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ARTICLE

Non-targeted lipidomics using LC-orbitrap HRMS and chemometrics to discriminate beef, pork, and goat meat for halal authentication

Lipidómica no dirigida mediante LC-Orbitrap HRMS y quimiometría para discriminar carne de vacuno, cerdo y cabra para la autenticación halal

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Abstract: This study presents a non-targeted lipidomic approach using liquid chromatography-high-resolution mass spectrometry (LC-HRMS) coupled with chemometric analysis to discriminate between beef, pork, and goat meat. Lipid extracts from the three meat types were analyzed using reversed-phase LC-HRMS, generating comprehensive lipid profiles. Chemometrics methods, including principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), were employed to identify distinct lipid signatures capable of differentiating the meat samples. The results revealed various lipid classes, and triglycerides became the highest percentage of lipids contained in pork obtained from the positive ionization mode. Compounds of TG (52:1COOH_18:0), PE (O_36:1) and, DG (17:0_18:2) showed good capacity for discriminating pork, and further potential as biomarker candidates. The resulting models demonstrated high classification accuracy, highlighting specific lipid species which contribute significantly to the discrimination. These findings directly support the potential application for halal authentication in the meat industry, as the potential lipid markers can be further validated in binary mixtures, processed products, and real-market samples.

Keywords: Chemometrics; LC-HRMS; Lipid biomarker; Non-targeted; lipidomics; Pork.

Resumen: Este estudio presenta un enfoque lipidómico no dirigido que utiliza cromatografía líquida-espectrometría de masas de alta resolución (LC-HRMS) acoplada con análisis quimiométrico para discriminar entre carne de res, cerdo y cabra. Los extractos lipídicos de los tres tipos de carne se analizaron mediante LC-HRMS de fase inversa, generando perfiles lipídicos completos. Se emplearon métodos quimiométricos, incluyendo análisis de componentes principales (PCA) y análisis discriminante de mínimos cuadrados parciales (PLS-DA), para identificar perfiles lipídicos distintivos capaces de diferenciar las muestras de carne. Los resultados revelaron varias clases de lípidos, siendo los triglicéridos el porcentaje mayoritario de lípidos contenidos en la carne de cerdo obtenida en el modo de ionización positiva. Los compuestos TG (52:1COOH_18:0), PE (O_36:1) y DG (17:0_18:2) tuvieron una alta responsabilidad en la discriminación de la carne de cerdo, con un potencial adicional como candidatos a biomarcadores. Los modelos resultantes demostraron una alta precisión de clasificación, destacando especies lipídicas específicas que contribuyen significativamente a la discriminación. Estos hallazgos respaldan directamente la posible aplicación de la autenticación halal en la industria cárnica, ya que los marcadores lipídicos potenciales pueden validarse en mezclas binarias, productos procesados y muestras reales del mercado.

Palabras clave: Biomarcador lipídico; Carne de cerdo; LC-HRMS; Lipidómica no dirigida; Quimiometría.

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Supplementary information ↓

1. INTRODUCTION

The increasing global demand for halal-certified food products has led to significant challenges in verifying the authenticity of meat sources, particularly beef, pork, and goat meat. The accurate identification and discrimination of these meats are essential for ensuring compliance with halal dietary laws (Al Olan and Yossouf, 2023). Various analytical methods have been developed to authenticate halal meat, such as beef from pork, focusing on distinguishing species-specific biomarkers (Fathima *et al*., 2024). DNA-based techniques, such as polymerase chain reaction (PCR) and real-time PCR, amplify species-specific genetic markers, providing a reliable method for meat identification. However, the DNA-based method cannot identify lipids because lipids do not contain DNA (Amaral *et al*., 2017; Siswara *et al*., 2022). Protein-based methods like enzyme-linked immunosorbent assays (ELISA) utilize antibodies to detect specific proteins unique to each meat type, offering a fast and straightforward approach. However, protein-based methods cannot directly identify lipids because proteins and lipids are fundamentally different types of biomolecules with distinct chemical structures and compositions (Jiang *et al*., 2020; Perestam *et al*., 2017). Lipid profiling using gas chromatography (GC) analyzes the fatty acid composition, which varies among meat samples. However, finding a definitive biomarker with GC-based fatty acid profiling can be difficult (Perez-Palacios *et al*., 2022). GC analysis typically requires hydrolysis and derivatization of lipids to fatty acid methyl esters, resulting in a loss of information about the intact lipid structure. The derivatization process, such as methylation, can introduce artifacts or require corrections (e.g., δ13C value adjustments), further complicating the analysis. Variability in extraction and derivatization methods (e.g., sodium methoxide vs. boron trifluoride) can lead to inconsistent results, although some studies report no significant differences. In addition, traditional one-dimensional GC often fails to resolve complex mixtures, such as isomers or co-eluting compounds, which can obscure biomarker identification (Johnston and Sobhi, 2017; Ferro *et al*., 2026). Consequently, GC-based approaches provide limited coverage of complex lipid classes, such as phospholipids, sphingolipids, and triglycerides, which may contain species-specific molecular features (Indelicato *et al*., 2023). Therefore, it is crucial to develop reliable and specific analytical authentication methods to ensure meats' halal status.

Lipidomic has emerged as a promising analytical approach for meat authentication due to its ability to capture the unique lipid profiles present in different meat types (Sidira *et al*., 2024; Wu *et al*., 2021). Unlike proteins or DNA, which can be altered through processing or cooking, lipids remain relatively stable and retain distinct signatures that can differentiate between species (Song *et al*., 2022). Each animal species, including beef, goat, and pork, has a characteristic lipid composition influenced by diet, metabolism, and genetics, making lipidomic profiling a powerful tool for species identification. Furthermore, lipidomics allows for the analysis of a wide range of lipid classes, including triglycerides, phospholipids, and fatty acids, providing a comprehensive view of the biochemical makeup of meat (Jia *et al*., 2023; Li *et al*., 2022).

Non-targeted lipidomics, combined with liquid chromatography-Orbitrap high-resolution mass spectrometry (LC-Orbitrap HRMS) and chemometrics, offer a powerful approach to distinguishing between beef, pork, and goat meat (Wang *et al*., 2021). LC-HRMS allows the direct detection of intact lipid species with high sensitivity, mass accuracy, and broader lipidome coverage, enabling more comprehensive lipid profile characterization and facilitating the discovery of specific lipid biomarkers for meat authentication (Ferro *et al*., 2026). This methodology leverages the comprehensive analysis of lipid profiles to identify unique lipid markers that can distinguish halal from non-halal meat products (Chiesa *et al*., 2022; Windarsih *et al*., 2023). The integration of chemometric techniques, such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), facilitates the interpretation of complex lipidomic data, enhancing the accuracy and efficiency of meat authentication processes (Qin *et al*., 2024; Wu *et al*., 2021). PCA and PLS-DA proved crucial for effectively interpreting the complex lipidomic data generated by LC-Orbitrap HRMS, ultimately improving the accuracy and efficiency of the meat authentication process. This study underscores the synergistic power of advanced analytical techniques and sophisticated data analysis in addressing critical food authentication challenges (García-Pérez *et al*., 2024).

A previous study was conducted on the halal authentication of pork and beef muscle parts, i.e., *Triceps barchii*, *Biceps femoris*, and *Longissimus dorsi* (Maritha *et al*., 2023). The results showed that the three muscles in pork and beef had different lipid compositions. Analysis using the untargeted

lipidomic method showed that under negative ions, compounds such as glycerolipids were not detected in pork but were detected in beef. Further analysis using PCA and PLSDA also showed differences in lipid profiles in the three pork and beef muscles. Another study analyzed lipids from pork and chicken using UPLC-TIMS-QTOF-MS for 4D lipidomic analysis using methyl tert-butyl ether and methanol in the extraction process (Martakos *et al*., 2024). The results showed that this technique could distinguish isomeric and isobaric compounds and identify 395 lipids. Some important compounds found include phospholipids, glycerolipids, and n-3 fatty acids.

Although previous studies have demonstrated the applicability of lipidomics for meat authentication, several important gaps remain. Most reported investigations have focused on pairwise discrimination, such as pork versus beef or pork versus chicken (Maritha *et al*., 2023; Martakos *et al*., 2024), and have primarily emphasized lipid profile differentiation without systematically identifying and validating discriminatory lipid biomarkers across multiple meat species. Furthermore, differences in analytical platforms, sample matrices, and data-processing strategies have limited the comparability and practical application of the reported findings. There are currently few thorough non-targeted lipidomic studies using LC-HRMS coupled with chemometric analysis for the simultaneous discrimination of goat, pork, and beef meat. In particular, there is limited information regarding the lipid species that consistently contribute to meat differentiation and could serve as reliable biomarker candidates for halal meat authentication. This study addresses these gaps by integrating high-resolution non-targeted lipidomics with multivariate chemometric approaches to comprehensively characterize lipid profiles, identify key discriminatory lipid markers, and evaluate their potential utility for the simultaneous authentication of beef, pork, and goat meat within a unified analytical framework.

2. MATERIALS AND METHODS

2.1. Meat collection

Beef (E), pork (L), and goat (A) meats were purchased from markets in Yogyakarta, Indonesia. Each type of meat was obtained from three different markets supplied by different slaughterhouses. Therefore, the meats used were obtained from three individuals and the loin part was used. The weight of the animals was 300-350 kg for beef (around 2 years of age), 80-85 kg for pork (around 1 year old), and 50-55 kg for goat (around 1 year old). The meats were immediately stored at -20 °C until analysis. They were thawed at 4 °C on the analysis day for 6 hours. The meats were sliced into small pieces and ground separately using different meat grinders to prevent cross-contamination. The ground meats were then used for the extraction of lipids.

2.2. Meat extraction

Samples were extracted using isopropanol for each sample. 1 mL of isopropanol was added to each tube and vortexed for 30 seconds. The sample was subjected to sonication for 30 min at room temperature to complete the extraction method. Following sonication, the sample was left at room temperature for around 30 minutes before being filtered using a 0.22 PTFE filter. The filtered supernatant was placed into a 2-mL LC-HRMS vial and was ready to be injected for non-targeted LC-HRMS lipidomic analysis (Jia *et al*., 2021).

2.3. Lipidomics analysis using LC-HRMS

Lipid analysis of the meat samples was performed using ultra-high performance liquid chromatography (UHPLC, binary pump, Thermo Scientific, USA) coupled with a high-resolution mass spectrometer detector (Q-Exactive, Orbitrap, Thermo Scientific, USA). 5 µL of sample were injected. The analytes were separated through a C-18 column (Accucore, Thermo Scientific, 100 mm x 2.1 mm x 2.6 µm) at a flow rate of 0.26 mL/min. The first mobile phase (A) contained acetonitrile: water (60:40), and the second mobile phase (B) contained 2-propanol: acetonitrile (90:10 v/v). A and B were added with 0.1% and 60 mM formic acid. In this study, the parameters of both UHPLC and HRMS used for lipidomic analysis were adopted from the method described by Windarsih *et al*. (2024).

2.4. Data processing

Xcalibur software processed the resulting total ion chromatogram (TIC) data. The TIC was subjected to data processing to extract important information and identify the lipid compositions in each meat sample using LipidSearch software (Thermo Scientific, USA). The compounds were matched using the MS2 spectra from the library. Lipids with mass error between -5 ppm and 5 ppm were selected. The identified compounds were exported into CSV files for further statistical analysis using the chemometric approach.

2.5. Chemometric analysis

The CSV data were used for chemometric analysis, consisting of principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The area of all selected lipid compounds was used as a variable to construct PCA and PLS-DA. The data were subjected to sum normalization followed by auto scaling before chemometric analysis. The PCA score plot was observed to identify a pattern. Then, PLS-DA was performed using latent variables to discriminate beef, pork, and goat meat samples. The PLS-DA score plot was identified along with the R2X, R2Y, and Q2 values to evaluate the accuracy and predictive power of the PLS-DA models. The model was validated using cross-validation employing 5 number of folds. Variable importance for projections (VIP) value analysis was performed in PLS-DA to investigate the discriminating metabolites responsible for the discrimination of beef, pork, and goat meat. Metabolites with a VIP value above 1.5 were important in discriminating between each meat (Paul *et al*., 2021). Then, the VIP and heatmap plots were analyzed to determine the distribution patterns of the discriminating metabolites. All the selected discriminating metabolites had a p-value < 0.05 and fold change > 2.0.

3. RESULTS AND DISCUSSION

3.1. Non-targeted LC-HRMS lipidomic analysis

The present study successfully employed a non-targeted lipidomic approach using LC-Orbitrap HRMS to identify comprehensive lipids in beef, pork, and goat meat. The strength of the non-targeted approach lies in its ability to capture a broad spectrum of lipid species, thereby providing a comprehensive overview of the lipidomic landscape of each meat sample (Yin *et al*., 2023). The LC-Orbitrap HRMS-based non-targeted lipidomic approach is highly effective for generating detailed and comprehensive lipid fingerprints of biological samples. The total ion chromatogram (TIC) generated from this approach can be considered a visual representation of the overall lipid fingerprint of a sample (Figure 1). Isopropanol (IPA) was deliberately chosen as a single-solvent extraction method based on its proven performance in non-targeted LC-HRMS lipidomics, particularly for complex food matrices such as meat. Compared with conventional biphasic methods (e.g., Folch or Bligh-Dyer), monophasic isopropanol extraction offers several advantages including greater simplicity and speed, reduced risk of lipid loss at the phase interface, higher throughput, and improved safety by eliminating the use of toxic chloroform. Importantly, isopropanol provides broad lipid coverage, effectively extracting both polar lipids (phospholipids, lysophospholipids) and non-polar lipids (triglycerides), which is well-suited for our LC-HRMS-based untargeted lipidomic workflow. The combination of isopropanol extraction with sonication and direct compatibility with our C18 UHPLC mobile phase (containing isopropanol) minimized sample loss and matrix effects, enabling comprehensive lipid profiling across beef, pork, and goat meat samples (Iriondo *et al*., 2019; Horing *et al*., 2022).

In this study, 493 lipids were identified in beef from positive ionization, followed by 186 lipids from negative ionization. Meanwhile, a total of 722 lipids were identified in goat meat, including 472 lipids from positive ionization and 250 lipids observed from negative ionization. In addition, the lipids contained in pork were 472 lipids from positive ionization and 147 lipids from negative ionization. Phosphatidylcholine (PC) exhibited the highest abundance among lipid classes in beef and goat meat in positive ionization, followed by triglycerides (TG). On the other hand, in negative ionization mode, PC represented the predominant lipid class in all three meat samples. The distribution of each lipid class is illustrated in Figure 2. To evaluate the differentiation between goat, beef, and pork lipids, the PCA and PLS-DA were conducted. Figure 3 shows the spontaneous, distinct clustering according to meat species, with no overlapping observed among the biological replicates for both ionizations.

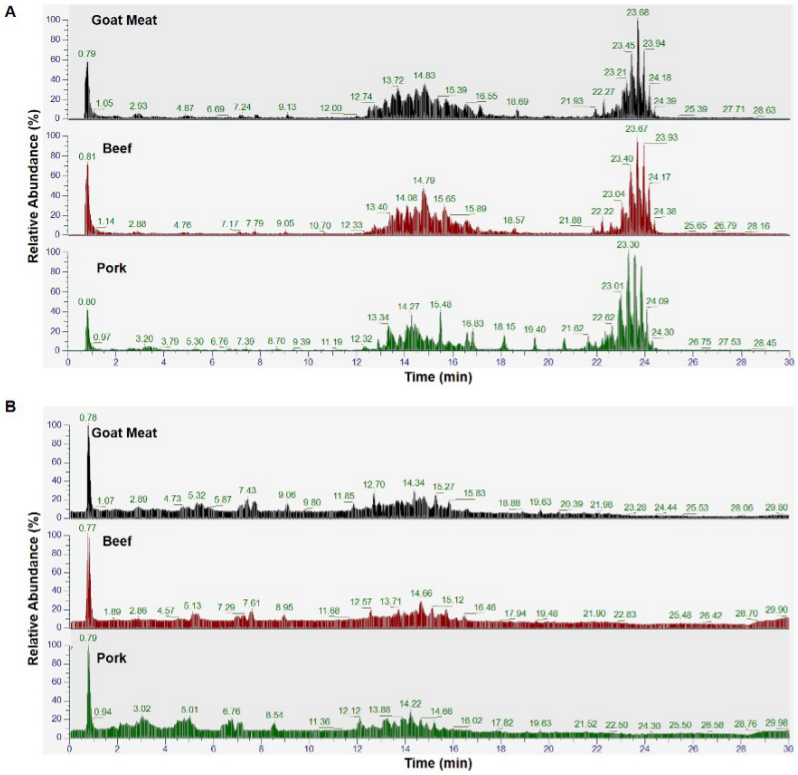


Figure 1. Total ion chromatogram of beef, pork, and goat meat obtained from positive (A) and negative ionization modes.

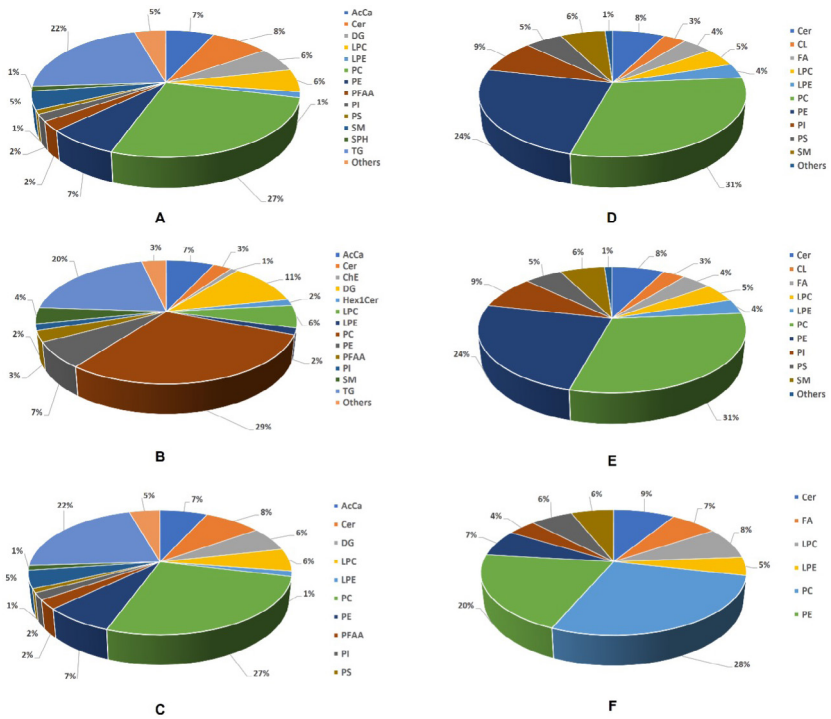


Figure 2. Lipid distribution in beef, goat meat, and pork obtained from positive ionization (A, B, C) and negative ionization (D, E, F) modes (AcCa = acylcarnitine, Cer = ceramide, ChE = cholesteryl hexadecyl ether, CL = cardiolipin, DG = diglyceride, FA = fatty acyl, Hex1Cer = hexosylceramide, LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PFAA = primary fatty acid amide, PI = phosphatidylinositol, PS = phosphatidylserine, SM = *sphingomyelin, SPH = sphingosine, TG = triglyceride). Each sample was measured in three replicates.*

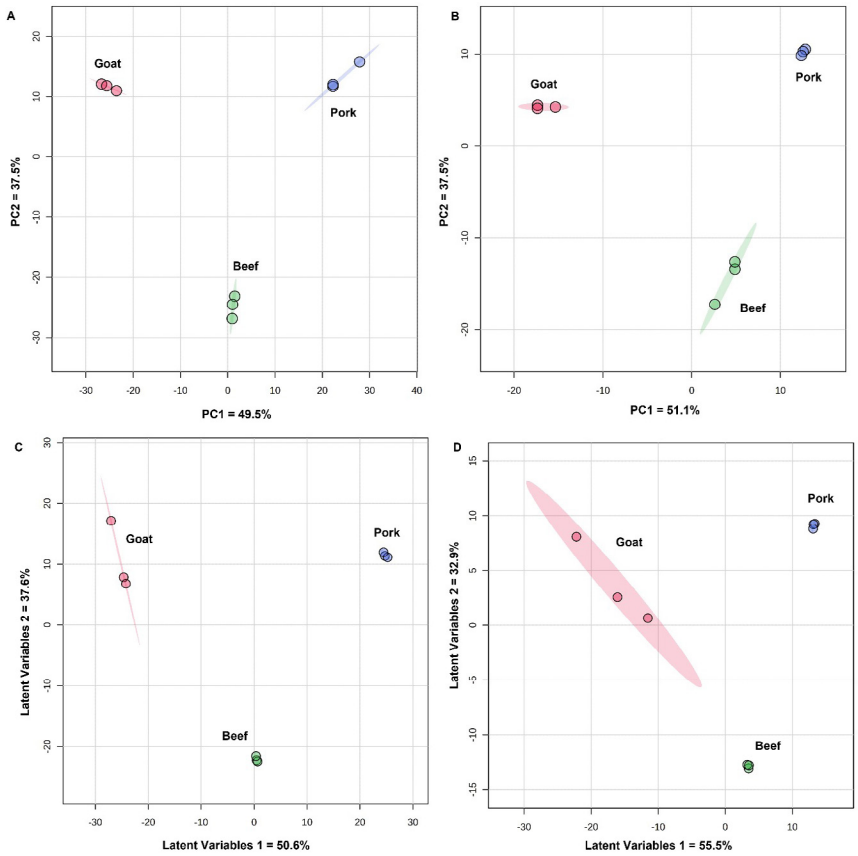


Figure 3. Differentiation of goat, beef, and pork through lipidomic analysis observed using the score plot of principal component analysis in positive (A) and negative (B) ionization along with the score plot of partial least square-discriminant analysis in positive (C) and negative (D) ionization.

Previous research has reported that pork contains triglycerides as a major lipid profile component, contributing to its sensory and nutritional qualities (Yi *et al.*, 2023). Meanwhile, beef consumption does not significantly affect triglyceride levels in the same way as pork. Studies have shown that beef has a non-significant effect on the biochemical parameters in the blood, including triglyceride concentrations (Igenbayev *et al.*, 2022). In addition, goat meat is recognized for its lower fat and cholesterol contents, which include lower triglyceride levels compared to pork (Wattanachant, 2018).

In a study conducted by (Bi *et al.*, 2023), UPLC-MS/ was successfully applied for the lipidomic analysis of the pork belly fatty layer with good results. The results showed that variations in the steaming process affected the lipid composition of pork belly. A total of 884 lipid species belonging to 35 different lipid classes were successfully identified. The compounds that play an important role as variables in the metabolism of glycerophospholipids and phosphoinositides are phospholipids. Lipidomic analysis has also been successfully carried out to determine the effects of frozen storage on pork (Feng *et al.*, 2022). The results showed that 688 lipids were successfully identified in pork samples. One type of lipid reduced during frozen storage is ether-linked glycerophospholipids, especially ether-linked phosphatidylcholine and phosphatidylcholine. Several compounds resulting from lipid oxidation include cis-11,14-eicosenoic acid, decanal, and cis-5,8,11,14,17-dicosapentaenoic acid. These compounds are likely to be used to determine the duration of frozen storage in pork samples.

Therefore, the non-targeted LC-Orbitrap HRMS approach generates a unique lipid fingerprint for beef, pork, and goat meat. When analyzed using chemometric techniques, these fingerprints reveal subtle yet consistent differences in lipid composition, enabling accurate discrimination and authentication of the meat species. The comprehensive nature of these lipid fingerprints makes them a powerful tool for distinguishing samples, even when specific single biomarkers are elusive.

3.2. Chemometrics analysis to discriminate between beef and pork

The application of multivariate statistical techniques, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), was instrumental in extracting meaningful patterns from the complex, high-dimensional lipidomic data. The dataset of lipid compounds, along with their peak area from beef, pork, and goat meat, was subjected to PCA and PLS-DA. First, PCA and PLS-DA were applied to differentiate among beef, pork, and goat meats using variables of lipids from positive and negative ionization in separate models. PCA provided an initial overview of the data structure, revealing inherent clustering tendencies according to meat type (Figures 3A and 3B). Subsequently, the supervised PLS-DA also demonstrated good discrimination among lipids from beef, pork, and goat meat (Figures 3C and 3D). These PCA and PLS-DA results suggested differences in lipid composition among beef, pork, and goat meats.

A deeper PCA analysis to differentiate between beef and pork showed that the PCA score plot using two principal components (PCs) showed different clusters between beef and pork, indicating differences in their lipid compositions (Figures 4A and 4B). Further PLS-DA analysis (Figures 4C and 4D) showed clear discrimination between beef and pork using the first two latent variables (LVs). The supervised PLS-DA models effectively maximized the separation between the groups, leading to high classification accuracies. The PLS-DA models were validated using cross validation employing 5 number of folds in both positive and negative ionization PLS-DA models. The results of cross validation in the PLS-DA model from positive ionizations had R2X of 1.0 and R2Y of 0.999, indicating a good model fitting, and Q2 of 0.953, indicating a good model predictability. On the other hand, PLS-DA, subjected to lipid data obtained from negative ionization, also demonstrated separate clusters between beef and pork, as observed through the PLS-DA score plot (Figure 4D). The results of cross validation in the PLS-DA model from negative ionization provided R2X of 1.0, R2Y of 0.999, and Q2 of 0.972. Furthermore, the result of confusion matrices to discriminate and classify beef and pork using the lipid compositions is presented in Table 1. As summarized in the provided tables, the PLS-DA models yielded a flawless classification accuracy of 100% for both positive and negative ionization modes. All three biological replicates of beef (class 1) and pork (class 2) were correctly categorized into their true biological classes, yielding zero false-positive or false-negative assignments.

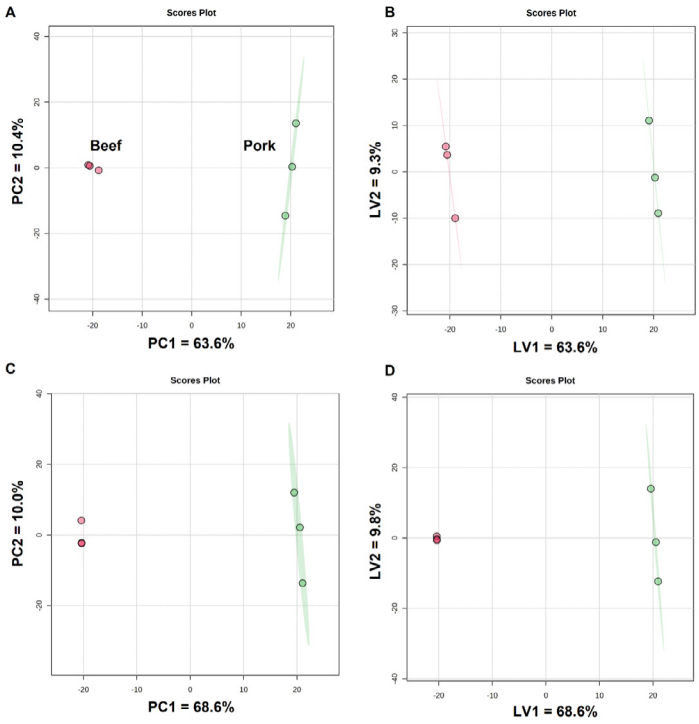


Figure 4. Principal component analysis in positive (A) and negative (C) ionization and partial least square-discriminant analysis in positive (B) and negative (D) ionization to differentiate beef and pork. Each sample was measured in three replicates.

TABLE 1. The confusion matrices of partial least square-discriminant analysis (PLS-DA) model to discriminate and classify beef and pork using the lipid compositions.

Class	Samples	Number	Correct (%)	1	2
Positive Ionization PLS-DA Model					
1	Beef_1	1	100	1	0
1	Beef_2	1	100	1	0
1	Beef_3	1	100	1	0
2	Pork_1	1	100	0	1
2	Pork_2	1	100	0	1
2	Pork_3	1	100	0	1
Total		**6**	**100**	**3**	**3**
Negative Ionization PLS-DA Model					
1	Beef_1	1	100	1	0
1	Beef_2	1	100	1	0
1	Beef_3	1	100	1	0
2	Pork_1	1	100	0	1
2	Pork_2	1	100	0	1
2	Pork_3	1	100	0	1
Total		**6**	**100**	**3**	**3**

Further investigations on the discriminating lipids through VIP analysis revealed lipid compounds which are responsible for discriminating beef and pork. VIP analysis offers valuable insights into the key lipid species driving the differentiation. These potential biomarkers warrant further investigation for their specificity and robustness across different breeds, diets, and processing conditions. Table 2 shows lipids which play and important role and are responsible for discriminating between beef and pork (VIP value above 1.50) from both positive and negative ionization modes. The lipids of TG (52:1COOH_18:0), PE (O-36:1), and TG (16:1CHO_18:1_18:2) had the highest VIP values of 1.88, 1.85, and 1.81, respectively, as obtained from the positive mode. The compounds of TG (52:1COOH_18:0) and PE (O-36:1) were highly distributed in pork, whereas the compound of TG (16:1CHO_18:1_18:2) was high in beef. In addition, from the negative mode, lipids of PE (O-17:1_18:2), Cer (d18:1_23:0) and PE (16:0_18:1) had VIP values of 1.94, 1.79, and 1.77, respectively. All three of these lipids were found to be highly distributed in pork.

TABLE 2. Lipid compounds which are responsible for discriminating lipids (*p* value < 0.05) to discriminate between beef and pork as biomarker candidates. Each sample was measured in three replicates.

No.	Lipids	VIP	Calculated m/z	Retention Time (min)	Ionization
1	"TG(52:1COOH_18:0)+NH_4"	1.88	1161.07300	24.58	+
2	"PE(O-36:1)+H"	1.85	732.59017	16.55	+
3	"TG(16:1CHO_18:1_18:2)+NH_4"	1.81	886.74943	19.82	+
4	"TG(8:0_18:1_18:2)+NH_4"	1.78	762.66062	20.58	+
5	"PC(14:0)+H"	1.77	482.28773	2.26	+
6	"PC(37:1)+H"	1.76	802.63203	17.53	+
7	"DG(18:1_20:1)+NH_4"	1.76	666.60310	18.03	+
8	"TG(18:0_18:0_19:0)+NH_4"	1.76	922.87972	24.47	+
9	"PE(P-18:1_18:2)+H"	1.75	726.54322	14.18	+
10	"PFAA(14:0)+H"	1.75	228.23219	2.62	+
11	"TG(16:0_18:0COOH_18:1)+NH_4"	1.75	908.79130	22.05	+

12	"TG(O-18:0_19:0_18:1)+NH_4"	1.74	906.88480	24.57	+
13	"TG(O-14:0_16:0_18:2)+NH_4"	1.74	806.75960	23.45	+
14	"SM(d43:1)+H"	1.74	829.71570	21.07	+
15	"TG(O-18:1_18:0_18:1)+NH_4"	1.73	890.85350	2426	+
16	"LPC(20:5)+H"	1.72	542.32412	1.54	+
17	"SM(d40:2)+H"	1.70	785.65310	16.52	+
18	"TG(O-18:0_18:0_22:4)+NH_4"	1.70	942.88480	24.37	+
19	"PE(O-18:4_18:1)+Na"	1.69	746.50951	13.51	+
20	"AcHexSiE (18:0)+NH_4"	1.69	860.73378	21.66	+
21	"TG(16:0_16:1_18:1)+NH_4"	1.68	848.77017	23.40	+
22	"LPC(O-14:0)+H"	1.68	454.32920	2.15	+
23	"Cer(d17:1_24:1)+H"	1.68	634.61327	17.97	+
24	"TG(16:0_18:0_26:0)+NH_4"	1.67	992.95797	24.94	+
25	"Cer(m18:2_24:2)+H"	1.67	628.60271	16.98	+
26	"PE(O-17:1_18:2)-H"	1.94	712.52867	14.64	-
27	"Cer(d18:1_23:0)+HCOO"	1.79	680.61985	19.20	-
28	"PE(16:0_18:1)-H"	1.77	716.52358	14.34	-
29	"PE(O-16:1_20:2)-H"	1.72	726.54432	14.72	-
30	"LPI(18:2)-H"	1.71	595.28889	1.38	-
31	"PI(16:0_20:4)-H"	1.67	857,51856	10.65	-
32	"PC(O-17:1_20:4)+HCOO"	1.66	824.58110	14.52	-
33	"PC(O-17:0_18:2)+HCOO"	1.65	802.59675	15.20	-
34	"PC(18:0_18:1)+HCOO"	1.64	832.60731	16.43	-
35	"PS(18:0_20:4)-H"	1.64	810.52906	12.50	-
36	"LPC(16:0)-CH3"	1.62	480.30957	2.49	-
37	"PE(O-18:1_20:5)-H"	1.61	748.52867	14.22	-
38	"PC(16:0_18:2)+HCOO"	1.61	802.56036	13.55	-
39	"PE(O-18:2_22:4)-H"	1.60	776.55997	14.30	-
40	"PC(O-15:1_18:2)+HCOO"	1.57	772.54980	13.25	-
41	"PC(18:2_18:2)+HCOO"	1.54	826.56036	12.45	-
42	"LPC(O-16:0)-CH3"	1.54	466.33030	3.25	-
43	"PC(O-16:0_18:1)+HCOO"	1.50	790.59675	15.99	-
44	"PC(16:0_17:1)+HCOO"	1.49	790.56036	13.96	-
45	"PE(O-17:1_22:5)-H"	1.49	762.54432	14.19	-
46	"PC(18:1_20:4)+HCOO"	1.48	852.57601	13.28	-
47	"CL(18:1_18:2_18:2_18:2)-H"	1.47	1449.98060	22.39	-
48	"PI(18:1_18:1)-H"	1.47	861.54986	12.44	-
49	"PS(18:0_22:5)-H"	1.46	836.54471	13.01	-
50	"PC(O-14:0_18:2)-CH3"	1.45	700.52867	13.76	-

Through further investigation using a heatmap plot (Figure 5), the lipids of LPC (O-14:0), PC (14:0), PE (P-18:1_18:2), TG (52:1COOH_18:0), PE (O018:4_18:1), TG (16:1CHO_18:1_18:2), TG (8:0_18:1_18:2), DG (18:1_20:1), PE (O-36:1), Cer (m18:2_24:2), TG (O-14:0_16:0_18:2), and TG (16:0_18:0COOH_18:1) were found to have high peak areas in pork and low in beef, observed in the positive mode. Meanwhile, in the negative ionization modes, a higher number of lipids were found to be in high peak areas in pork, including PE (O-18:2_22:4), PI (16:0_20:4), PC (O-17:0_18:2), LPI (18:2), LPC (16:0), PS (18:0_20:4), PC (O-14:0_18:2), PE (O-16:1_20:2), Cer (d18:1_23:0), CL (18:1_18:2_18:2_18:2), PE (O-18:1_20:5), PC (18:0_18:1), LPC (O-16:0), PE (16:0_18:1), PE (O-17:1_18:2), PC (O-17:1_20:4), PC (18:1_20:4), PC (18:2_18:2), and PC

(16:0_18:2). Therefore, these lipids may be further investigated as potential indicators of the adulteration of beef with pork by using broader samples and the targeted lipidomic approach.

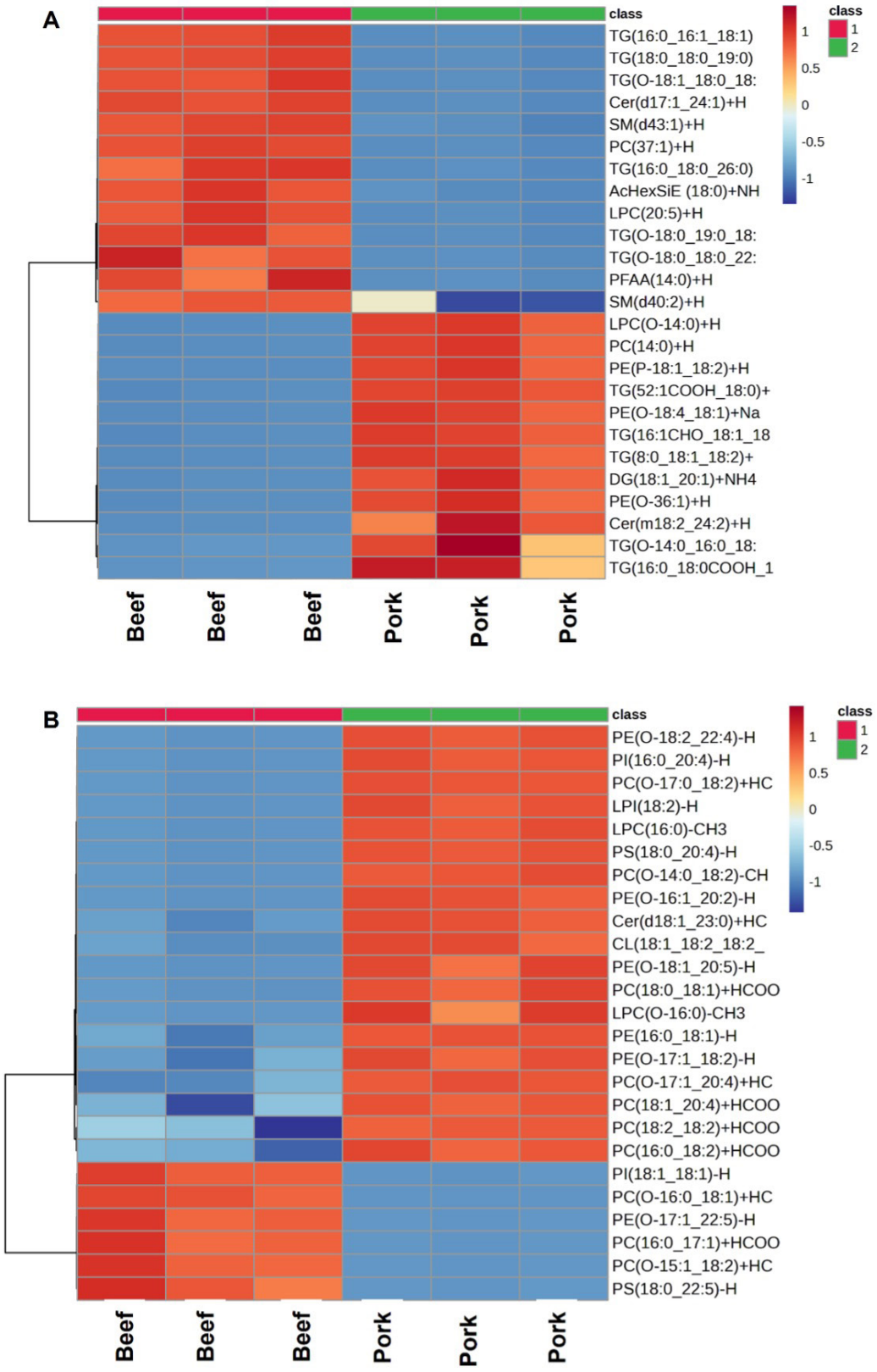


Figure 5. Heatmap of the discriminating metabolites (*p* value < 0.05) to discriminate beef and pork in positive (A) and negative (B) ionization modes. Each sample was measured in three replicates.

Triacylglycerols (TGs) and diacylglycerols (DGs) are major subclasses of complex glycerolipids composed of a glycerol backbone esterified with fatty acids (FAs). TGs, including TG (52:1COOH_18:0), the most abundant glycerolipid, consist of three FAs attached at sn-1, sn-2, and sn-3 positions, serving as essential energy reserves and key carriers of FAs in foods and biological systems (Song *et al*., 2022; Tietel *et al*., 2023). The fatty acid composition of TGs, such as capric and lauric acids,varies across species and tissue types (Li *et al*., 2022). TGs dominate the lipid profile in lard, accounting for over 50%, followed by DGs (Windarsih *et al*., 2023). As nonpolar lipids, TGs and DGs are dissolved in organic solvents like chloroform and are commonly detected in positive ion mode as ammonium adducts (Maritha *et al*., 2023).

Phosphatidylethanolamine (PE), such as PE (O-36:1), belongs to the glycerophospholipid class of complex lipids, which are key structural components of biological membranes (Tietel *et al*., 2023). Glycerophospholipids (GPs) are abundant in animal and plant cells and are primarily composed of a glycerol backbone, two fatty acid chains, and a phosphate group. The major subclasses include phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (SM) (Song *et al*., 2022). These lipids contribute to membrane structure, fluidity, and function by forming a semi-permeable barrier that maintains cell integrity. Phospholipids, polar lipids, are also involved in diverse cellular processes and can be found across various tissues, including the brain and muscle. While triglycerides and diglycerides dominate the lipid profile in lard (Windarsih *et al*., 2023), phospholipids such as PE have also been identified in beef, highlighting their broader distribution in animal-derived lipids.

A previous study reported that the lipidomic profiles of beef and pork differ significantly. For instance, under negative ion mode, beef contains fatty acyls, glycerophospholipids, glycerolipids, and sphingolipids, whereas pork lacks glycerolipids in this mode. Specific lipids have been identified as potential markers for distinguishing beef from pork. For example, a study identified 12 potential lipid profiles that can be used for halal authentication of beef and pork (Maritha *et al*., 2023). Another study found that pork has a higher polyunsaturated fatty acid (PUFA) content than beef, which can also be a distinguishing factor. The differences in lipid classes and molecular levels between beef and pork have been observed. For instance, beef and pork have different compositions of triglycerides and phospholipids, which can be used as markers (Li *et al*., 2021).

3.3. Chemometrics analysis to discriminate between goat meat and pork

In the second part, chemometric techniques were employed to discriminate between goat meat and pork samples based on their lipid profiles in both positive and negative ionization. The application of PCA and PLS-DA enables the extraction of relevant information from complex datasets, facilitating the identification of subtle yet consistent differences between meat types. This section presents the outcomes of PCA and PLS-DA, highlighting the effectiveness of the applied models in classifying the two meat species in lipidomics analysis. PCA's first two PCs (PC1 and PC2) captured a substantial proportion of the total variance and were sufficient to visually differentiate between goat meat and pork samples. The resulting score plots revealed a clear clustering which corresponded to each meat type, indicating inherent compositional differences captured by the model in both positive and negative ionization modes (Figures 6A and 6B). These results suggest that, even without class labels, PCA effectively highlights the underlying variability distinguishing goat from pork, supporting its utility as a preliminary tool for meat authentication.

PLS-DA was subsequently performed to further enhance classification and address the supervised nature of the discrimination task. Unlike PCA, PLS-DA incorporates class information to maximize the separation between predefined groups, such as goat meat and pork. The first two LVs (LV1 and LV2) accounted for most of the variation relevant to class discrimination. They provided clear separation between the meat types in positive and negative ionization modes (Figures 6C and 6D). The score plot based on these LVs showed distinct clustering, confirming that PLS-DA is a powerful tool for accurately distinguishing between goat and pork samples. The PLS-DA model's good accuracy and predictability were obtained in positive ionization mode, as indicated by the five-fold cross-validation results, with R^2X, R^2Y, and Q^2 values of 1.000, 0.999, and 0.987, respectively. Meanwhile, the R^2X, R^2Y, and Q^2 values of the PLS-DA model from cross validation results using 5 number of folds in the negative ionization mode were 1.0, 0.999, and 0.990, respectively. Table 3 shows the confusion matrices of PLS-DA models from both ionizations. A good classification accuracy (100%) for both positive and negative ionization modes were obtained. All three biological replicates of goat meat (class 1) and pork (class 2) were correctly categorized into their classes, without false-positive or false-negative assignments.

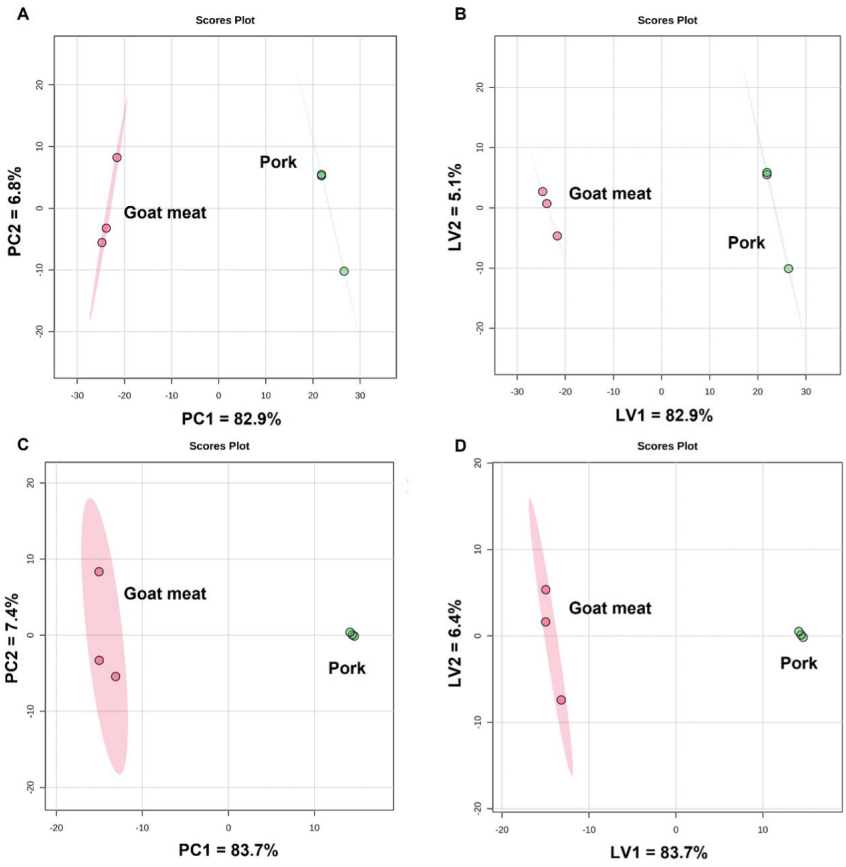


Figure 6. Principal component analysis in positive (A) and negative (C) ionization and partial least square-discriminant analysis in positive (B) and negative (D) to differentiate goat meat and pork. Each sample was measured in three replicates.

TABLE 3. The confusion matrices of partial least square-discriminant analysis (PLS-DA) model to discriminate and classify goat meat and pork using their lipid compositions.

Class	Samples	Number	Correct (%)	1	2
		Positive Ionization PLS-DA Model			
1	Goat_1	1	100	1	0
1	Goat_2	1	100	1	0
1	Goat_3	1	100	1	0
2	Pork_1	1	100	0	1
2	Pork_2	1	100	0	1
2	Pork_3	1	100	0	1
Total		**6**	**100**	**3**	**3**
		Negative Ionization PLS-DA Model			
1	Goat_1	1	100	1	0
1	Goat_2	1	100	1	0
1	Goat_3	1	100	1	0
2	Pork_1	1	100	0	1
2	Pork_2	1	100	0	1
2	Pork_3	1	100	0	1
Total		**6**	**100**	**3**	**3**

Following the PLS-DA modelling, VIP analysis was conducted to identify the most influential variables contributing to the discrimination between goat meat and pork. The VIP scores highlighted specific lipid components that played a significant role in differentiating the two meat types. These discriminating lipids exhibited consistent abundance or spectral feature variations, reflecting the biochemical differences between goat and pork. Identifying these key markers enhances the interpretability of the PLS-DA model. It provides valuable insight into the lipidomic profiles characteristic of each meat species, supporting their use in targeted authentication strategies. According to Table 4, lipids belonging to various lipid classes identified through both positive and negative ionization modes were found to be responsible for differentiating goat meat and pork. The presence and intensity patterns of these lipid species, as revealed by VIP scores greater than 1.0, strongly contributed to the classification model, reinforcing their role as key biomarkers. These findings highlight the metabolic distinctions between goat and pork and demonstrate the potential of lipid profiling combined with chemometrics for robust meat authentication and species differentiation.

TABLE 4. Lipid compounds which are responsible for discriminating lipids (*p* value < 0.05) to discriminate between goat meat and pork as biomarker candidates. Eeach sample was measured in three replicates.

No.	Lipids	VIP Value	Calculated m/z	Retention Time (min)	Ionization
1	“DG(17:0_18:2)+NH_4“	1.10	624.55615	16.18	+
2	“SPH(d16:1)+H“	1.10	272.25841	1.19	+
3	“DG(16:0_17:0)+NH_4“	1.10	600.55615	17.49	+
4	“PC(O-36:7)+H“	1.10	762.54322	12.04	+
5	“PC(42:9)+H“	1.10	856.58508	12.25	+
6	“AcCa(21:0)+H“	1.10	470.42039	8.21	+
7	“PC(O-14:1_20:3)+H“	1.10	740.55887	14.96	+
8	“PI(18:1_18:1)+NH_4“	1.10	880.59096	12.73	+
9	“PC(37:2)+H“	1.10	800.61638	15.68	+
10	“PFAA(24:0)+H“	1.10	368.38869	11.25	+
11	“PC(O-34:4)+Na“	1.10	762.54081	13.45	+
12	“SM(d35:2)+H“	1.10	715.57485	12.58	+
13	“TG(16:1_18:1_18:1CHO)+NH_4“	1.10	888.76508	19.78	+
14	“TG(16:0_18:0_18:1)+H“	1.10	861.79057	23.86	+
15	“PC(O-14:2_20:5)+H“	1.10	734.51192	12.44	+
16	“DG(O-13:0_19:0)+NH_4“	1.10	572.56124	18.75	+
17	“DG(O-18:0_18:2)+NH_4“	1.10	624.59254	18.32	+
18	“PE(P-17:0_18:3)+H“	1.10	712.52757	13.66	+
19	“PC(38:5)+H“	1.10	808.58508	13.21	+
20	“TG(25:0_18:1_18:1)+NH_4“	1.10	100.94230	24.64	+
21	“TG(18:0_18:1_22:4)+NH_4“	1.10	954.84842	23.87	+
22	“PE(P-15:0_18:2)+H“	1.10	686.51192	13.46	+
23	“MG(O-19:4)+H“	1.10	351.28937	7.78	+
24	“Cer(m18:2_22:0)+H“	1.10	604.60271	18.48	+
25	“PC(O-14:1_22:6)+H“	1.10	762.54322	14.05	+
26	“PE(O-18:1_20:5)-H“	1.09	748.52867	14.36	-
27	“PC(O-15:0_20:4)+HCOO“	1.09	798.56545	13.60	-
28	“PC(O-16:0_18:3)+HCOO“	1.09	786.56545	13.68	-
29	“PE(O-17:1_22:5)-H“	1.09	762.54432	14.34	-

30	"PE(O-16:1_20:5)-H"	1.09	720.49737	12.93	-
31	"CL(18:1_18:1_18:2_18:2)-H"	1.09	145.99630	22.85	-
32	"PC(O-16:0_20:5)+HCOO"	1.09	810.56545	13.44	-
33	"PE(O-15:1_20:5)-H"	1.09	706.48172	12.15	-
34	"FA(20:1)-H"	1.09	30.27990	8.56	-
35	"LBPA(18:2_18:3)-H"	1.09	767.48686	10.05	-
36	"LBPA(18:2_18:2)-H"	1.09	769.50251	10.96	-
37	"PC(16:0_17:1)+HCOO"	1.09	790.56036	14.05	-
38	"PS(18:0_22:5)-H"	1.09	836.54471	13.12	-
39	"PC(17:0_22:5)+HCOO"	1.09	866.59166	14.25	-
40	"PC(18:0_18:1)+HCOO"	1.09	832.60731	16.46	-
41	"PE(18:0_22:6)-H"	1.09	790.53923	14.25	-
42	"PC(18:0_22:5)+HCOO"	1.09	880.60731	15.06	-
43	"PE(18:1_22:5)-H"	1.09	790.53923	13.38	-
44	"PE(18:0_20:5)-H"	1.09	764.52358	13.72	-
45	"PI(17:0_20:5)-H"	1.09	869.51856	11.11	-
46	"PI(18:0_20:2)-H"	1.09	889.58116	13.42	-
47	"CL(18:1_18:2_18:2_18:2)-H"	1.09	1449.9806	22.45	-
48	"PC(O-18:1_22:6)+HCOO"	1.09	862.59675	15.25	-
49	"PC(15:0_20:5)+HCOO"	1.09	810.52906	11.64	-
50	"SM(d33:1)+HCOO"	1.09	733.55013	12.08	-

Using heatmap plots derived from the VIP analysis, the distribution of lipids responsible for discriminating between goat meat and pork can be observed more clearly (Figures 7A and 7B).

The heatmap visually highlights the differential expression patterns of key lipid species across the two groups, with distinct clustering patterns corresponding to goat and pork samples. For instance, lipids upregulated in pork showed consistently higher signal intensities across pork replicates, while certain lipids were notably elevated in goat meat. In this study, lipids of TG (16:0_18:0_18:1), TG (16:1_18:1_18:1CHO), DG (17:0_18:2), Cer (m18:2_22:0), and DG (O-18:0_18:2) detected in the positive mode, were upregulated in pork. In addition, the lipids of PI (18:0_20:2) and FA (20:1) were upregulated in pork and observed in the negative mode. This visual representation reinforces the discriminatory power of the selected lipid biomarkers. It supports the robustness of the PLS-DA model in distinguishing the two meat types based on their lipidomic profiles.

DGs, including DG (17:0_18:2), are neutral lipids that have been identified as bioactive compounds with anti-inflammatory, antimicrobial, and antitumor potential (Tietel *et al.*, 2023). Together with TGs and other lipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), these molecules are frequently observed in lipidomic studies on animal fats and meats. Notably, certain DG species, such as DG (4:0/17:2)+NH_4, have been reported exclusively in beef, highlighting their potential as species-specific lipid biomarkers (Maritha *et al.*, 2023).

Previous studies have reported that goat meat has been characterized by low lipid and cholesterol contents, with specific lipid biomarkers identified for quality assessment and authentication. For goat meat, lipidomic studies have identified processes involving the biosynthesis of unsaturated fatty acids, lipid oxidation, and lipid hydrolysis as key factors in meat quality, which can be used for authentication (Jia *et al.*, 2023). Meanwhile, specific lipid classes such as glycerolipids, glycerophospholipids, sterol lipids, sphingolipids, polyketides, fatty acyls, and prenol lipids have been identified as potential markers in pork (Mi *et al.*, 2019).

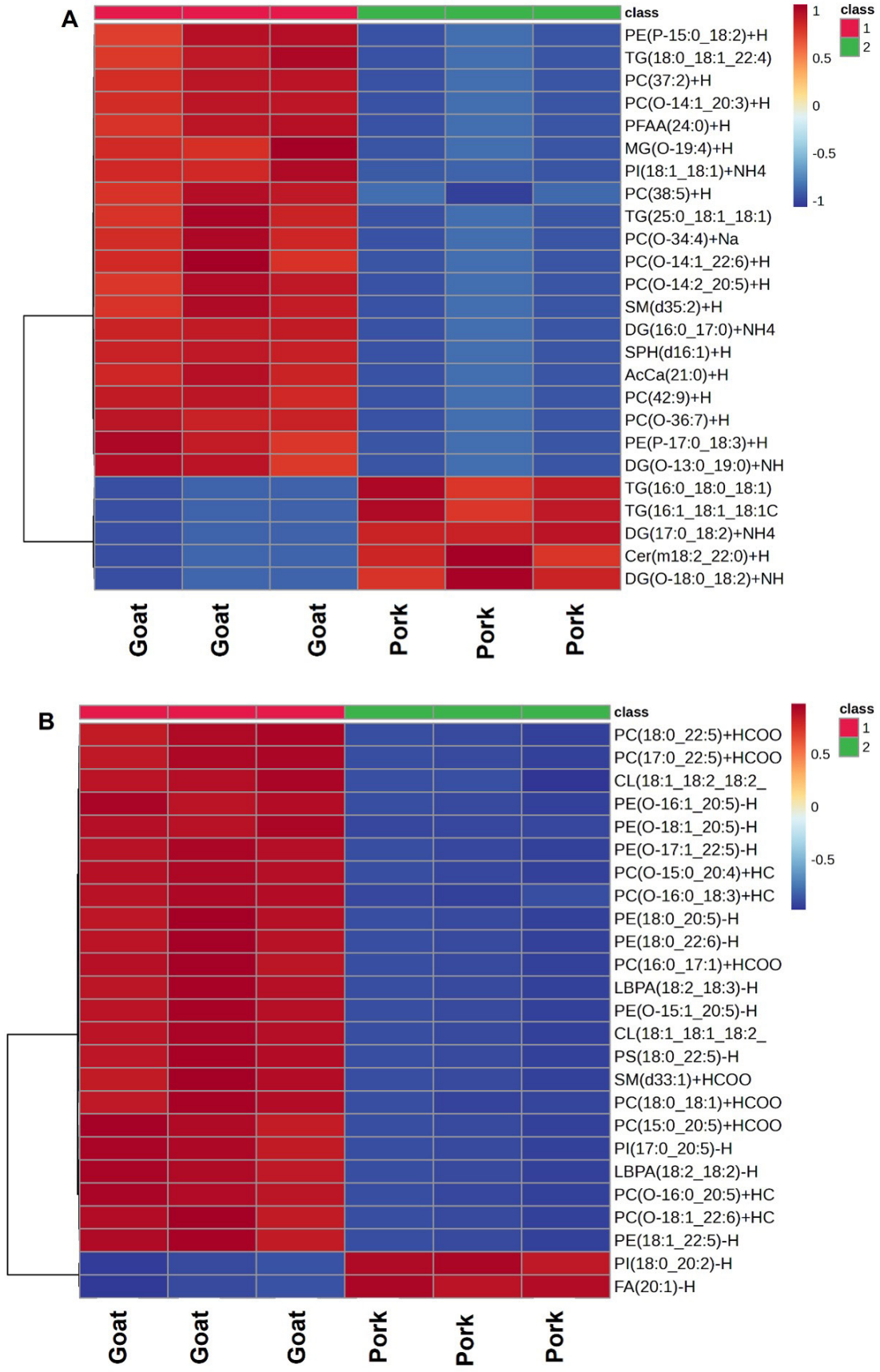


Figure 7. Heatmap of the discriminating metabolites (*p* value < 0.05) to discriminate goat meat and pork in positive (A) and negative (B) ionization modes. Each sample mas measured in three replicates.

3.4. Future perspectives for targeted assays in processed foods and adulteration scenarios

The lipid biomarkers identified in this study, particularly TG (52:1COOH_18:0), PE (O-36:1), DG (17:0_18:2), PE (O-17:1_18:2), and several other high-VIP lipids, show strong potential for the development of targeted lipidomic assays (e.g., using triple quadrupole or Orbitrap PRM mode). These assays could be adapted for routine halal authentication by monitoring selected multiple reaction monitoring (MRM) transitions or accurate mass with retention time. To address real-world applications, future validation should include: (1) binary and multiple adulteration models (e.g., pork in beef at 1–50% w/w), (2) various processed meat products (minced meat, meatballs, sausages, jerky) subjected to cooking, freezing, and storage, and (3) market samples collected from different regions. Matrix effects, lipid oxidation during processing, and the stability of candidate biomarkers under thermal and oxidative stress will need to be systematically evaluated. Such targeted approaches would offer higher sensitivity, faster analysis time, and greater robustness compared to non-targeted methods, thereby facilitating practical implementation in halal certification laboratories and regulatory enforcement (Harlina *et al*., 2025).

4. CONCLUSIONS

The findings of this study demonstrate the efficacy of non-targeted lipidomics, utilizing LC-Orbitrap HRMS coupled with chemometric analysis, as a powerful approach for the discrimination of beef, pork, and goat meat for halal authentication purposes. The distinct lipid profiles identified through multivariate statistical analysis enabled accurate classification of the three meat types, highlighting the potential of specific lipid species as biomarkers for authentication. This methodology offers a sensitive and reliable analytical tool that can be applied to ensure the integrity of halal food products and protect consumers from fraudulent mislabelling. The compounds of TG (52:1COOH_18:0), PE (O-36:1), and DG (17:0_18:2) were found to play important roles in discriminating pork from beef and goat meat and, therefore, represent potential biomarker candidates. Further research could focus on validating these findings in processed meat products and exploring the application of this approach to authenticating other food commodities.

Supplementary information ↑

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Supplementary material

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Data availability

Not applicable.

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Authorship contribution statement

NAF: Investigation, Data curation, Writing original draft, Writing-review & editing; AW: Conceptualization, Data curation, Investigation, Formal analysis, Supervision, Writing original draft, Writing-review & editing; HDW: Formal analysis, Writing original draft, Writing-review & editing; AWI: Formal analysis, Writing original draft, Writing-review & editing; LR: Data curation, Writing original draft, Writing-review & editing; SDV: Data curation, Writing original draft, Writing-review & editing; ASA: Formal analysis, Writing original draft, Writing-review & editing; S: Data curation, Writing original draft, Writing-review & editing; AR: Formal analysis, Writing-review & editing; RAS: Formal analysis, Writing-review & editing.

Competing interests

The authors declare that there is no conflict of interest.

Statement on the use of Artificial Intelligence

Not applicable.

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