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Nutritional and Sensory Characteristics of Plant-Based Analog Meatballs Made from Wood Ear Mushroom (*Auricularia auricula*) with the Addition of Soybean Flour (*Glycine max* (L.) Merr.)

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Abstract: Plant-based analog meatballs have emerged as a healthy food alternative, with wood ear mushroom (*Auricularia auricula*) serving as a texture former and soybean flour (*Glycine max* (L.) Merr.) as a source of plant-based protein. This study highlights the combined use of wood ear mushroom and soybean flour in developing plant-based analog meatballs to improve both nutritional and sensory characteristics. The objective of this study was to analyze the effect of the ratio of wood ear mushroom to soybean flour on the nutritional characteristics and sensory acceptance of analog meatballs. This study used a Randomized Block Design (RBD) with six treatments, namely a control (100% meat meatballs) and five treatments of wood ear mushroom to soybean flour ratios (80%:20% to 60%:40%), each with three replications. Proximate data were analyzed using One-Way ANOVA and Duncan's test, whereas sensory data were analyzed using the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction and descriptive analysis. The best treatment was determined using the De Garmo effectiveness index, which integrates nutritional and sensory parameters into a comprehensive evaluation to select the optimum formulation. Treatment had a significant effect ($p<0.05$) on all proximate parameters: moisture content (60.036%–67.518%), ash (2.206%–4.313%), fat (3.978%–5.057%), protein (5.632%–7.331%), and carbohydrate (18.258%–26.448%). The control obtained the highest hedonic score, while among the analog meatballs, J3K3 (70%:30%) showed the best sensory acceptance. Based on the De Garmo effectiveness index, J5K5 (60%:40%) was identified as the optimum formulation because it achieved the best overall balance between nutritional quality and sensory acceptance. These findings indicate that additional protein enrichment is still required before commercialization while demonstrating the potential of wood ear mushrooms and soybean flour for developing plant-based analog meatballs.

Keywords: Analog Meatball; Nutritional Characteristics; Soybean Flour; Sensory Evaluation; Wood Ear Mushroom.

Introduction

Meatballs are a popular processed meat product widely consumed by Indonesians of all ages. According to SNI 01-3818:2014, meatballs are defined as a processed meat product mixed with starch and seasonings, shaped into balls, and cooked until done [1]. Growing public concern for health and environmental sustainability has driven the development of plant-based foods as alternatives to animal-based products. The plant-based food and beverage market in Indonesia is projected to reach USD 1.04 billion by 2025, with an annual growth rate of approximately 7.02% during the 2025–2030 period [2]. Plant-based meatballs are considered more environmentally friendly and have the potential to serve as a healthy food alternative, including for vegetarian consumers and individuals with high cholesterol levels [3].

Plant-based meatball analogs are designed to replicate the texture, flavor, and overall sensory characteristics of conventional meatballs using ingredients derived entirely from plants. One of the major challenges in developing these products is achieving a desirable chewy texture while maintaining adequate nutritional quality. Wood ear mushroom (Auricularia auricula) is a promising ingredient because of its naturally chewy texture, high dietary fiber content, and low fat content [4], [5]. However, its relatively low protein content limits its nutritional value when used as the primary ingredient. Therefore, incorporating plant-based protein sources, such as soybean flour, may improve the nutritional quality of meatball analogs while maintaining their desirable textural properties.

Soybean (*Glycine max* (L.) Merr.) is a valuable source of plant-based protein with functional properties that enhance gel formation and water-holding capacity in meat analog products. Incorporating soybean flour into meatball analog formulations has been shown to improve protein content while contributing to desirable textural properties [6]. Previous studies have explored various formulations of plant-based meatball analogs using ingredients such as oyster mushrooms and Bogor peanut flour [7]. However, these studies investigated different plant-based ingredients and formulations, and none evaluated the combined use of wood ear mushroom and soybean flour while simultaneously assessing nutritional characteristics, sensory acceptance, and formulation optimization using the De Garmo effectiveness index. Consequently, information regarding the integrated optimization of nutritional quality and sensory acceptance in wood ear mushroom-based analog meatballs remains limited. Therefore, this study was conducted to evaluate the effects of different proportions of wood ear mushroom

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(*Auricularia auricula*) and soybean flour on the nutritional characteristics (moisture, ash, fat, protein, and carbohydrate contents) and sensory acceptance of meatball analogs. The novelty of this study lies in the combined utilization of wood ear mushroom and soybean flour to develop plant-based analog meatballs while integrating nutritional and sensory evaluations to determine the optimum formulation using the De Garmo effectiveness index. The most favorable formulation was identified using De Garmo's effectiveness index based on nutritional characteristics and sensory acceptance. The findings of this study are expected to contribute to the development of nutritionally improved plant-based meatball analogs and provide scientific evidence for optimizing plant-based food formulations using a multi-criteria evaluation approach.

Research Methods

Study Site and Period

This study was conducted from February to March 2026. The preparation of meatball analogs was carried out at the Food Processing Laboratory, Faculty of Technology, Bali Institute of Technology and Health (ITEKES Bali), Denpasar, Bali, Indonesia. Proximate analyses were performed at the Agricultural Laboratory, Udayana University, Badung, Bali, Indonesia.

Ingredients and Equipment

The main ingredients used in this study were fresh chopped wood ear mushrooms (*Auricularia auricula*) as the primary texture-forming ingredient, soybean flour as the plant-based protein source, and tapioca flour as the binding agent. Additional ingredients included low-protein wheat flour, garlic, salt, mushroom broth powder, sodium tripolyphosphate (STPP), vegetable oil, and ice water. Chemicals used for proximate analysis were prepared according to AOAC (2016) methods and included sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), boric acid (H_3BO_3), phenolphthalein indicator, bromocresol green–methyl red indicator, 0.01 N hydrochloric acid (HCl), and petroleum ether. The equipment used in this study included a digital balance, a cooking pot, a stove, a Memmert oven, a desiccator, porcelain crucibles, a Soxhlet extraction apparatus, a Kjeldahl distillation unit, a volumetric flask, and an Erlenmeyer flask.

Research Design

The experiment was conducted using a Randomized Block Design (RBD) with a single treatment factor, namely the proportion of wood ear mushroom (*Auricularia auricula*) and soybean flour. Blocking was based on production time because not all samples could be prepared simultaneously. Six treatment formulations with three replications were evaluated: control (J0K0; 100% chicken meat), J1K1 (80:20), J2K2 (75:25), J3K3 (70:30), J4K4 (65:35), and J5K5 (60:40). The total amount of the main ingredients was fixed at 100 g (48.6% of the total dough weight of 205.6 g) for each formulation, whereas the quantities of the remaining ingredients were kept constant, consisting of 20 g tapioca flour, 15 g low-protein wheat flour, 5 g garlic, 3 g salt, 2.5 g mushroom broth powder, 0.1 g sodium tripolyphosphate (STPP), 55 g ice water, and 5 g vegetable oil. The formulation ratios were established based on preliminary trials and modified from the method reported by [8].

Procedure for Preparing Meatball Analogs

The preparation of meatball analogs was carried out following the method described by [7] with slight modifications. Fresh wood ear mushrooms were washed thoroughly and finely chopped according to the formulation. The chopped mushrooms were then mixed with soybean flour based on the treatment proportions, followed by the addition of tapioca flour, low-protein wheat flour, garlic, salt, mushroom broth powder, sodium tripolyphosphate (STPP), vegetable oil, and ice water. The mixture was blended until a homogeneous dough was obtained. The dough was shaped into meatballs weighing approximately 15 g each and boiled at 90–95°C for 10–15 min until they floated, indicating that they were fully cooked. The cooked meatball analogs were then removed, drained, cooled to room temperature, and subjected to further analyses.

Parameters Observed

The evaluated parameters included proximate composition and sensory acceptance. Proximate analyses were performed according to AOAC (2016) methods, including moisture content determined by the oven-drying method at 102–105°C, ash content by dry ashing at 550°C, protein content by the micro-Kjeldahl method using a nitrogen conversion factor of 6.25, fat content by Soxhlet extraction with petroleum ether as the solvent, and carbohydrate content calculated by difference (100% minus the percentages of moisture, ash, fat, and protein).

Sensory acceptance was evaluated using a hedonic test involving 30 untrained panelists recruited from students of the Bali Institute of Technology and Health (ITEKES Bali). The number of panelists was determined based on the Indonesian National Standard (SNI 2346) [27], which recommends a minimum of 30 untrained panelists for hedonic sensory evaluation. Prior to the sensory evaluation, all prospective panelists were informed about the purpose of the study and the ingredients used in the formulations, including soybean flour, to ensure that individuals with food allergies could decline participation. Participation was entirely voluntary, and only individuals who agreed to participate were included in the sensory evaluation. Five sensory attributes, namely color, aroma, taste, texture, and overall acceptance, were evaluated using a five-point hedonic scale ranging from 1 (dislike very much) to 5 (like very much). Each sample was assigned a random three-digit code and served in a randomized order to minimize order bias. Mineral water was provided as a palate cleanser between samples.

Data Analysis

Data from the proximate analyses were subjected to a one-way analysis of variance (ANOVA) in SPSS at a significance level of 5% ($\alpha = 0.05$). When significant differences were detected among treatments, Duncan's multiple range test (DMRT) was performed to determine

differences between treatment means. Hedonic test data were analyzed using the Kruskal-Wallis test to determine overall differences among treatments, followed by Dunn's post-hoc test with Bonferroni correction for pairwise comparisons ($\alpha = 0.05$), in addition to descriptive analysis of mean scores for each sensory attribute.

Results and Discussion

Moisture Content

The analysis of variance showed that the proportion of wood ear mushroom and soybean flour significantly affected the moisture content of the meatball analogs ($p < 0.05$). As shown in Table 1, the moisture content ranged from 60.036% (J5K5) to 67.518% (J2K2), whereas the control sample had a moisture content of 62.171%. All formulations complied with the Indonesian National Standard for meatballs [1], which specifies a maximum moisture content of 70% (w/w) [1].

Table 1. Moinsture content of analog meatballs

Treatment	Average Moisture Content (%)
Control (0%)	62.171±0.243[a]
J1K1 (80%:20%)	66.741±0.861[b]
J2K2 (75%:25%)	67.518±2.113[b]
J3K3 (70%:30%)	65.673±1.797[b]
J4K4 (65%:35%)	60.812±1.253[a]
J5K5 (60%:40%)	60.036±0.983[a]

Note: Different letters in the notation column indicate significant differences among treatments ($p < 0.05$) based on Duncan's multiple range test (DMRT).

The moisture content obtained in this study was comparable to that reported by [7], who developed meatball analogs from oyster mushrooms and Bogor peanut flour and reported a moisture content of 66.05% for the selected formulation. Variations in moisture content among treatments may be attributed to differences in the proportions of the main ingredients, as wood ear mushrooms contain considerably more water than soybean flour. Consequently, increasing the proportion of soybean flour reduced the moisture content of the meatball analogs. This finding is related to the naturally high moisture content and water-retention capacity of fresh wood ear mushrooms, whereas soybean flour has a lower moisture content, resulting in a decrease in the overall moisture content as its proportion increased,

Compared with the study of [6], which formulated meatball analogs using oyster mushrooms and soybean flour, the moisture content in the present study was generally higher, particularly in treatments J1K1 and J2K2. [6] reported moisture contents ranging from 49.02% to 62.50%, whereas the highest moisture content in the present study reached 67.518%. This difference is likely attributable to the use of the wood ear mushroom (Auricularia auricula), which has a naturally higher moisture content (93.7 g/100 g fresh weight) and hydrophilic polysaccharides with high water-binding capacity [9], [10]. Consequently, formulations containing a higher proportion of wood ear mushroom, particularly J1K1 and J2K2, exhibited higher moisture contents. In contrast, increasing the proportion of soybean flour in J4K4 and J5K5 reduced the moisture content because soybean flour contains considerably less water than fresh wood ear mushrooms.

Ash Content

The analysis of variance showed that the proportion of wood ear mushroom and soybean flour significantly affected the ash content of the meatball analogs ($p < 0.05$). As presented in Table 2, the ash content ranged from 2.206% (J5K5) to 4.313% (J1K1), whereas the control sample had an ash content of 3.328%. Among the formulations, J3K3 (3.215%), J4K4 (3.029%), and J5K5 (2.206%) met the maximum ash content specified in the Indonesian National Standard for meatballs [1], whereas J1K1 slightly exceeded the permissible limit of 3.0% [1]. Duncan's multiple range test (DMRT) showed that J1K1 had the highest ash content and differed significantly from all other treatments, whereas J5K5 exhibited the lowest ash content.

Table 2. Ash Content of Analog Meatballs

Treatment	Average Ash Content (%)
Control (0%)	3.328±0.298[bc]
J1K1 (80%:20%)	4.313±0.067[d]
J2K2 (75%:25%)	3.418±0.069[c]
J3K3 (70%:30%)	3.215±0.148[bc]
J4K4 (65%:35%)	3.029±0.215[b]
J5K5 (60%:40%)	2.206±0.148[a]

Note: Different letters in the notation column indicate significant differences among treatments ($p < 0.05$) based on Duncan's multiple range test (DMRT).

The ash content generally decreased as the proportion of soybean flour increased. This trend may be attributed to the relatively high mineral content of wood ear mushroom, which contains calcium, phosphorus, potassium, sodium, and iron [9]. Similarly, [11] reported that *Auricularia* spp. are rich in minerals and other bioactive compounds that contribute to their nutritional value. In contrast, soybean flour contains proportionally higher amounts of protein and carbohydrates than minerals, resulting in a lower ash content as its proportion increased in the formulation. [12] also reported that differences in the composition of plant-based ingredients significantly influence the ash content of food products because each ingredient has a distinct mineral composition. Since ash content reflects the total mineral content of a food product [13], the observed variation among treatments indicates that the ratio of wood ear mushroom to soybean flour directly influenced the mineral composition of the meatball analogs.

Fat Content

The analysis of variance showed that the proportion of wood ear mushroom and soybean flour significantly affected the fat content of the meatball analogs ($p < 0.05$). As shown in Table 3, the fat content ranged from 3.918% (control) to 5.057% (J1K1). All formulations complied with the Indonesian National Standard for meatballs [1], which specifies a maximum fat content of 10% (w/w) [1]. Duncan's multiple range test (DMRT) indicated that the control and J5K5 were not significantly different, whereas J1K1 showed a significantly higher fat content than the control.

The relatively higher fat content observed in J1K1 may be attributed to the greater proportion of wood ear

mushroom combined with the addition of vegetable oil during processing. Although wood ear mushroom is generally classified as a low-fat ingredient, it still contains approximately 0.6 g fat per 100 g of fresh material [9]. In contrast, the lower fat content observed in J5K5 may be explained by the functional properties of soybean protein, which acts as a natural emulsifier. The amphiphilic characteristics of soybean protein promote the formation of a stable protein–water matrix and enhance fat binding, thereby reducing the amount of extractable fat during Soxhlet analysis [14].

Table 3. Fat Content of Analog Meatballs

Treatment	Average Fat Content (%)
Control (0%)	3.918±0.080[a]
J1K1 (80%:20%)	5.057±0.137[c]
J2K2 (75%:25%)	4.801±0.197[bc]
J3K3 (70%:30%)	4.497±0.436[bcd]
J4K4 (65%:35%)	4.242±0.544[ab]
J5K5 (60%:40%)	3.978±0.396[a]

Note: Different letters in the notation column indicate significant differences among treatments ($p < 0.05$) based on Duncan's multiple range test (DMRT).

Compared with the study by [6], which reported a fat content of 5.37% in the best formulation, J5K5 in the present study exhibited a lower fat content (3.978%). Meanwhile, [7] reported a lower fat content (1.42%) in meatball analogs formulated with oyster mushrooms and Bogor peanut flour, which may be attributed to differences in the composition of the raw materials. Nevertheless, all formulations in the present study remained well below the maximum limit specified by SNI 01-3818:2014. These findings are consistent with [8], who reported that meatball analogs formulated with mushrooms and soybean flour generally have lower fat contents than conventional meatballs, making them a promising alternative for consumers seeking foods with reduced fat intake.

Protein Content

The analysis of variance showed that the proportion of wood ear mushroom and soybean flour significantly affected the protein content of the meatball analogs ($p < 0.05$). As presented in Table 4, the protein content ranged from 5.632% (J1K1) to 7.331% (J5K5), whereas the control sample had the highest protein content (13.498%). None of the formulations met the minimum protein requirements specified by SNI 01-3818:2014 for either meatballs (11.0%) or combination meatballs (8.0%) [1]. Duncan's multiple range test (DMRT) indicated that the control differed significantly from all treatments, whereas J1K1–J5K5 did not differ significantly from one another, despite the increasing trend in protein content.

Protein content increased as the proportion of soybean flour increased, confirming soybean flour as the primary contributor of protein in the formulations. Soybeans contain considerably higher protein levels than fresh wood ear mushrooms, approximately 30.2 g and 3.8 g per 100 g of fresh material, respectively [9]. Likewise, [15] reported that soybeans contain approximately 36.5% protein, highlighting their potential as an important plant-based protein source. A similar trend was reported by [6], [16], who observed increased protein content with increasing proportions of soybean flour in meatball analog formulations.

Table 4. Protein Content of Analog Meatballs

Treatment	Average Protein Content (%)
Control	13.498±0.245[b]
J1K1 (80%:20%)	5.632±0.190[a]
J2K2 (75%:25%)	5.984±0.927[a]
J3K3 (70%:30%)	6.909±1.641[a]
J4K4 (65%:35%)	6.559±0.805[a]
J5K5 (60%:40%)	7.331±0.913[a]

Note: Different letters in the notation column indicate significant differences among treatments ($p < 0.05$) based on Duncan's multiple range test (DMRT).

Despite the increasing trend, the protein content remained below the SNI minimum requirement. This finding is likely attributable to the relatively high proportion of wood ear mushrooms, which have a lower protein content than the soybean flour and chicken meat used in the control formulation. Similar findings were reported by [12], who observed protein levels below 8% in several formulations of plant-based meatballs. Compared with the study by [6], which reported a protein content of 14.46% in the best formulation, the lower protein content observed in the present study may be explained by differences in formulation and in the proportion of protein-rich ingredients. In addition, although increasing the proportion of soybean flour improved the protein content, the amount incorporated in the present formulation was limited to maintain desirable sensory characteristics. Further increases in soybean flour could potentially enhance protein content but may also intensify the beany flavor and alter the texture of the product, which could negatively affect consumer acceptance. Therefore, achieving both nutritional improvement and acceptable sensory quality remains one of the major challenges in developing plant-based meatball analogs. Although the formulations developed in this study have not yet fulfilled the minimum protein requirement specified by SNI for commercial meatballs, the findings provide valuable information for future formulation optimization and demonstrate the potential of wood ear mushroom and soybean flour as ingredients for developing plant-based meatball analogs. Therefore, increasing the proportion of soybean flour or incorporating other plant-based protein sources, such as soy protein isolate or textured vegetable protein, may further improve the protein content while maintaining the characteristics of the meatball analogs [17].

Carbohydrate Content

The analysis of variance showed that the proportion of wood ear mushroom and soybean flour significantly affected the carbohydrate content of the meatball analogs ($p < 0.05$). As presented in Table 5, the carbohydrate content ranged from 17.084% (control) to 26.448% (J5K5). Duncan's multiple range test (DMRT) indicated that the control, J1K1, J2K2, and J3K3 did not differ significantly, whereas J4K4 and J5K5 showed significantly higher carbohydrate contents, indicating

that increasing the proportion of soybean flour to 35–40% significantly increased the carbohydrate content.

Table 5. Carbohydrate Content of Analog Meatballs

Treatment	Average Carbohydrate Content (%)
Control (0%)	17.084±0.025[a]
J1K1 (80%:20%)	18.258±0.754[a]
J2K2 (75%:25%)	18.223±2.644[a]
J3K3 (70%:30%)	19.706±2.703[a]
J4K4 (65%:35%)	25.357±1.991[b]
J5K5 (60%:40%)	26.448±1.769[b]

Note: Different letters in the notation column indicate significant differences among treatments ($p < 0.05$) based on Duncan's multiple range test (DMRT).

The carbohydrate content generally increased with the proportion of soybean flour. This trend may be attributed to the higher carbohydrate content of soybean flour, which contains polysaccharides, oligosaccharides, and dietary fiber [15], In addition, carbohydrate content was determined using the by-difference method; therefore, reductions in moisture and protein contents at higher soybean flour proportions contributed to a proportional increase in calculated carbohydrate content. Wood ear mushrooms also contribute polysaccharides, including β-glucans and heteropolysaccharides, whereas soybean flour provides additional complex carbohydrates and dietary fiber, resulting in a higher total carbohydrate fraction [10], [11].

Compared with the control, the meatball analogs exhibited higher carbohydrate contents because carbohydrates were supplied not only by tapioca starch but also by wood ear mushroom polysaccharides and soybean flour. Similar findings were reported by [12], who observed higher carbohydrate contents in plant-based meatballs than in conventional meatballs due to the contribution of plant-derived carbohydrates and polysaccharides. In addition to increasing carbohydrate content, the dietary fiber contributed by both wood ear mushroom and soybean flour may provide functional health benefits, including improved digestive health and potential prebiotic effects.

Sensory (Hedonic) Test

Table 6 shows the control sample received the highest hedonic score for every attribute: color (4.50), aroma (4.50), taste (4.73), texture (4.10), and overall acceptance (4.83), for a total of 22.63. The analog meatball formulations (J1K1 to J5K5) totaled 14.27 to 14.90, so panelists preferred the control.

A Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction ($\alpha = 0.05$) found significant differences between the control and the analog formulations for color, aroma, taste, and overall acceptance. The analog formulations did not differ from one another on these attributes. Texture followed a different pattern: J1K1 (3.23) and the control (4.10) shared a significance group, while the other formulations scored lower. J3K3 had the highest total score (14.90) and overall acceptance (3.00) among the analogs, but the gap to the other analogs was not statistically significant. Differences in ingredient composition may explain the lower analog scores, since ingredients shape color development, volatile aroma compounds, flavor, and texture. Future work should optimize ingredient ratios and processing conditions to raise taste and aroma scores while keeping the nutritional and functional properties of the product.

Table 6. Hedonic Test Scores for Analog Meatballs on

Mean Hedonic Scores	Sample Code					
	control	J1K1	J2K2	J3K3	J4K4	J5K5
Color	4.50[a]	3.00[b]	2.93[b]	3.03[b]	3.03[b]	2.87[b]
Aroma	4.50[a]	2.77[b]	2.83[b]	2.90[b]	2.90[b]	3.10[b]
Taste	4.73[a]	2.77[b]	2.63[b]	2.90[b]	2.80[b]	2.80[b]
Texture	4.10[a]	3.23[a]	3.07[b]	3.10[b]	2.93[b]	2.63[b]
Overall Acceptance	4.83[a]	2.93[b]	2.93[b]	3.00[b]	2.90[b]	2.90[b]
Total	22.63	14.70	14.39	14.90	14.53	14.27

Note: Rating scale from 1 (strongly dislike) to 5 (strongly like). Different superscript letters within the same row indicate a significant difference (Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction, $\alpha = 0.05$).

Color

The descriptive analysis showed that the mean color scores of the meatball analogs ranged from 2.87 (J5K5) to 3.03 (J3K3 and J4K4), whereas the control sample received a score of 4.50. The meatball analogs were generally rated between “neither like nor dislike” and “like slightly,” while the control was rated between “like” and “like very much.” These findings indicate that the analog formulations were less preferred in terms of color than the conventional meatball control.

Color is the first sensory attribute perceived by consumers and therefore plays an important role in determining initial product acceptance [18]. The darker brown colour of the meatball analogues can be attributed to the natural pigments of the wood ear mushroom, particularly melanin and phenolic compounds, as well as the Maillard reaction during cooking [11], [19]. Increasing the proportion of soybean flour slightly intensified the brown color because the Maillard reaction between soybean proteins and reducing sugars becomes more pronounced during heating. Although this darker appearance may reduce consumer acceptance, especially among consumers unfamiliar with mushroom-based products, improvements such as blanching the mushrooms before processing or optimizing the cooking conditions may help produce a more desirable color.

Aroma

The descriptive analysis showed that the mean aroma scores of the meatball analogs ranged from 2.77 (J1K1) to 3.10 (J5K5), whereas the control sample received a mean

score of 4.50. Among the analog formulations, J5K5 (60%:40%) achieved the highest aroma score, suggesting that increasing the proportion of soybean flour improved aroma acceptance compared with formulations containing higher proportions of wood ear mushroom.

Aroma is an important sensory attribute because it strongly influences consumer acceptance and flavor perception. The aroma of meatball analogs is determined by volatile compounds generated during processing, including aldehydes, ketones, and esters formed through the thermal degradation of proteins and fatty acids [19]. Wood ear mushroom contributes characteristic volatile compounds, such as 1-octen-3-ol and terpenoids [11], whereas soybean flour contains compounds responsible for the characteristic beany flavor produced by lipoxygenase-catalyzed oxidation of unsaturated fatty acids [19]. Heat treatment during cooking reduces lipoxygenase activity, thereby decreasing the intensity of the beany flavor and improving aroma acceptance [16]. Consequently, the higher aroma score observed in J5K5 suggests that the aroma of cooked soybean flour partially masked the characteristic odor of wood ear mushroom, resulting in a more balanced aroma profile.

Taste

The descriptive analysis showed that the mean taste scores of the meatball analogs ranged from 2.63 (J2K2) to 2.90 (J3K3), whereas the control sample received the highest mean score of 4.73. Among the analog formulations, J3K3 (70%:30%) achieved the highest taste score. However, all analog meatball formulations were generally rated between “dislike slightly” and “neither like nor dislike,” indicating that their flavor had not yet matched that of conventional meatballs.

Taste is one of the most important sensory attributes influencing consumer acceptance because it is determined by the composition of raw materials, processing conditions, interactions among food components, and individual sensory perception. The relatively low taste scores of the meatball analogs may be attributed to the absence of animal-derived flavor compounds, particularly free amino acids and nucleotides responsible for the characteristic umami taste of meat [20]. Although mushroom broth was incorporated into the formulation to enhance umami flavor, its contribution was insufficient to reproduce the flavor profile of conventional meatballs. The superior taste score of J3K3 suggests that this formulation provided the most balanced combination of wood ear mushroom and soybean flour among all treatments. Previous studies have shown that flavor acceptance of plant-based meat products can be further improved through the incorporation of natural flavor enhancers, such as yeast extract or plant-derived nucleotides, or by applying fermentation prior to processing [21], [5].

Texture

The descriptive analysis showed that the mean texture scores of the meatball analogs ranged from 2.63 (J5K5) to 3.23 (J1K1), whereas the control sample received a mean score of 4.10. Among the analog formulations, J1K1 (80%:20%) achieved the highest texture score, indicating that a higher proportion of wood ear mushroom contributed to a more acceptable texture, while J5K5, containing the highest proportion of soybean flour, received the lowest texture score.

Texture is one of the most important quality attributes influencing consumer acceptance of meatballs, with chewiness and firmness being the primary characteristics. In conventional meatballs, the characteristic chewy texture results from interactions between myofibrillar proteins and starch during heating [22]. Replicating this texture in plant-based meatball analogs remains challenging because plant-derived ingredients differ in composition and functional properties from animal proteins.

The higher texture score observed in J1K1 may be attributed to the hydrophilic polysaccharides present in wood ear mushroom, particularly β-glucans and heteropolysaccharides, which can bind water and form an elastic gel network during heating [10]. Consequently, formulations containing higher proportions of wood ear mushroom produced a chewier texture that more closely resembled conventional meatballs. In contrast, increasing the proportion of soybean flour gradually reduced texture acceptance. High concentrations of soybean protein tend to form a denser and more rigid protein network, resulting in a firmer and less elastic texture that was less preferred by the panelists [23]. These findings indicate that optimizing the ratio of wood ear mushroom to soybean flour is essential to produce meatball analogs with desirable textural characteristics.

Overall Acceptance

The descriptive analysis showed that the mean overall acceptance scores of the meatball analogs ranged from 2.90 (J4K4 and J5K5) to 3.00 (J3K3), whereas the control sample received the highest mean score of 4.83. Among the analog formulations, J3K3 (70%:30%) achieved the highest overall acceptance score, followed by J2K2 and J1K1, while J4K4 and J5K5 received the lowest scores. These findings indicate that J3K3 provided the most acceptable balance of sensory attributes among the meatball analog formulations, although overall consumer acceptance remained lower than that of the conventional meatball control.

Overall acceptance reflects consumers’ integrated evaluation of all sensory attributes, including color, aroma, taste, and texture. The relatively low acceptance scores observed for the meatball analogs were primarily associated with their lower ratings for taste and aroma, which had not yet closely resembled those of conventional meatballs. Similar findings were reported by [7], [12], who found that consumer acceptance of plant-based meatball products generally remained lower than that of conventional meatballs because of differences in sensory characteristics.

The superior overall acceptance of J3K3 suggests that the 70:30 ratio of wood ear mushroom to soybean flour provided the most balanced combination of texture, flavor, and nutritional characteristics among the tested formulations. Further improvements in consumer acceptance may be achieved through optimization of seasoning, modification of processing techniques, such as marination or extrusion, and the incorporation of natural plant-based flavor enhancers to improve aroma and taste [20], [21].

Potential of Dietary Fiber in Analog Meatballs Made from Wood Ear Mushrooms

Although dietary fiber content was not directly determined in this study, the formulation used suggests that the analog meatballs have the potential to provide dietary fiber because wood ear mushroom (*Auricularia auricula*) is recognized as a rich source of functional polysaccharides and dietary fiber. Fresh wood ear mushrooms contain approximately 5.1 g of dietary fiber per 100 g, which is higher than that of oyster mushrooms (3.6 g/100 g) and straw mushrooms (2.4 g/100 g) (Ministry of Health of the Republic of Indonesia, 2019). In addition, [4] reported that black wood ear mushrooms contain approximately 43.2% polysaccharide dietary fiber, highlighting their potential as a functional food ingredient.

The dietary fiber fraction of wood ear mushrooms consists mainly of uronic acid, water-soluble polysaccharides such as mannan and glucan, pectin, chitin, and cellulose [24]. These components have been associated with various physiological functions, including modulation of glucose and cholesterol absorption, prebiotic activity, immune regulation, and maintenance of gastrointestinal health [4], [25], [11]. In addition, the hydrophilic nature of these polysaccharides enables them to bind water and form hydrocolloid-like structures, which may also contribute to the desirable texture of plant-based meat products [10].

The relatively high carbohydrate content observed in the present study may therefore be partly attributed to the polysaccharides naturally present in wood ear mushrooms rather than solely to digestible carbohydrates. However, because dietary fiber was not directly quantified, this assumption requires confirmation through enzymatic dietary fiber analysis, such as the AOAC 985.29 method, in future studies.

Determination of the Best Analog Meatball Formulation

The best formulation was determined using the De Garmo effectiveness index method, considering all measured parameters simultaneously, including moisture, ash, fat, protein, and carbohydrate contents, as well as overall sensory acceptance. Each parameter was assigned a weighting factor according to its relative importance: protein content (0.25), carbohydrate content (0.20), overall acceptance (0.20), fat content (0.15), moisture content (0.10), and ash content (0.10). In this method, weighting factors reflect the relative priority of each parameter to the study's specific objective, allowing greater emphasis on parameters most relevant to the intended product characteristics [26]. In the present study, greater weight was assigned to protein and carbohydrate contents because the primary objective of the research was to develop an analog meatball with nutritional characteristics approaching those of conventional meat products. Parameters with desirable higher values (protein, carbohydrate, and overall acceptance) were treated as benefit attributes, whereas parameters with desirable lower values (moisture, ash, and fat) were treated as cost attributes. The effectiveness score of each parameter was multiplied by its respective weighting factor to obtain the product value, and the sum of all product values yielded the Total Product Value (TPV) for each formulation.

Based on the De Garmo effectiveness index, J5K5 (60% wood ear mushroom: 40% soybean flour) achieved the highest TPV, indicating it was the optimal formulation among the treatments evaluated. This formulation exhibited the highest protein (7.331%) and carbohydrate (26.448%) contents, while also showing the lowest moisture (60.036%), ash (2.206%), and fat (3.978%) contents. Although J3K3 showed the highest overall sensory acceptance, the determination of the optimum formulation in this study was based on a multi-criteria evaluation using the De Garmo effectiveness index rather than sensory acceptance alone. Notably, the Kruskal–Wallis and Dunn's post-hoc tests indicated that none of the analog formulations, including J3K3 and J5K5, differed significantly from one another in overall sensory acceptance (Table 6), whereas the significant differences were attributable solely to the meat control. This finding indicates that the higher descriptive sensory score of J3K3 did not correspond to a statistically superior sensory performance, and that selecting J5K5 for its superior nutritional characteristics did not result in a statistically significant reduction in overall sensory acceptance among the analog formulations. Therefore, J5K5 was selected as the optimal formulation because it achieved the highest Total Product Value following a comprehensive multi-criteria evaluation integrating nutritional quality and sensory acceptance.

This study has several limitations that should be considered when interpreting the findings. Instrumental analyses, such as Texture Profile Analysis (TPA), dietary fiber determination, instrumental color measurement, and shelf-life evaluation, were not performed. Consequently, texture and color were evaluated only through sensory assessment, whereas the discussion regarding dietary fiber was based on previous literature rather than direct experimental measurement. Future studies should incorporate these analyses to provide a more comprehensive evaluation of the quality, nutritional value, and storage stability of plant-based meatball analogs and to support their potential commercial application.

Conclusion

This study demonstrates that combining wood ear mushroom (*Auricularia auricula*) and soybean flour is a viable strategy for developing plant-based analog meatballs with improved nutritional value, offering a practical formulation approach for the plant-based meat analog industry. Increasing the proportion of soybean flour consistently enhanced protein and carbohydrate contents while reducing moisture, ash, and fat contents, underscoring soybean flour's functional role in improving the nutritional profile of mushroom-based meat analogs. Although the resulting formulations did not yet meet the SNI minimum protein requirement, the consistent improvement in protein content with increasing soybean flour proportion indicates a clear pathway toward closing this gap through further formulation optimization or supplementation with concentrated soy protein sources. Beyond its nutritional findings, this study contributes methodologically by applying the De Garmo effectiveness index to integrate nutritional and sensory data into a single, evidence-based formulation decision — an approach that offers a replicable framework for optimizing other plant-based meat analog

products. J5K5 (60% wood ear mushroom : 40% soybean flour) was identified as the optimum formulation, balancing superior nutritional characteristics with sensory acceptance that was not statistically inferior to the other analog formulations, while J3K3 (70%:30%) offered the most favorable sensory profile among the analogs. These findings support the potential of wood ear mushroom–soybean flour combinations as a functional, environmentally friendly alternative to conventional meatballs, particularly for vegetarian consumers and individuals managing cholesterol intake, and provide a scientific foundation for further protein enrichment and product optimization prior to commercial-scale development.

Author's Contribution

E.L. Magi: Conceptualization, methodology, investigation, formal analysis, data curation, writing-original draft. A.A.A.W. Putra: Supervision, methodology, validation, writing-review and editing**.** I.G.A.Y. Rabani RS: Supervision, validation, writing-review and editing.

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