirable sensory profile and potential nutritive and health benefits. This research is significant because it provides a new approach to producing tempeh by using plant-based protein sources, which is crucial in meeting the growing demand for alternative protein sources.

Conflict of interest

The authors do not have any conflicts of interest regarding the content of the present work.

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