Indonesian Journal of Halal Research
E-ISSN: 2657-0165
P-ISSN: 2656-3754

ARTICLE

Indonesian Journal of HALAL RESEARCH

Comparative Volatilomic Profiling of Beef and Pork Sausages Using HS-GCMS and Chemometric Analysis

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Abstract: *Verifying the species origin of processed meat is important for both genuineness and halal compliance. This study aims to identify volatile organic compounds (VOCs) in fresh and processed meat using a volatilomic metabolomics approach. Fresh beef, fresh pork, beef sausage, and pork sausage are collected from supermarkets. For sample preparation, each meat sample was homogenized in phosphate buffer and placed in headspace vials. The VOCs were analyzed with HS-GCMS, and principal component analysis (PCA) was used to interpret the data. Fresh beef contained 50 volatile compounds, while fresh pork had 30. Beef sausage, pork sausage, and mixed beef–pork sausages (25:75 and 50:50) contained 41, 30, 38, and 50 volatile compounds, respectively. PCA results showed clear differences between beef and pork based on their chemical profiles. Hexanal and 1-octen-3-ol were strong indicators of pork, while nonanal, octanal, and benzaldehyde were typical for beef. In sausages, PC1 separated pork and beef by the types of volatile compounds from fat compared to those from protein or heme. PC2 reflected the phenolic and terpene compounds from smoke seasonings. Some VOCs, like n-hexane and N-tert-butylhydroxylamine, are commonly used in pork sausage production. The VOC profiles of mixed sausages depended on the beef-to-pork ratio. Some markers were present only in small amounts; for example, 1-octen-3-ol was detected only in the 25:75 mixture, and benzaldehyde was detected only in the 50:50 mixture. These results show that species-specific volatile compounds can still be detected in mixed sausage samples, supporting the use of volatilomics to detect adulteration.*

Keywords: *volatile organic compounds, sausage, meat adulteration, HS-GCMS, chemometric analysis*

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Received: January 30, 2025	Accepted: February 28, 2026	Published: February 28, 2026

How to cite this article (APA 7th Edition Reference Style): Hermanto, S., Fathoni, A., Siregar, B. J., & Alfarus, D. (2026). Comparative Volatilomic Profiling of Beef and Pork Sausages Using HS-GCMS and Chemometric Analysis. *Indonesian Journal of Halal Research*, *8*(1), 68–85. https://doi.org/10.15575/ijhar.v8i1.44234

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1. Introduction

Meat is also one of the most consumed protein sources and is valued for its taste and nutritional value. It offers complete and balanced essential amino acids with high-quality protein and includes numerous vitamins and minerals (Stadnik, 2024). Over the last few years, due to the high market price of beef, there have been fraudulent activities where partial or full replacement with cheaper meats, such as pork or boar, has occurred. These replacements are occasionally identified by physical dissimilarity in appearance, including hue, fibre design, and touch. Several studies have shown that pork tends to be less brown compared to beef, and beef fibers are more pronounced (Nafiasari & Handayani, 2019). However, it becomes difficult to differentiate when the products are mixed or processed into derivatives.

To protect consumers, the Indonesian government launched the policy of safe, healthy, intact, and halal that addresses various aspects. These include (1) the circulation of non-health and non-halal products of animal origins, (2) food-related illnesses and poisoning incidents, some of which are not reported, (3) insufficient production and distribution facilities, and (4) a lack of consumer awareness about the safety of products and their halal nature (Lusianti et al., 2025). According to this policy, Law No. 33 of 2014 requires halal certification of food products, medicines, cosmetics, and some goods. Halal certification became mandatory for all producers who sold products in Indonesia starting in October 2024. It is a process that involves document-based audits, validation of production plants, and, in certain instances, laboratory tests to give a correct halal audit, especially in animal-based products (Lusianti et al., 2025). In Indonesia, adulteration of beef with pork or boar (Sus scrofa) is an issue that is prevalent both in fresh meat and processed meat items, including meatballs, sausages, and jerky. These activities are a serious threat to halal integrity, indicating that effective detection mechanisms must be established to facilitate certification and discourage fraud in the market.

Several analytical methods have been developed to identify pork derivatives in meat products. Genomic DNA and RNA analyses with polymerase chain reaction (PCR) and real-time PCR are commonly utilized in the detection of contamination using molecular methods (Che Man et al., 2007; Erwanto et al., 2012). Other approaches are focused on lipid compounds (Kurniawati et al., 2014; Rohman & Fadzillah, 2018) or proteins and antibody ELISA (Chen & Hsieh, 2000). The advantages and limitations of each approach are unique and depend on the complexity of the samples and processing conditions. Previous studies have also explored marker-based methods, with (Indrasti et al., 2010) finding 3 different fatty acids in lard and (Zhang et al., 2009) reporting marker peptides distinguishing beef and porcine gelatin. The electrophoresis and densitometry protein profiling have been used to process meat products, including sausages (Hermanto et al., 2022). However, such methods are not always effective because the samples can become degraded through cooking or heating.

Another alternative that has a lot of potential in halal authentication of meat products is volatilomics. This method is based on the volatile organic compounds (VOCs) that are easy to evaporate and add a species-specific flavor profile (Lytou et al., 2019). The flavor of meat differs across species and depends on cooking practices (Kosowska et al., 2017). Previous studies have shown the potential of volatilomics for species differentiation. For instance, Yang et al., (2022) characterized and identified pork flavor compounds and their precursors in Chinese indigenous pig breeds using volatile profiling and multivariate analysis. Other studies have differentiated fresh meat types, such as pork, beef, chicken, and goat (Ahamed et al., 2024); Pavlidis et al., 2019). Indrasti & Ramadhina, (2025) analyzed volatile profile and its application on species authentication of meat-based food. Ratel et al., (2022) identified hydrocarbon, oxygenated, sulfur, and aromatic markers associated with livestock exposure to α-hexabromocyclododecane.

Recent studies have further demonstrated the efficacy of volatilomics in distinguishing processed meat types. (Andryani et al., 2025) analyzed chicken and pork *Urutan*, a traditional Balinese sausage, using SPME-GC-MS and OPLS-DA. The study identified markers such as 1-octen-3-ol specific to pork *Urutan* and 2,5-dimethyl-furan for chicken *Urutan*. (Amalia et al., 2022) also characterized VOCs, texture, and color in meatballs made from beef, rat, and wild boar. The results showed that volatile profiles, rather than texture or color, could reliably detect adulteration even at 20% inclusion, with markers, such as (Z)-2-amino-5-methyl-benzoic acid and 2-heptenal differentiating species. (Pranata et al., 2021) used volatilomics with SPME-GC-MS to differentiate halal beef, chicken, and non-halal wild boar meatballs, identifying key markers including b-cymene, benzaldehyde, and 5-ethyl-m-xylene via PLS-DA analysis.

In volatilomic studies, gas chromatography-mass spectrometry (GC-MS) is popular because of its high sensitivity, resolution, rapid analysis, and low sample needs (Tranchida et al., 2012); (Seo et al., 2025). Headspace extraction, simultaneous distillation-extraction (SDE), and solid-phase

microextraction (SPME) are some of the common methods used for preparing samples. Previous studies have shown that headspace extraction can reduce the consumption of solvents, minimize the interference of non-target compounds, and provide better sensitivity (Cavalli et al., 2003; Song et al., 2021). In a recent study, headspace GC-MS was used to conduct the analysis of VOCs in beef and pork sausages to determine possible markers for halal authentication. Data obtained were further analyzed through multivariate chemometric analysis using Principal Component Analysis (PCA), to effectively determine the difference between the 2 types of sausages.

Despite the wide variety of analytical methods applied to identify pork adulteration, including DNA and protein-based approaches as well as lipid profiling, their ability can be reduced when using highly processed meat samples, including sausages. This is primarily because thermal treatments, spices, and additives can modify or mask the target biomolecules. Although recent volatilomics studies have shown promising potential for species authentication, most have been carried out using fresh meats or a small range of products. There is also limited information on the stability of volatile markers in complex, spice-laden sausage formulations. Current literature rarely evaluates adulteration at low concentrations that are applicable to halal audits and fails to suit practical authentication procedures. Therefore, systematic exploration of VOC profiles in beef and pork sausages with sensitive analytical methods, such as headspace GCMS, along with multivariate analysis, is needed to find reliable, process-resistant markers that can be used to differentiate and justify halal verification.

The present study can be considered a new contribution because it defines a volatilomics-based authentication method specifically targeted at processed meat products, such as beef and pork sausages. In comparison with the previous literature on fresh meat or small sets of products, this study shows that headspace GC-MS with multivariate chemometrics can generate strong, process-resistant volatile markers for differentiating species despite the inclusion of spices, additives, and thermal modification.

2. Materials and Methods

2.1. Sample Collection

Fresh beef, pork, and commercial pork sausages were purchased from a supermarket in Serpong, South Tangerang, Banten, Indonesia. Beef sausages were obtained from a traditional market in Jakarta. The meat cuts included pelvic (flank), ribs, and thigh (shank) portions. Equal amounts of meat were weighed, mixed, vacuum-packed in plastic bags, and stored at −20 °C until analysis. Additional ingredients for sausage preparation included tapioca flour and eggs. Analytical-grade chemicals used in this study included internal standards of n-alkanes (C7–C30, 1000 µL; Merck), methanol, and sodium chloride

2.2. Instrumentation

Volatile compounds analysis was carried out using a Shimadzu QP-2020 GC–MS system (Shimadzu, Japan) equipped with a quadrupole mass analyzer and coupled to a Shimadzu HS-20 NX Headspace Autosampler. Separation was achieved on an RTX-5MS capillary column (Restek, USA; 60 m × 0.25 mm i.d., 0.25 µm film thickness) with a diphenyl dimethyl polysiloxane stationary phase. Sample preparation and handling used a Millipore syringe, Tokebi homogenizer, micropipettes, a Bio-Rad microcentrifuge, an analytical balance, and a meat grinder. Data acquisition and chemometric analysis were performed using SIMCA®-online software version 14.0 (Sartorius, Germany).

2.3. Sausage Preparation and Volatile Compound Extraction

Sausages were prepared by weighing 100 g of meat (beef or pork) and mixing it with 1 g of tapioca flour and 2 whole eggs. The mixture was homogenized until uniform, packed into plastic casings, and sealed. The sausages were subsequently boiled for 30 minutes and immediately cooled in ice water to prevent wrinkling. Each sausage type (pork and beef) was prepared in duplicate. (Hermanto et al., 2022). All sausages were cut into small pieces, and 5 g of each sample was homogenized with 50 mL of saturated sodium chloride solution. The homogenates were transferred into headspace vials and incubated at 80 °C for 30 minutes. Volatile compounds were extracted and analyzed using headspace gas chromatography–mass spectrometry (HS-GC/MS). Fresh beef, fresh pork, beef sausages, and commercial pork sausages were included as controls and subjected to the same extraction and analytical procedures (Song et al., 2021).

2.4. GC-MS Analysis of Volatile Compounds

Meat extracts were injected into the HS-20 NX Headspace Autosampler equipped with a hydrocarbon trap, with a split ratio of 10:1 at 250 °C. Separation was achieved using an RTX-5MS column under the following temperature program: initial temperature 55 °C, increased at 3 °C/min to 150 °C (held for

2 min), then at 5 °C/min to 200 °C (held for 2 min), and finally to 250 °C (held for 4 min). The interface temperature was maintained at 250 °C. Mass spectrometry was carried out in electron ionization mode (70 eV) with a scanning range of 35–350 m/z and an ion source temperature of 230 °C. Raw chromatographic data were obtained, and peaks were deconvoluted to obtain retention time, peak area, and spectral information. Peak detection parameters (signal-to-noise threshold, minimum peak width) were also optimized using test samples and pooled quality controls (Lammers et al., 2007).

2.5. Peak Alignment and Curation

The retention time was consistent correct chromatographic drift between different runs to allow consistent feature matching. Blanks or poor spectral qualities were eliminated. A spectral matching was performed with the NIST MS 14.0 library and retention indices to verify compound identity.

2.6. Data Normalization and Scaling

Internal standards were used to quantify and normalize peak areas to correct injection variability. Probabilistic quotient normalization (PQN) and total area normalization were used to explain the effect of dilution. Before chemometric analysis, data were log-transformed to stabilize variance, mean-centered, and autoscaled (unit variance).

2.7. Quality Control

Regular injections of pooled quality control samples were performed to check instrument drift. Features with large variability (relative standard deviation > 30% in quality control replications) were eliminated. Non-detects were replaced with small values to impute missing values.

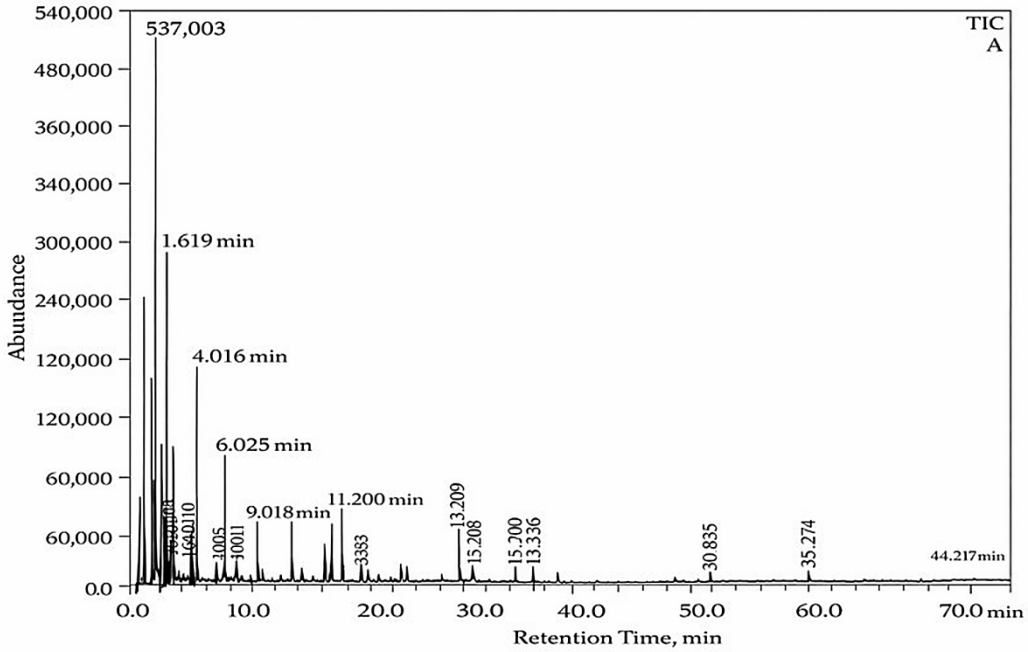
2.8. Chemometric Analysis

Multivariate statistical analysis was conducted on the processed data matrix. Unsupervised exploration of the clustering and outlier identification of samples was performed using PCA. Supervised classification of meat types was performed using partial least squares-discriminant analysis (PLS-DA K-fold cross-validation and permutation testing were used to validate model performance. Discriminant volatile markers were identified using Variable Importance in Projection (VIP) scores.

3. Results and Discussion

3.1. Volatile Constituents in Fresh Beef and Pork

The GC-MS analysis of fresh beef and pork showed that there were several chromatographic peaks, as shown in **Figure 1**. The volatile compounds identified were mainly aldehydes, ketones, aromatic hydrocarbons, alkanes, alkenes, terpenoids, and carboxylic acids. These compounds were mostly formed as a result of the degradation of fatty acids, proteins, carbohydrates, and other macronutrients found in meat (**Table 1**).



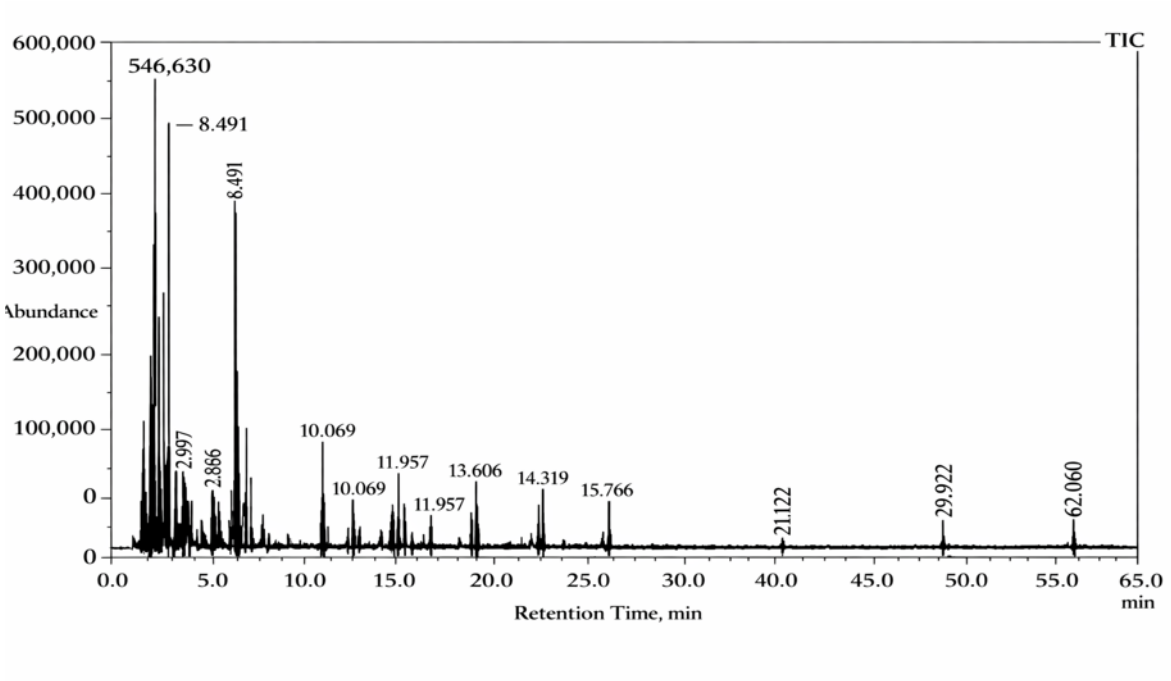


Figure 1. GCMS result of fresh beef (A) and pork (B)

A total of 50 volatile compounds in beef and 30 volatile compounds in pork were observed. Remarkably, 34 compounds were only found in beef, and 17 compounds were found in pork, as indicated in Table 1. These results showed that species-related metabolism and lipid oxidation, Maillard reaction products, and amino acid degradation were different, leading to the characteristic aroma and flavor of each meat type.

Table 1. Composition of Volatile Compounds in Fresh Beef and Pork Samples

No	RT	Fresh Beef	No	RT	Fresh Pork
1	1,619	*2-p-nitrobenzoyl-1,3,5-tribenzyl-.alpha.-d-ribose*	1	1,560	*6e, 10z, 14e)-6 oxo-2-octahydrocyclotetra 1,4-dimethyl-3-methylidene*
2	1,672	*carbonyl sulfide*	2	1,593	*3,4-dimethyl-5-methoxy-isoxazole*
3	1,895	*2-methyl-2-propanamine*	3	1,658	*thiosemicarbazone cyclododecanone*
4	1,975	*boric acid*	4	1,829	*3,3'-oxybis-1-propyne*
5	2,044	*tetracarbonylbis(hexamethylphosphorus triamide-p)-(oc-6-22)*	5	1,855	*2,4,4-trimethyl-2-pentanamine*
6	2,115	*2-methyl-propanal*	6	1,981	*carbon disulfide*
7	2,304	*n-hexane*	7	2,125	*phytol, e, tbdms derivative*
8	2,840	*carbonic acid, ethyl 2-ethylhexyl ester*	8	2,278	*isobutyl vinylacetate*
9	2,870	*acetic acid, (dodecahydro-7-hydroxy-1,4b,8,8-tetramethyl-10-oxo-2(1h)-phenanthrenylidene*	9	2,304	*n-hexane*
10	2,927	*2-methyl-butanal*	10	2,445	*tungsten[1,2-bis(methylthio)ethane-s,s']tetracarbonyl-(oc-6-22)*
11	3,180	*trans-3-methoxycarbonyloxy-4- methoxy cinnamic acid*	11	2,515	*di-dodecylphosphine oxide*
12	3,269	*glysine, n-methyl-n-propoxycarbonyl-hexadecyl ester*	12	3,397	*2-methyl-heptanal*
13	3,301	*acetic acid*	13	3,610	*3-methyl-isovaline*
14	3,428	*4-methyl- trans-cyclohexanol*	14	4,865	*1,3,5-cyclo heptatriene*
15	3,597	*2-methylamino-3-methyl butanoic acid*	15	5,504	*o-3-deoxy-4-c- methyl-3-(methylamino)-.beta.-l-arabinopyranosyl d-strepttamine*
16	3,680	*monoethylmalonate monoamide*	16	5,891	*1,1-dimethylethyl)-cyclohexane*
17	3,727	*methyl (z)-octadec-11-enoyl-l- leucianate*	17	10,089	*Heptanal*
18	4,166	*1,3,18,20-tetraazabicyclo [18.8.8]hexatriacontan- 2,19-dithion*	18	13,967	*bicyclo[3.1.0]hexane, 4-methylene-1- (1-methylethyl*

Table 1. Composition of Volatile Compounds in Fresh Beef and Pork Samples (continued)

No	RT	Fresh Beef	No	RT	Fresh Pork
19	4,216	*n-glycil-l-isoleucine,*	19	14,162	*beta.-myrcene*
20	4,269	*methoxycarbonyl-, methyl ester ile-pro-ile, trymethylsylil ester*	20	14,591	*6-oxa-bicyclo[3.1.0]hexan-3-ol*
21	4,326	*dimethyl-silanediol*	21	15,024	*octamethyl-cyclotetrasiloxane*
22	4,375	*2-methyl-2-hexen-4-yne*	22	15,806	*1-hexanol, 2-(hydroxymethyl)-*
23	4,550	*digitoxose*	23	15,961	*3-carene*
24	4,570	*5,5-dimethyl-2,7 dioxabicycl o[4.1.0]heptan-3-on*	24	16,854	*1-methyl-3-(1-methylethyl)-benzene*
25	4,625	*3-methyl-isovaline*	25	17,119	*d-limonene*
26	4,898	*toluene*	26	21,322	*Nonanal*
27	5,891	*1,1-dimethylethyl-cyclohexane*	27	23,325	*decamethyl- ethanone, 1-[3-(5-chlorooxazolo [4,5-cyclopentasiloxane*
28	6.207	*ethylene glycol monoisibutyl ether*	28	29,925	*1-[3-(5-chlorooxazolo[4,5-h]quinolin-2ylsulfanylmethyl)-4-methoxyphenyl]-ethanone*
29	6,345	*4-piperidinecarboxamide*	29	57,510	*phorbol 12,13-dihexanoate*
30	6,462	*ethylene glycol monoisibutyl ether*	30	63,304	*adenosine, 4'-de(hydroxymethyl-4-) [n-ethylaminoformyl*
31	6,605	*1,1'-[oxybis(2,1- ethanediyloxy)]bis butane*			
32	8,618	*pentadecafluorooctanoic acid, hexyl ester*			
33	9,350	*benzaldehyde, 3-(2-phenoxyethoxy)-,1-phenilsemicarbazone*			
34	9,474	*2-heptanone*			
35	10,081	*heptanal*			
36	11,411	*methyl 23-methyl-tetracosa-5,9- dienoate*			
37	13,260	*bis[4acetamidophenylsulfonyl]phenyl methane*			
38	14,552	*1-octen-3-ol*			
39	14,830	*2-isopropyloxan-4-one*			
40	15,019	*furan, 2-pentyl-*			
41	15,799	*1-hexanol, 2-(hydroxymethyl)-*			
42	16,848	*benzene, 1-methyl-3-(1-methylethyl)-*			
43	17,125	*nickel, (1,6-dimethylcycloocta-1,5-diene)[methyl trans-.beta.-*			
44	18,710	*eicosane, 1-iodo-*			
45	21,314	*nonanal*			
46	21,598	*chlorobis(1,2,3,4,5,6-.eta.)-methylbenzene]bis(.eta.3-2propenyl)di-*			
47	23,336	*cyclopentasiloxane, decamethyl-*			
48	24,200	*(3e, 10z)-oxacyclotrideca-3,10-diene-2,7-dione*			
49	29,885	*nonane, 3-methyl-5-propyl-*			
50	34,274	*satratoxin h*			

The presence of unique compounds such as *2-methyl butanal* in beef, which was absent in pork and other samples, emphasized the role of branched-chain aldehydes derived from amino acid catabolism in beef flavor development. Such compounds were known to impart malty or nutty notes, which were often associated with beef aroma (Smit et al., 2009). Meanwhile, pork-specific volatiles originated from higher levels of unsaturated fatty acids, leading to distinct lipid-derived oxidation products that influenced its sensory attributes. A study analyzing different pork breeds confirmed that volatile flavor compounds correlate strongly with unsaturated fatty acids such as C18:1n9c (oleic acid) and C18:2n6c (linoleic acid), indicating lipid oxidation as a major source of pork volatiles (Huang et al., 2025). These volatile profile differences were of great importance in meat authentication and quality

control. Species-specific markers were identified and formed the basis of the development of rapid methods of product analysis to detect adulterated meat and to assure product integrity.

Pavlidis et al. (2019) detected 53 and 41 volatile compounds in fresh beef and fresh pork, while Pranata et al. (2021) detected 69 and 62 compounds in beef and pork meatballs, respectively. Variations in compound numbers across studies could be attributed to differences in extraction and analytical techniques, particularly the use of SPME in those studies. Pranata et al. (2021) showed 5 major differentiating compounds between beef and pork: toluene, 2-heptanone, o-xylene, indene, and m-xylene—all cyclic aromatic compounds. Sun et al. (2021) suggested that aromatic cyclic compounds in fresh meat originate from the decomposition of aromatic amino acids during post-mortem processes. Discrepancies between studies resulted from differences in animal genotype, habitat, and diet (Skobrák et al., 2011), as well as variations in analytical methods and targeted compound profiles (Cavalli et al., 2003).

3.2. Volatile Constituents of Beef, Pork, and Adulterated Sausages

GC-MS analysis of simulated beef and pork sausages revealed multiple chromatographic peaks, as illustrated in **Figure 2a** and **2b,** while adulterated sausages can be seen in **Figure 3a** and **3b**.

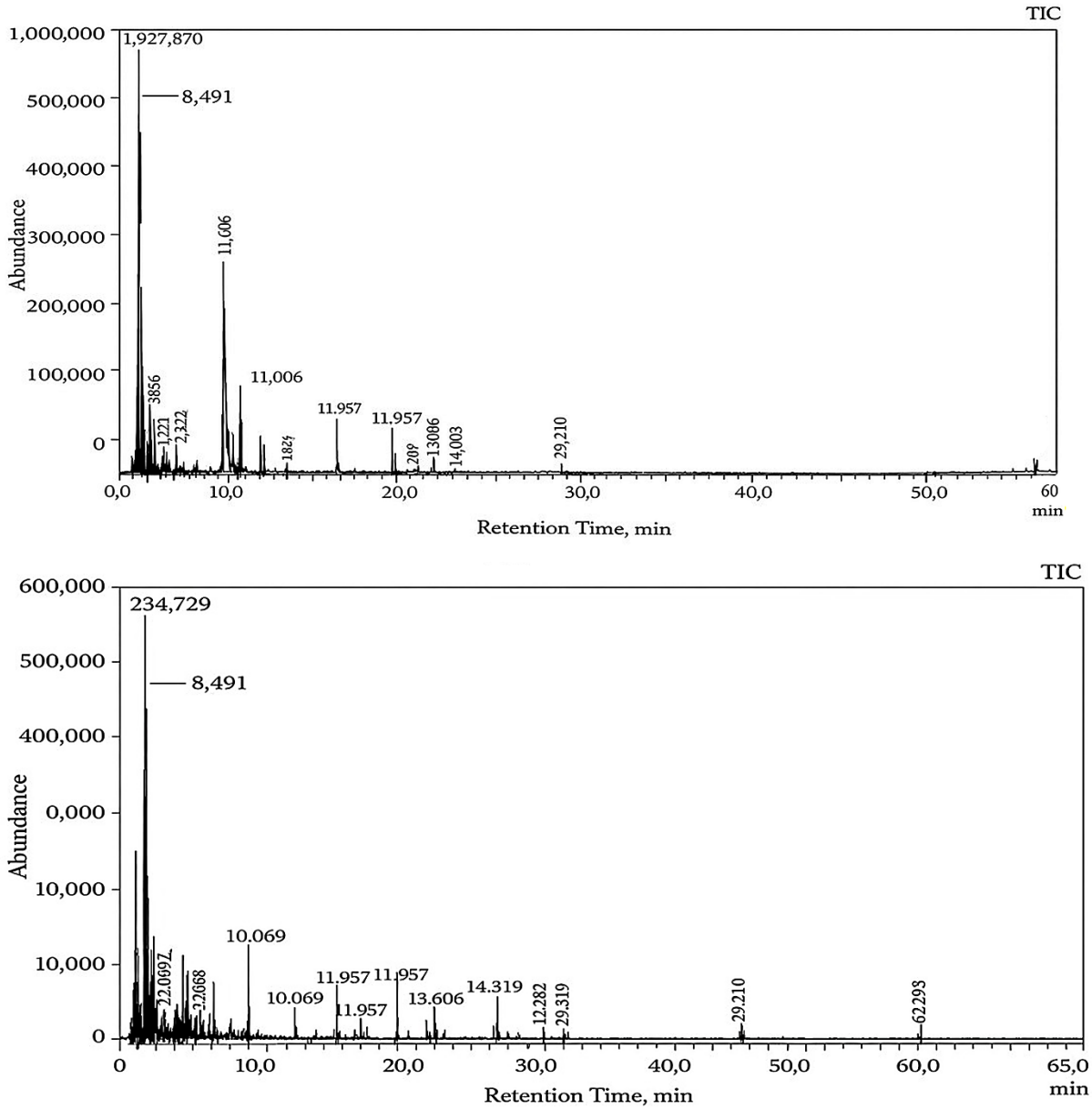


Figure 2. GCMS peaks of volatile compounds of (A) Beef Sausage, (B) Pork Sausage

As shown in **Figure 2**, simulated sausages prepared from 100% beef and 100% pork contained 41 and 33 VOCs, respectively. Mixed sausages with beef-to-pork ratios of 25:75 and 50:50 contained 38 and 40 VOCs, respectively, showing that both species composition and processing conditions affect

VOC diversity (**Figure 3**). The thermal processing during the sausage production triggered a cascade of chemical reactions, such as lipid oxidation, Maillard reactions, and protein degradation, which, in combination, lead to the creation of more volatile compounds. Kosowska et al. (2017) reported that meat processing significantly influences the composition and abundance of volatile components.

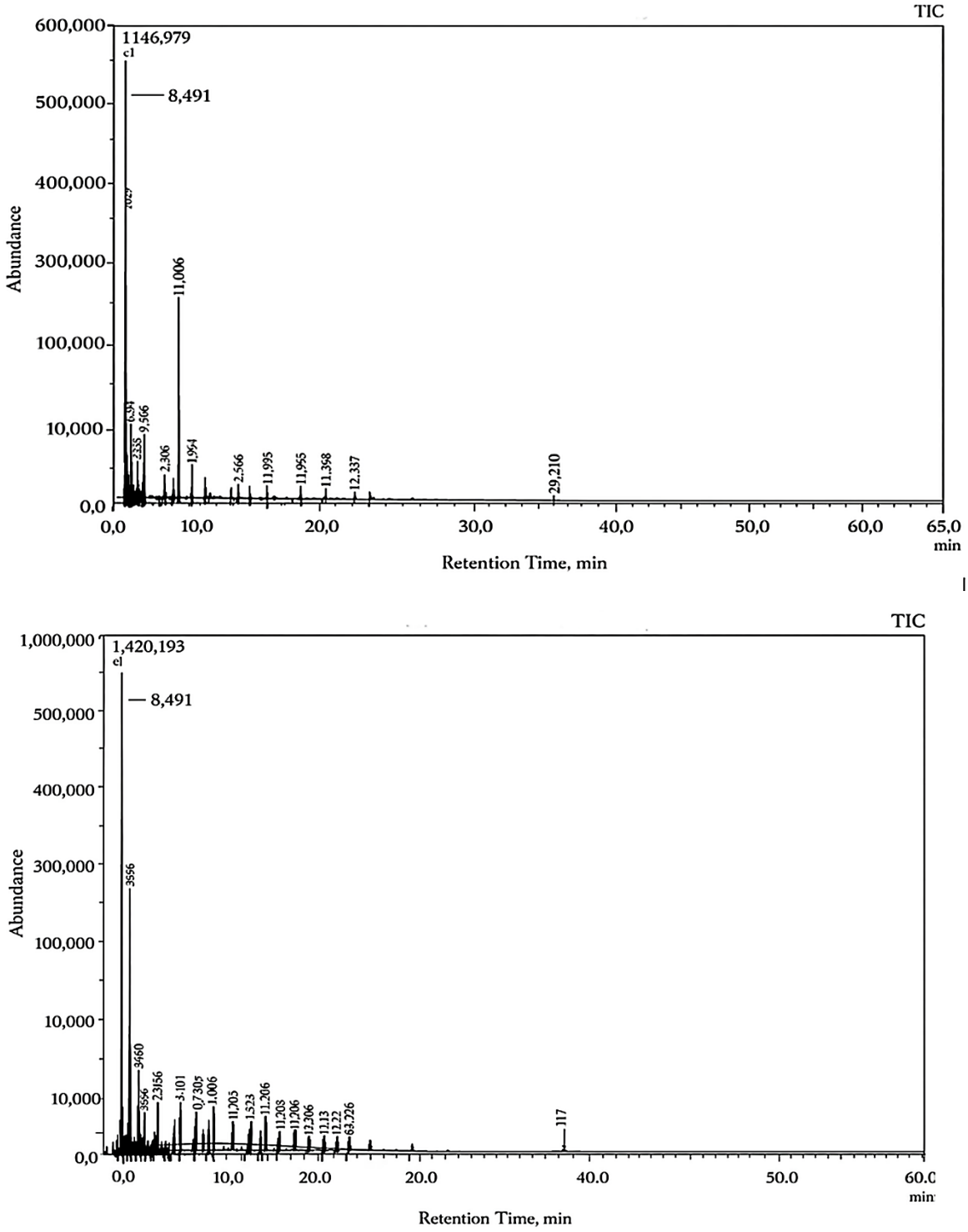


Figure 3. GCMS peaks of volatile compounds of (A) Mixed sausage (25% pork;75% beef)
B) Mixed sausage (50% pork;50% beef)

Table 2 shows that the beef sausage sample had 41 volatile compounds, and 17 of these were unique to this sample, while the pork sausage sample had 33 volatile compounds, and 18 were unique to pork. The identification of marker compounds of pork sausage was made by comparing the spread of distinct compounds on homemade pork sausages and mixed formulations and multivariate analysis using PCA. In fresh pork, sulfur-containing compounds were reduced to only 1 compound, n-decyl sulfone, while processed pork sausages had other sulfur volatiles, such as dimethyl disulfide and diallyl

sulfide. These were identified as major contributors to cooked meat aroma (McKee et al., 2012) and formed as a result of thiamine degradation and cysteine-related reactions (Dordevic et al., 2023). Beyond sulfur compounds, processing significantly increased the abundance of other volatile classes, such as acids, heterocyclics, ketones, esters, alcohols, aromatic hydrocarbons, aliphatic hydrocarbons, aldehydes, and terpenes. Notably, the major classes of organic compounds responsible for meat aroma formation included aldehydes, alcohols, esters, ketones, carboxylic acids, furans, ethers, pyrazines, pyridines, pyrroles, oxazoles and oxazolines, thiazoles and thiazolines, thiophenes, and other sulfur-containing compounds (Wojtasik-Kalinowska et al., 2023).

Table 2. Composition of Volatile Compounds in Beef, Pork, and Mixed Sausages

No	RT	beef sausage	RT	pork sausage	RT	mixed sausage (25,75%)	RT	mixed sausage (50, 50%)
1	1,615	*alpha.--(hydroxymethyl)-alpha.-(1- acetyl-3-ethylidene-4-piperidyl)- 3-(indole-2-acetid acid.*	1,610	*2,4',5-tribromobiphenyl*	1.643	*1-[3-dimehylamino]propylimino-[1.3.4.10-tetrahydro-7-trifluoromethyl-acridine*	1,621	*1-[3-dimehylamino]propylimino-[1.3.4.10-tetrahydro-7-trifluoromethyl-acridine*
2	1,640	*rhodium, di-.mu.-chlorobis(.eta.4-1,2-diethenylcyclobutane)*	1,640	*3-etenyl-methyl-2-oxazolidinone,*	1.693	*4-chloro-butanoic acid*	*1,716*	*4-chloro-butanoic acid*
3	1,710	*4-chloro-butanoic acid*	1,717	*acetamide, n,n'-thiobis-*	1.912	*n,n-dimethyl hexacosylamine*	*1.900*	*oxirene,methyl-(5)*
4	1,885	*propanoic acid, 2-chloro-, 1,1-dimethylethyl ester*	1,869	*methylcyclopropanemethanol propylcarbamate*	1.962	*tetra methylammonium perchlorate*	*1.994*	*boric acid*
5	1,855	*acetone*	1,895	*2-methyl-2-propanamine*	2.010	*4(3,5-diiodo-2-methoxybenzidineamino 1,2,4-triazole*	*2.045*	*bis(n-tert-butyl dihydrocarbamate*
6	2,015	*urethane*	2,000	*2-(3,4-dichlorophenyl)-5-propylcarbamate*	2.050	*n-4-(aminosulfonyl)phenyl}-2-pyrolidinyl acetamide*	*2.317*	*methyl urea*
7	2,083	*nitric acid pentyl ester*	2,030	*(3,5-dichlorosalicylideneamino) benzoxazole*	2.200	*tetrahydro-thiophene*	*2.336*	*methyl thiopene*
8	2,165	*2-methyl-3-(1-methylethoxy)- 1-propene*	2,072	*allyl chloride*	2.325	*n-tert-butylhydroxylamine*	*2.442*	*difluorophosphoric acid*
9	2,190	*3-(1-methylethyl)-oxetane*	2,353	*2-amino-2-methyl-1,3-propanediol*	2.462	*difluorophosphoric acid*	*2.485*	*tetrahydro-6-methyl 2h-pyran-2-acetic acid*
10	2,320	*2-(1,1,2,3,3,3-hexafluoro-propylsulfanyl)-3-methyl-but-2 enenitrle*	2,442	*difluorophosporic acid*	2.500	*2,4-dicloro-5-fluorobenzyl alcohol*	*2.629*	*1,1,3,3-tetrachloro 2-propanone*
11	2,340	*n-tert-butylhydroxylamine*	2,480	*(2,6-dibromo4-methylphenoxy) acetic acid*	2.635	*2-(3-methylphenoxy)octahydro 1h-1,3,2-benzodiazophosphole 2 -oxide*	*2.715*	*difluorophosphoric acid*
12	2,442	*difluorophosporic acid*	2,615	*ethyltrimethyl-stannane,*	2.730	*difluorophosphoric acid*	*2.919*	*3-methyl butanal*
13	2,480	*2',4',5'-trichloroacetanilide*	2,815	*n,n-diethyl-2-phenyl cyclopropane-1-carboxamide*	3.443	*butoxymethyl-oximene*	*3.426*	*methyl alyl sulfide*

Table 2. Composition of Volatile Compounds in Beef, Pork, and Mixed Sausages (continued)

No	RT	beef sausage	RT	pork sausage	RT	mixed sausage (25,75%)	RT	mixed sausage (50, 50%)
				n, n'-bis(trifluoroacetyl)-n-[3-(dimethylamino) propyl]piperidine-4-carboxamide		*toluene*	*4.219*	*2-pentene*
14	2,615	*decaborane(14)*	2,902		4.920			
15	2,693	*3- methylbut-2-enoic acid, 4-nitrophenyl ester*	3,030	*4-methyl-1-hexene,*	5.585	*butanoic acid*	*4.898*	*l-fucose trifluoroacetate*
16	2825	*z, tetradec-11-en-1-yl 2,2,3,3,3-pentafluoropropanoate*	3,419	*allyl methyl sulfide*	5.620	*2-butan-1ol propanoate*	*4.931*	*1,3,5-cylcoheptatriene*
17	2,910	*cyclopropyl methyl carbinol*	3,610	*2-(3,4-dichlorophenyl)-5-1,3,5-cycloheptatriene*	5.928	*2,4-dimethyl-1-hexene*	*5.923*	*3-methyl 4-hydroxy butanal*
18	3,419	*sulfide, allyl methyl*	4,868	*(3,5-dichlorosalicylidene amino) benzoxazole*	7.550	*3-methyl butanoic acid*	*7.983*	*dialyl sulfide*
19	4,870	*dihydroxy-2-methyl-cycloheptano[d] 1,3-imidazolidine,*	5,891	*(1,1-dimethylethyl)-cyclohexane*	7.984	*dialyl sulfide*	*8.105*	*4-amino-3-methyl2-thioxo-2,3-dihydrothiazole*
20	5,891	*1,1-dimethylethyl)-cyclohexane*	7,975	*diallyl sulfide*	10.103	*heptanal*	*9.417*	*styrene*
21	7,975	*diallyl sulfide*	10,081	*heptanal*	13.967	*bicyclo [3,1,1] 4-methylene-1-(1-methylalyl) hexane*	*10.116*	*heptanal*
22	9,384	*styrene*	10,693	*methyl 2-propenyl disulfide*	14.163	*beta pinene*	*10.719*	*methyl 2-prophenyl disulfide*
23	10,081	*heptanal*	11,732	*n-(2,3-dihydrobenzo[b] [1,4]dioxin6-yl)-3-oxo-4-pyridinylmethyl)-2,3,4,5-tetrahydroben*	14.560	*1-octan-3-ol*	*11.736*	*alpha-pinene*
24	10,693	*methyl 2-propenyl disulfide*	13,697	*4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane*	15.802	*1-hexanol, 2-(hyroxymethyl){*	*13.234*	*benzanaldehyde*
25	11,696	*alpha.-pinene*	14,147	*beta.-pinene*	15.961	*3-carene*	*13.964*	*1,1 methylethyl bicycle (3,1,1)hextane*
26	11,714	*(1r)-2,6,6-trimethylbicyclo [3.1.1]hept-2-ene*	15,825	*1-methylene-4-(1-methylethenyl)-cyclohexane*	16.852	*p-cymene*	*14.169*	*6,6 dimethyl bicycle (3,1,1)heptane*
27	13,253	*benzaldehyde*	15,961	*3-carene*	17.120	*d-limonene*	*14.835*	*dihexadecyl phosphate*
28	13,952	*beta.-phellandrene*	16,848	*1-methyl-3-(1-methylethyl)-benzene*	21.313	*pentadecafluoro octanoic acid heptyl ester*	*15.829*	*alpha phelandrene*
29	14,147	*beta.-pinene*	17,119	*d-limonene*			*15.966*	*alpha pinene*
30	15,804	*2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene*	19,773	*diallyl disulphide*			*16.442*	*ethyl 2-hexatriade-3,3,3,-trifluoro-2-(3-metoxybenzyl amino propionate*

Table 2. Composition of Volatile Compounds in Beef, Pork, and Mixed Sausages (continued)

No	RT	beef sausage	RT	pork sausage	RT	mixed sausage (25,75%)	RT	mixed sausage (50, 50%)
31	16,425	*4terpinenyl acetate*	20,209	*(+)-4-carene*			*16.625*	*o-cymene*
32	16,848	*1-methyl-3-(1-methylethyl)-benzene*	21,325	*2-nonen-1-ol bicyclo[7.2.0]undec-4-ene*			*16.858*	*p-cymene*
33	17,119	*d-limonene*	36,163	*4,11,11-trimethyl-methylene,[1r-(1r*,4z,9s*)]-*			*17.124*	*d-limonene*
34	18,712	*1-chloro-tetradecane*					18.751	gamma terpinene
35	19,773	*diallyl disulphide*					19.793	dialyl bisulphate
36	20,012	*1-methyl-4-(1-methylethylidene)-cyclohexene*					20.197	1-methyl 4-(1-methylethylene cyclohexene,
37	20,189	*ethyl 2-benzamido-3-3-3-trifluoro-2-(3-methoxy benzylamino)propionate*					20.678	4-heptafluorobutyl oxyhexadecane
38	20,321	*1,1-bis(dodecyloxy)-hexadecane,*					21.067	3,7-dimethyl 1,6-octadiene-3-ol
39	21,052	*beta,-pinene molybdenum, di-.mu.*					21.329	pentadecafluorooctanoic acid heptyl ester
40	21,598	*bis(.eta.3-2propenyl)di-chlorobis(1,2,3,4,5,6-.eta.)-methylbenzene*					36.157	caryophylene
41	36,126	*caryophyllene*						

Multivariate Analysis (PCA)

The differentiation of fresh meat and sausage samples was evaluated using PCA models. PCA loading plots and biplots for fresh meat (pork and beef) are presented in **Figure 4A** and **4 B.** The PCA loading plot (p[1], p[2]) for fresh meat samples provided insight into the variables driving separation between pork and beef in the score space. Principal Component 1 (PC1) and Principal Component 2 (PC2) together explained the majority of variance in the dataset. Examination of the loadings revealed distinct patterns associated with chemical composition and quality attributes of the 2 meat types. Variables with high positive loadings on PC1 are strongly associated with pork samples (red), suggesting that these features represent characteristics typical of pork, such as higher intramuscular fat content, specific lipid-derived volatiles, or fatty acid profiles. Meanwhile, the variables that have a high negative correlation with PC1 were associated with beef samples (blue), which had more protein-related compounds, lean muscle markers, and volatile compounds formed as a result of amino acid degradation. This showed that PC1 was mainly used to capture a lipid-to-protein gradient, which was one of the major differences between pork and beef. PC2 was found to encode secondary differences, which could be associated with oxidation status, freshness indicators, or small volatile compounds. Strong positive loadings on PC2 of variables corresponded to aldehydes and alcohols related to lipid oxidation, and negative loadings were sulfur-containing or branched-chain volatiles related to species-specific metabolic processes. These results indicated that PC2 indicated stability to oxidation and species-specific aroma compounds. The similarity of variables in the loading plot indicated co-variation patterns, including those of lipid-derived volatiles clustering together, while protein degradation products were in a different cluster. This strengthened the fact that pork and beef were not only different in the formation of macronutrients, but also in their oxidative and flavor chemistry profiles. In general, the loading plot validated that PCA was able to determine chemical drivers of species differentiation. The variables that exhibited the highest absolute value in PC1 and PC2 must be taken as the possible biomarkers used to verify meat authenticity and quality.

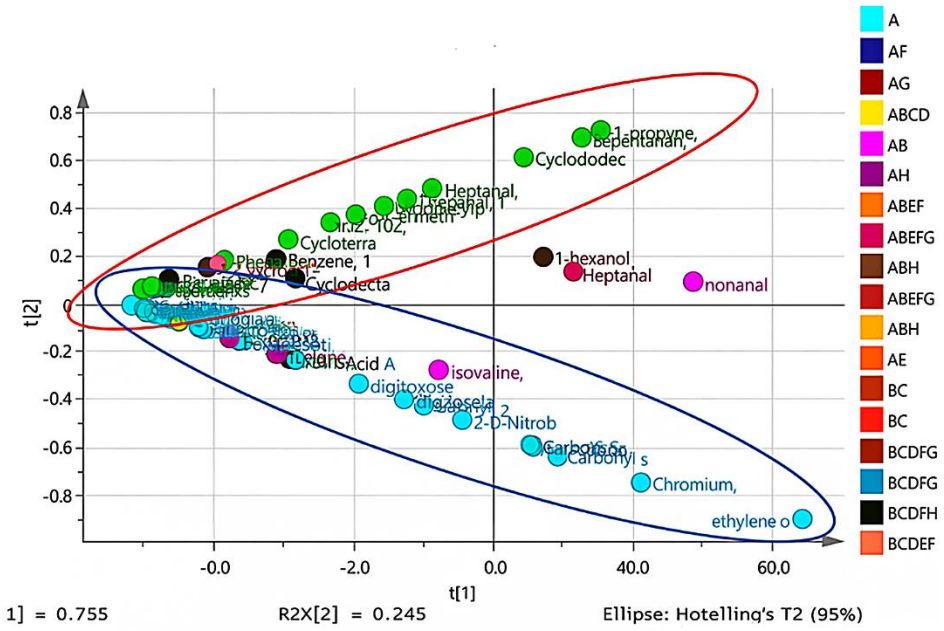


Figure 4A. Loading plot PCA for fresh meat (pork: red, beef: blue)

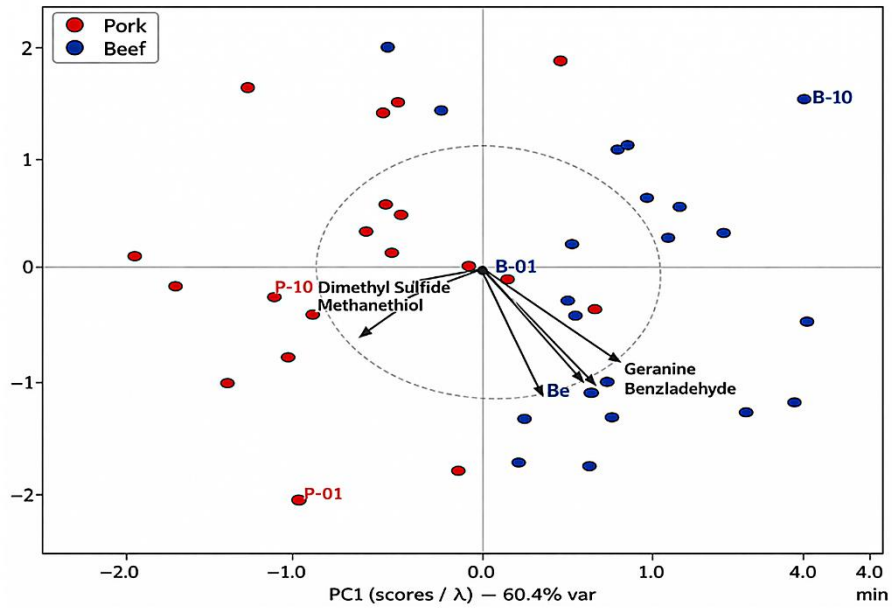


Figure 4B. PCA correlation biplot of fresh meat samples (Pork: red; Beef: blue)

PC1 and PC2; arrows denote variable loadings, plotted on the unit correlation circle. Lipid-derived aldehydes and sulfur volatiles (hexanal, nonanal, octanal, dimethylsulfide, methanethiol) accumulate on the pork side of PC1, as compared to protein/hemeplicitent related markers (creatine, carnosine, lactate, benzaldehyde, 2,3-butanedione) on the beef side. The strength of correlation to the PCs was shown by arrow length, allowing to identify chemical drivers of species differentiation.

PC1, as shown in **Figure 5A**, represented the compositional difference that was the strongest between pork and beef sausages. The high positive loading of variables on PC1 was anticipated to be associated with pork-specific features, including increased fat-derived volatiles (e.g., aldehydes such as hexanal and nonanal and sulfur compounds) and lipid oxidation indicators. Meanwhile, high negative loadings of PC1 variables indicated beef-specific features such as protein degradation products (e.g., creatine, carnosine) and volatiles of the Maillard reaction (e.g., 2,3-butanedione, benzaldehyde). This implied that PC1 was an indicator of lipid-protein gradient in fresh meat, but with processing and seasoning. PC2 was found to represent secondary differences, possibly associated with spice-related volatiles, curing agents, and oxidative stability. Sausages were frequently mixed with additional substances (salt, nitrites, spices), and new chemical indicators were born. Strong positive loadings on PC2 were on the spice-related compounds (e.g., terpenes, phenolics), and negative loadings might be on

the curing-related volatiles or smoke-derived phenols. This demonstrated that PC2 captured processing and seasoning effects, differentiating pork and beef sausages on top of their underlying meat composition.

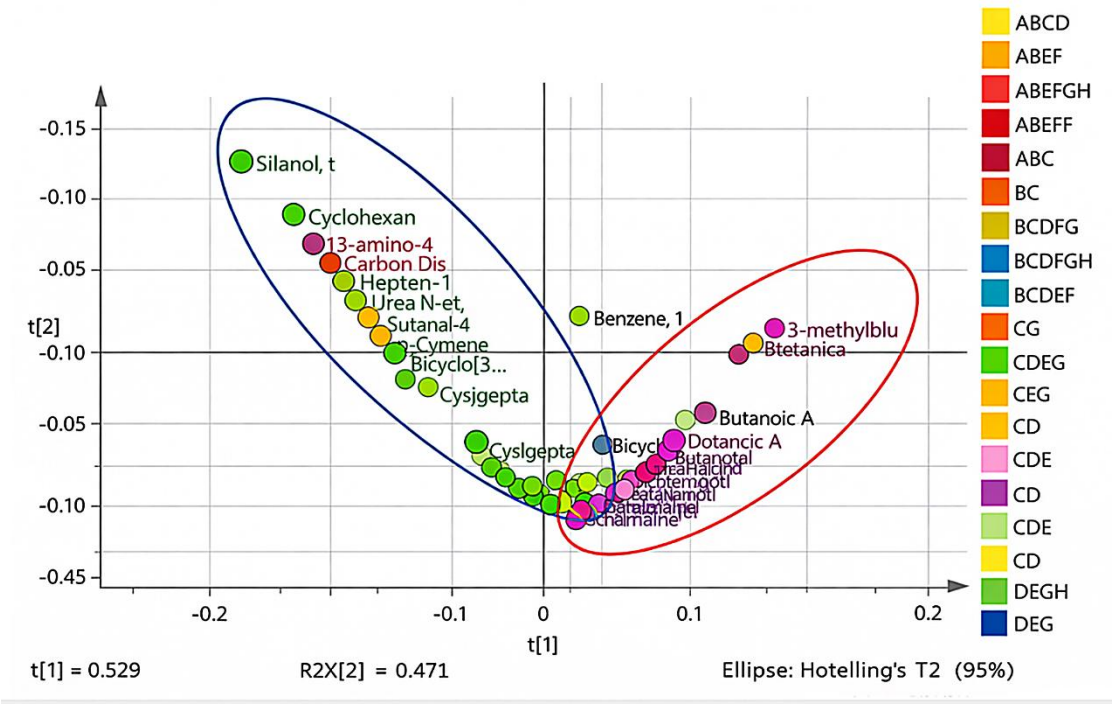
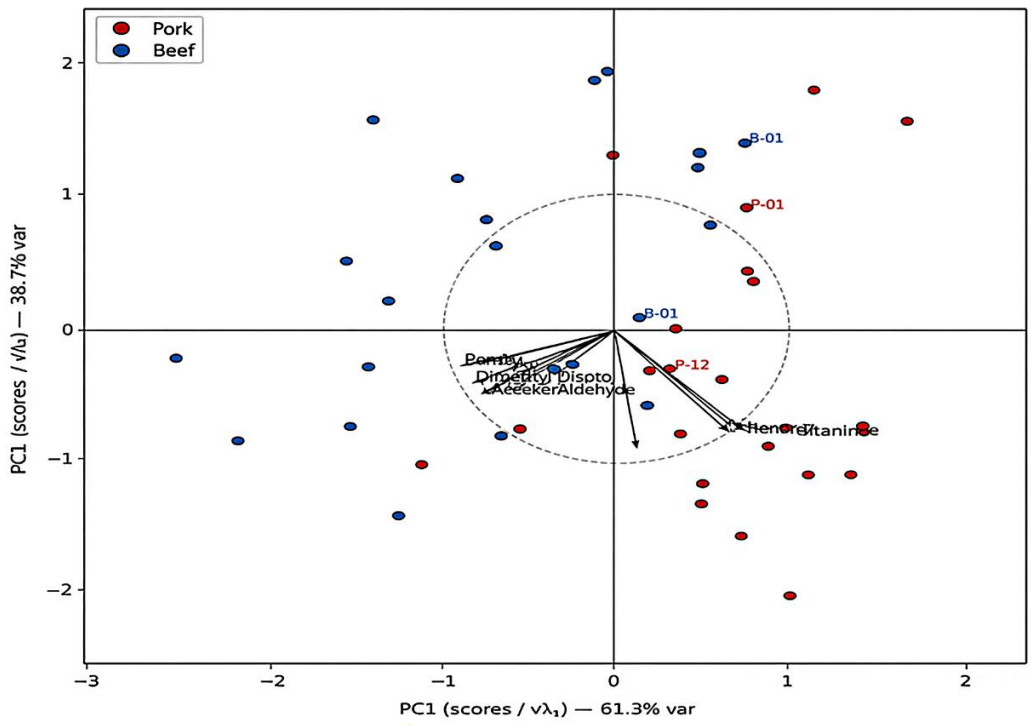


Figure 5A. PCA loading plot PCA for sausages (pork: red, beef: blue)

The long ellipse showed that variable variance was concentrated around a single axis (PC1), most discrimination was along the lipid or protein axis, while PC2 had a lower variance ratio, but introduced a refining dimension in the processing. Patterns of co-variation indicated by clusters of variables in the loading plot included the lipid oxidation volatiles clustering together and another group of compounds related to proteins, which was consistent with species and formulation differences. Therefore, the loading plot validates that PCA observes chemical drivers of species differentiation even in processed products. The variables having the highest absolute loadings on PC1 and PC2 must be taken into account as the possible markers of sausage authentication and quality control.



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Figure 5B. PCA correlation biplot Sausages (Pork: red; Beef: blue)

Figure 5B is a PCA correlation biplot of sausage samples, which represented the sample scores on PC1 and PC2. Arrows were used to show variable loadings, and these are drawn in the unit correlation circle. The LDAs included lipid derived aldehydes and sulfur volatiles (hexanal, heptanal, nonanal, octanal, dimethylsulfide and methanethiol) which load on the pork face of PC1, and protein/heme/Maillard markers (creatine, carnosine, 2,3 butanedione, pyrazines, aldehydes) loading on the PC2 records the seasoning/smoke effects and the formulations are differentiated by terpenes and phenols (limonene, eugenol, guaiacol, 4 methylguaiacol). The strength of correlation with the PCs was reflected in the strength of arrows, which revealed chemical factors driving species differentiation in processed meat (Figure 5B). The PCA correlation biplot did not look the same when comparing fresh meat and sausage due to the significant changes in the volatile profile as a result of the processing. In fresh meat, PCA separation was mainly caused by natural biochemical composition. Lipid oxidation aldehydes (hexanal, 1-octen-3-ol) and sulfur volatiles were present in pork, while protein/heme-derived products (benzaldehyde, 2,3 butanedione) are found in beef. Thermal processing, spices, and smoke add an additional class of compounds (phenolics, terpenes, Maillard products), generating new discriminatory variables in sausages. As a result, PC1 still indicates patterns of species related to lipid vs protein, but PC2 is dominated by the influence of processing, including guaiacol, 4-methylguaiacol, eugenol, and limonene. Such chemical alterations cause a shift in the clustering of the samples and generate PCA biplots that are distinctly different compared to fresh-meat models.

The PCA score plot (Figure 6) showed that there was a sharp division of the sample groups on the first 2 principal components, indicating the presence of different volatilomic signatures between meat types. The model explained 38.0% of the total variance on PC1 ($R^2X[1] = 0.38$) and 25.5% on PC2 ($R^2X[2] = 0.255$), which was within an acceptable range for exploratory chemometric analysis of complex VOC datasets. Pure beef sausage samples were a separate cluster that was found in the upper-left quadrant, with higher p[2] values. However, the pure pork sausage was located in the lower-left quadrant, with a considerably dissimilar profile of VOCs than beef. This separation implied that PC2 interacts with chemical properties of relatively strong association with species-specific volatile compounds.

The mixed sausage samples had a middle ground in comparison to the two pure meat groups. The 25:75 (beef: pork) blend was plotted nearer to the pork sausage cluster, while the 50:50 blend was in a central area between the beef and pork clusters. This gradational distribution showed the PCA model was sensitive enough to show compositional variations based on the percentage of each meat type. Consequently, the results of the PCA indicated that the headspace VOC profiling with chemometric analysis could be effectively used to distinguish between beef, pork, and mixed sausages. The clear grouping of PC1 and PC2 also substantiated the possibility of volatilomics as a quick screening assay of meat authentication and adulteration detection.

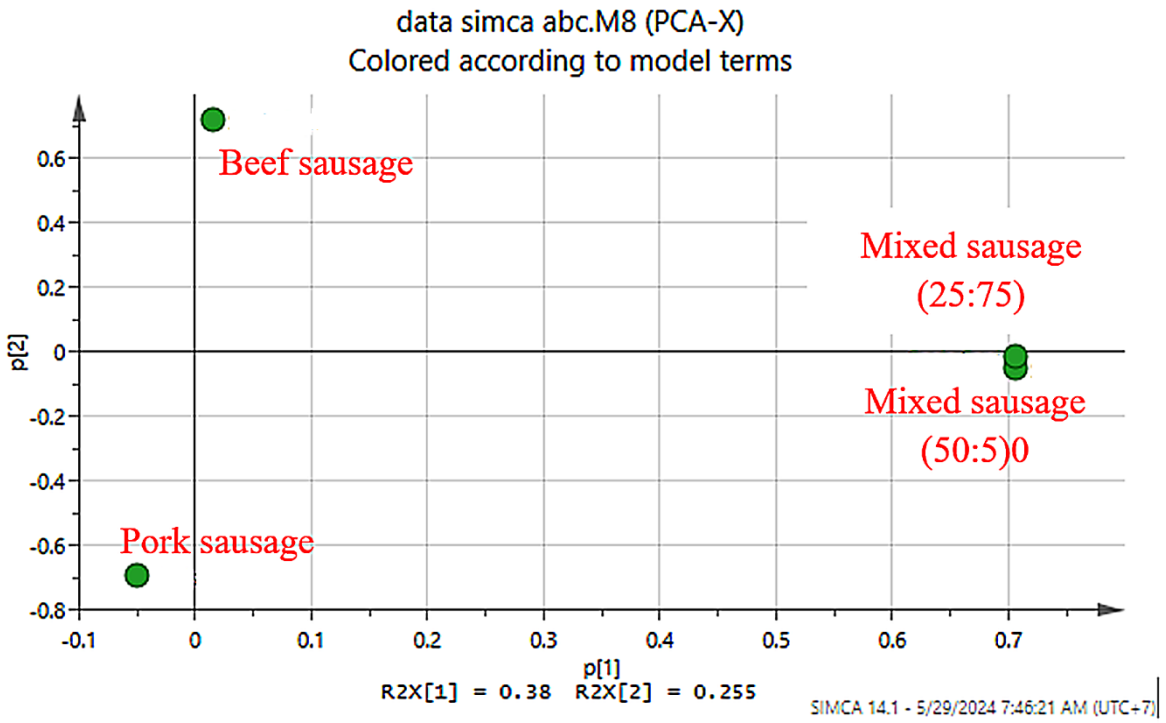


Figure 6. Score plot PCA for beef sausage, pork sausage, and mixed sausage

According to the PCA, PC2 was a processing input that depended on the formulation and smoking conditions, in particular, smoke phenolics (guaiacol, 4-methylguaiacol, eugenol) and spice/terpene markers (e.g., limonene), which were reported in controlled smoking experiments and GC IMS/PCA comparisons of commercial products (Zhao et al., 2024). These results suggested that species-driven chemistry (PC1) was still strong even in processed matrices, while process-specific volatiles created an orthogonal dimension (PC2).

In sausages, PC1 was still able to separate pork and beef on the basis of the same lipid vs protein/heme comparison. However, PC2 was motivated by phenolic smoke components (guaiacol, 4-methylguaiacol, syringol, eugenol) and terpenes (e.g., limonene), which are a result of seasoning and smoking. These phenolics are observed to be the predominant smoke markers in controlled studies in both raw and fermented sausages, where their levels are dependent on the density of smoke, the moisture content of the wood used, and ventilation conditions. PCA loadings can capture such features, and unit circle biplots in which the phenolics are directed towards processed formulations. Moreover, GC-IMS in conjunction with PCA showed significant disparity in brand or formulation differences in commercial sausages, with phenolics/ketones as some of the essential volatiles, which validated the interpretation of PC2 as a processing dimension (Yoo et al., 2005). In species discrimination, hexanal, 1-octen-3-ol (pork), and nonanal are used as candidates; in process monitoring, guaiacol, 4-methylguaiacol, eugenol, and limonene reflect contributions of smoke/spices. These choices were consistent with published VIP selections and Random Forest selections in SPME-GC-MS datasets and need to be checked in the dataset through OPLS-DA (VIP > 1), cross-validation, and permutation testing (Pranata et al., 2021). Since the phenolics of smoke change with the processing conditions, their PC2 loadings could be exploited to optimize the processes with consideration of PAH minimization principles (Ahamed et al., 2024).

The combination of pork and beef in sausage formulations leads to observable changes in volatile compound profiles because of the differences in lipid oxidation pathways, protein chemistry, and matrix-release behavior. These changes were mainly caused by lipid oxidation because pork and beef fats do not have the same fatty acid composition and oxidative stability, which causes different patterns of aldehyde and ketone formation upon interaction. Moreover, the presence of sulfur in volatiles is exacerbated in mixed sausages as a result of the interactions between various amino acid pools and the muscle protein system. Mixed matrices also promoted the Maillard reaction, showing that there was more availability of combined precursors. Volatile release was subject to matrix effects, with beef proteins showing a higher binding affinity to particular aroma compounds, while pork fat had a higher propensity to entrap volatiles. This suggested that the volatiles were inhibited and enhanced,

respectively, by the mixing process. Phenolics and terpenes in spices and smoke also partition differently in pork-rich and beef-rich systems, further complicating the aroma properties of mixed-meat sausages.

Mixed pork-beef sausages produced typical volatile signatures that can not be simply explained as a combination of pork and beef volatile signatures. Numerous pork and beef-specific volatiles were retained, while others were lost by dilution, thermal enrichment, the suppression of the volatile by the matrix, or overshadowing of the volatile by other markers. Dehydration resulted in the formation of new volatiles, particularly sulfur compounds and Maillard-formed aldehydes due to the interaction of pork with high PUFA levels, beef with more protein/heme ratios, and spice/smoke constituents during heating. Consequently, a 25:75 mixture was more pork-like, and the 50:50 mixture generated a unique intermediate volatilome, which produced unique PCA clustering patterns.

4. Conclusion

In conclusion, this study shows that volatilomic analysis can be used to substantially distinguish between beef and pork and the corresponding sausage products in terms of volatile compounds composition. Headspace-GC-MS with the multivariate analysis (PCA) identifies several compounds that are used to authenticate a species and differentiate products. The PCA of volatile profiles of fresh meat and sausages shows that species differentiation (pork vs. beef) is primarily due to a lipid oxidation/protein/heme/Maillard gradient reflected by PC1, and that PC2 is due to formulation and processing effects (smoke phenolics and spice terpenes). In fresh meat, pork samples are correlated with aldehydes and sulfur volatiles (e.g., hexanal, nonanal), but beef samples with nitrogenous and Maillard volatiles (e.g., pyrazines, 2,3 butanedione, benzaldehyde). Some VOCs identified in this study, such as N, N dimethyl hexacosylamine, tetrahydrothiophene, methyl thiophene and tetrahydro 6 methyl 2H pyran 2 acetic acid were only found in the mixed pork-beef sausages prepared in a 25:75 and 50:50 ratio showing that the volatilome These distinctive VOCs show that novel chemical reactions between pork- and beef derived lipid, protein, and sulfur bearing precursors do happen throughout thermal processing leading to the generation of mixed matrix-specific volatile compounds. The mentioned markers provide a viable potential for authentication and quality control of meat, and the orthogonal processing dimension emphasized the necessity to consider variability in formulation when developing multivariate models.

CRediT Authorship Contribution Statement

Sandra Hermanto : Writing – original draft, Writing–review & editing, Formal analysis, Methodology. **Ahmad Fathoni :** Writing – original draft, Writing – review & editing, Formal analysis, Methodology. **Barita Juliano Siregar:** Writing – review & editing, Methodology. **Denny Alfarus:** Writing – review & editing, Methodology.

Acknowledgments

The authors are grateful to the Research and Community Service Institute of the Syarif Hidayatullah State Islamic University (UIN) Jakarta for funding this study through Grant Number: B-303/LP2M-PUSLITPEN/TL.02/2023. The Head of Puslitpen (Research and Publishing Center) and the Head of the Integrated Laboratory Center, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta and the Head of the Biotechnology Laboratory at BRIN (National Research and Innovation Agency) are also appreciated to provide facilities and support of the study activities and sample analyses.

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Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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