



# Volatilomics and Physical Characteristics of Chicken and Pork “Urutan” (Traditional Balinese Sausage)

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**Abstract**— “Urutan” is a traditional Balinese fermented sausage prepared from pork or chicken. Information on chemical and physical characteristics of “Urutan” is important, specifically for Muslims unable to consume pork. Therefore, this study aimed to characterize volatile compounds and physical properties of chicken and pork “Urutan” available in Balinese market. Volatile compounds were analyzed using Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (SPME/GC-MS) and multivariate data analysis. Physical characteristics assessed were texture, color, and water holding capacity (WHC). Multivariate analysis was conducted for the data collected to determine distinct volatile compounds as well as physical profiles of chicken and pork “Urutan”. The score plots of orthogonal projection to the least square-discriminant analysis (OPLS-DA) of volatile data showed distinct grouping among chicken and pork “Urutan”. Based on Variable Importance in Projection (VIP) scores and Correlation Coefficients, the most significant volatile markers in chicken “Urutan” were formic acid, 2,5-dimethyl-furan, and 6-methyl-tridecane, while 1-octen-3-ol, 3,6-dimethyl-octane, and 1-Octen-3-one were identified as markers of pork “Urutan”. The results of physical analysis showed significant differences between springiness, lightness, and WHC of both products. There were no significant differences between hardness, cohesiveness, gumminess,  $a^*$ , and  $b^*$  values. Volatilomics was found to be a more reliable method for differentiating chicken “Urutan” from pork “Urutan”, compared to using physical characteristics only.

**Keywords**— Halal; Physical analysis; “Urutan”; Volatilomics

Manuscript received Nov 12, 2024; revised May 25, 2025; accepted July 12, 2025. Available online July 26, 2025  
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## I. INTRODUCTION

“Urutan” is a traditional Balinese food served during Hindu celebrations such as Galungan and Kuningan [1]. This is often prepared from raw materials, including pork, salt, and spices, which are mixed and fermented in pig intestines, then sun-dried for 2 to 5 days. “Urutan” is not popular among Muslims because of pork content [2], leading to commercial versions recently using chicken and synthetic casings for a broader market appeal [3]. Ensuring halal food is crucial in Indonesia to prevent pork adulteration, which is hard to detect visually in processed products such as sausage or “Urutan” [4].

Current methods for detecting meat adulteration include Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA) [5], Liquid Chromatography–Mass Spectrometry (LC-MS) [6], and Fourier Transform Infrared Spectroscopy (FTIR) [7]. However, these require strict sample preparation and high technical expertise, leading to the less suitability for routine use [8]. Metabolomics, particularly by focusing on volatile compounds (volatilomics), offers a promising alternative. Meat from different species is empirically known to have a unique aroma released by the content of various volatile compounds. Solid-phase Microextraction–GC–MS (SPME/GC–MS) can be used to comprehensively detect volatile

metabolites. Subsequently, different multivariate data analyses, such as principal component analysis (PCA) and orthogonal projection to the least square-discriminant analysis (OPLS-DA) are used to distinguish volatile compounds in various meat products [8] [9]

Some studies performed physical analysis to differentiate processed meat products in addition to using volatile profiles. Physical analysis is necessary for authenticating the type of meat and products. This is conducted because consumers generally assess meat quality based on water holding capacity (WHC) as well as appearance [10], including texture and color. A similar method was previously used to distinguish between meatballs manufactured from halal and non-halal animals [8].

Multiple physical and chemical interactions occur during fermentation, leading to changes in the flavor, texture, color, and shelf life of "Urutan" [11], [12]. Various enzymes are released during fermentation, causing proteolysis and lipolysis. Proteolysis produces free amino acids, peptides, and ammonia, which contribute to typical fermented sausage aroma. Meanwhile, lipolysis generates free fatty acids that are transformed into aromatic compounds, such as ketones, aldehydes, and esters, giving fermented sausage a richer, more nuanced aroma than raw meat. Fermentation reduces the relative moisture content of "Urutan", producing a firmer texture.

In this study, volatile components and physical characteristics are analyzed to differentiate between chicken and pork "Urutan" sold in Balinese market. The analysis of volatile components, known as volatilities, is conducted comprehensively using SPME, combined with GC-MS. The obtained data are analyzed using OPLS-DA, while distinctive volatile compounds in various "Urutan" samples are determined based on Variable Importance in Projection (VIP) and Correlation Coefficient value. Physical analyses performed include the assessment of texture, color, and WHC.

## II. MATERIAL AND METHOD

### A. Material

"Urutan" samples used in this study were uncooked (raw), while the fermentation duration and processing might vary depending on the methods implemented by each producer in Bali, Indonesia. Pork and chicken "Urutan" (four each) were purchased directly from four different traditional producers in Badung area, then two other chicken "Urutan" samples were purchased from two supermarkets in Denpasar. The samples, weighing 500 g each, were individually wrapped in layered plastic and transported to IPB University, Bogor, using a refrigerated truck (-2°C). During arrival, the samples were stored in a separate freezer (-33°C) in a box until the time of analysis [8]. The necessary chemicals for analysis included alkane standards (C10-C40) (Polyscience, Illinois, USA).

The samples were coded as AP1 to AP4 representing chicken "Urutan" from traditional producers 1 to 4. Additionally, AK1 and AK2 were chicken "Urutan" from Supermarkets 1 and 2.

BP1 to BP4 are pork "Urutan" from traditional producers 1 to 4. The U letter and number at the last position of the code signified replication for each sample. In total, there were 30 "Urutan" samples, specifically 15 each of chicken and pork "Urutan".

### B. Methods

#### Volatilomics Procedure

All frozen samples were thawed at room temperature, 4 g was cut into smaller pieces ( $\pm 1$  cm x 1 cm x 1 cm), and transferred into a vial and tightly closed with the lid. Volatile compounds profiling was conducted using SPME connected to GC-MS. SPME apparatus included a DVB/CAR/PDMS 2 ml fiber (DVB/Car/PDMS 50-30  $\mu$ m, Supelco, Sigma Aldrich, USA), while GC-MS used was Shimadzu GCMS-QP 2010 Plus. SPME extraction and GC-MS analytical procedure followed previous studies [8] [9]. The extraction process was carried out by positioning a clean SPME fiber into a sample vial bottle at headspace for 30 minutes. This vial bottle was repositioned on a heating plate at a constant temperature of 50°C, and injected into the injection port of GC.

Multivariate data analysis was conducted using SIMCA-P software (v. 16.0, Sartorius-Umetrics, Umeå, Sweden). Furthermore, data pre-processing was performed using As1-Smoothing filtering and Pareto scaling. OPLS-DA was used to assess classification patterns among different types of "Urutan" evaluated with the predictive coefficient value  $Q^2_X$ . Model validation was conducted with permutation and cross-validated analysis of variance (CV-ANOVA) tests to assess the reproducibility as well as predictive strength of OPLS-DA model based on  $R^2$  and  $Q^2$  values. Minimum values of  $R^2_X$ ,  $Q^2$ , and  $R^2_Y$  at 0.5 were considered acceptable [13]. Meanwhile, correlation coefficient and VIP values were used to identify volatile compounds contributing to broth separation. VIP values greater than 1 and positive correlation coefficient were considered significant.

#### Texture Profile Analysis

The samples were thawed at room temperature, cut into uniform sizes of 2 x 2 x 2 cm modified to match the size of "Urutan", and analyzed using a Texture Analyzer (TA-XT2i, SMS United Kingdom). The analysis procedure followed the previously published study [14].

#### Color Analysis

Color measurement was conducted using a Minolta CR-400 Chromameter calibrated using a standard white paper provided with the instrument. The analysis of color profile was performed by positioning 5-10 mg of the samples in the container, which was then illuminated with light using the Chromameter. Light was reflected three times at different points on the samples to obtain representative results. The data obtained were in CIELAB color system with L,  $a^*$ , and  $b^*$  values [15]. The Hue Degree ( $h^\circ$ ) was calculated based on the following formula:

$$h^\circ = \tan^{-1} (b^*/a^*) \quad \text{eq (1)}$$

### WHC Analysis

WHC measurement followed a previously published study [16], where 5-g samples were centrifuged at 4500 rpm for 15 minutes. Subsequently, the supernatant was separated, and the solid portion was weighed (W1). The solid portion was dried in an oven at 105°C for 6 hours and reweighed (W2). The analysis was performed in triplicate, and WHC was expressed using the formula:

$$\text{WHC (\%)} = ((W1-W2)/W1) \times 100 \quad \text{eq (2)}$$

### Data Analysis (Physical Characteristics)

The data obtained from physical analysis were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) with a 5% confidence interval. The results were further analyzed using the Mann-Whitney test when the data were abnormally distributed and the t-test in case of normal distribution. Initially, normality and homogeneity were assessed using the Shapiro-Wilk and Levene's tests, respectively. Normality and homogeneity assumptions were considered to be met when the p-value > 0.05.

## III. RESULT AND DISCUSSION

### C. Volatile Compounds Profiles of Chicken and Pork "Urutan"

A total of 353 volatile compounds were successfully identified from both chicken and pork "Urutan", as presented in **Table S1** (Supplementary Data, available upon request). The 353 consisted of 117 aliphatic hydrocarbons, 80 terpenes, 30 alcohols, 26 cyclic hydrocarbons, 16 ketones, 15 organic acids, 14 esters, 12 aromatic hydrocarbons, 12 sulphuric compounds, 11 aldehydes, 11 terpenoids, 4 phenols, 1 amine, 1 ether, 1 heteroatomic, and 1 heterocyclic. Chicken and pork meatballs respectively contained 150 and 141 volatile compounds, compared to other unfermented products. These were grouped into 11 categories, including acids, alcohols, aldehydes, aliphatic hydrocarbons, aromatic cyclic hydrocarbons, esters, heterocyclic compounds, ketones, sulfur compounds, terpenoids, and mixed or other compounds [8]. The representative chromatograms (total ion chromatogram, TIC) of chicken and pork "Urutan" are presented in **Figure 1**. Chicken "Urutan" chromatogram contained more peaks, signifying that volatile profiles of both products were different. To further extract the information about the discriminating volatile compounds, multivariate data analysis was conducted.

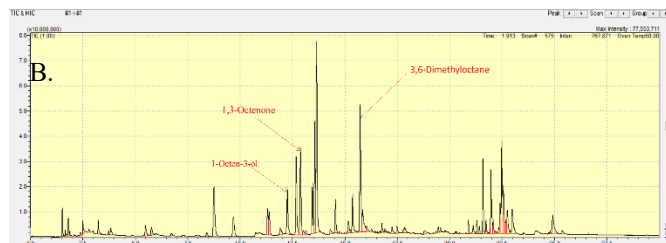
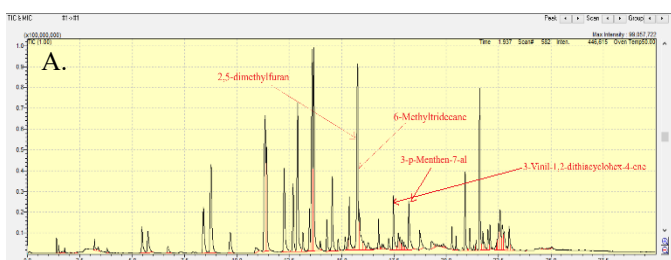


Fig. 1. Representative chromatograms and several identified marker compounds A. Chicken "Urutan". B. Pork "Urutan", obtained from GC-MS analysis.

### D. Multivariate Analysis of Volatile Data

The score plot of OPLS-DA model showed the clear grouping of chicken and pork "Urutan" (**Figure 2**). All pork "Urutan" samples were categorized in Group 3 and distinctly separated from other products. However, chicken "Urutan" samples were clustered into Group 1 (AP4) and Group 2 (AP1, AP2, AP3, AK1, AK2). Distinct classifications suggested significant differences in volatile compound profiles and the presence of characteristic volatile compounds for each sample type. A similar observation was previously reported where differences in volatile compounds were identified among chicken, beef, and wild boar meatballs, with each clustering according to the respective meat type [8]. Pork "Urutan" was categorized separately from the two chicken groups, signifying that there was strong discriminant volatiles from the main raw materials (chicken and pork) responsible for the grouping.

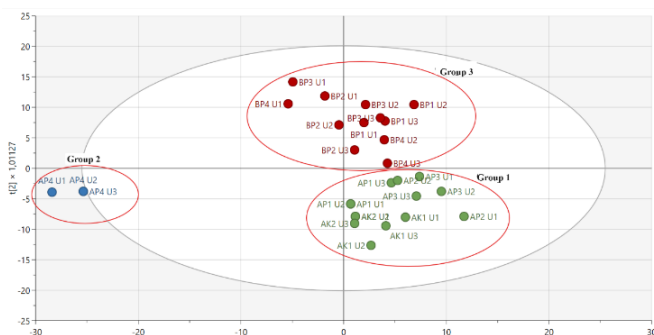


Fig. 2 OPLS-DA Score Plot of Group 1 chicken "Urutan" AP1, AP2, AP3, AK1, AK2 (all replications), Group 2 chicken "Urutan" AP4 (all replications), and Group 3 pork "Urutan" BP1, BP2, BP3, BP4 (all replications)

The division of chicken "Urutan" into different groups despite using the same main materials was clarified by observing discriminating volatile compounds for each group and tracing the possible origin. The discriminating compounds were selected using VIP and correlation coefficients. These should have a positive coefficient correlation and VIP value >1, with the error bar in VIP plot not intersecting the x-axis. The three most important volatile compounds contributing significantly to the group separation are presented in **Table 1**, and others are shown in **Table S2** which is a supplementary file available upon request.

The most significant volatile marker compound for Group 1 was formic acid produced from the metabolism of lactic acid originating from glucose metabolism [17]. This compound has been found in chicken breast meat [18], where formic acid can form through mixed acid fermentation by gastrointestinal bacteria in animals [19]. Formic acid is present in fermented chicken meat among the acids produced from carbohydrate fermentation [20], corresponding with this study because “Urutan” is a fermentation product. The second marker, 2,5-dimethylfuran, is a volatile compound that forms in food during heating. Furans can develop in food from various precursors, including ascorbic acid, amino acids, carbohydrates, unsaturated fatty acids, and carotenoids [21]. An investigation detected 2,5-dimethylfuran compound in chicken legs [22]. The presence of the third marker, 6-methyltridecane, in processed meat, has not been previously reported. However, tridecane is among the 12 hydrocarbons found in higher concentrations in fresh chicken breast cuts [23]. The stated markers in chicken “Urutan” were reported in other meats, such as formic acid identified in goat meat [24], and 2,5-dimethylfuran found in yak meat [25]. These compounds are not exclusive to chicken and the concentrations can vary. Therefore, some may serve as distinguishing factors for certain species based on the relative concentrations.

TABLE 1  
 MARKER VOLATILE COMPOUNDS FOR GROUPS 1, 2, AND 3

Group	Compound	VIP value
1	Formic acid	1,30787
	Furan, 2,5-dimethyl-	1,30732
	Tridecane, 6-methyl-	1,30398
2	3-Octadecene, (E)-	1,29646
	3-p-Menthen-7-al	1,29229
	3-Vinyl-1,2-dithiacyclohex-4-ene	1,28655
3	1-Octen-3-ol	1,08189
	Octan, 3,6-dimethyl-	1,07478
	1-Octen-3-one	1,07133

AP4 was separated from chicken “Urutan” purchased from other producers, and well-separated from pork “Urutan”. The discriminating compound for AP4 group with the highest VIP value was (E)-3-Octadecene. This is an intermediate in the Maillard reaction during the browning process that produces aroma in food [26]. (E)-3-Octadecene is an aliphatic hydrocarbon found as a major component in grilled chicken oil when cooked over charcoal [27]. Additionally, four aliphatic hydrocarbon compounds with high VIP values were detected in AP4 samples, namely (E)-3-Octadecene, 3-Vinyl-1,2-4dithiacyclohexene,  $\beta$ -Patchoulene, and 4a,8-Dimethyl-2-(2,1-propenyl)-1,2,3,4,4a,5,6,7-octahydronaphthalene). Chicken “Urutan” AP4 was suspected to be prepared through smoking using charcoal included among the common modification processes in “Urutan” production. The added spices might be the origin of some of the marker compounds in Group 2 (not presented in Table 1), such as 3-p-Menthen-7-al (VIP value = 1,29229) found in cumin [28],[29] and 3-Vinyl-1,2-dithiacyclohex-4-ene (VIP value = 1,28655) found in garlic

[30]. The presence of these compounds signified that AP4 samples were combined with more diverse spices as compared to other chicken “Urutan” in Group 1. The categorization of chicken “Urutan” into two groups but still well-separated from pork “Urutan” showed volatile composition.

The strongest marker volatile compound in Group 3 consisting of all pork “Urutan” was 1-Octan-3-ol. This is a product of the oxidation of polyunsaturated fatty acids [31], and is also found in frozen pork “Urutan” [32] as well as fermented pork [33]. The second marker, 3,6-Dimethyl-octane, was reported as a degradation product of pork fatty acids, with the concentration increasing over extended storage time [34]. Additionally, 3,6-Dimethyl-octane has been previously detected in raw pork [34]. Another identified marker for pork “Urutan” was 1-Octan-3-one, which was reported as a ketone biomarker for pork [9]. Aliphatic compounds and alcohols are the dominant groups found in pork during storage [35], which corresponds with the results of this study showing five aliphatic and alcohol compounds identified as markers.

Naturally occurring volatile compounds and those formed during fermentation are detected in the products using SPME. Naturally occurring volatile compounds include 3,6-dimethyloctane [32] and 1,3-octanone in pork [9], while fermentation processes produce 1-octan-3-ol [29]. These include 2,5-dimethylfuran in chicken [20], and fermentation generates formic acid [18]. Volatile compounds in processed meat represent a complex combination of aroma volatiles, depending on the precursors and heat used during processing. This suggests that the identified volatile compounds may be influenced by variations in thermal processing, different extraction conditions, and sample storage conditions [33]. The effects could be observed in this study, where chicken “Urutan” samples were clustered in two different groups due to the differences in processing methods (charcoal grill) and added ingredients (spices). However, the influence of discriminating volatile compounds was stronger, leading to both chicken groups being well-separated from pork group.

### E. Physical Characteristics

#### Texture Profile Analysis

Texture characteristics of chicken and pork “Urutan” were observed based on the variables of hardness, springiness, cohesiveness, and gumminess (Table 2). Both samples showed a significant difference in springiness, with pork “Urutan” presenting a higher springiness value. However, no significant difference was found between the two samples in terms of hardness, cohesiveness, and gumminess.

The lack of a significant difference in hardness, cohesiveness, and gumminess can be due to the direct correlation among these variables. Fat may contribute to hardness, acting as a plasticizer that influences the moisture content in the product [36]. Gumminess directly correlates with hardness [37] and is calculated as the product of hardness and cohesiveness [38]. Previous studies reported a triacylglycerol composition of palmitooleolein (POO) reaching 21.55 in chicken fat, which

was similar to 23.52 measured in pork fat [39]. This similarity in fat composition between the two types of “Urutan” plays a key role in balancing the characteristics of hardness, cohesiveness, and gumminess. Another study [38] observed no significant difference in hardness, cohesiveness, and gumminess between chicken breast and pork ham.

TABLE 2  
 TEXTURE ANALYSIS OF CHICKEN AND PORK “URUTAN”

Variable	Sample	Mean ± SD	P-value
Hardness (N)	Chicken	15.032 ± 1.70	0.083
	Pork	24.257 ± 5.04	
Springiness	Chicken	0.224 ± 0.04	0.011
	Pork	0.494 ± 0.12	
Cohesiveness	Chicken	0.414 ± 0.04	0.083
	Pork	0.351 ± 0.004	
Gumminess (N)	Chicken	6.216 ± 0.17	0.083
	Pork	2.683 ± 1.59	

Note. Significantly different ( $p < 0.05$ ). The above values were the average from three replications.

The significantly higher springiness of pork “Urutan” as compared to chicken “Urutan” suggests differences in muscle fiber structure or other genetic factors [40]. Pork has a thicker muscle sheath (perimysium) of approximately 23  $\mu\text{m}$  [41] than the 4–10  $\mu\text{m}$  measured in chicken [42]. Pork “Urutan” samples showed springiness values identical to those reported by Chorizo and Salami [43]. Several factors can influence the texture parameters of “Urutan”, including production and cooking conditions, ingredient composition, as well as inherent characteristics of chicken and pork [44]. “Urutan” is produced without added emulsifiers, compared to other sausage types. Emulsifiers enhance the general texture of “Urutan” [45], which can explain the observed lower springiness, compared to other fermented pork products with added emulsifiers, such as Chorizo (0.26–0.60), Mini-fuet (0.23–0.33), Fuet (0.31–0.45), Salami (0.32–0.45), and Salchichon (0.39–0.54) [46].

#### Color Analysis

The data in Table 3 showed a significant difference in the average lightness (L) levels between chicken and pork “Urutan” samples. However, there was no significant difference in the variables  $a^*$ ,  $b^*$ , and hue degree.

TABLE 3  
 COLOR ANALYSIS OF CHICKEN AND PORK “URUTAN”

Variable	Sample	Mean ± SD	P-value
L	Chicken	60.788 ± 5.30	0.019
	Pork	50.581 ± 5.58	
$a^*$	Chicken	-1.359 ± 4.04	0.303
	Pork	6.621 ± 2.69	
$b^*$	Chicken	29.887 ± 9.28	0.626
	Pork	27.357 ± 3.93	
Hue <sup>o</sup>	Chicken	1.034 ± 0.258	0.171
	Pork	10.651 ± 2.036	

Note. Significantly different ( $p < 0.05$ ). The above values were the average from three replications.

A significant difference in the lightness values (L) of chicken and pork “Urutan” could be due to the various myoglobin content in the sarcoplasm of muscle fibers of different species [47]. This was consistent with previous studies showing significant differences in lightness between chicken and pork, where chicken “Urutan” had significantly higher brightness levels [48]. The visual appearance of sliced chicken and pork “Urutan” can be seen in Figure 3.



Fig. 3. Visual appearance of A. Pork “Urutan”. B. Chicken “Urutan”

#### WHC Analysis

Significant differences were observed between the average WHC values of chicken and pork “Urutan”. WHC analysis results are shown in Table 4, where chicken “Urutan” presents a higher WHC value than pork “Urutan”. This difference may be attributed to the varying fat and meat composition used in the production process. Pork “Urutan” primarily has a fat-to-meat ratio of 60:40 [2], while chicken “Urutan” comprises a ratio of 70:30 [49]. Based on the ratio, chicken sausage contains a higher meat content relative to fat, potentially increasing the protein content.

TABLE 4  
 WHC ANALYSIS OF CHICKEN AND PORK “URUTAN”

Variable	Sample	Mean ± SD	P-value
Water holding capacity (%)	Chicken	63.717 ± 3.40	0.001
	Pork	54.525 ± 0.97	

Note. Significantly different ( $p < 0.05$ ). The above values were the average from three replications.

Myofibrillar proteins play a crucial role in binding or “holding” water in meat structure [50], hence, a higher protein content supports greater WHC. The denser, higher-meat-content chicken “Urutan” has a higher WHC value. In addition to fat and meat composition, fermentation process significantly influences WHC in meat products. Natural fermentation primarily includes lactic acid bacteria, naturally present in meat or introduced from the environment, which produces lactic acid as a metabolic byproduct, lowering meat pH. This decrease in pH can lead to protein denaturation, enhancing WHC, as denatured proteins have a greater capacity to retain water [51]. At pH levels below the isoelectric point of meat proteins (5.0–5.1), WHC increases because charged meat molecules repel each other, generating voids that could accommodate water molecules. “Urutan” production process uses natural fermentation lasting 3 to 5 days [2].

#### IV. CONCLUSION

In conclusion, the results showed that volatilomics successfully differentiated volatile compounds between chicken and pork "Urutan". These included marker compounds identified in chicken "Urutan", namely formic acid, 2,5-dimethylfuran, and 6-methyltridecane. The marker compounds in chicken "Urutan" AP4 suspected to have passed through a smoking process were (E)-3-Octadecen, 3-p-menthen-7-al, and 3-Vinyl-1,2-dithiacyclohex-4-ene. Meanwhile, volatile compounds in pork "Urutan" identified as markers were 1-Octen-3-ol, 3,6-dimethyloctane, and 1-octen-3-one. Chicken and pork "Urutan" were shown to have significantly different physical characteristics, including springiness, lightness, and WHC. There was no significant difference between texture parameters such as hardness, cohesiveness, and gumminess. For color analysis, only L showed significantly different values, while a\* and b\* of chicken and pork "Urutan" were not significantly different. The conclusion derived in this study applied to the conditions of the samples used, specifically raw (uncooked) chicken and pork "Urutan".

#### ACKNOWLEDGMENT

The authors are grateful for the support provided by IPB University through International Research Collaboration with contract number [3341/IT3.L1/PT.01.03/P/B/2022].

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### USE OF ARTIFICIAL INTELLIGENCE (AI) TOOLS STATEMENT

We used Grammarly (Grammarly Inc., 2025) to improve the clarity and grammar of the manuscript. The authors reviewed and approved all changes.

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