

## Rapid discrimination of beef, pork and rabbit meat using HS-SPME coupled with GC-MS and chemometric analysis

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### ABSTRACT

Species adulteration in meat products remains a persistent challenge for food safety, regulatory compliance, and consumer confidence, especially in products labeled as rabbit, pork, or beef. This study developed and validated a rapid, non-targeted volatilomic strategy based on headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME–GC–MS) for the authentication of minced samples from these three species. A total of 28 volatile compounds (VOCs), predominantly aldehydes and fatty acids associated with lipid oxidation pathways, exhibited statistically significant interspecies differences and distinct species-specific distribution patterns. Multivariate data exploration using t-Distributed Stochastic Neighbor Embedding (t-SNE) demonstrated complete cluster separation among rabbit, pork, and beef samples. Supervised machine-learning algorithms, including Random Forest (RF), eXtreme Gradient Boosting (XGBoost), and Artificial Neural Network (ANN), achieved high classification performance, with overall accuracies ranging from 90.3 to 94.4%; the ANN model yielded the best predictive performance. Rabbit meat was distinguished with near-perfect precision, primarily attributable to distinctive fatty acid-derived volatile markers. The proposed method requires less than 60 min per sample, involves minimal sample preparation, and relies on widely available analytical instrumentation, supporting its applicability as a practical, cost-effective tool for routine meat authenticity assessment in both raw and processed products.

### 1. Introduction

Meat constitutes a fundamental component of the human diet, providing highly bioavailable complete proteins, long-chain polyunsaturated fatty acids, essential minerals (e.g., zinc, selenium, iron, and phosphorus), and B-complex vitamins that collectively contribute to immune competence and overall physiological function (Zhao et al., 2025). With increasing global living standards and heightened consumer awareness of nutritional quality and product origin, demand for premium and specialty meat products has grown substantially, reinforcing the economic importance of the global meat sector (Zhang et al., 2023). Flavor is among the most influential attributes determining consumer acceptance of meat products. Volatile organic compounds (VOCs), particularly those generated during thermal processing, are key chemical contributors to aroma and overall flavor perception (Li et al., 2024). These compounds arise primarily from complex heat-induced reactions,

including the Maillard reaction, lipid oxidation, and interactions between these pathways (Wang et al., 2024). The qualitative and quantitative composition of VOCs is strongly influenced by species-specific factors such as fatty acid profile, muscle structure, and endogenous enzyme activity, making volatilomic profiling a promising approach for meat differentiation and authentication.

Meat and meat products have historically been susceptible to both intentional and unintentional adulteration, including the substitution or undeclared addition of lower-value species (Pavlidis et al., 2019). Such practices pose economic, ethical, and, in certain cases, health-related risks, and they undermine consumer trust. Consequently, regulatory authorities require standardized, validated analytical methods capable of verifying species authenticity with high sensitivity and specificity (Ruiz Orduna et al., 2017). A wide range of analytical techniques has been employed for this purpose, including immunoassays, enzymatic assays, DNA-based methods (e.g., PCR), spectroscopic and

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chromatographic approaches, and electronic nose systems (Ahamed et al., 2024).

Hyperspectral imaging (HSI) has recently gained attention as a non-destructive tool for evaluating meat quality and authenticity. Applications, including muscle-type discrimination and quality prediction in lamb (Kamruzzaman et al., 2013), classification of cooked and sliced turkey ham, prediction of physicochemical and microbial attributes in beef (e.g., water-holding capacity, pH, color, tenderness, and microbial spoilage), grading, and sensory prediction in pork (Qiao et al., 2007), detection of defects and contaminants in poultry (Park et al., 2007), and estimation of water and fat content in fish fillets (Kamruzzaman et al., 2013).

Despite its advantages, the broader implementation of HSI in routine industrial settings remains limited by high equipment costs and computational demands associated with large spectral datasets.

GC-MS represents a highly robust and sensitive analytical technique for the separation, identification, and semi-quantification of VOCs in complex food matrices. When combined with headspace solid-phase microextraction (HS-SPME), GC-MS enables efficient, solvent-free extraction of volatile compounds with minimal sample preparation. This approach offers excellent reproducibility and sensitivity for detecting trace-level compounds derived from lipid oxidation and Maillard chemistry, facilitating both quality assessment and authenticity verification across fresh, processed, and stored meat products (Xu et al., 2025).

To date, although HS-SPME-GC-MS has been extensively applied to characterize volatile profiles of individual meat species and to detect adulteration in binary mixtures (e.g., pork in beef or vice versa), no previous study has systematically investigated its integration with advanced unsupervised visualization techniques (such as t-SNE) and supervised machine-learning classifiers for the simultaneous discrimination and authentication of three distinct species—rabbit, pork, and beef—in minced form. These three species are widely consumed and have been implicated in documented adulteration incidents, particularly in Spain and other European markets, where rabbit meat, as a premium and niche product, is frequently susceptible to substitution with more common and lower-cost species such as pork or beef. Accordingly, the objective of the present study was to develop and validate a rapid, non-targeted volatilomics-based methodology combining HS-SPME-GC-MS with multivariate statistical analysis and these advanced machine-learning algorithms for the accurate classification and authentication of rabbit, pork, and beef meat. By establishing species-specific volatile fingerprints—especially capturing unique markers such as medium- and long-chain saturated fatty acids in rabbit—and rigorously evaluating predictive model performance, this work aims to provide a scientifically robust, operationally feasible, and highly performant framework for routine meat authenticity testing in regulatory and industrial contexts.

## 2. Materials and methods

### 2.1. Meat sample preparation

Four independent fresh samples each of rabbit, pork, and beef were sourced from different butcher shops and local markets across the city of Orihuela (Alicante, Spain) to incorporate natural biological and commercial variability. All samples were transferred to the laboratory under refrigerated conditions and processed within 2 h of purchase. Visible connective tissue and external fat were trimmed to minimize non-muscle variability. Each samples were minced using a commercial meat grinder with a 4-mm plate aperture, thoroughly homogenized to ensure uniformity, and were analyzed within 2 h after mincing while stored at 4 °C (Pavlidis et al., 2019).

### 2.2. HS-SPME preparation for volatile compounds extraction

Volatile organic compounds were extracted using HS-SPME based on

the method of Pavlidis et al., 2019, with minor modifications. Briefly, 2.5 g of homogenized minced meat was transferred into a 20 mL vial. Subsequently, 5 mL of 25% (w/v) NaCl solution was added to promote salting-out effects and enhance VOCs partitioning into the headspace. The mixture was vortexed for 2 min to ensure homogeneous dispersion. A 1 cm fused-silica SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm thickness) was employed for extraction, owing to its broad affinity for both low- and mid-molecular-weight volatiles. Headspace extraction was conducted at 60 °C for 30 min under continuous agitation at 500 rpm. All extractions were performed using a Shimadzu AOC-6000 Plus autosampler (Shimadzu Corporation, Kyoto, Japan), which provided precise temperature control, agitation, and automated fiber handling, thereby ensuring analytical reproducibility. After extraction, the SPME fiber was immediately introduced into the GC injection port for thermal desorption.

### 2.3. GC-MS analysis of VOCs

Gas chromatographic separation was carried out using an SLB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Teknokroma, Barcelona, Spain). Helium (purity ≥ 99.999%) was used as the carrier gas at a flow rate of 1 mL/min. Injections were performed in split mode (split ratio 1:2) at 250 °C using a narrow-bore SPME liner (0.75 mm i.d.; Agilent Technologies). The oven temperature program was as follows: initial temperature 40 °C (held for 5 min), increased to 150 °C at 4 °C/min, then ramped to 250 °C at 30 °C/min, and held at 250 °C for 5 min. The transfer line temperature was maintained at 280 °C.

Mass spectrometric detection was conducted in electron ionization (EI) mode at 70 eV under full-scan acquisition ( $m/z$  29–350), with a scan rate of 4.37 scans/s. The ion source and quadrupole temperatures were set to 230 °C and 150 °C, respectively. Volatile compounds identification was achieved using a combination of complementary approaches: (i) calculation of retention indices based on analysis of a C8–C24 n-alkane standard mixture (Sigma-Aldrich, Steinheim, Germany); (ii) comparison of retention times with those of authentic reference standards analyzed under identical conditions; (iii) matching of experimental mass spectra with reference spectral databases (Wei et al., 2023). Only compounds meeting retention index agreement and spectral similarity criteria were considered reliably identified.

### 2.4. Chemometric analysis

Supervised machine-learning techniques are particularly well-suited for processing complex volatilomic datasets (chromatographic data) that exhibit only minor differences between classes. Accordingly, the most commonly employed classification algorithms in similar studies include Linear Discriminant Analysis (LDA), Partial Least Squares–Discriminant Analysis (PLS-DA), Random Forest (RF), and Decision Tree (DT) (Pourmoradian et al., 2025).

All statistical and machine-learning analyses were performed using Python 3.7. For supervised classification, three algorithms were implemented: RF, XGBoost, and ANN. Prior to model training, raw GC-MS data underwent standard preprocessing including baseline correction, retention index-based peak alignment, and normalization of peak areas to the total ion current (TIC). Missing values were imputed with column means, and all features were scaled to zero mean and unit variance using StandardScaler.

#### 2.4.1. Exploratory analysis

Unsupervised visualization of the high-dimensional volatilomic dataset was conducted using t-SNE, a non-linear dimensionality-reduction technique that preserves local similarity structure in complex datasets. This approach enabled visual assessment of natural clustering patterns among rabbit, pork, and beef samples.

### 2.4.2. Supervised classification

The supervised machine-learning algorithms implemented to evaluate species classification performance: Random Forest (RF), eXtreme Gradient Boosting (XGBoost), and Artificial Neural Network (ANN). The dataset was randomly divided into training (70%) and test (30%) subsets. A fixed random\_state (42) was applied to ensure reproducibility. No hyperparameter optimization (e.g., grid search or cross-validation tuning) was performed; default parameters were used as described below.

**Random forest (RF):** Implemented using scikit-learn's RandomForestClassifier (n\_estimators = 100, criterion = 'gini', random\_state = 42).

**XGBoost:** Implemented using XGBClassifier, with use\_label\_encoder = False, eval\_metric = 'mlogloss', and random\_state = 42; other [arameters were set to default values.

**ANN:** Constructed using MLPClassifier with one hidden layer containing 100 neurons (hidden\_layer\_sizes = 100), maximum iterations = 500 (max\_iter = 500), and random\_state = 42. All other parameters, including activation function and solver, were kept at their default values. Default activation function and solver settings were retained.

### 2.4.3. Model evaluation

Model performance was evaluated on the independent test set using accuracy, sensitivity (true positive rate), and specificity (true negative rate), providing complementary measures of classification reliability (Egido et al., 2024). These metrics were calculated according to standard definitions (Ropciuc et al., 2023; Pourmoradian et al., 2026):

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \quad (1)$$

$$\text{Sensitivity} = \frac{TP}{TP + FN} \quad (2)$$

$$\text{Specificity} = \frac{TN}{TN + FP} \quad (3)$$

Where TP, TN, FP, and FN represent true positive, true negative, false positive, and false negatives, respectively. Collectively, this chemometric framework enabled robust discrimination of meat species based on their volatilomic fingerprints and provided a reproducible pipeline for routine authenticity assessment. Also, stratified 3-fold cross-validation was applied to the training set to select optimal hyperparameters.

### 2.5. Repeatability and sample stability

Repeatability (intra-day precision) was evaluated by analyzing one randomly selected sample from each species (rabbit, pork, and beef) in three independent replicate extractions performed on the same day using the same SPME fiber and GC-MS conditions. The relative standard deviations (RSDs) of peak areas for major volatile compounds (hexanal, nonanal, 1-octen-3-ol, and total aldehydes) were calculated. All RSDs were below 12%, indicating acceptable repeatability.

The stability of volatile profiles in minced meat stored at 4 °C was evaluated at 0, 2, 4, and 6 h post-mincing. All samples were analyzed in duplicate. No statistically significant changes in the relative abundance of major VOCs were observed within the first 4 h.

As this study employs a non-targeted volatilomic fingerprinting approach for class discrimination rather than absolute quantitation of specific markers, formal limits of detection (LOD) and quantitation (LOQ) were not calculated. For routine authentication applications, relative peak areas normalized to total ion current (TIC) are sufficient for species differentiation, as demonstrated by the clear clustering and high classification accuracy.

## 3. Results and discussion

### 3.1. Volatile compound fingerprinting by GC-MS

HS-SPME-GC-MS was used to characterize the VOCs contributing to the aroma profiles of fresh minced rabbit, pork, and beef. A total of 28 compounds were positively identified across the three species (Table 1). These compounds belonged predominantly to aldehydes, alcohols, ketones, carboxylic acids, and furan derivatives- chemical classes commonly associated with lipid autoxidation and early Maillard-type reactions in raw muscle tissues.

Aldehydes represented the most abundant and consistent class across all samples. Hexanal, heptanal, octanal, nonanal, and their unsaturated aldehydes (E)-2-octenal, (E)-2-nonenal, and (E)-2-decenal dominated the chromatographic profiles. As shown in Table 1, beef exhibited substantially higher relative abundances of both straight-chain and unsaturated aldehydes compared with pork and rabbit. Hexanal, a well-established marker of linoleic acid oxidation, accounted for 45.1%, 23.3%, and 52.0% of the total chromatographic peak area in rabbit, pork, and beef, respectively. Among alcohols, 1-octen-3-ol (characterized by a mushroom-like aroma) and 1-hexanol were prominent in all three species, again reaching their highest relative concentrations in beef (Table 1). Pronounced species-specific differences were observed. Medium- and long-chain saturated fatty acids (decanoic, dodecanoic, tetradecanoic, and pentadecanoic acids) were detected almost exclusively in rabbit meat, whereas pork displayed the lowest overall abundance of both aldehydes and fatty acids among the three species. The exclusive presence of these fatty acids in rabbit provides chemically distinctive markers that enhance its discriminability.

The present finding is consistent with previously published volatilomic studies of individual meat species. The pork profile closely aligns with the dominant compounds (pentanal, hexanal, heptanal, octanal, nonanal, 1-pentanol, 1-hexanol, 1-octen-3-ol, 2,3-octanedione, and E-2-octenal) reported by Li et al. (2022) using HS-SPME-GC-MS. Similarly, the beef volatile fingerprint observed here corresponds well with lipid-derived aldehydes and alcohols identified as major aroma contributors in roasted beef (Zhang et al., 2023), confirming that these

**Table 1**  
Volatile compounds detected in rabbit, pork and beef by HS-SPME-GC-MS (%).

Volatile Compounds	RT	Beef	Pork	Rabbit
Pentanal	3.65	0.81	1.14	1.71
Acetoin	3.87	2.32	9.08	3.44
1-Pentanol	5.62	0.80	1.62	1.30
Hexanal	6.76	51.99	23.27	45.05
1-Hexanol	9.75	0.65	1.81	1.36
2-Heptanone	10.58	0.61	0.51	0.36
Heptanal	11.11	4.67	2.22	2.32
1-Heptanol	14.20	0.36	0.31	0.30
1-Octen-3-ol	14.61	5.87	2.21	3.24
2,3-Octanedione	14.82	7.77	3.68	4.96
Furan, 2-pentyl-	15.00	1.76	-	0.62
2,4-Heptadienal, (E,E)-	15.30	-	-	0.21
Octanal	15.58	4.76	1.62	1.29
1-Hexanol, 2-ethyl-	16.68	-	-	3.43
2-Octenal, (E)-	17.88	0.24	0.49	0.86
2-Octen-1-ol, (E)-	18.28	0.63	0.21	0.24
1-Octanol	18.46	0.57	0.80	0.43
2-Hexen-1-ol, acetate, (E)-	18.90	0.26	-	-
Nonanal	19.79	7.87	4.60	3.01
2-Nonenal, (E)-	21.94	0.23	0.19	0.13
Decanal	23.71	0.15	0.15	0.09
2,4-Nonadienal, (E,E)-	24.05	-	-	0.20
2-Decenal, (E)-	25.75	0.10	-	0.12
2,4-Decadienal, (E,E)-	27.72	-	-	0.15
n-Decanoic acid	29.33	-	-	1.53
Dodecanoic acid	34.20	-	-	0.18
Tetradecanoic acid	35.80	-	-	0.15
Pentadecanoic acid	37.01	0.11	-	0.17

compounds are already present in significant amounts in the raw state as oxidation precursors. For rabbit meat, the detection of medium- and long-chain fatty acids together with elevated hexanal and 1-octen-3-ol levels corroborates the characteristic volatile pattern previously described by Xie et al. (2016). Overall, these results demonstrate that each species exhibits a reproducible and chemically distinctive volatile fingerprint, primarily governed by differences in fatty acid composition and oxidative susceptibility. The interspecies differences in volatile profiles were primarily assessed through multivariate visualization (t-SNE) and supervised machine-learning classification rather than univariate statistical tests, consistent with current approaches in non-targeted volatilomics. Additionally, further controlled feeding trials would be beneficial to fully confirm the genetic/species origin of these markers independent of environmental factors.

### 3.2. Chemometric analysis of volatilomic data

#### 3.2.1. Unsupervised visualization by t-SNE

t-SNE is a non-linear dimensionality-reduction algorithm designed to project high-dimensional data into a low-dimensional space while preserving local neighborhood structure. By transforming pairwise Euclidean distances into conditional probability distributions and optimizing a corresponding Student's t-distribution in the embedded space, t-SNE effectively reveals clusters and natural separations in complex analytical datasets, such as chromatographic or mass-spectral profiles (Saito et al., 2024). To assess the intrinsic separability of the volatilomic dataset without using class labels, t-SNE was applied to the normalized peak-area matrix comprising all identified VOCs. The resulting two-dimensional projection (Fig. 1) revealed three distinct, non-overlapping clusters corresponding precisely to rabbit, pork, and beef samples. Beef samples formed a compact and cohesive cluster, indicating limited intra-species variability. Pork samples occupied a slightly more dispersed region yet remained clearly separated from the other species. Rabbit samples formed a tight and well-defined cluster, completely distinct from both pork and beef. The absence of cluster overlap underscores the presence of strong species-specific signatures within the volatile profiles. These findings demonstrated that even

without supervised learning, the chemical information contained in the VOCs dataset is sufficient to enable clear discrimination among the three species. Similar results have been reported by Sani et al. (2025), who obtained clear t-SNE-based separation of anatomical parts from different animal species, further supporting the suitability of this method for food authenticity applications.

#### 3.2.2. Supervised machine-learning classification

Three supervised machine-learning algorithms- RF, XGBoost, and ANN- were evaluated using 30% of the dataset as an independent test set. Performance metrics are summarized in Table 2, and confusion matrices are presented in Fig. 2.

The ANN (MLPClassifier) achieved the highest overall accuracy of (94.44%), followed by XGBoost (90.50%), and RF (90.28%). All models demonstrated strong discriminatory capability, with class-wise F1-

**Table 2**  
Comparative performance of classification models.

Model	Evaluation metric	Samples		
		Beef	Pork	Rabbit
RF	Precision	0.88	0.85	1.00
	Recall	0.83	0.97	0.88
	F1-score	0.86	0.90	0.94
	Sensitivity	0.83	0.96	0.88
	Specificity	0.96	0.88	1.00
	<b>Accuracy= 90.28%</b>			
XGBoost	Precision	0.89	0.90	0.92
	Recall	0.89	0.90	0.92
	F1-score	0.89	0.90	0.92
	Sensitivity	0.88	0.89	0.92
	Specificity	0.96	0.93	0.95
	<b>Accuracy= 90.50%</b>			
ANN	Precision	0.89	0.94	0.92
	Recall	0.97	0.97	0.97
	F1-score	0.96	0.92	0.94
	Sensitivity	0.94	0.96	0.92
	Specificity	0.96	0.97	0.97
	<b>Accuracy= 94.44%</b>			

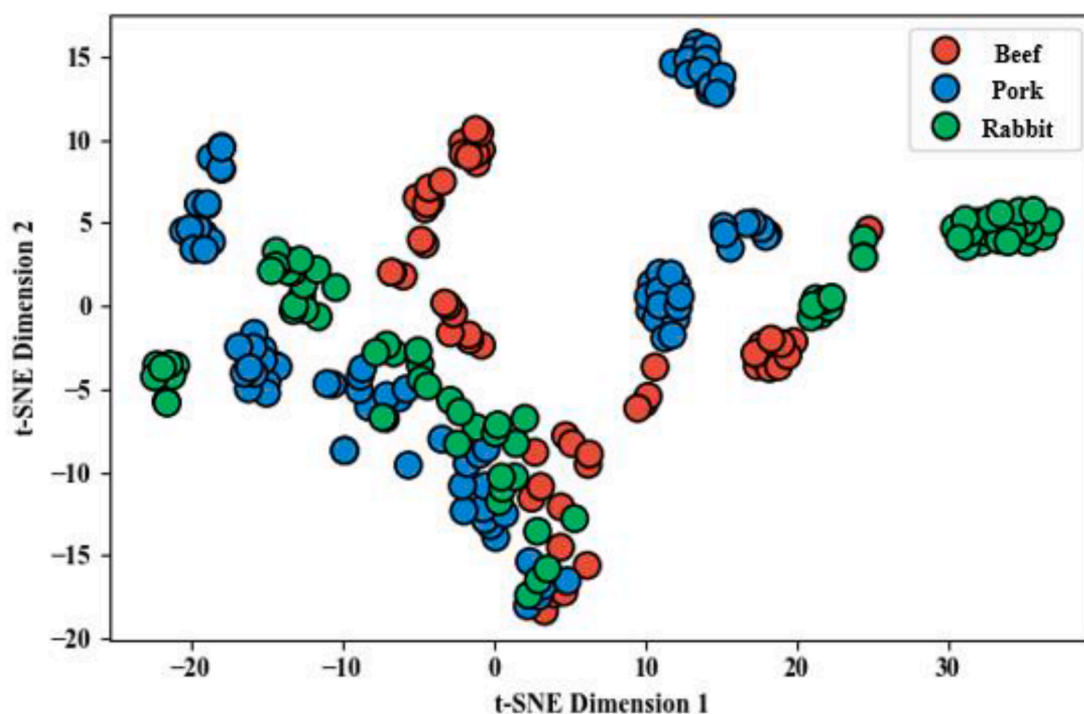


Fig. 1. t-SNE plot showing clustering of different meat samples.

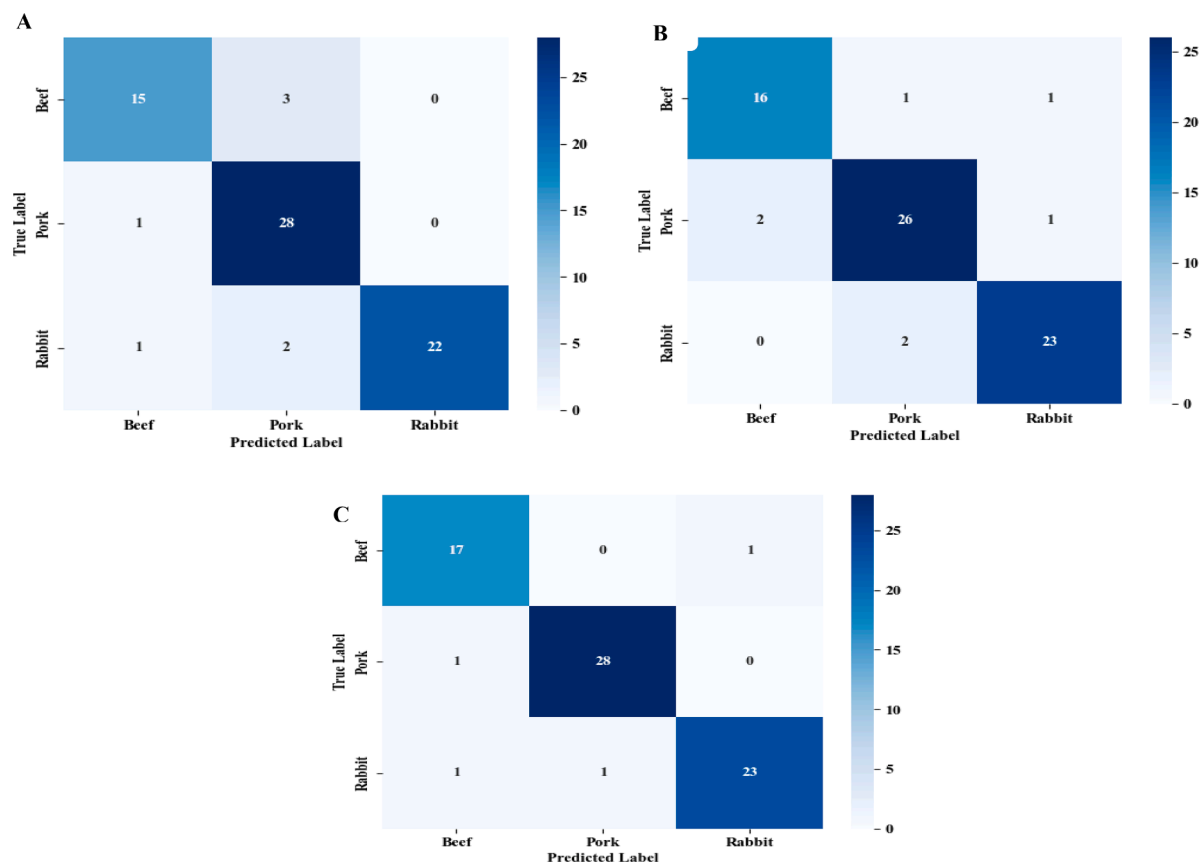


Fig. 2. Confusion matrices A: Random forest (RF); B: XGBoost; C: Artificial neural network (ANN).

scores ranging from 0.86 to 0.96, indicating balanced performance across species. The ANN model exhibited the most consistent results, with recall and sensitivity values  $\geq 0.94$  for beef and pork, and  $\geq 0.92$  for rabbit. Precision and F1-scores remained uniformly high (0.89–0.97), and specificity exceeded 0.96 for all classes, reflecting outstanding ability to correctly identify non-target species. Comparable performance of ANN for meat classification has been reported by Bristy et al. (2025), who achieved 90% accuracy in discriminating cooked broiler and duck meat.

XGBoost demonstrated highly balanced performance across all species (precision, recall, and F1-scores between 0.89 and 0.92), with minimal deviation among class-wise metrics, underscoring its robustness and resistance to overfitting in high-dimensional biological datasets. Similar robustness of XGBoost has been observed in livestock monitoring studies involving heterogeneous physiological and environmental data (Ma et al., 2024).

RF performed particularly well in identifying rabbit meat (precision = 1.00, specificity = 1.00, F1-score = 0.94), benefiting from the presence of distinctive medium- and long-chain fatty acids. A slightly lower recall for beef (0.83) limited its overall accuracy relative to ANN and XGBoost. RF has similarly demonstrated strong performance in meat classification and microbial population studies (Hanif et al., 2022).

Across all algorithms, rabbit meat was classified with the highest precision and specificity, consistent with its unique volatile fingerprint. Pork and beef, which share more similar aldehyde-dominated profiles, required the enhanced non-linear modeling capacity of ANN and XGBoost to achieve optimal separation.

These results confirm that HS-SPME-GC-MS-derived volatile profiles contain sufficient species-specific information to enable accurate, rapid, reliable, and objective authentication of minced rabbit, pork, and beef using standard machine-learning techniques, even without extensive hyperparameter optimization. The ANN model, achieving 94.44%

overall accuracy with no class F1-score below 0.92, is recommended as the most effective classifier for routine screening applications. Additionally, Cross-validation using three stratified folds confirmed the robustness of all models, with mean accuracies of 90.3% (RF), 90.5% (XGBoost), and 94.4% (ANN), as shown in Fig. 3. The low standard deviations (<4%) indicate minimal performance variation across folds, supporting the generalizability of the proposed approach

#### 4. Discussion

The present study robustly demonstrates that HS-SPME-GC-MS, when integrated with advanced chemometric and machine-learning tools, offers a rapid, non-targeted, and highly reliable strategy for the simultaneous authentication of minced rabbit, pork, and beef. The unequivocal complete cluster separation achieved via t-SNE visualization, coupled with classification accuracies reaching up to 94.44% (particularly with the superior ANN model), confirms the existence of highly reproducible, species-specific volatilomic fingerprints even in raw minced meat samples—highlighting the method's exceptional discriminatory power with minimal sample preparation and analysis time around 60 min.

The dominant drivers of this discrimination stem from distinct patterns in lipid-derived volatile compounds, reflecting fundamental differences in intramuscular fat composition and oxidative susceptibility across species. Beef and pork exhibited markedly higher relative abundances of straight-chain and unsaturated aldehydes—such as hexanal (a primary marker of linoleic acid oxidation), nonanal, (E)-2-octenal, and (E)-2-decenal—consistent with their richer content of oleic and linoleic acid triglycerides. In striking contrast, rabbit meat was distinguished by the exclusive or near-exclusive presence of medium- and long-chain saturated fatty acids (C10:0–C16:0, including decanoic, dodecanoic, tetradecanoic, and pentadecanoic acids), compounds rarely detected in

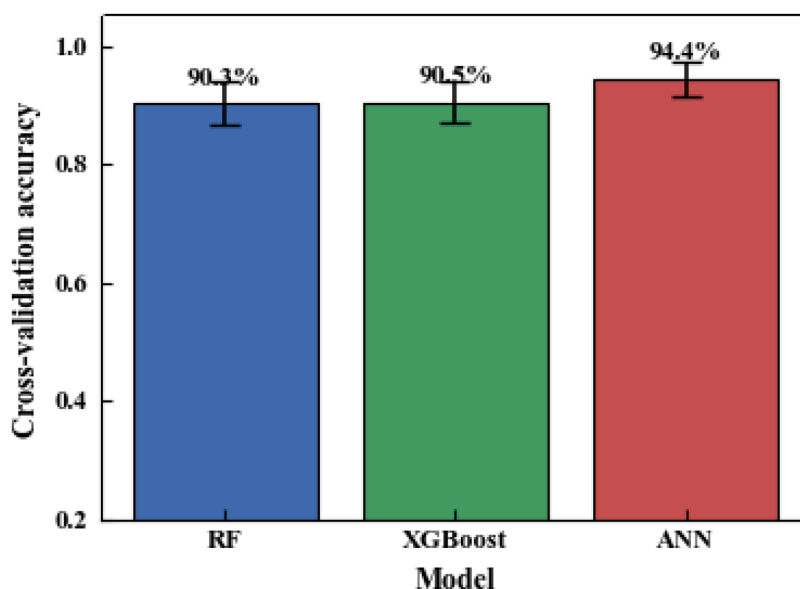


Fig. 3. Three-fold cross-validation accuracy of RF, XGBoost, and ANN models for meat species authentication.

pork or beef. This unique signature aligns with rabbit meat's characteristically lean profile and higher proportion of polyunsaturated fatty acids (PUFAs), which promote alternative oxidative pathways favoring these distinctive markers rather than the aldehyde-dominated patterns seen in beef and pork.

The outstanding performance of non-linear classifiers—particularly ANN and XGBoost—further underscores the presence of intricate, non-linear interrelationships within the volatilomic dataset that linear methods alone might overlook. These advanced models effectively captured subtle synergistic effects among volatiles, resulting in balanced, high-precision classification across all three species (F1-scores  $\geq 0.92$  in the top-performing ANN). The minor residual confusion between beef and pork is biochemically anticipated, given their closer compositional similarities; nonetheless, overall misclassification rates remained consistently below 10%, reinforcing the method's robustness for routine screening in quality control, regulatory enforcement, and anti-fraud applications.

In essence, by leveraging these species-specific lipid oxidation fingerprints—bolstered by rabbit's distinctive saturated fatty acid markers—this volatilomics approach bridges a key gap in multi-species authentication while delivering a scientifically rigorous, operationally efficient, and cost-effective alternative to more complex targeted methods (e.g., DNA-based or spectroscopic techniques), with strong potential for extension to processed or mixed-meat products.

#### 4.1. Industrial relevance and scalability for routine quality control

The proposed HS-SPME-GC-MS workflow is well-suited for industrial meat authentication. Total analysis time is around 60 min per sample with minimal sample preparation. In addition, developed method does not need any organic solvents, making it cost-effective and environmentally friendly. Per-sample consumable costs are low, and the method uses standard GC-MS instrumentation already available in most food quality control laboratories, avoiding expensive dedicated platforms such as DNA analysis and PCR.

For high-throughput industrial environments (e.g., incoming raw material screening), the method can be integrated by: (i) using auto-samplers with multiple fiber trays for parallel extraction, (ii) reducing GC run time via faster temperature ramps with minimal loss of discriminatory power, or (iii) transferring validated volatile markers to portable ion mobility spectrometry (IMS) units for on-site rapid screening. The default machine-learning models (ANN, XGBoost, RF)

require no hyperparameter tuning and can be pre-trained and embedded into laboratory information management systems (LIMS), allowing non-expert operators to obtain species predictions with confidence scores. This enables real-world scenarios such as rapid authentication of raw minced meat upon delivery, screening of processed products (burgers, sausages, meatballs), or verification of labels in contract testing laboratories. Future validation on larger sample sets and thermally processed matrices will further support full industrial deployment.

## 5. Conclusions

This work establishes HS-SPME-GC-MS-based volatilomics, combined with machine-learning classification, as a rapid, reliable, and economically viable tool for differentiating and authenticating minced rabbit, pork, and beef. The method exploits inherent species-specific differences in lipid oxidation products and fatty-acid-derived volatiles to achieve classification accuracies between 90% and 94% using standard laboratory instrumentation and minimal sample pretreatment comparing to recent studies using alternative techniques such as FTIR, NIRS-HSI, or NMR (summarized in Table 3). Among the evaluated models, the ANN achieved the highest performance (94.44% accuracy; F1-score  $\geq 0.92$  for all classes), followed closely by XGBoost and RF, confirming methodological robustness across distinct algorithmic principles. Given its speed, objectivity, minimal sample preparation, and applicability to both fresh and potentially processed meats, the proposed volatilomic fingerprinting strategy represents a significant advancement in meat species authentication. It provides regulatory authorities and the meat industry with a practical analytical tool to combat species substitution and fraudulent mislabeling, particularly in markets where rabbit, pork, and beef are frequently implicated. Several recent studies employing different analytical platforms and chemometric approaches for meat species authentication and classification are summarized in Table 3, highlighting the growing importance and diversity of rapid, non-targeted methodologies in this field. Future studies should validate the method using larger and geographically diverse sample sets, and assess performance in complex multi-species mixtures, and evaluate robustness in commercially processed and thermally treated products to confirm industrial applicability under real-world conditions.

**Table 3**  
Several studies on meat authenticity and classification.

Technique/ methods	Chemometrics model	Aims	Performance	Ref.
FTIR	PLS-DA	Meat authenticity predictive classification	Achieved 92.86 % classification accuracy	(Dimitriou et al., 2025)
NIRS-HSI	PLDR	Assessment of chemical, textural, and structural characteristics of meat	Captured unique chemical and structural characteristics of meat.	(Reis et al., 2018)
HSI	PLS-DA	Classification of beef <i>longissimus thoracis</i> muscle tenderness	Accuracy from 54.79 to 74.67%	(León-Ecay et al., 2022)
<sup>1</sup> H NMR	ANN	Lipid profiling of irradiated beef	Classification accuracy of 81.9%	(Zanardi et al., 2013)
FTIR	PLS	Lard differentiation	Achieved accuracy of 84.6%	(Siddiqui et al., 2023)
HS-SPME-GC-MS	t-SNE-RF-XGBoost-ANN	Rapid and reliable differentiating in three meat samples	Achieved accuracy of 94.4%	Our study

### Declaration of generative AI and AI-assisted technologies in the writing

During the preparation of this work, the authors used TalkAI in order to edit the draft of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

### Ethical Statement

This study does not involve any human or animal testing.

### CRedit authorship contribution statement

**Amir Pourmoradian:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Mohsen Barzegar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ángel A. Carbonell-Barrachina:** Writing – review & editing, Resources, Formal analysis, Conceptualization. **Luis Noguera-Artiaga:** Writing – review & editing, Methodology, Formal analysis.

### 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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### Data availability

Data will be made available on request.

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