Research Article

BIOTECHINTELLECT, 2025,2 (1) e22 (1-10)
https://jbiotechintel.com/index.php/biotechintel

eISSN: 3115-7920

OPEN ACCESS

Boosting Chicken Sausage Quality with Javanese Grasshopper Flour: Enhanced Nutrition, Antioxidant Benefits, and Microbial Safety

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Article history:
Received 20 Oct 2025
Revised 12 Nov 2025
Accepted 24 Dec 2025
Published online 30 Dec 2025
Keywords: Edible Insect Fortification, Javanese Grasshopper Flour, Chicken Sausage Nutrition, Antioxidant Activity, Microbial Safety, Sustainable Protein Source, *Valanga nigricornis*, Sensory Evaluation, Mineral Enrichment
How to cite this article: Iwansyah AC, Kristanti D, Setiaboma W, Azzahra FL, Nur Huda I, Palupi E, et al. Boosting Chicken Sausage Quality with Javanese Grasshopper Flour: Enhanced Nutrition, Antioxidant Benefits, and Microbial Safety. BiotechIntellect, 2025: 2 (1); e22 (1-10).
https://doi.org/10.66224/biotechintellect.2.1.6
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Abstract

This research explores the potential of Javanese grasshopper (Valanga nigricornis Burm.) flour (JGF) as a sustainable fortification ingredient in chicken sausages, focusing on nutritional, functional, and sensory enhancements. A completely randomized design was employed to assess four formulations: a control (F0, 0% JGF) and three JGF levels at 5% (F3), 10% (F2), and 15% (F1). JGF-fortified sausages exhibited significantly higher protein, fat, and mineral content (calcium, zinc, magnesium, iron) compared to the control ($p<0.05$), alongside reduced carbohydrate and energy levels. All formulations achieved protein digestibility above 98%. Antioxidant activity, measured via DPPH and ABTS assays, was markedly elevated in JGF-fortified sausages, with F1 demonstrating the highest potency. Microbial analysis revealed lower total bacterial counts and no E. coli in JGF formulations, indicating improved food safety. Sensory analysis showed that F2 (10% JGF) was most preferred, despite slight reductions in color and texture appeal compared to the control. Principal component and hierarchical clustering analyses confirmed that JGF-fortified sausages outperformed the control in nutritional and antioxidant properties. These findings underscore the value of edible insects as innovative, nutrient-rich ingredients for advancing meat product quality and sustainability.

What is "already known":

- Grasshoppers are rich in essential minerals, making them a promising alternative mineral source to fulfill and supplement nutritional needs.

What this article adds:	• High protein digestibility was maintained across all fortified sausage formulations. • Improved antioxidant activity (DPPH and ABTS assays) was observed, with the 15% JGF formulation showing the highest capacity. • Enhanced microbial safety: no *E. coli* or mold/yeast contamination detected in JGF-fortified sausages. • Trade-off in sensory acceptance: while nutrition improved, panelists rated texture and color of fortified sausages lower than the control. • Edible insects as a sustainable, nutrient-dense ingredient: JGF offers a promising alternative to fortify meat products with protein and essential minerals.Modern biological systems exhibit multiscale, nonlinear, and higher-order structure that cannot be faithfully captured by pairwise or Euclidean models alone.

1. Introduction

The use of poultry meat such as chicken as a source of protein is widely practiced in the world (1)]. Chicken can be processed into frozen food products, such as sausage (2)]. Chicken is not only a good source of protein but also contains a relatively complete range of minerals. However, its mineral content-including Ca, Mg, P, Na, K, Zn, Fe, and Mn-is lower compared to that of red meat (pork and beef) [(3)]. To enhance the mineral content in chicken sausages, fortification methods can are employed (4)].

Edible insects have been widely consumed as food sources in various regions worldwide. They are highly nutritious, particularly rich in protein and minerals. The protein content of insects is higher than that of both plant- and animal-based protein sources. Additionally, edible insects exhibit high protein digestibility, exceeding 90%. Among edible insects, those belonging to the order *Orthoptera*, such as grasshoppers, locusts, and crickets, have the highest protein content (5)]. Grasshoppers have high protein content and digestibility (6)]. O*rthopterans* (crickets and grasshoppers) contain higher levels of minerals such as Na, Ca, P, Mg, Fe, Zn, Cu, Mn, and I compared to chicken breast and drumstick (7)]. Grasshoppers are rich in essential minerals, including Cu, Fe, Mg, Co, Zn, Na, and K, making them a promising alternative mineral source to fulfill and supplement nutritional needs (8)].

The application of edible insects through fortification in food products has been explored in several studies. Fortifying food products with edible insects enhances their nutritional profile, physical properties, and chemical characteristics. Examples of such products include biscuits, ice cream, sausages, meat analogs, patties, noodles, and pasta. Some of these studies show the potential of edible insects to improve the characteristics of food products. Therefore, this study aims to evaluate nutritional composition-particularly mineral content-, protein digestibility, antioxidant activity, microbial contamination, and sensory properties of chicken sausages fortified with Javanese grasshopper (*Valanga nigricornis* Burm.) flour.

2. MATERIAL AND METHODS

2.1. Materials

The material used in this study was fresh Javanese grasshopper (*Valanga nigricornis*) purchased from Extreme Food MSME, located in Wonosari, Gunung Kidul, Yogyakarta. Other ingredients needed for making the sausage, including chicken, salt, pepper, sugar, vinegar, onion, tapioca, and cow fat, were obtained from Agrosari Market, also in Wonosari, Gunung Kidul, Yogyakarta. Sodium tripolyphosphate (STPP) and fermented brown rice (angkak) were sourced from Ben Abadi Sejahtera. The research was conducted from May to September 2023 at the Food Product Development Laboratory, Research Center for Food Technology and Processing, National Research and Innovation Agency in Yogyakarta, Indonesia.

2.2. Preparation of samples

Javanese grasshopper flour (JGF) was produced by first removing the wings, hind legs, and internal organs of the grasshoppers. The remaining bodies were thoroughly washed with running water and then soaked in a 0.3% $NaHCO_3$ solution for approximately 15 mins. This was followed by blanching the grasshoppers at a temperature of 70-80 °C for 1-2 mins. After blanching, they were dried using a tray dryer at 50-60 °C for 24 h. Once dried, the Javanese grasshoppers were ground in a blender and sifted through a 60-mesh sieve to create a homogeneous flour.

To make the sausage, start with 1000 g of chicken meat, 132 g of ice cubes, 80 g of beef fat, and 0.5 g of red yeast rice, which should be ground together for 1 minute. Next, add the following ingredients: 10 g of vinegar, 12 g of ground pepper, 18 g of salt, 20 g of shallots, and 20 g of garlic powder. Grind the mixture for an additional minute. Then, incorporate tapioca flour and/or grasshopper flour, along with 15 g of sodium tripolyphosphate (STPP), and grind the mixture for one more minute. Finally, pour the sausage mixture into casings and cook in the oven at 100°C for 30 mins (9)]. This study employed an experimental method with a completely randomized design (CRD), varying the formulation of Javanese grasshopper flour in the chicken sausage: F0 (without JGF), F1 (15% JGF), F2 (10% JGF), and F3 (5% JGF), with three repetitions across 12 experimental units. Javanese grasshopper sausage is shown on Fig. 1.

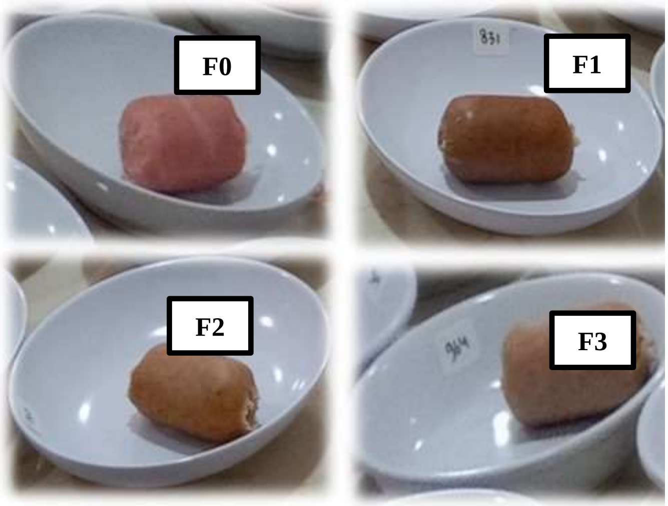

Figure 1. Javanese grasshopper sausage. F0:0%, F1:15%, F2:10%, and F3:5% JG flour.

2.3. Nutritional composition

Nutritional composition *viz.*, protein, moisture, fat, ash, carbohydrate of sausage samples was determined. Moisture and ash content was determined using the gravimetric method. The Kjedahl equipment was used to measure the protein content, fat was measured by the Weibull method, and carbohydrates were calculated. Energy was measured by At-water factor, where 1 g of carbohydrates equal to 4 kcal; 1 g of protein equal to 4 kcal; and 1 g of fat equal to 9 kcal. The contents of iron, zinc, calcium, and magnesium were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.4. Protein digestibility

Protein digestibility of Javanese grasshopper sausage was evaluated by in vitro digestibility assay. 200 mg of the sausage was dissolved in 9 mL Walpole's buffer solution (pH 2.0; 0.2 N). 1 mL of 2% Pepsin solution was added and was incubated at 37°C for 1.5 hours in a water bath shaker. Digested samples were centrifuged at 3,000 rpm for 20 mins. 5 mL of the supernatant was transferred into a test tube and 5 mL of 20% trichloroacetic (TCA) was added to the supernatant. The mixture was incubated at an ambient temperature for 1.5 hours and filtered using Whatman filter paper (No. 41). 5 mL of the filtrate was used for micro-Kjeldahl analysis to determine the crude protein content in the samples after digestion. In vitro protein digestibility was calculated by the Eq. 1.

$$\text{In vitro protein digestibility } (\%) = \frac{(A - B)}{A} \quad \text{......... Eq. 1}$$

A = Crude protein content before digestion

B = Crude protein content after digestion

2.5.Antioxidant activity

The DPPH assay was conducted following the procedure outlined by (10) with slight modification. 40 µL of the sample was combined with 160 µL of an ethanolic DPPH (60 µM) solution. After a 30mins incubation period, the absorbance was measured at 517 nm using a microplate spectrophotometer (Thermo Scientific, Finland).

The ABTS assay was performed according to Espitia-Hernández, Ruelas-Chacón (10)] with minor modification. Subsequently, 40 µL of the extracts were mixed with 160 µL ABTS cation radicals. The absorbance was then recorded at 734 nm after 10 mins in a microplate spectrophotometer (Thermo Scientific, Finland). The percentage inhibition was calculated using Eq 2.

$$\text{Inhibition\%} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}\ x\ 100\% \quad \text{.........Eq.2}$$

2.6. Microbiological analysis

Microbiological analysis consists of several tests including bacterial contamination, fungal contamination and yeast and Escherichia detection procedures. Bacteria contamination testing required a 5 g Javanese grasshopper sausage sample and dissolved in 45 mL sterile aquades using a 400 Circulator Stomacher, the sample solution was stratified dilution, and the last 3 dilutions were taken as much as 1 mL and added to the plate count media so that they were then incubated at 37 ° C for 48 hours. Mold and yeast contamination requires a sample of Javanese grasshopper sausage 5 g and dissolved in 45 mL sterile aquades using a Stomacher 400 Circulator. The sample solution is stratified dilution, and the last 3 dilutions are taken as much as 1 mL and added to potato dextrose media so that they are then incubated at 37 ° C for 48 h. Detection of *Escherichiae coli* was carried out using 1 mL of sample solution Javanese grasshopper sausage inoculated into 9 mL EC broth and incubated at 37 °C for 24 h. Then, loop from the aseptically enriched sample was etched into the EMB agar and incubated at 37°C for 24 hours. Colonies are characterized by their metallic green color.

2.7. Sensory characteristics

Sensory or organoleptic tests were performed on 35 untrained panelists at the Research Center for Food Technology and Food Processing. Panelists were given four sausage samples and asked to rank their likeliness on color, texture, taste, aftertaste, and smell on the 6 levels scoring method, (1 was extremely dislikes to 6 was extremely like). Approval of the research code was obtained from the Ethics Commission, National Research and Innovation Agency, Indonesia (Number:002/KE.04/SK/01/2023).

2.8. Data and statistical analysis

The data collected was analyzed with a *R-studio x64. 4.1.1* statistical program and Microsoft Excell for windows. The normality of the data was tested. Data was analyzed using analysis of variance (ANOVA) followed by Duncan multiple comparison test. Characterization of each sausage was performed using Principal component analysis (PCA) and hierarchical cluster analysis (HCA).

3. Results and Discussion

3.1.Nutritional composition

The nutritional value of the chicken sausage based on Javanese grasshopper flour (JGF) can be seen in Table 1. The addition of JGF as the substitute for wheat flour significantly increased the moisture, crude protein, and mineral content in sausage products (p<0.05). In contrast, carbohydrates and calories significantly decreased in grasshopper-fortified sausage products (p<0.05), meanwhile ash, fat, and protein digestibility content were not affected significantly (p>0.05).

These findings were in line with several studies that incorporated edible insects into food products. Roncolini, Milanović (11)] reported that protein content in snacks increased by 99% after the addition of mealworm powder. 10% substitution of wheat flour with cricket powder increased the protein content in bread (12)]. Increased protein content in sausage products was related to the high protein content in JGF. In this study, we found that JGF contains 64.75% crude protein. These studies were in line with (13)] who reported that JGF contained 72.5% crude protein, and 1016.3 ppm water soluble protein. Another research reported the Javanese grasshopper (*V. nigricornis*) contained 76.69% crude protein higher than other insects such as paddy locust (*Nomadacris succinta*), cricket (*Gryllus* sp.), yellow mealworm (*Tenebrio molitor*) and silkworm (*Bombyx mori*)(14)]. Table 1 shows that the carbohydrate control (F0) sausage was almost twice the amount of carbohydrates compared with grasshopper-fortified sausage. This is due to the high content of carbohydrates in flour which is around 75.24–77.40% (15)]. While JGF only contains 7.96% of carbohydrates (13)]. The low carbohydrate and high moisture content in grasshopper-fortified sausages resulted in lower calories level. In this study, the addition of JGF increased the amount of mineral Fe, Mg, Zn, and Ca. Mineral content in control sausage was significantly lower because of mineral scarcity in chicken meat. Chicken meat only contains 0.4-0.7 mg/100 g Fe, 5-8 mg/100 g Ca, 26-33 mg/100 g Mg, and 0.5-1.4 mg/100 g Zn depending on the part of the chicken (7)]. This value was much lower than the mineral content of edible insects from the Orthoptera order. Black cricket (*Gryllus bimaculatus*) contains 9.5

mg/100 g Fe, 105.14 mg/100 g Ca, 72.94 mg/100 g Mg, and 14.39 mg/100 g Zn (16)].

Table 1 Nutritional composition of Javanese grasshopper sausage

Nutritional Aspects	F0	F1	F2	F3
Moisture (%)	51.97±4.97[b]	57.51±2.10[ab]	56.40±2.23[ab]	59.56±1.07[a]
Ash (%)	2.98±0.07[a]	2.89±0.25[a]	3.25±0.01[a]	3.23±0.17[a]
Protein (%)	17.18±1.69[b]	22.14±0.76[a]	20.85±0.48[a]	20.92±2.68[a]
Fat (%)	5.62±0.60[a]	5.67±0.20[a]	5.97±0.74[a]	5.11±0.70[a]
Carbohydrates (%)	22.24±7.17[a]	11.78±2.73[b]	13.52±1.21[b]	11.17±2.39[b]
Energy (Kcal)	213.91±17.47[a]	191.83±10.35[ab]	196.51±12.44[ab]	179.15±7.29[b]
Protein digestibility (%)	97.57±2.24[a]	98.26±0.48[a]	98.81±0.59[a]	98.26±0.79[a]
Minerals				
Fe (µg/g)	-*	5.81±0.50[a]	-*	-*
Ca (µg/g)	102.55±0.06[d]	185.16±2.24[a]	143.06±1.24[b]	130.43±5.75[c]
Zn (µg/g)	4.80±0.07[d]	15.15±0.11[a]	9.42±0.13[c]	9.94±0.15[b]
Mg (µg/g)	217.38±0.75[d]	283.14±1.38[b]	271.35±2.12[c]	336.09±2.21[a]

Data are presented as mean ± standard deviation (s.d). a>b>c>d, values in the same rows followed by different alphabets are significantly different by Duncan's test ($p < 0.05$). Note: *mineral contents less than 10 ppb cannot be detected

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3.2. Microbial contamination

The microbial contamination of Javanese grasshopper sausage is summarized in Table 2. Javanese grasshopper sausages that had gone through the heating and processing process were analyzed to determine the level of microbial contamination, *E. coli*, mold and yeast. The results of this study reported that the lowest total plate count value of Javanese grasshopper sausage was the addition of 10% (F2). Javanese grasshopper sausage with a TPC value of 3.0 x 10^2 CFU/mL, while the highest TPC value was in the formula without the addition of JGF (F0) with a TPC value of 2.6 x 10^4 CFU/mL. In the results of the analysis of mold and yeast, it was reported that there was no contamination of mold and yeast in the treatment of Javanese grasshopper sausages. While the detection results of pathogenic bacteria *E. coli* showed positive results in the formula without the addition of Javanese grasshopper flour (F0), namely >3 colony/g, while the addition of JGF F1, F2 and F3 negative or no contamination of pathogenic bacteria *E. coli*.

Table 2 Microbial contamination of Javanese grasshopper sausage

Sample	Total plate count (CFU/mL)	Molds and yeast (CFU/mL)	E.coli
F0	2.6 x 104	n.d	+
F1	2.9 x 103	n.d	-
F2	3.0 x 102	n.d	-
F3	3.4 x 103	n.d	-

Data presented as means. CFU: colony forming units; nf: not detected; (+) positive; (-) negative

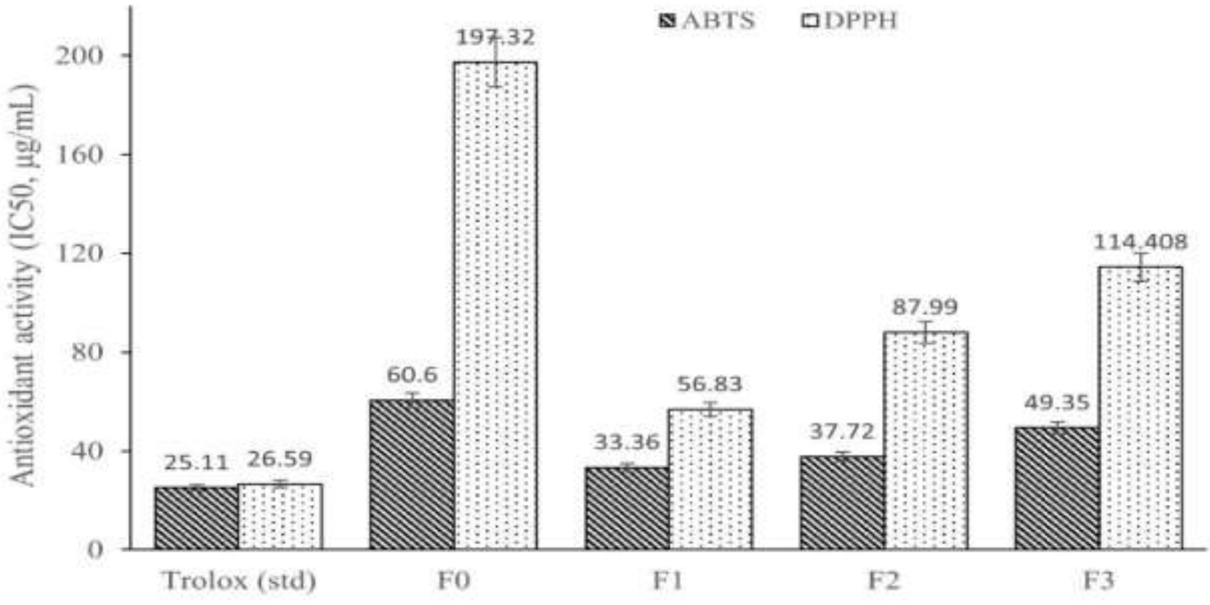

Figure 2: Antioxidant activity of sausage with Javanese grasshopper flour. Data presented as mean ± standard errors (s.e). TE: trolox equivalent. a>b>c>d, Values in the graphics followed by different alphabets are significantly different by Duncan's test ($p < 0.05$).

Microbial contamination levels (total plate count, mold and yeast, and pathogenic bacteria contamination of *Javanese* grasshopper sausages were within the acceptable limits. In general, the total plate count ranged from 3.0×10^2 to 2.6×10^4 CFU/mL, which can be classified as satisfactory. According to international guidelines, TPCs below 10^5 CFU/g are regarded as satisfactory (17)]. Further, no yeast and mold contamination were found, consistent with acceptable levels, which specify that such counts should be below 10^4 CFU/g (18)]. Moreover, sample containing Javanese grasshopper flour (F1, F2, and F3) were also negative for *E. coli* and considered satisfactory microbiological quality since *E. coli* should be absent in 25 g food Ssausages that were processed through the heating and cooking process reduce the value of microbial contamination (19)]. The heating process was not enough to completely kill the microbiota, therefore through additional processing procedures such as blanching before grinding can reduce the number of organisms (19)].

3.3. Antioxidant activities

The antioxidant activities of several edible insect flours were demonstrated in previous studies, due to the amino acids, proteins, and polysaccharides in the edible insect flours (19)]. Two experiments were condicted to evaluate the antioxidant properties of sausage variations (F0–F3), utilizing the DPPH radical and ABTS cation radical assays (Fig.2). Fig. 2 shows that significant differences in DPPH antioxidant activity were observed among the four sausage mixtures ($p<0.05$). It indicates that the addition of JGF contributed to an improvement in antioxidant capacity. The sausage mixture with the highest DPPH antioxidant activity was F1, containing 15% JGF, exhibiting an IC_{50} value of 56.83 µg/mL and the IC_{50} trolox as positive control was 26.60 µg/mL. In comparison to the control (F0), sausages F3, F2, and F1 exhibited enhanced antioxidant activity, with improv-ements of 1.77, 2.27, and 3.46 times, respe-ctively.

Similarly, the highest ABTS antioxidant activity was observed in sausage F1, containing 15% JGF, with an

IC_{50} value of 33.36 µg/mL, and the IC_{50} value for trolox was 25.11 µg/mL (see Fig. 3). In comparing sausages with JGF to the control (F0), a significant increase in ABTS antioxidant activity was observed in samples F1 to F3 ($p<0.05$). This resulted in an enhancement of 1.85, 1.60, and 1.18 times in ABTS antioxidant activity for F1, F2, and F3, respectively. Zielińska, Pečová (20)] reported similar findings, demon-strating the enriched muffins with edible insect flour increased antioxidant capacity against ABTS and DPPH radicals. In addition, several recent studies also showed the enhancement of antioxidant activities after enriching with edible insects, for example, nut bars, cookies, and snack pellets (21)].

3.4. Sensory characteristics

Sensory characteristics of Javanese grasshopper sausage can be seen in Table 3. It could be observed that the panelists significantly preferred F1 color less. The color in the F1 sample was the darkest, so it was known that consumers did not really like sausages with too dark colors. The preferred sausage color was F2 which was given an additional 10% JGF. Some panelists reasoned that the color of F2 was like sausages commonly sold in the market.

From the aroma attribute, panelists preferred samples without JGF. Panelists mentioned that their level of liking was influenced by the strong aroma of pepper in the sample, so it was not necessarily related to the addition of JGF. In the table, it could also be observed that F1 and F2 samples were preferred over F3 which had a lower JGF content. In terms of texture, sausages fortified with JGF are significantly less likable. Panelists stated that the texture of JGF flour-fortified sausages was less chewy, too mushy, and disintegrates easily. This result was consistent with the water-holding capacity of JGF of around 30%, much lower than wheat flour which was worth 50-60%. This caused a decrease in elasticity in sausage products. When viewed from the taste aspect, panelists tend to like both control and grasshopper-fortified sausage products.

3.5 Principal component (PCA) and hierarchical cluster (HCA) analysis

Nutritional composition, antioxidant properties and sensory characteristics of Javanese grasshopper (JG) sausages were described by principal component analysis. PCA analysis results shown in Fig. 3 consist of two principal components (PC), namely PC1 and PC2. These PCs constituted 67.30% of the total variability. PCA successfully separated four samples of JG sausages, F0 was in the second and third quadrants, F1 and F2 were in the first quadrant, and F3 was in the fourth quadrant.

Each quadrant was characterized by different traits. Calcium, zinc, moisture, protein contents, and protein digestibility were the dominant traits in the first quadrant. While in the second quadrant was characterized by sensory texture, smells, color, aftertaste, fat, and mineral iron. Characters that were outstanding in the third quadrant were antioxidant properties (DPPH and ABTS), carbohydrates and energy. Describing characteristics for the fourth quadrant were magnesium and ash content.

From this result, it could be concluded that control sausage (F0) had better carbohydrate content, energy value, overall texture, and sensory likeliness. While the JG sausages (F1, F2, and F3) had a better content of minerals, protein, antioxidant activity and protein digestibility. From this information, we know that JG sausages needed some enhancement in terms of sensory attribute, especially in the texture aspect, integrity of the sausages' structure and the color of the sausages.

Table 3 Sensory characteristics of Javanese grasshopper sausage

Sample	Color	Aroma	Texture	Taste	Aftertaste	Overall
F0	4.80±1.18a	4.63±1.00a	5.23±0.73a	5.17±0.75a	4.91±1.06a	5.17±0.78a
F1	3.89±1.10b	4.31±0.96ab	3.23±1.23c	4.03±1.15b	3.89±1.13b	3.86±0.84b
F2	4.89±0.72a	4.31±0.90ab	3.86±1.26b	4.40±0.95b	4.34±0.94b	4.20±0.93b
F3	4.49±1.01a	4.11±1.02b	2.54±1.09d	2.97±1.12c	2.97±1.10c	3.00±1.11c

Data are presented as mean ± standard deviation (n = 3). a>b>c>d, values in the same columns followed by different alphabets are significantly different by Duncan's test ($p < 0.05$). (F0): control without JGF, (F1): 15% of JGF, (F2): 10% of JGF, (F3): 5% of JGF.

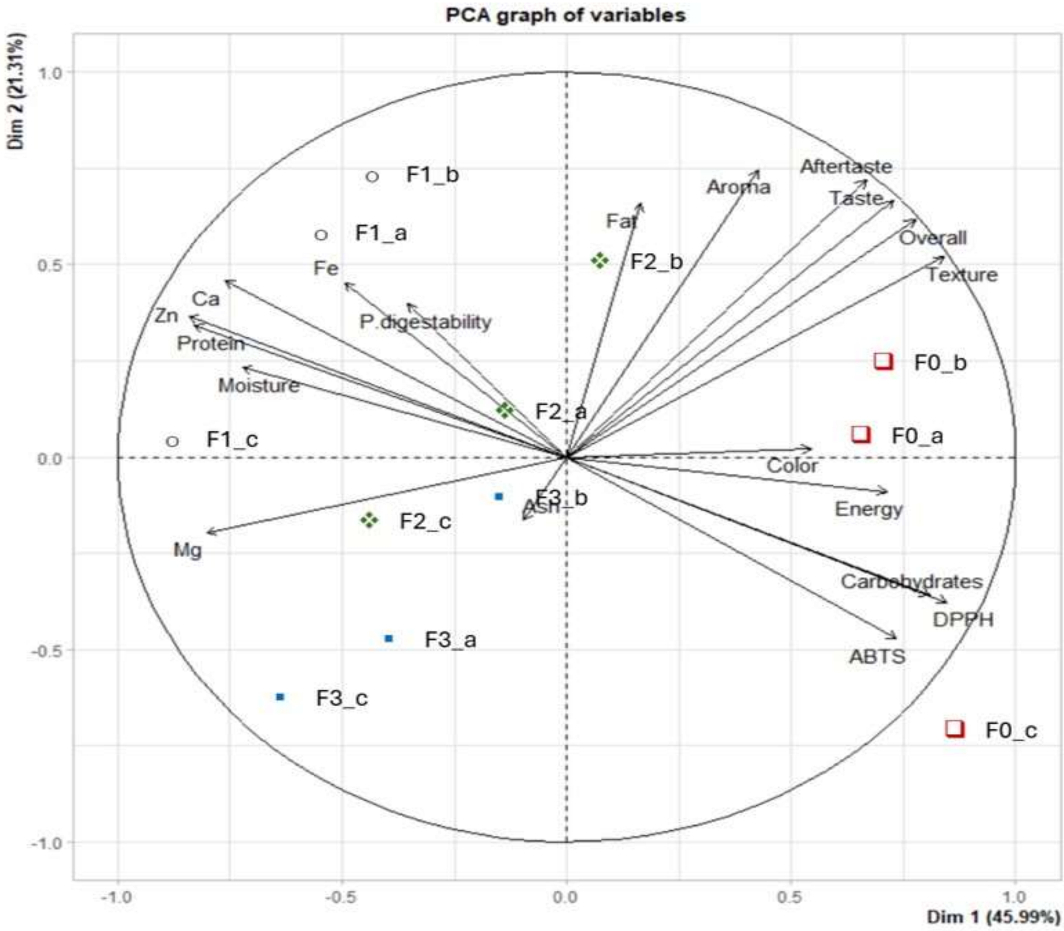

Figure 3: Biplots of Javanese grasshopper sausages (F1, F2, and F3) and control sausage without JGF (F0) characterized by nutritional composition, antioxidant activity and sensory characteristics.

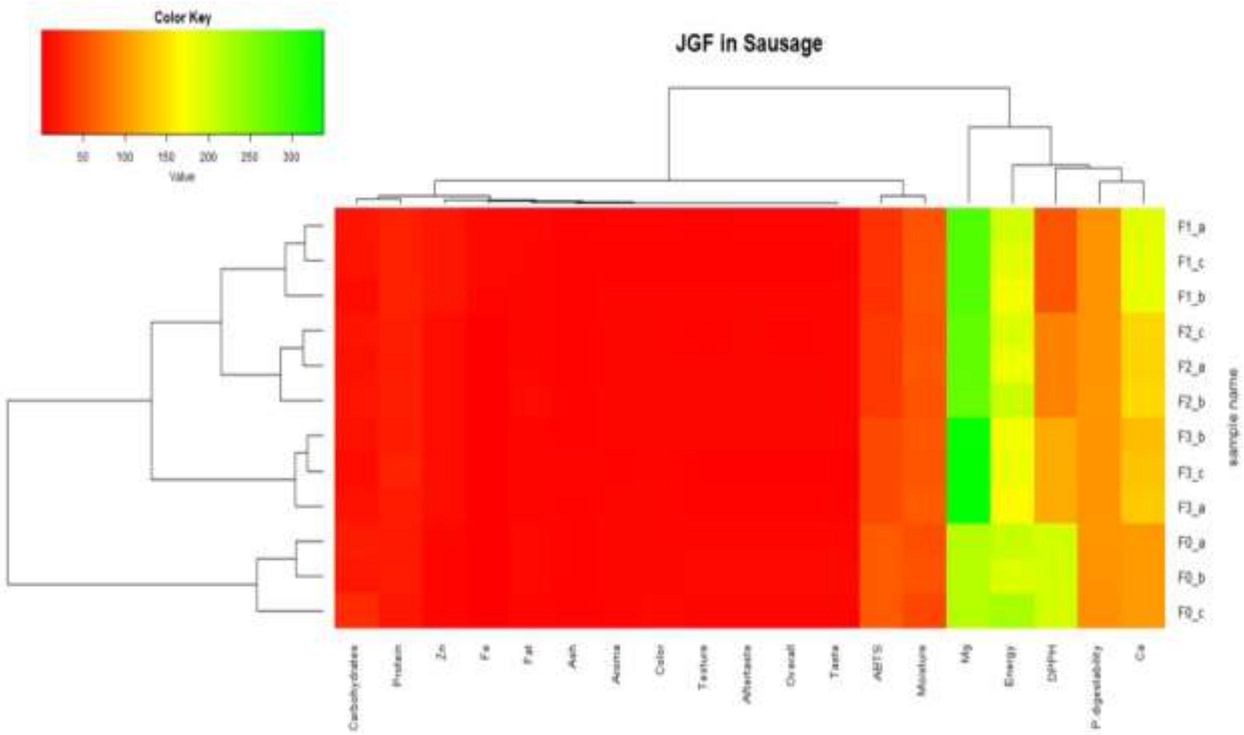

Figure 4. Heat map of the relationships between treatments and response variables of Javanese grasshopper sausage, as well as the clustering of these variables. The color represents the direction of the treatment impact, ranging from the lowest (red) to the highest (green).

Hierarchical clustering analysis identified distinct and overlapping nutrient content, antioxidant activity, and sensory characteristics in JGF fortified chicken sausage formulations, emphasizing the impact of various ingredient combinations. The heatmap also illustrates the relationship of all variables of treatments. The interaction between the nutritional composition, antioxidant properties and sensory characteristics of the Javanese grasshopper sausage is shown in Fig. 4, along with variable clustering and treatment based on parameter similarity. Chicken sausages containing 15% (F1) and 10% (F2) JGF are grouped together due to their high mineral content, which includes magnesium and calcium, as well as their energy levels and antioxidant activity (measured by DPPH IC_{50}). In contrast, F3 shows a trend of being negatively correlated with some of these parameters.

4. CONCLUSION

Fortifying chicken sausages with Javanese grasshopper flour (JGF) significantly enhances their nutritional, functional, and safety profiles, positioning edible insects as a sustainable solution for innovative food production. JGF-fortified sausages exhibited superior protein, fat, and mineral content (calcium, zinc, magnesium, iron), alongside enhanced antioxidant activity and excellent protein digestibility (>98%). Microbial safety was improved, with reduced bacterial counts and no E. coli detected in fortified samples. However, sensory attributes, particularly color and texture, require optimization to improve consumer acceptance. These findings highlight JGF's potential as a nutrient-dense, eco-friendly ingredient for meat products, paving the way for further research into sensory enhancements and broader applications in sustainable food systems.

5.Declarations

5.1. Acknowledgments

The author would like to thank the Research Organization of Agriculture and Food, Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta for financial (No. 9/III.11/HK/2023) and supporting research activities through e-science services (ELSA-BRIN). We would like to extend our appreciation to the Universiti Malaysia Pahang Al-Sultan Abdullah for their support and for providing the grant for this research, RDU243303 to Hamid HA.

5.2. Competing interests

The authors declare that they have no competing interests.

5.3. Funding

Not applicable

5.4. *Using Artificial Intelligent*

Not Used.

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