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Formulation and impact of soy protein isolate on white oyster mushroom (*Pleurotus ostreatus*) sausage: Palatability evaluation, nutritional and economic value

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Abstract. This study investigated the utilization of white oyster mushrooms (*Pleurotus ostreatus*) and soy protein isolate in developing vegetarian sausages. Two formulations were evaluated: a control (100% mushrooms) and an experimental (80% mushrooms, 20% soy protein isolate). Sensory assessment using a 5-point hedonic scale with 25 trained panellists revealed no significant differences ($p > 0.05$) in aroma, taste, texture, or colour between formulations. Chemical analysis indicated that the experimental sample showed a significant increase in protein content from 0.42% to 2.24%, though this still falls short of the Indonesian National Standard (SNI 3820:2015) minimum requirement of 8% for sausages. Moisture, fat, and ash content were comparable between formulations and within SNI limits. Carbohydrate content was slightly lower in the experimental sausages but presumably exceeded the SNI minimum. Microbiological testing demonstrated that both formulations met safety standards, with total plate count, coliform count, and *Salmonella* levels well below the limits set by SNI 3820:2015. Cost estimation revealed that the vegetarian sausages had a lower selling price compared to traditional meat sausages, offering an economically viable alternative. This research highlights the potential of mushrooms and plant proteins in developing nutritious, safe, and cost-effective meat alternatives though further formulation improvements are needed to meet protein content standards for sausage products.

Keywords and phrases: meat analogue, alternative protein sources, sensory evaluation, hedonic test

1. Introduction

Sausage is a popular processed food, usually made from meat or a combination of several types of meat through a process of mashing and mixing with herbs or spices. The main ingredients of sausages usually come from beef, pork, chicken, fish, and rabbit (*Leroy et al.*, 2006). From year to year, the demand for ready-to-cook food is getting higher. This is validated by survey data obtained in the framework of the National Economy Survey in 2018, suggesting that the consumption of processed meat, including sausages, in some regions of Indonesia takes place almost on a monthly basis (*Rasyda & Santosa*, 2023). The demand for meat also increased along with the high demand for sausages in the market. The Chairman of the Indonesian Meat Processing Industry Association and National Meat Processor Association stated that the income of the meat processing industry per day had a demand for chicken meat of 75 metric tons and an income range of one trillion Indonesian Rupiah (IDR) per year (*Hugo & Hugo*, 2015).

The other side of the popularity of consuming processed meat products, such as sausages, is that people who consume them excessively contribute to increasing the mortality rate in favour of cardiovascular diseases and cancer. Faced with such a reality, people want to live healthier but do not want to change their consumption patterns such as reducing processed meat products to consume healthier foods. Vegetarianism is currently one of the healthy lifestyle choices adopted in various countries, including Indonesia. It can be defined as avoiding foods that contain meat or animal products. Therefore, avoiding and reducing animal products is part of a healthy lifestyle (*Nezlek & Forestell*, 2020; *Rosenfeld*, 2018). However, there are also contrary opinions stating that a balanced, moderate consumption of processed meats can be part of a healthy diet. Critics argue that eliminating food groups is unnecessary and can lead to nutritional deficiencies if not properly planned. They advocate for reasonable portion control of meat products rather than strict avoidance. Nevertheless, developing appetizing plant-based meat alternatives provides options for those looking to reduce processed meat intake without drastically altering their dietary patterns.

The obstacles experienced in following a vegetarian diet are the temptation of non-vegetarian food and the influence of a non-vegetarian environment. Vegetarians feel confused to find a substitute for the protein found in meat, which is their biggest problem (*Permana & Dewanto*, 2021). It can be concluded that people tend to want to have a healthier lifestyle while still wanting to eat foods whose consumption they should reduce, namely meat products or their preparations. One of the vegetarian food ingredients that can be used as a substitute for meat products in animals is oyster mushrooms (*Giawa*, 2023).

Oyster mushrooms have beneficial effects because they do not contain cholesterol. Oyster mushrooms can be a food alternative to meat raw materials (*Gizatova et* *al.*, 2020). To increase the protein content in oyster mushrooms, other ingredients are needed, one of which is soy protein isolate flour. Soy protein isolate is a food product resulting from the separation process of oil, water, starch, and carbohydrate components of soybeans with the aim of achieving protein levels (*Munasir & Sekartini*, 2020). Soy protein isolate is one of the numerous ingredients that can be utilized as a binder in the production of processed meat products due to its ability to bind both water and oil. It can stabilize emulsions and help to maintain the structure or shape of processed meat products (*Gao et al.*, 2015).

Sausage is one of the processed meat products the demand for which is increasing year by year in the community. On the contrary, excessive consumption of processed meat products such as sausages is associated with increased risk of mortality, and there is a limited choice of alternative protein sources for meat products. The authors aimed to formulate a white oyster mushroom vegetarian sausage using soy protein isolate as alternative vegetarian sausage products.

2. Materials and methods

Materials

White oyster mushroom, isolate protein soybeans (Para Agribusiness), salt (Segitiga Biru,® Indonesia), sugar (Gulaku,® Indonesia), ground white pepper (Abrofood,® Indonesia), tapioca (Rosebrand,® Indonesia), crystal ice (Air Beku,® Indonesia), palm cooking oil (Bimoli,® Indonesia), garlic powder (Koepoe Koepoe,® Indonesia), sodium tripolyphosphate (Aditya Birla,® Indo Food Chem, Indonesia), polyamide sausage casings (skin) (Devro,® Czech Republic), and butchers cotton twine (Librett Durables,® USA) were purchased through online trading. The product was prepared in the kitchen laboratory at Polimedia Campus, Jakarta.

Methods

This research uses quantitative methods with random sampling techniques. Normality was assessed using the Shapiro–Wilk test. The validity of the measurement instrument used was assessed by examining the significance level of each item. An item was considered valid if its significance level was below 0.05. The reliability of the instrument was evaluated using Cronbach's alpha (α), with a value greater than 0.6 indicating acceptable reliability.

The vegetarian sausage products were prepared (*Table 1*) using two different formulations, A and B. Formula A1/A2 consists of 100% white oyster mushrooms and 0% soy protein isolate. Formula B1/B2 is composed of 80% white oyster mushrooms and 20% soy protein isolate. The numbers in parentheses next to each formula variation (745, 467, 905, and 275) are random numbers associated with the formulas.

Table 1. Composition of the sausage formula

No	Ingredient name (English)	Unit	Control	Experiment
1.	White oyster mushroom	g	100	80
2.	Soy protein isolate (SPI)	g	0	20
3.	Tapioca flour	g	15	15
4.	Sodium tripolyphosphate (STPP)	g	0.5	0.5
5.	Garlic powder	g	0.2	0.2
6.	Salt	g	3	3
7.	Pepper	g	0.2	0.2
8.	Sugar	g	1	1
9.	Cooking oil	ml	20	20
10.	Distilled water	ml	25	25

Sensory evaluation

The outcomes of this experiment were evaluated using a hedonic test to assess the sensory attributes and consumer acceptance. This evaluation included the level of liking with a numerical representation that incorporated aspects of product evaluation from the human sensory indicators of aroma, taste, texture, and colour. The test was conducted in a provisional laboratory at Jakarta State Polytechnic of Creative Media Campus, located in Srengseng Sawah, South Jakarta. A total of 25 trained panellists, consisting of students from the Culinary Arts Study Programme, participated in the study. These panellists had previously undergone a triangle test, which is a discriminatory test in sensory evaluation. In the triangle test, a set of three samples is presented to the panellists, who must identify the odd sample without using a reference, to assess their sensory discrimination abilities.

Data measurement and analysis

The hedonic test was conducted according to the favourability level and scored accordingly: dislike extremely (1), dislike (2), quite like (3), like (4), like extremely (5) (*Mello et al.*, 2019). The mean was obtained using the formula:

$$\overline{x} = \frac{\sum f(x)}{n} \tag{1}$$

Notation:
$\overline{x}$ = mean (average),
$\Sigma f(x)$ = number of frequencies multiplied by the value,
n = number of panellists.

The average score criteria, as a method by *Lim* (2011), was done by calculating the interval value, that is, the highest score minus the lowest score, and the result was divided by the number of types of assessment criteria. The result is 0.8 – hence, the intervals are 1–1.8 (dislike extremely), 1.9–2.6 (dislike), 2.7–3.4 (moderately like), 3.5–4.2 (like), and 4.3–5 (like extremely).

Product development and work steps

The experimental process consisted of two formulations, with two repetitions. The production process was based on the following flowchart (*Figure 1*).

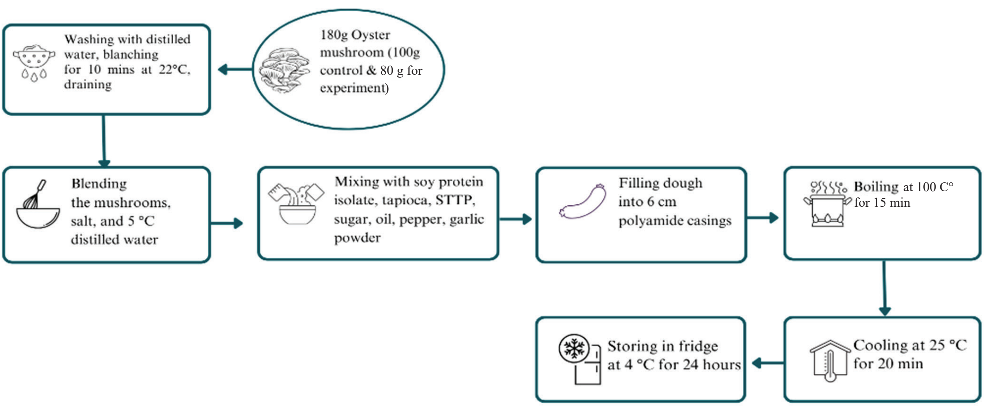

Figure 1. Flowchart of white oyster mushroom sausage formulation

Data processing and analysis

The hedonic data were collected and analysed with SPSS (Statistical Package for the Social Sciences) version 25. The data were not normally distributed; hence, the Mann–Whitney U test was performed.

Number of trial units

Experiments were performed twice for this formulation. The number of experimental units (n) was expressed as a product between the number of repetitions (r) and the number of treatments (t).

Chemical analysis and microbiological evaluation

Moisture content determination (SNI 2354.2:2015)

2 g of sausage sample was weighed into a pre-weighed aluminium dish. The sample was dried in an oven at 105 °C for 3 hours or until constant weight was achieved. The dish was cooled in a desiccator and reweighed. Moisture content was expressed as mass percent.

Ash content determination (SNI 2354.1:2010)

2 g of sausage sample was weighed into a pre-weighed porcelain crucible. The sample was incinerated in a muffle furnace at 550 °C for 5 hours or until white ash was obtained. The crucible was cooled in a desiccator and reweighed. Ash content was expressed as mass percent.

Protein content determination (Kjeldahl method)

1 g of sausage sample was weighed into a Kjeldahl flask. 15 ml of concentrated sulphuric acid and a catalyst tablet ($K_2SO_4 + CuSO_4$) were added. The mixture was digested at 420 °C for 1 hour or until the solution became clear. After cooling, the sample was distilled with 40% NaOH solution. The distillate was collected in 4% boric acid solution with indicator and titrated with standardized 0.1 N HCl. Nitrogen content was calculated and multiplied by 6.25 to obtain protein content.

Fat content determination (Soxhlet extraction method)

5 g of dried sausage sample was weighed into an extraction thimble. The sample was extracted with petroleum ether in a Soxhlet apparatus for 6 hours. The extract was dried and weighed. Fat content was expressed as mass percent.

Total plate count (TPC) (SNI 01-2332.3:2006)

Serial dilutions of the sausage sample were prepared in sterile peptone water. 1 ml of appropriate dilutions was inoculated onto Plate Count Agar using the pour plate method. The plates were incubated at 35 °C for 48 hours. Colonies were counted and reported as CFU/g.

Escherichia coli detection (SNI 01-2332.1:2006)

1 ml of appropriate sausage sample dilutions was inoculated into Lauryl Sulphate Tryptose (LST) broth. The broth was incubated at 35 °C for 24-48 hours. A loopful from positive LST tubes was transferred to EC broth and incubated at 44.5 °C for 24 hours. Positive EC tubes were streaked onto Eosin Methylene Blue (EMB) agar. Typical colonies were confirmed with biochemical tests. Results were reported as MPN/g.

Salmonella detection (SNI 01-2332.2:2006)

25 g of sausage sample was pre-enriched in 225 ml Buffered Peptone Water at 35 °C for 24 hours. 0.1 ml was transferred to 10 ml Rappaport-Vassiliadis (RV) broth and 1 ml to 10 ml Tetrathionate (TT) broth. RV was incubated at 42 °C and TT at 35 °C for 24 hours. The broths were streaked onto Xylose Lysine Deoxycholate (XLD) and Bismuth Sulphite (BS) agars and incubated at 35 °C for 24 hours. Typical colonies were confirmed with biochemical and serological tests. Results were reported as presence or absence in 25 g.

3. Results and discussions

The development of the vegetarian sausage focused on creating a plant-based alternative that mimics the sensory and nutritional qualities of traditional meat sausages. Two formulations were developed: a control formulation using 100% white oyster mushrooms (*Pleurotus ostreatus*) and an experimental formulation comprising 80% white oyster mushrooms and 20% soy protein isolate. White oyster mushrooms were chosen as the primary ingredient due to their meat-like texture and umami flavour profile (*Giawa*, 2023), while soy protein isolate was selected to enhance the protein content and improve the binding properties of the mixture (*Gao et al.*, 2015). Additional ingredients common to sausage production were incorporated, including tapioca starch (5%) as a binder and texture enhancer (*Mazumder et al.*, 2023), salt (1.5%) for flavour enhancement and preservation, sugar (0.5%) to balance flavours, white pepper (0.3%) and garlic powder (0.2%) for seasoning, and sodium tripolyphosphate (0.3%) to improve water retention and texture (*Hugo & Hugo*, 2015).

The sausage production process involved several steps: preparation of ingredients, mixing, emulsification, stuffing into polyamide casings (19 mm diameter), linking to

6 cm lengths, cooking in water at 80 °C for 30 minutes until an internal temperature of 72 °C was reached, cooling in an ice bath, and, finally, vacuum-packing and refrigerating at 4 °C. The final products had a length of 6 cm, a diameter of 19 mm, and weighed approximately 15 g per link. Visually, both formulations resembled traditional sausages, with the experimental formulation showing a slightly firmer texture due to the soy protein isolation. The colour of both formulations was light beige before cooking, turning to golden brown after frying, similar to the colour change observed by *Kang et al.* (2022) in their study on plant-based sausages.

During development, several challenges were addressed. Initial formulations were too moist, leading to a soft texture, which was corrected by adjusting the ratio of mushrooms to dry ingredients and optimizing the cooking process. The control formulation initially had poor binding properties, which was improved by increasing the tapioca starch content and fine-tuning the emulsification process. Early versions lacked the umami flavour associated with meat sausages, addressed by adjusting the seasoning profile and incorporating a small amount of yeast extract (0.1%) to enhance savoury notes. The experimental formulation initially had a slightly grainy texture due to the soy protein isolate, which was resolved by hydrating the latter before mixing. These optimizations resulted in two formulations that closely resembled traditional meat sausages in appearance and texture, while offering the nutritional benefits of plant-based ingredients.

The Shapiro–Wilk test results indicate a non-normal distribution ($p < 0.05$) for the sensory attributes of aroma, taste, texture, and colour in both the control and experimental groups. Specifically, p-values for aroma were 0.003 for both groups, while for taste they were 0.003 (control) and 0.001 (experimental). Texture p-values were 0.009 (control) and 0.000 (experimental), and for colour both groups had a p-value of 0.000. In contrast, overall acceptability was normally distributed in both groups, with p-values of 0.402 (control) and 0.883 (experimental). These findings imply the necessity of using non-parametric statistical tests for analysis of aroma, taste, texture, and colour data, while parametric tests can be applied to overall acceptability. Consequently, the data were not considered to be fully normally distributed, necessitating the use of the Mann–Whitney U test as an alternative to the independent t-test to determine significant differences between formulations.

Treatment of products was tested by frying sausages in both product formulations. Each product sample, measuring 3 cm in length and 19 mm in width, is characterized by the following features: a smooth surface texture, vibrant colour indicative of its ingredients, consistent weight for uniformity, and a balanced flavour profile that appeals to a diverse consumer base.

Figure 2 presents a comprehensive sensory evaluation of four product samples (A1, A2, B1, and B2) across five key attributes: aroma, taste, texture, colour, and overall impression. The data include mean scores and standard deviations for each attribute, providing insights into the performance and consistency of the samples.

Sample A2 stands out as the top performer, achieving the highest mean scores in aroma (3.88 ± 0.83), taste (3.72 ± 0.98), and overall impression (3.7 ± 0.82). In contrast, sample B1 consistently receives the lowest ratings, particularly struggling in the taste category with a mean score of 2.72 ± 1.03. However, B1 unexpectedly excels in colour, with the highest mean score of 3.8 ± 0.92 among all samples. Samples A1 and B2 generally fall between the performance extremes of A2 and B1, with B2 showing slightly stronger overall ratings.

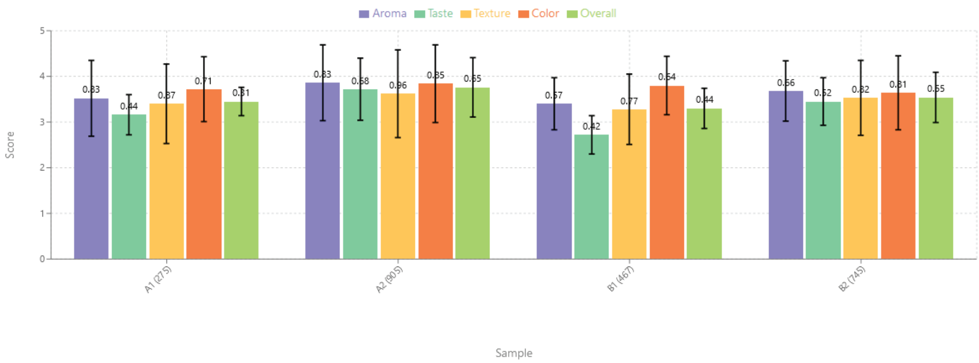

Figure 2. Results of the organoleptic test of the quality of white oyster mushroom sausage

Interestingly, the colour attribute exhibits the least variation in mean scores across samples, while taste shows the widest range and generally high standard deviations (0.98 to 1.23), suggesting diverse consumer responses. Sample B2 often demonstrates the lowest standard deviations, such as 0.75 for both aroma and texture, indicating more consistent evaluations. In contrast, B1 shows high variation in several attributes, with standard deviations ranging from 0.92 to 1.03. The overall scores align well with the patterns observed in the individual attributes, with A2 leading (3.7 ± 0.82) and B1 trailing (3.3 ± 0.98). This nuanced dataset highlights the strengths and areas for improvement of each sample, providing valuable insights for product development and optimization. The standard deviations offer additional information about the consistency of panellist responses, which can guide further refinements and targeted improvements in the sensory profiles of the samples.

Aroma plays a crucial role in the overall sensory experience of food products, significantly influencing consumer preference and acceptance (*Berčík et al.*, 2023). Within the food industry, understanding and optimizing aroma profiles are essential for developing successful products. In this study, the aroma of soy protein isolates in vegetarian sausages incorporating white oyster mushrooms was evaluated. The distribution of aroma scores, based on the average of two replicates, is presented in *Figure 2* (a. aroma).

Table 2. Hedonic data and Mann–Whitney results of the aroma indicator

Parameters	Control		Experiment		Significance 5%
	A1	A2	B1	B2	
Aroma	3.52	3.88	3.40	3.68	Not significant
Mean	3.7		3.54		
Acceptability					
Asymp. sig. (2-tailed)	0.496				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* ≥ *Alpha*, no significant difference between control and experimental formulations				

The analysis in *Table 2* reveals no significant difference in aroma between control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.496, $p > 0.05$). However, a subtle difference was noted: the experimental formulation, containing soy protein isolate, exhibited a stronger soy aroma compared to the control. This aligns with the mean favourability scores from *Table 2*, where the control (3.7, "like") slightly exceeded the experimental (3.54, "like"). This observation is consistent with prior research by *Flores & Piornos* (2021), who demonstrated that sausage aroma is influenced by both spices and binders. In our study, the increased soy protein isolate content in the experimental formulation likely contributed to the slightly lower aroma favourability.

Table 3. Hedonic data and Mann–Whitney U test results of taste indicators

Parameters	Control		Experiment		Significance 5%
	A1	A2	B1	B2	
Taste	3.16	3.72	2.73	3.40	Not significant
Mean	3.44		3.06		
Acceptability					
Asymp. sig. (2-tailed)	0.075				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

The Mann–Whitney U test results presented in *Table 3* indicate no significant difference in taste between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.075, $p > 0.05$). Although not statistically significant, a slight difference in mean favourability scores was observed, with the control formulation (3.44, "quite like") rating higher than the experimental formulation (3.06, "quite like"). This trend may be attributed to the addition of soy protein isolate in the experimental formulation, which could impart a slightly bitter taste, as reported in a similar study by *Maya et al.* (2023).

The taste of sausages can be influenced by various factors, including fillers, binders, and seasonings, with soy protein isolate potentially contributing to a subtle decrease in taste acceptability in this case.

Texture is a property resulting from a combination of several physical properties, consisting of food shape, size, amount, and material-forming elements that can be felt by the senses of touch and taste, as well as through the mouth and eyes. Food products are made not only to increase nutritional value but to obtain characteristics that suit the organoleptic tests of consumers (*Guiné et al.*, 2020).

Table 4. Hedonic data and Mann–Whitney U test results of texture indicators

Parameters	**Control**		**Experiment**		**Significance** 5%
	A1	A2	B1	B2	
Texture	3.40	3.44	3.28	3.44	Not significant
Mean	3.42		3.36		
Acceptability					
Asymp. sig. (2-tailed)	0.782				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between the control and experimental formulations				

The statistical analysis (Mann–Whitney U test) revealed no significant difference in texture between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.782, $p > 0.05$). Although mean favourability scores for texture were slightly higher in the control (3.42, "quite like") compared to the experimental group (3.36, "quite like"), this difference was not statistically significant (*Table 4*). Both formulations exhibited a dense and chewy texture, attributed to the amylose and amylopectin content in the tapioca flour used as a binder. While soy protein isolate, added to the experimental formulation, is known to enhance emulsion properties in meat products (*Mazumder et al.*, 2023), it did not significantly alter the perceived texture in this study.

Colour is the initial sensory attribute perceived by consumers, and it plays a crucial role in shaping their expectations and perceptions of food quality. A product's colour, if it deviates significantly from what is expected for that type of food, can influence a panellist's assessment. Conversely, a colour that closely resembles the natural or expected hue often creates a positive impression of quality and freshness (*Sipos et al.*, 2021).

Table 5. Hedonic data and Mann–Whitney U test results of colour indicators

Parameters	**Control**		**Experiment**		**Significance** 5%
	A1	A2	B1	B2	
Colour	3.72	3.76	3.80	3.64	Not significant
Mean	3.74		3.72		
Acceptability					
Asymp. sig. (2-tailed)	0.894				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

The Mann–Whitney U test results in *Table 5* indicate no significant difference in colour between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.894, $p > 0.05$). This aligns with the observed mean favourability scores, which were similar for both the control (3.74, "like") and experimental (3.72, "like") groups. The frying process, used for both formulations, resulted in a comparable brown colour. The addition of soy protein isolate in the experimental formulation did not appear to substantially alter the final colour of the sausages, consistent with findings in previous studies (*Kang et al.*, 2022; *Serdaroglu & Ozsumer*, 2003).

Overall acceptability represents the panellists' holistic evaluation of the white oyster mushroom vegetarian sausage enriched with soy protein isolate. It encompasses their integrated perception of all sensory attributes, including aroma, taste, texture, and colour, for each formulation. The hedonic assessment of overall acceptability serves as a crucial indicator of consumer liking, reflecting their comprehensive judgment of the product's sensory appeal (*Schouteten et al.*, 2018).

The statistical analysis (Mann–Whitney U test) revealed no significant difference in overall acceptability between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.251, $p > 0.05$), as shown in *Table 6*. However, a subtle preference for the control formulation (mean = 3.57, "like") was observed compared to the experimental formulation (mean = 3.42, "quite like"). Panellists noted a stronger soy aroma and taste in the experimental sausages containing 20% soy protein isolate, which may have influenced their overall preference. Additionally, the texture and colour, also affected by the soy protein content, contributed to the panellists' overall assessment.

Table 6. Hedonic data and Mann–Whitney U test results of the overall indicator

Parameters	**Control**		**Experimental**		**Significance** 5%
	A1	A2	B1	B2	
Overall acceptability	3.45	3.70	3.30	3.54	Not significant
Mean	3.57		3.42		
Acceptance parameters					
Asymp. sig. (2-tailed)	0.251				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

These findings suggest that while the addition of soy protein isolate did not significantly alter the overall acceptability of the vegetarian sausages, it did introduce subtle sensory changes that some panellists found less desirable compared to the control. This aligns with the research by *Sari et al.* (2021), who reported that sausages with lower concentrations of soy protein isolate, closer to the control, were generally preferred.

The price of vegetarian sausages per serving with a length of 6 cm and 19 mm has a selling price of 1,088 IDR, and the selling price of white oyster mushroom vegetarian sausages with the addition of soy protein isolate has a selling price of 1,235 IDR. White oyster mushroom vegetarian sausage with the addition of soy protein isolate has a slightly more expensive selling price compared to white oyster mushroom vegetarian sausage. However, the selling price of vegetarian sausages is cheap because the price of white oyster mushrooms and soy protein isolate is cheaper than meat. Making sausages with ingredients including white oyster mushrooms can reduce the selling price as compared to cases using meat (*Mazumder et al.*, 2023).

Table 7. Results of proximate chemical analysis

Composition	**Control sausage (%)**	**Experimental sausage (%)**
Moisture	65.00 ± 0.23^{a}	63.50 ± 0.19^{b}
Protein	0.42 ± 0.04^{a}	2.24 ± 0.05^{b}
Fat	2.28 ± 0.08^{a}	2.26 ± 0.07^{b}
Ash	2.15 ± 0.02^{a}	2.27 ± 0.03^{b}
Carbohydrate	30.15 ± 0.35^{a}	29.73 ± 0.34^{b}

Note: Numbers followed by the same letter in the same row indicate no significant difference at the 5% significance level.

The chemical analysis results presented in *Table 7* demonstrate statistically significant differences between the control and experimental samples across all measured parameters. This conclusion is supported using superscripts *a* and *b*, which indicate that the Mann–Whitney U test revealed significant differences at the 5% level for moisture, protein, fat, ash, and carbohydrate content. The experimental sample showed a notable increase in protein content (from 0.42% to 2.24%) and slight decreases in moisture (from 65.00% to 63.50%) and carbohydrate content (from 30.15% to 29.73%). Meanwhile, fat and ash content remained relatively stable with minor increases in the experimental sample. These consistent statistical differences across all parameters suggest that the experimental treatment had a substantial and measurable impact on the product's composition. However, it is important to note that despite these significant changes, particularly in protein content, the experimental sample still falls short of meeting the minimum protein requirements set by the SNI standard for meat sausage. This indicates that while the experimental treatment resulted in meaningful compositional changes, further modifications would be necessary to fully comply with regulatory standards for meat sausage products.

Based on the SNI 3820:2015 standard for meat sausage, both the control and experimental samples show mixed compliance with the requirements. While they meet the standards for fat content (max. 20%), moisture content (max. 67%), and ash content (max. 3.0%), they fall significantly short in protein content. The standard requires a minimum of 13% protein for meat sausage and 8% for combined meat sausage, but the control sample contains only 0.42% protein and the experimental sample 2.24%. This is a critical deficiency, as protein is a key nutritional component in meat products. The slight reduction in moisture content in the experimental sample (63.50% vs 65.00% in the control) is within the allowable range and could potentially contribute to a longer shelf life. The marginal increase in ash content in the experimental sample (2.27% vs 2.15%) is also within limits and suggests a slightly higher mineral content. There is no specific requirement for carbohydrate content in the SNI standard, so the slight decrease observed in the experimental sample (29.73% vs 30.15%) is not a compliance issue. To meet the SNI Standard and be classified as either meat sausage or combined meat sausage, significant product reformulation would be necessary to substantially increase the protein content while maintaining the current compliance in other areas.

Statistical analysis using the Mann–Whitney U test at the 5% significance level indicated no significant differences between control and experimental groups for any measured components. While the samples appear to meet SNI standards for fat and ash content and exceed requirements for carbohydrates, the protein levels are notably below the required minimum. This suggests a critical need for reformulation to increase protein content to meet the SNI 01-3820:1995 standard for sausage products. Future studies should report results in both percentages and grams to facilitate direct comparison with SNI standards.

Table 8. Results of microbial analysis

Analysis type	Experimental sausage	Control sausage	SNI 3820:2015	Conclusions
Total Plate Count (TPC)	7.4×10^2 CFU/g	1.3×10^3 CFU/g	max. 10^5 CFU/g	Safe for consumption
Coliform test	< 3 MPN/g	< 3 MPN/g	max. 10 MPN/g	Safe for consumption
Salmonella sp.	absent	absent	absent in 25 g	Safe for consumption

The microbial test results presented in *Table 8* provide a comprehensive analysis of the safety of both experimental and control sausage samples, evaluated against the Indonesian National Standard 3820:2015 for sausage products (*Badan Standardisasi Nasional*, 2015).

Three key microbial parameters were assessed: Total Plate Count (TPC), coliform presence, and *Salmonella* sp. detection, which are standard indicators of food safety and hygiene (*Arroyo-López et al.*, 2014). The experimental sausage showed a TPC of 7.4×10^2 CFU/g, while the control sausage had a slightly higher count at 1.3×10^3 CFU/g, both being well below the maximum allowable limit of 10^5 CFU/g.

For coliform determination, both samples registered less than 3 MPN/g, comfortably under the standard's maximum of 10 MPN/g. Additionally, *Salmonella* sp. was absent in both sausage types, meeting the requirement of absence in 25 g of sample. These results collectively indicate that both the experimental and control sausages are safe for consumption, successfully passing all three microbial safety criteria (*FDA*, 2001). Notably, the experimental sausage demonstrated a marginally lower bacterial count in the TPC test, suggesting that the experimental production method maintains, if not slightly improves, microbial safety standards compared to the control method. This aligns with recent studies indicating that innovative processing techniques can enhance food safety in meat products (*Zhang et al.*, 2010). Overall, these findings affirm that both sausage samples align with the established food safety regulations and are suitable for human consumption, reflecting adherence to good manufacturing practices in sausage production (*Heinz & Hautzinger*, 2007).

4. Conclusions

This study investigated the utilization of white oyster mushrooms (*Pleurotus ostreatus*) and soy protein isolate in the development of vegetarian sausages. Two formulations were evaluated: a control containing 100% white oyster mushrooms and an experimental formulation with 80% white oyster mushrooms and 20%

soy protein isolate. Sensory evaluation using a 5-point hedonic scale revealed no significant differences in aroma, taste, texture, or colour between the control and experimental sausages. The experimental formulation showed promising overall acceptability.

The chemical analysis revealed that the experimental formulation achieved a protein content of 2.24%, a significant increase compared to the control's 0.42%. However, both values fell short of the Indonesian National Standard (SNI 3820:2015), which mandates a minimum protein content of 8% for combined meat sausages. The moisture, fat, and ash levels were consistent with SNI guidelines, and the slight variations observed did not impact compliance. Additionally, microbiological assessments confirmed that both formulations adhered to safety standards, with TPC, coliform counts, and absence of *Salmonella* meeting SNI requirements.

In conclusion, white oyster mushroom vegetarian sausages enriched with soy protein isolate present a nutritionally and economically promising plant-based alternative to conventional meat products. The incorporation of soy protein isolates significantly enhanced the protein content without compromising sensory attributes or microbiological safety. This research underscores the potential of utilizing mushrooms and plant proteins in developing sustainable, healthy, and affordable meat substitutes.

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