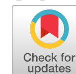
p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, September 2024, 47(3):381-391
DOI: https://doi.org/10.5398/tasj.2024.47.3.381
Available online at https://journal.ipb.ac.id/index.php/tasj



Chemometrics Assisted LC-HRMS Non-Targeted Metabolomics for Discrimination of Beef, Chicken, and Wild Boar Meats

A. Windarsih[a], A. Rohman[b,c,*], Y. Khasanah[a], Y. Erwanto[c,d], & N. K. Abu Bakar[e]
[a]Research Center for Food Technology and Processing (PRTPP), National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia 55861
[b]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
[c]Center of Excellence, Institute of Halal Industry and Systems (IHIS), Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
[d]Faculty of Animal Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
[e]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia 50603
*Corresponding author: abdulkimfar@gmail.com
(Received 16-02-2024; Revised 03-07-2024; Accepted 04-07-2024)

ABSTRACT

Meat authentication is very important to avoid adulteration, substitution, and mislabeling of meats and meat-based products to protect consumers by ensuring quality, safety, and halal status. This research aimed to employ metabolomics approach using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to identify metabolites of beef (BM), chicken meat (CM), and wild boar meat (WBM) as well as to identify the discriminating metabolites of BM-WBM and CM-WBM. The chemometrics of principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were used to differentiate BM, CM, and WBM. The orthogonal projection to latent structures-discriminant analysis (OPLS-DA) was used to discriminate and identify discriminating metabolites of BM-WBM and CM-WBM through the variable importance for projections (VIP) value analysis (VIP>1.50, p<0.05). The heatmap plot showed the distribution of discriminating metabolites in BM, CM, and WBM samples. The results of this study suggested that untargeted LC-HRMS successfully identified metabolites in meats. In addition, chemometrics could be used to discriminate between BM, CM, and WBM clearly. In summary, the combination of LC-HRMS and chemometrics is promising for the authentication of meats to ensure the quality as well as halal status of meats.

Keywords: *chemometrics; halal authentication; LC-HRMS; metabolomics*

INTRODUCTION

The authenticity of meat is important to ensure its quality, safety, and halal status. Due to economic reasons, the unethical practice of meat adulteration has been found in markets of several countries such as Indonesia, Malaysia, Thailand, China, and even in several European countries (Siswara *et al.,* 2022). In addition, meat and meat products become the most common food products susceptible for adulteration, as reported by the United States Pharmacopeia (USP) in 2021 (Owolabi & Olayinka, 2021). Certain meats have similar physical properties causing some difficulties in detecting adulteration or mislabeling only using visual investigation, especially in mince meats (Hrbek *et al.,* 2020). The incidence of horse meat scandal in Europe forces authorities to increase awareness on meat authenticity by applying more stringent regulations on meat adulterations (Premanandh, 2013; Sentandreu & Sentandreu, 2014). In addition, beef has a higher price than other meats, which often triggers unethical traders to mix it with lower-priced meats. Wild boar is widely consumed by people in some countries because of its availability in the market. Therefore, it is often used to replace or substitute beef for more profits. On the other hand, chicken meat is also widely spread and consumed by most of people. Although adulteration of chicken with wild boar maybe rare because the report is limited, however, we do not know in reality. It is possible that there are adulteration cases of chicken meat with wild boar. As a consequence, adequate analytical methods for the authentication of meats from adulteration and mislabeling are truly important to ensure the quality of meats.

There are a lot of analytical methods that have been developed by many researchers aimed for meat authentication, including spectroscopic and chromatographic-based methods. Fingerprinting analytical techniques using vibrational spectroscopy, such as Fourier transform infrared (FTIR) spectroscopy, near infrared (NIR) spectroscopy, and Raman spectroscopy offered fast analysis without requiring many chemical

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solvents and non-destructive techniques (Cozzolino *et al.,* 2023; Dashti *et al.,* 2022; Qu *et al.,* 2022). However, the vibrational spectroscopy techniques could not be used for the identification of compounds in meat; therefore, they cannot be used to identify the discriminating metabolites for each meat (Zia *et al.,* 2020). On the other hand, chromatographic-based techniques, such as gas chromatography (GC) and liquid chromatography (LC), allow for the analysis of targets of compounds specific in meat. Hyphenated with mass spectrometry (MS) as the detectors, LC-MS allows for analysis of as many as compounds/metabolites in meat samples called metabolomics. Currently, the metabolomics approach has attracted great interest in food research, including meat authentication, due to its ability to reveal metabolites in different meat samples (Böhme *et al.,* 2019; Selamat *et al.,* 2021).

Metabolomics is the analysis on metabolite composition in samples, including food, plant, and biological samples, to identify the types and content of metabolites. Several analytical techniques are available for metabolomics, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), liquid chromatography-high resolution mass spectrometry (LC-HRMS), and nuclear magnetic resonance (NMR) spectroscopy (Wang *et al.,* 2020; Zeki *et al.,* 2020). Among the metabolomics techniques, liquid chromatography-high resolution mass spectrometry (LC-HRMS) has a great capacity to reveal metabolites in food samples due to its high sensitivity and high specificity. In addition, the high-resolution mass spectrometer allows for the identification of compounds with high mass measurement accuracy (López-Ruiz *et al.,* 2019; Mialon *et al.,* 2023). Assisted with chemometrics, which can analyze and interpret the huge number of metabolites resulting from LC-HRMS techniques, the important information could be extracted using chemometrics (Paul *et al.,* 2021). Principal component analysis (PCA) is one of the chemometrics techniques which is mostly used in metabolomics analysis through unsupervised pattern recognition (Wang *et al.,* 2021). In addition, the supervised technique such as partial least square-discriminant analysis (PLS-DA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) is widely used due to its strong ability to discriminate and classify samples (Dinis *et al.,* 2023).

The metabolomics approach using LC-HRMS in combination with PCA and PLS-DA has been successfully used to discriminate beef from different origins (Windarsih *et al.,* 2023). The adulteration of beef with pork was also successfully detected using LC-HRMS metabolomics and PLS-DA (Trivedi *et al.,* 2016). In addition, the utilization of PCA and PLS-DA for analysis of LC-HRMS metabolomics data was successfully used to differentiate pork, tuna meat, and tuna meat adulterated pork (Suratno *et al.,* 2023). The volatilomics using headspace-solid phase microextraction-GC-MS (HS-SPME-GC-MS) has been used to discriminate meatballs made from beef, chicken, and wild boar meats (Pranata *et al.,* 2021). However, volatilomics only focuses on volatile compounds, which is not as comprehensive as non-volatile metabolites. Therefore, a study on metabolite profiling of non-volatile metabolites from beef, chicken, and wild boar meats is urgently needed to obtain potential biomarkers from non-volatile metabolites. To our best knowledge, no previous report was found associated with the use of LC-HRMS untargeted metabolomics focusing on non-volatile metabolites aided with chemometrics for discriminating of beef, chicken meat, and wild boar meat. There is no report on the comprehensive identification of non-volatile metabolites of beef, wild boar, and chicken meat using LC-HRMS metabolomics, which will provide useful information on the composition of non-volatile metabolites. In addition, there is no report on the utilization of chemometrics to discriminate BM, CM, and WBM for investigating the non-volatile metabolites potential as biomarker candidates for halal authentication purposes. Therefore, the purpose of this study was to identify non-volatile metabolites composition in beef, chicken meat, and wild boar meat using an LC-HRMS untargeted metabolomics technique and to apply chemometrics for discriminating beef, chicken meat, and wild boar meat and to identify the discriminating metabolites (non-protein markers) responsible for discriminating wild boar meat from beef and chicken meat. We focused on the non-volatile metabolites, not protein, because non-volatile metabolites analysis using LC-HRMS offers more efficiency in terms of time of analysis and cost of chemicals.

MATERIALS AND METHODS

Materials

Methanol for LC-MS, water for LC-MS, formic acid p.a., and methanol for HPLC was obtained from Merck (Darmstadt, Germany), whereas Pierce ESI (+) and Pierce ESI (-) calibration solution were obtained from Thermo Fisher (Thermo Scientific, Rockford, USA).

Samples Collection and Preparation

Beef meat (BM), chicken meat (CM), and wild boar meat (WBM) were obtained from three different markets in Yogyakarta, Indonesia. The number of samples for each meat was 5 samples obtained from different animals. The loin part was used for BM and WBM. Meanwhile, boneless breast meat was used for the CM. All samples were stored in a freezer (-20 ^{o}C) until used for analysis. The samples were immediately used for analysis the day after the day of collection. On the day of analysis, samples were thawed in a chiller (4 ^{o}C) for about 6 hours. Then, samples were chopped into small pieces and ground using a separate meat grinder for each meat. The obtained ground meat was used for metabolite extraction.

Metabolite Extraction

Extraction of metabolites from meat samples followed the method by Windarsih *et al.* (2022). The

amount of 100 mg of raw meat was weighed using an analytical balance and placed into a 2 mL centrifuge tube, and then 1 mL of methanol for LC-MS was added for sample extraction. Subsequently, samples were sonicated for 30 min at room temperature for metabolite extraction. Then, the protein was precipitated to avoid interfering with metabolite determination by placing samples in at freezer (-20 ^{o}C) for 30 min. After that, centrifugation was performed by using a centrifuge (Megafuge, Thermo Scientific, USA) at 5000 g for 10 min. The supernatant was taken and filtered using a PTFE 0.22 µm filter.

Metabolomics Analysis using LC-HRMS

An ultra-high performance liquid chromatography (Vanquish, Thermo Scientific, USA) connected to an Orbitrap high resolution mass spectrometer (Q-Exactive, Thermo Scientific, USA) was used for metabolomics analysis. Metabolomics analysis followed the untargeted workflow based on Suratno *et al*. (2023) with slight modifications. A reverse phase chromatography technique was chosen by using a C18 column. Metabolite separation was conducted using the gradient elution technique with two mobile phases of MS grade water (A) and MS grade methanol (B), both of which contained 0.1% formic acid. The elution started with 5% B and continued to 90% B from 5-20 min. Then, the condition was maintained at 90% B from 20-30 min and set to 5% B at 30-35 min. During the elution of compounds, the temperature of column was set at 40 ^{o}C. The sample was injected at 10 µL and eluted using a flow rate of 0.3 mL/min. The compounds were detected in an Orbitrap HRMS at positive and negative ionization modes. The formation of ions was performed using heated electrospray ionization (HESI) aided with a sheath gas flow rate of 32 arbitrary units (AU) followed by an auxiliary gas flow rate of 8 AU. Compounds were screened at 66.7-1000 m/z with a resolution of 70,000 for MS1 and 17,500 for MS2. Calibration of the mass spectrometer was performed using Pierce ESI (+) and Pierce ESI (-) to ensure the accuracy of the mass measurement.

Data Processing

The total ion chromatogram from LC-HRMS was analyzed using Compound Discoverer software (Thermo Scientific, USA) to identify the metabolites. The steps for processing the TIC included spectrum selector, retention time alignment, background subtraction, peak integration, and analysis of compounds against the Chemspider and MzCloud databases. The metabolites were filtered according to the parameters: only metabolites with names, metabolites with mass error between 5 ppm, and metabolites from data for preferred ion.

Chemometrics Analysis

The results of the metabolites that were obtained were exported into a table in Microsoft Excel. The metabolites and their peak area were used as the variables to build chemometrics models using Metaboanalyst 6.0 online platform. Prior to analysis, the data were subjected to sum normalization and Pareto scaling. Unsupervised pattern recognition analysis of principal component analysis (PCA) was initially used to identify samples pattern in BM, CM, and WBM. Then, supervised pattern recognition using orthogonal partial least square-discriminant analysis (OPLS-DA) was applied to discriminate BM-WBM and CM-WBM. The values of R^2 and Q^2 were observed to evaluate model fitness and model predictivity of PCA, respectively. In addition, the performance of OPLS-DA was evaluated through R^2Y and Q^2 values. The discriminating metabolites of beef-wild boar and chicken-wild boar were investigated through the variable importance for projections (VIP) value analysis. Metabolites with VIP≥1.50 and $p<0.05$ were selected. Both OPLS-DA models were subjected to permutation tests for model validation purposes. The Heatmap analysis was also performed using variables of discriminating metabolites from VIP analysis of both OPLS-DA model of BM-WBM and CM-WBM to demonstrate the distribution of metabolites in each class.

RESULTS

LC-HRMS was successfully used to identify metabolites in BM, CM, and WBM using the untargeted metabolomics approach. Employing reverse-phase chromatography technique, various metabolites mostly consisting of amino acids, fatty acids, and other lipids, are found in meat samples. Previous research reported that reverse-phase chromatography was more suitable for metabolomics analysis in meat samples than using HILIC (Zhang *et al*., 2021). Figure 1 shows the obtained result of the total ion chromatogram (TIC) from LC-HRMS measurement on beef, chicken meat (CM), and wild boar meat (WBM). According to Figure 1, TIC of CM had a quite similar TIC pattern with WBM. It might be associated with the number of metabolites that are similar in CM and WBM. Therefore, the identification of metabolites against the databases is important to reveal the composition of metabolites among BM, CM, and WBM.

In this study, various amino acids such as acetylcholine, D-(+)-Proline, DL-Arginine, DL-Glutamine, DL-Histidine, DL-Lysine, DL-Tryptophan, L(-)-Carnitine, L-(-)-Methionine, L-(-)-Threonine, L-Glutamic acid, L-Isoleucine, L-Phenylalanine, Prolyl leucine, and L-Tyrosine were found in BM, CM, and WBM since amino acids become one of the important metabolites contained in meats. In addition, various fatty acids were also found including oleic acid, palmitic acid, linolenelaidic acid, ethyl palmitoleate, ethyl oleate, ethyl myristate, eicosapentaenoic acid, docosatrienoic acid, docosahexaenoic acid, docosapentaenoic acid, 3-oxopalmitic acid, and 3-oxooctaenoic acid. Amino acids and fatty acids become the most common metabolites found in BM, CM, and WBM. Besides, other metabolites from organic acids and lipid classes were also found.

The metabolites extracted from TIC were used for chemometrics analysis to differentiate beef, chicken

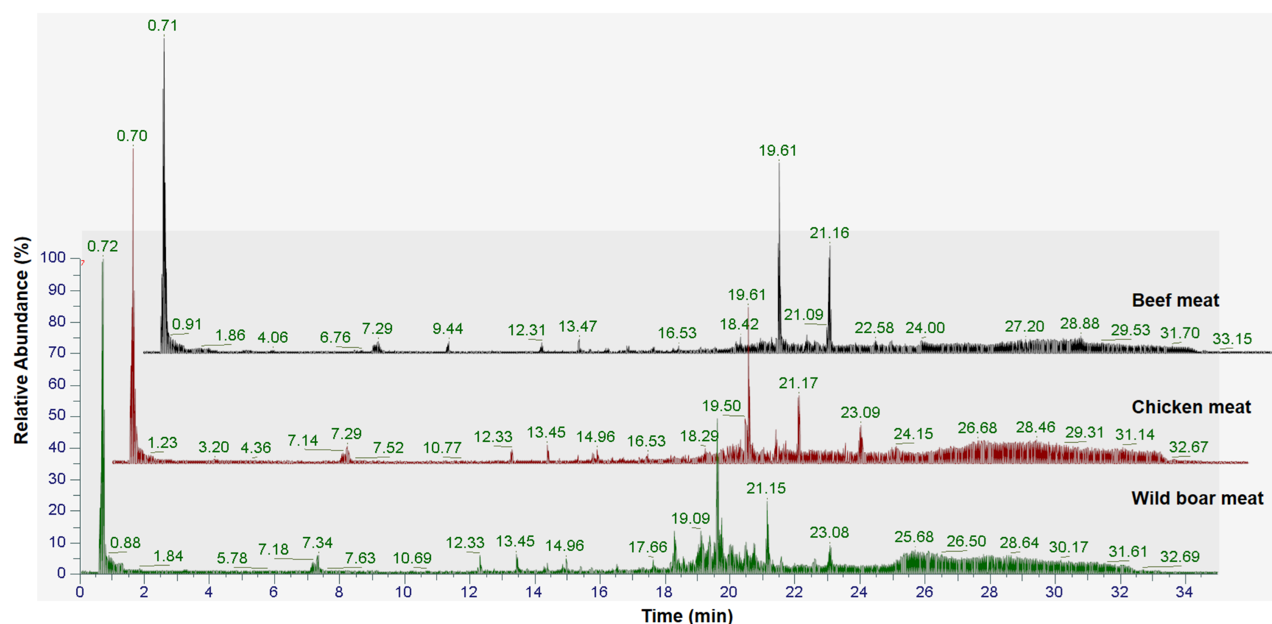

Figure 1. Total ion chromatogram of beef, chicken meat, and wild boar meat

meat, and wild boar meat. A total of 448 metabolites were used as variables for chemometrics analysis. BM, CM, and WBM could be differentiated by using two principal components (PCs, PC1=56.4%, PC2=28.1%) in PCA, as shown in Figure 2A. The score plots of BM, CM, and WBM appeared in a different cluster, indicating the difference in metabolites composition of BM, CM, and WBM. PCA had a good of fitness (R^2=0.845) and good of predictivity (Q^2=0.805). Confirming using PLS-DA (Figure 2B), the score plots of PCA were similar to the score plots of PLS-DA. The PLS-DA was built using 2 latent variables (LVs), resulting in good accuracy (R^2X=0.845, R^2Y=0.992) and good predictivity (Q^2=0.990).

To further discriminate between BM-WBM and CM-WBM, supervised chemometrics of OPLS-DA were applied. In this study, OPLS-DA was successfully used to discriminate BM and WBM clearly (Figure 3A). The OPLS-DA model had R^2Y value of 0.995 and Q^2 of 0.941, associating to the good of fitness and good predictivity model, respectively. Permutation employing 100 permutations of the validation test showed that the p-value of Q2 and R2Y was lower than 0.01, indicating the validity of the OPLS-DA as a discrimination model. Table 1 shows the discriminating metabolites responsible for discriminating BM and WBM observed through variable importance for projections (VIP) analysis (VIP≥1.50, $p<0.05$). Different metabolites were found in the discriminating metabolites, such as amino acids, peptides, fatty acids, and lipids. The distribution of each discriminating metabolite in BM and WBM samples is illustrated in Figure 4 through the heatmap plot. The five strongest discriminating metabolites to discriminate BM and WBM are 3-Hydroxy-3-[(3-methylbutanoyl)oxy]-4-(trimethylammonio)butanoate, L(-)-Carnitine, pipecolic acid, 1-Stearoylglycerol, and arachidonic acid. Metabolite of 3-Hydroxy-3-[(3-methylbutanoyl)oxy]-4-(trimethylammonio)butanoate has the highest VIP value to discriminate beef and wild boar. It is one of the carnitine compounds which has a synonym of (2R)-3-Hydroxyisovaleroyl Carnitine. The second highest discriminating metabolite is also a carnitine compound, L(-)-Carnitine. Carnitine is reported to be found in meats, including red meat such as beef, pork, and wild boar as well as other meats such as chicken meat. The third largest VIP value was pipecolic acid, a metabolite of lysine. Pipecolic acid is also reported to be found in meats, including beef. The 1-Stearoylglycerol, the fourth strongest discriminating metabolite, is a long chain fatty alcohol and it is widely found in meat samples. Then, the fifth strongest discriminating metabolite is arachidonic acids. Meats such as beef, pork, chicken, and wild boar are reported to contain arachidonic acids. Therefore, the amounts of arachidonic acids in each type of meat could be used to discriminate each other.

On the other hand, OPLS-DA was also successfully used to discriminate CM and WBM (Figure 3B). The good of fitness of OPLS-DA model was shown by its R^2Y (0.992) whereas the model predictivity was stated by the Q^2 (0.882) value. The obtained results from the OPLS-DA score plots showed that CM was clearly discriminated into a separate cluster with WBM samples. The OPLS-DA model was valid evaluated through permutation test using 100 permutations with significant p-value of both Q^2 and R^2Y ($p<0.05$). Table 2 shows the discriminating metabolites responsible to discriminate CM and WBM observed through variable importance for projections (VIP) analysis (VIP≥1.50, $p<0.05$). The heatmap plot in Figure 5 clearly shows the high distribution of metabolites in CM and WBM samples. The five strongest discriminating metabolites to differentiate CM and WBM mostly consisted of 1-Palmitoyl-sn-glycero-3-phosphocholine, 3-hydroxydecanoyl carnitine, cortisol, LysoPC(18:3(9Z,12Z,15Z)), and stearic acid. Compounds of 1-Palmitoyl-sn-glycero-3-phosphocholine and LysoPC(18:3(9Z,12Z,15Z)) are lipid metabolites. It

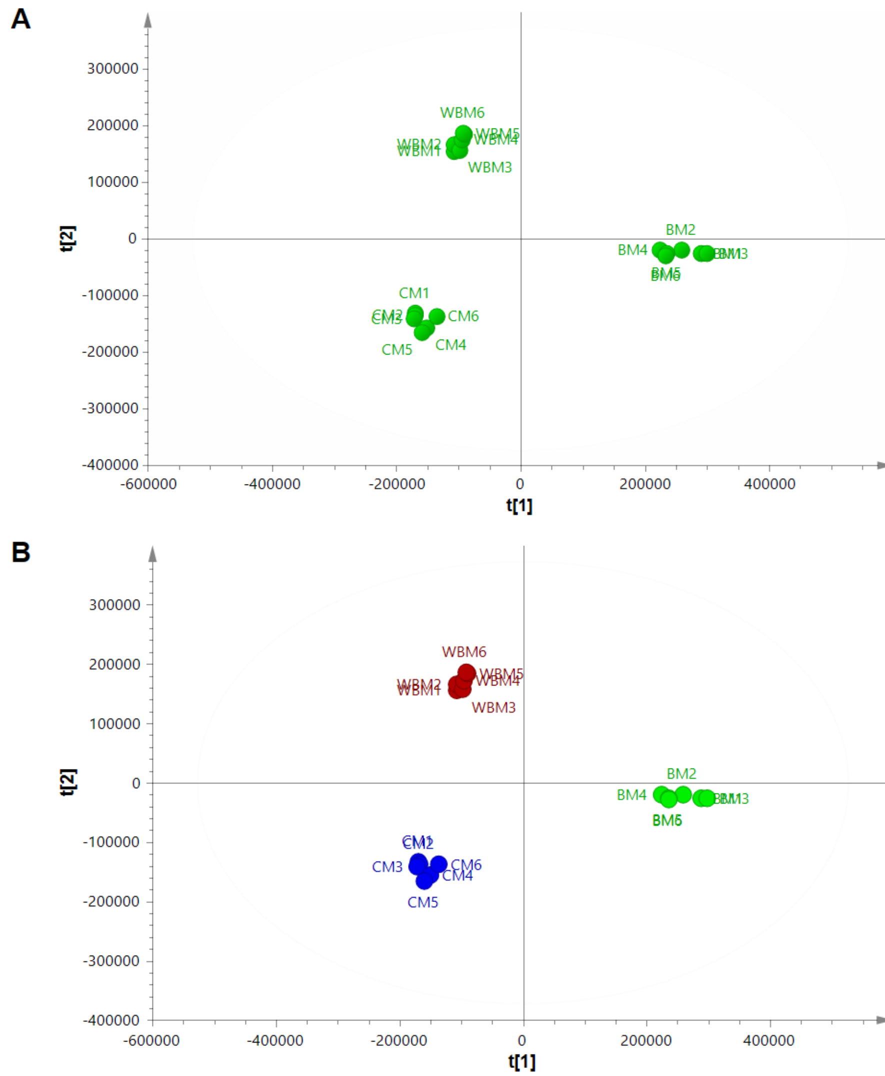

Figure 2. The principal component analysis (A) and partial least square-discriminant analysis (B) of beef, chicken meat, and wild boar meat. BM=beef meat, CM=chicken meat, WBM=wild boar meat, t[1]=principal component 1, t[2]=principal component 2.

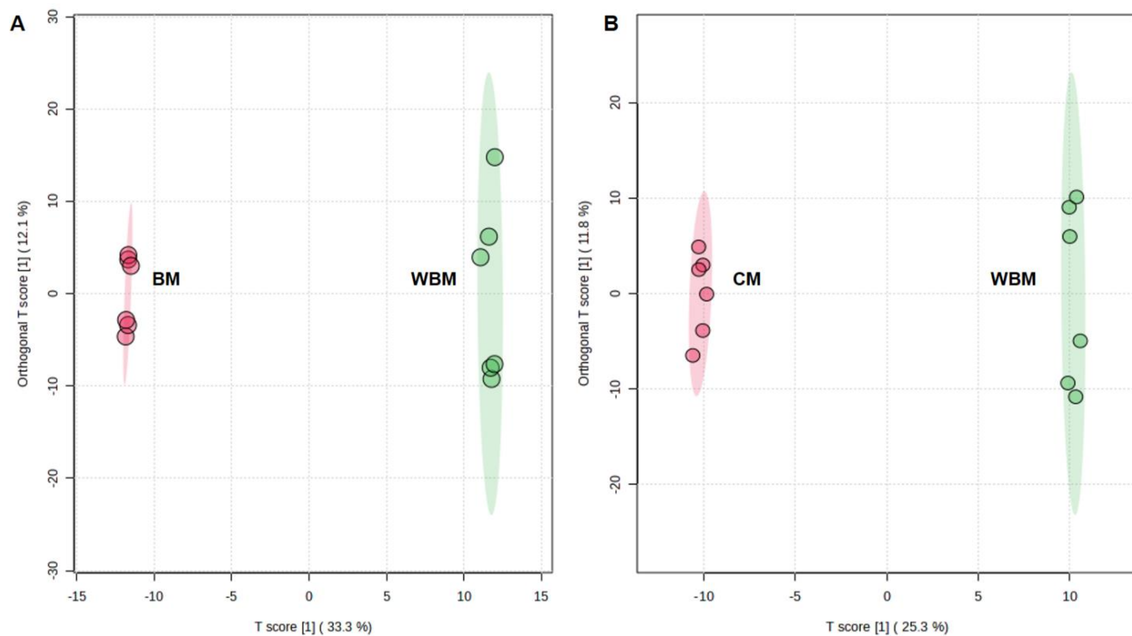

Figure 3. The orthogonal projection to latent structures-discriminant analysis (OLPLS-DA) to discriminate beef-wild boar meat (A) and chicken meat-wild boar meat (B). BM=beef meat, CM=chicken meat, WBM=wild boar meat.

Table 1. The discriminating metabolites to discriminate beef and wild boar meat (VIP≥1.50, p<0.05)

No.	Compounds	VIP	Molecular formula	Calculated m/z	Retention time (min)
1	3-Hydroxy-3-[(3-methylbutanoyl)oxy]-4-(trimethylammonio) butanoate	1.72	$C_{12}H_{23}NO_5$	26.115.774	1.249
2	L(-)-Carnitine	1.71	$C_7H_{15}NO_3$	16.110.506	0.689
3	Pipecolic acid	1.71	$C_6H_{11}NO_2$	12.907.890	0.686
4	1-Stearoylglycerol	1.71	$C_{21}H_{42}O_4$	35.830.766	21.159
5	Arachidonic acid	1.71	$C_{20}H_{32}O_2$	30.424.042	19.651
6	1-heptadecanoyl-sn-glycero-3-phosphocholine	1.71	$C_{25}H_{52}NO_7P$	50.934.801	19.815
7	(2E,4Z)-N-Isobutyl-2,4-octadecadienamide	1.70	$C_{22}H_{41}NO$	33.531.841	21.463
8	8-hydroxy-deoxyguanosine	1.70	$C_{10}H_{13}N_5O_5$	28.309.176	1.289
9	1-Hydroxy-2-eicosapentaenoyl-sn-glycero-3-phosphoethanolamine	1.69	$C_{25}H_{42}NO_7P$	49.927.025	17.630
10	2-Hexenoylcarnitine	1.69	$C_{13}H_{23}NO_4$	25.716.309	5.237
11	b-Ala-Lys	1.68	$C_9H_{19}N_3O_3$	21.714.245	0.644
12	Uridine	1.68	$C_9H_{12}N_2O_6$	24.406.931	1.013
13	2,3-Dihydroxy-4-decynoic acid	1.68	$C_{10}H_{16}O_4$	20.010.511	9.655
14	1-Oleoyl-2-hydroxy-sn-glycerol-3-phosphocholine	1.67	$C_{26}H_{52}NO_7P$	52.134.810	19.341
15	9,10-Dihydroxy-11-(3-pentyl-2-oxiranyl) undecanoic acid	1.66	$C_{18}H_{34}O_5$	33.024.049	14.491
16	(+/-)-Muscone	1.66	$C_{16}H_{30}O$	23.822.950	19.617
17	Oleic acid alkyne	1.66	$C_{18}H_{30}O_2$	27.822.468	19.108
18	Oleamide	1.66	$C_{18}H_{35}NO$	28.127.146	19.520
19	N-homo-γ-linolenoylethanolamine	1.65	$C_{22}H_{39}NO_2$	34.929.774	20.846
20	2-Tetradecylcyclobutanone	1.64	$C_{18}H_{34}O$	26.626.085	21.159
21	Monoolein	1.63	$C_{21}H_{40}O_4$	35.629.211	19.837
22	L-Isoleucine	1.63	$C_6H_{13}NO_2$	13.109.460	1.114
23	(11-eicosenoyl)-lysophosphatidylethanolamine	1.63	$C_{25}H_{50}NO_7P$	50.733.264	18.469
24	α-Linolenyl carnitine	1.60	$C_{25}H_{43}NO_4$	42.131.873	17.336
25	D-Glucose 6-phosphate	1.59	$C_6H_{13}O_9P$	26.002.973	0.755
26	9-Oxo-10(E),12(E)-octadecadienoic acid	1.59	$C_{18}H_{30}O_3$	29.421.927	17.161
27	Leu-arg	1.58	$C_{12}H_{25}N_5O_3$	28.719.591	1.162
28	(2-(9Z,12Z)- octadecadienoyl-sn-glycero-3-phosphocholine	1.58	$C_{26}H_{50}NO_7P$	51.933.239	18.313
29	Carnosine	1.58	$C_9H_{14}N_4O_3$	22.610.644	0.655
30	3,4-Dimethyl-5-propyl-2-furantridecanoic acid	1.56	C22H38O3	35.028.133	21.277
31	1-[(9Z)-hexadecenoyl]-sn-glycero-3-phosphocholine	1.56	$C_{24}H_{48}NO_7P$	49.331.701	18.185
32	DL-Histidine	1.55	$C_6H_9N_3O_2$	15.506.930	0.662
33	N-(2-hydroxypentadecanoyl)-4-hydroxysphinganine	1.54	$C_{33}H_{67}NO_5$	55.750.158	19.679
34	2-[(5Z)-5-tetradecenyl]cyclobutanone	1.54	$C_{18}H_{32}O$	26.424.526	19.588
35	1-Adrenoyl-glycero-3-phosphocholine	1.51	$C_{30}H_{54}NO_7P$	57.136.318	19.463
36	D-lysopine	1.51	$C_9H_{18}N_2O_4$	21.812.664	1.905
37	L-α-Palmitin	1.50	$C_{19}H_{38}O_4$	33.027.640	19.617

Note: VIP=variable importance for projection

can be found in meats, including BM, CM, and WBM. Meanwhile, 3-hydroxydecanoyl carnitine is one of the carnitine groups that is commonly found in meats, especially red meat. Meanwhile, cortisol is a hormone that is also reported to be found in meats. The last is stearic acid, a fatty acid compound contained in various meats.

DISCUSSION

PCA and PLS-DA become the most used chemometrics techniques in pattern recognition analysis because both techniques can be coupled with various types of chemical data from different analytical instruments. A previous study reported that PCA and PLS-DA were successfully used to differentiate beef meat with different qualities. The high marble and low marble meats could be differentiated using PCA and PLS-DA (Jeong *et al.*, 2020). The accuracy is evaluated by using R^2 for PCA whereas for PLS-DA uses R^2Y. The R^2 and R^2Y close to 1 indicated high accuracy associated to the good of fitness. Meanwhile, a Q^2 larger than 0.40 is associated with good model predictivity (Worley & Powers, 2013). In this study, supervised chemometrics of OPLS-DA were used for the discrimination of beef-wild boar meat and beef-chicken meat. OPLS-DA is known for its strong ability to differentiate two classes of samples. The orthogonal algorithm could filter the variables with less importance for discrimination, thereby resulting in more adequate models to discriminate groups (Song *et al.*, 2021).

In this study, the obtained discriminating metabolites from VIP analysis consisted of amino acids, fatty

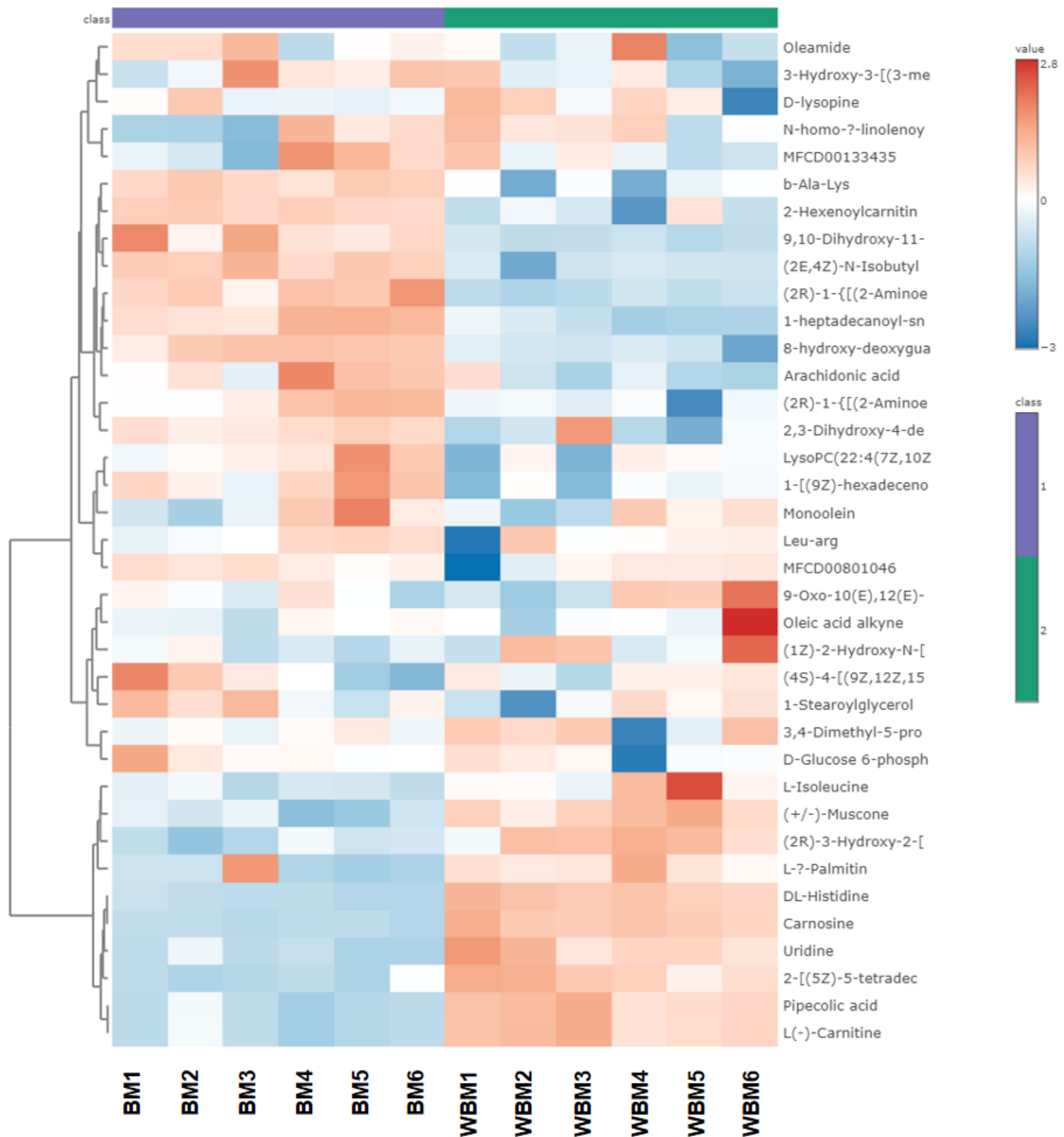

Figure 4. Heatmap analysis to identify the distribution of discriminating metabolites to discriminate beef meat and wild boar meat. BM=beef meat, WBM=wild boar meat, the number of 1, 2, 3, 4, 5, and 6 indicates the number of sample replications.

acids, peptides, and carnitines. Amino acids play an important role in growth and in regulating metabolic pathways (Zotte *et al.*, 2020). In addition, amino acids are also responsible for meat flavor. In this study, the branched-chain amino acids (BCAA), such as leucine and isoleucine, were also found. BCAA has various roles, such as signaling molecules in metabolic pathways and regulating lipolysis (Castillo & Gatlin, 2018; Nie *et al.*, 2018). On the other hand, meats also contain various fatty acids. Red meat, such as beef, is reported to be a valuable source of various fatty acids, especially polyunsaturated fatty acids (PUFA). Different meats might contain different fatty acids. In this study, the different types and numbers of fatty acids could be very useful for discriminating BM, CM, and WBM (Cama-Moncunill *et al.*, 2021).

Meanwhile, meats are also rich in short peptides such as Ala-Lys, Leu-Val, and Ala-Leu. Peptides are contained in red meat and other meats, including chicken meat. Peptides play an important role and sometimes some of them are active compounds, thereby called bioactive peptides. In this study, the different contents of peptides were potential as discriminating metabolites to differentiate BM and WBM as well as CM and WBM (López-Pedrouso *et al.*, 2023; Ryan *et al.*, 2011). On the other hand, carnitines were also found as discriminating metabolites with high VIP value. Carnitines are important to help the body convert fat into energy. Carnitines are highly found in red meat; besides, carnitine is also found in chicken meat. Therefore, in this study, carnitines found in BM and WBM were higher than those found in CM. Several types of carnitine are L-Carnitine, acetyl-L-carnitine,

Table 2. The discriminating metabolites to discriminate chicken meat and wild boar meat (VIP≥1.50, p<0.05)

No.	Compounds	VIP	Molecular formula	Calculated m/z	Retention time (min)
1	1-Palmitoyl-sn-glycero-3-phosphocholine	1.98	$C_{24}H_{50}NO_7P$	49.533.229	19.117
2	3-hydroxydecanoyl carnitine	1.97	$C_{17}H_{33}NO_5$	33.123.599	11.460
3	Cortisol	1.95	$C_{21}H_{30}O_5$	36.220.952	11.626
4	LysoPC(18:3(9Z,12Z,15Z))	1.95	$C_{26}H_{48}NO_7P$	51.731.691	17.716
5	Stearic acid	1.95	$C_{18}H_{36}O_2$	28.427.167	22.586
6	DL-Histidine	1.95	$C_6H_9N_3O_2$	15.506.930	0.662
7	D-(-)-Fructose	1.94	$C_6H_{12}O_6$	18.006.301	0.686
8	3-hydroxyhexadecanoylcarnitine	1.94	$C_{23}H_{45N}O_5$	41.532.986	17.443
9	Citric acid	1.94	$C_6H_8O_7$	19.202.635	0.746
10	Benzoyl cyanide	1.94	C_8H_5NO	13.103.728	6.574
11	L-Tyrosine	1.94	$C_9H_{11}NO_3$	18.107.354	1.024
12	D-(+)-Proline	1.93	$C_5H_9NO_2$	11.506.352	0.707
13	Prolylleucine	1.92	$C_{11}H_{20}N_2O_3$	22.814.724	0.734
14	L-alpha-lysophosphatidylcholine	1.91	$C_{22}H_{46}NO_7P$	46.730.151	17.880
15	((3β,4α,5α)-3-Hydroxy-4-methylcholest-7-ene-4-carbaldehyde)	1.91	$C_{29}H_{48}O_2$	42.836.515	26.451
16	2-methylbutyrylcarnitine	1.91	$C_{12}H_{23}NO_4$	24.516.308	4.155
17	5-Ethyl-4-hydroxy-3(2H)-furanone	1.90	$C_6H_8O_3$	12.804.747	1.252
18	α-Linolenyl carnitine	1.90	$C_{25}H_{43}NO_4$	42.131.873	17.336
19	Carnosine	1.90	$C_9H_{14}N_4O_3$	22.610.644	0.655
20	Uridine	1.90	$C_9H_{12}N_2O_6$	24.406.931	1.013
21	Propionylcarnitine	1.90	$C_{10}H_{19}NO_4$	21.713.143	1.061
22	LysoPC(22:4(7Z,10Z,13Z,16Z))	1.89	$C_{30}H_{54}NO_7P$	57.136.318	19.463
23	Arachidyl carnitine	1.87	$C_{27}H_{53}NO_4$	45.539.745	21.260
24	2-Methyl-1-sulfanyl-1-butanol	1.87	$C_5H_{12}O_S$	12.006.107	0.659
25	C14-Carnitine	1.85	$C_{21}H_{41}NO_4$	37.130.344	17.181
26	D-Gluconic acid	1.84	$C_6H_{12}O_7$	19.605.772	0.724
27	Palmitoylcarnitine	1.83	$C_{23}H_{45}NO_4$	39.933.446	18.433
28	3-Hydroxy-(9Z)-hexadecenoylcarnitine	1.83	$C_{23}H_{43}NO_5$	41.331.428	16.630
29	((3β,4α,5α)-3-Hydroxy-4-methylcholest-7-ene-4-carbaldehyde)	1.82	$C_{29}H_{48}O_3$	44.436.012	24.546
30	Cholest-4-en-3-one	1.82	$C_{27}H_{44}O$	38.433.872	32.053
31	Xanthine	1.8	$C_5H_4N_4O_2$	15.203.294	0.989
32	Dibenzylamine	1.79	$C_{14}H_{15}N$	19.712.047	7.242
33	Corchorifatty acid F	1.78	$C_{18}H_{32}O_5$	32.822.553	13.493
34	Ala-Leu	1.77	$C_9H_{18}N_2O_3$	20.213.166	1.246
35	Pipecolic acid	1.76	$C_6H_{11}NO_2$	12.907.890	0.686
36	Oleamide	1.75	$C_{18}H_{35}NO$	28.127.146	19.520
37	Z-Leu-OH	1.75	$C_{14}H_{19}NO_4$	26.513.179	4.766
38	Linoleyl carnitine	1.75	$C_{25}H_{45}NO_4$	42.333.498	17.951
39	p-Cresylsulfate	1.74	$C_7H_8O_4S$	18.801.386	5.157
40	2-[(5Z)-5-tetradecenyl]cyclobutanone	1.72	$C_{18}H_{32}O$	26.424.526	19.588
41	LysoPC(22:5(7Z,10Z,13Z,16Z,19Z))	1.70	$C_{30}H_{52}NO_7P$	56.934.803	18.849
42	N-[(2S)-2-Hydroxypropanoyl]-L-phenylalanine	1.70	$C_{12}H_{15}NO_4$	23.710.032	8.162
43	MFCD00037215	1.67	$C_8H_{14}N_2O_5$	21.808.992	0.720
44	Terminaline	1.64	$C_{23}H_{41}NO_2$	36.331.310	23.344
45	14(Z)-Eicosenoic acid	1.64	$C_{20}H_{38}O_2$	31.028.744	22.797
46	1-(1Z-hexadecenyl)-sn-glycero-3-phosphocholine	1.64	$C_{24}H_{50}NO_6P$	47.933.755	19.712
47	(2E,4Z)-N-Isobutyl-2,4-octadecadienamide	1.64	$C_{22}H_{41}NO$	33.531.841	21.463
48	3-hydroxyoctanoylcarnitine	1.62	$C_{15}H_{29}NO_5$	30.320.474	7.596
49	α-Eleostearic acid	1.61	$C_{18}H_{30}O_2$	27.822.420	17.251
50	1-Stearoyl-2-hydroxy-sn-glycero-3-PE	1.60	$C_{23}H4_{8N}O_7P$	48.131.747	20.095
51	D-Sedoheptulose 7-phosphate	1.59	$C_7H_{15}O_{10}P$	29.004.035	0.746
52	N-(Carboxymethyl)norleucine	1.59	$C_8H_{15}NO_4$	18.909.963	5.639
53	Phosphatidylserine(18:0/20:0)	1.59	$C_{44}H_{86}NO_{10}P$	81.959.799	21.606
54	Creatinine	1.59	$C_4H_7N_3O$	11.305.907	0.693
55	3-Oxo-2-[(2E)-2-pentenyl]-1-cyclopenten-1-yl)octanoic acid	1.58	$C_{18}H_{28}O_3$	29.220.393	16.588
56	1-Oleoyl-sn-glycero-3-phosphorylcholine	1.57	$C_{26}H_{52}NO_7P$	52.134.810	19.341

Table 2. Continued

No.	Compounds	VIP	Molecular formula	Calculated m/z	Retention time (min)
57	Pro-Pro	1.56	$C_{10}H_{16}N_2O_3$	21.211.653	0.948
58	L-(-)-Threonine	1.56	$C_4H_9NO_3$	11.905.848	0.719
59	Uracil	1.56	$C_4H_4N_2O_2$	11.202.750	0.986
60	1-(5-O-Phosphonopentofuranosyl)-2,4(1H,3H)-pyrimidinedione	1.56	$C_9H_{13}N_2O_9P$	32.403.591	0.760
61	11-(5-Ethyl-3,4-dimethyl-2-furyl)undecanoic acid	1.55	$C_{19}H_{32}O_3$	30.823.482	18.104
62	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	1.55	$C_9H_{19}NO$	15.714.659	12.329
63	Crotonic acid	1.53	$C_4H_6O_2$	8.603.711	1.003
64	Methyl palmitate	1.53	$C_{17}H_{34}O_2$	27.025.579	19.144
65	1,2-Dioctanoyl-sn-glycerol	1.53	$C_{19}H_{36}O_5$	34.425.643	15.080
66	(+/-)-Muscone	1.51	$C_{16}H_{30}O$	23.822.950	19.617
67	Alverine	1.50	$C_{20}H_{27}N$	28.121.426	19.222

Note: VIP=variable importance for projection

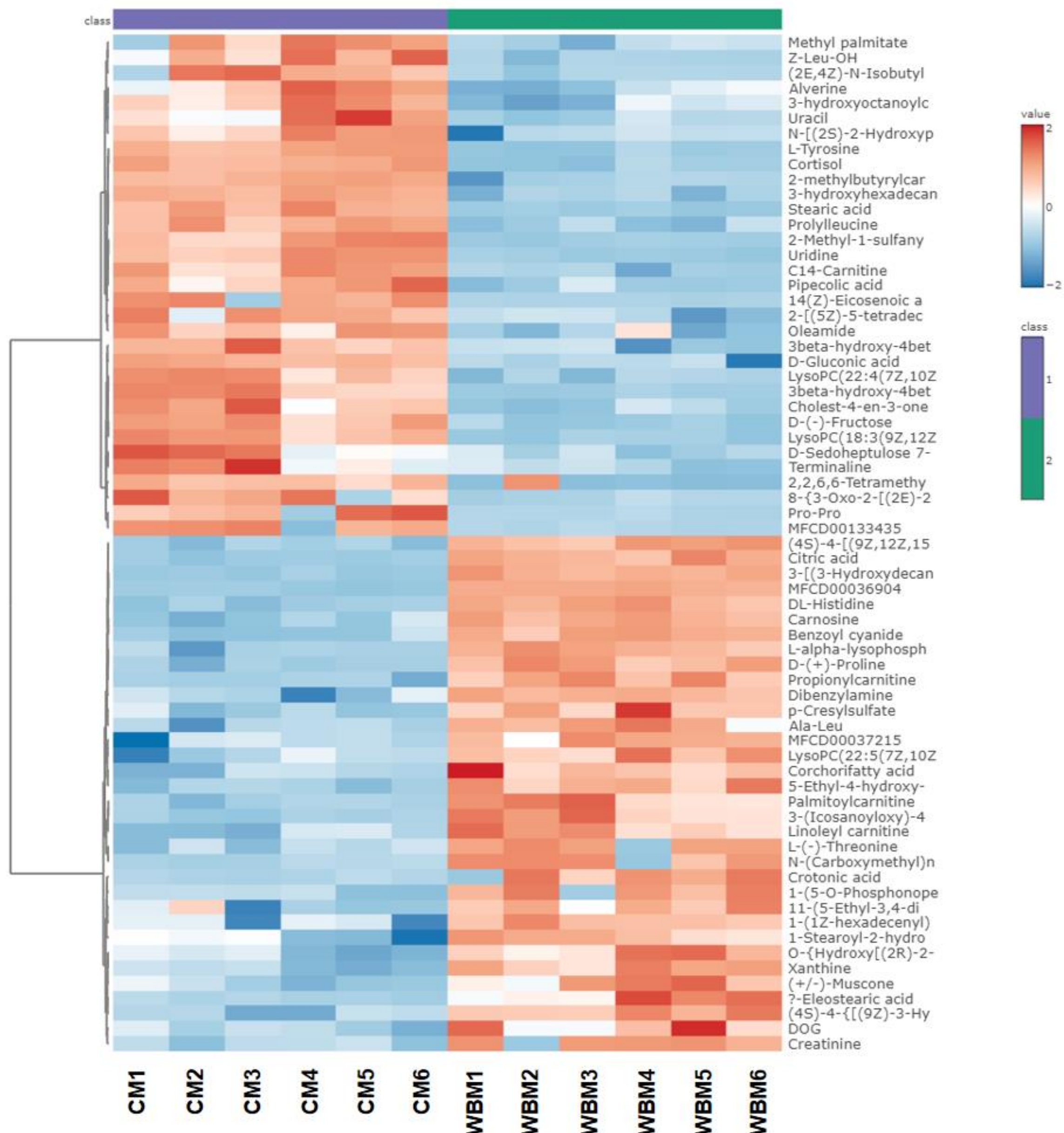

Figure 5. Heatmap analysis to identify the distribution of discriminating metabolites to discriminate chicken meat and wild boar meat. CM=chicken meat, WBM=wild boar meat, the number of 1, 2, 3, 4, 5, and 6 indicates the number of sample replications.

propionyl carnitine, and DL-Carnitine (Delgado *et al.*, 2021).

The results of this study indicated that the metabolomics approach using LC-HRMS has a powerful ability to identify metabolites in meat samples with high sensitivity and high specificity. Combined with chemometrics such as PCA and PLS-DA, resulting useful insight in investigating discriminating metabolites potential as biomarkers.

CONCLUSION

The untargeted LC-HRMS metabolomics revealed various metabolites in beef, chicken meat, and wild boar meat. Chemometrics of principal component analysis could be used to differentiate BM, CM, and WBM. Supervised pattern recognition chemometrics of OPLS-DA successfully discriminated WBM from BM and CM. Various discriminating metabolites responsible for the discrimination of BM-WBM and CM-WBM were obtained through VIP analysis. It can be concluded that the utilization of LC-HRMS metabolomics untargeted aided with chemometric techniques provided satisfactory results for the authentication of meats, including halal authentication purposes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

We thank LPDP for supporting this research through the Riset Indonesia Maju scheme, which was awarded to Prof. Dr. Abdul Rohman. This research was funded by Riset Inovasi untuk Indonesia Maju (RIIM) LPDP-BRIN 2023-2024 with contract number 172/IV/KS/11/2023 and 6815/UN1/DITLIT/Dit-Lit/KP.01.03/2023. The authors acknowledge Laboratorium BRIN Kawasan Yogyakarta for facilitating LC-HRMS instruments through ELSA BRIN.

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