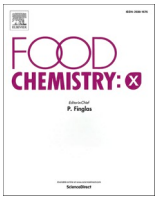
Food Chemistry: X 21 (2024) 101113

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Relationship between phospholipid molecules species and volatile compounds in grilled lambs during the heating process

Kexin Cheng [1], Teng Liu [1], Cong Yang, Hui Yang, Dengyong Liu [*]

College of Food Science and Technology, Bohai University, Jinzhou 121013, China

ARTICLE INFO

Keywords:
Lipidomics
Volatile compounds
Grilled lamb
Mechanism of flavor formation

ABSTRACT

The present study used a comprehensive analysis combining headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) and ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) to investigate changes in volatile compounds and phospholipid molecules in grilled lambs. The results revealed 19 key volatile compounds (OAV > 1) involved in the grilling process of lambs. Additionally, UPLC-ESI-MS/MS analysis detected 142 phospholipid molecules in grilled lamb, with phosphatidylcholine exhibiting the highest content (36.62 %), followed by phosphatidyl ethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidyl glycerol, and phosphatidic acid. Through partial least squares analysis, 63 key differential phospholipids were identified. Principal component analysis of the key differential phospholipids and volatile compounds indicated that phosphatidylcholine and phosphatidyl ethanolamine phospholipids are the key substrates in forming volatile compounds in grilled lambs. This information is essential for precisely regulating the flavor profile, enhancing the grilling process, and minimizing the production of harmful compounds in grilled meat products.

1. Introduction

Grilled lamb is a popular choice among consumers as a roasted meat product because of its pleasant aroma. However, under high-temperature heating conditions, complex chemical events such as protein degradation, lipid oxidation, and the Maillard reaction generate the most volatile chemicals in grilled lambs (Bassam, Noleto-Dias, & Fara, 2022). Studies have shown that the most volatile compounds in grilled lamb are aldehydes, alcohols, ketones, and furans, produced by the hydrolysis and oxidation of lipids (Mottram, 1998). Lipids hydrolyze to form free fatty acids, which are oxidized to form aromatic compounds, such as hexanal and nonanal (Frank, Kaczmarska, Paterson, Piyasiri, & Warner, 2017). Therefore, lipids play a crucial role in producing the aroma of grilled lambs.

Lipids are small hydrophobic or amphiphilic molecules classified into eight types of lipid MAPS: glycerophospholipids (GP), fatty acyls (FA), glycerolipids (GL), sphingolipids (SP), prenol lipids (PR), sterol lipids (ST), polyketides (PK), and saccharolipids (SL) (Fahy et al., 2005). Phospholipids (PL), on the other hand, can be divided into two main categories based on their molecular structure: GP and SP. Typical glycerophospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylglycerol (PG) (Fahy et al., 2005).

Phospholipids are enriched in unsaturated fatty acids, particularly polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFA), especially C18:2n-6 and C20:4n-6, are readily oxidized to produce volatile compounds such as hexanal, non-hexene, and 1-octen-3-ol, which are positively correlated with the lipid content (Li et al., 2020). In a sturgeon study, 173 lipids were found to change significantly during heating, of which phospholipids accounted for 67.05 % (Li et al., 2022). As a result, phospholipids are susceptible to oxidative degradation, and the oxidative degradation products directly affect the composition of volatile compounds in grilled lambs. Several studies have investigated phospholipids' role in producing volatile compounds in meat products. Phospholipids significantly affect the aromatic characteristics of pork, and variations in intramuscular phospholipid content cause differences in the aromatic characteristics of different pork breeds (Huang, Li, He,

* Corresponding author at: College of Food Science and Technology, Bohai University, No. 19, Keji Rd., New Songshan District, Jinzhou 121013, Liaoning Province, China.
E-mail address: jz_dyliu@126.com (D. Liu).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.fochx.2023.101113
Received 6 October 2023; Received in revised form 19 December 2023; Accepted 31 December 2023
Available online 5 January 2024

Wang, & Qin, 2010). Adding phospholipids to chicken significantly increases key volatile compounds, such as 2, 4-decadienal (lipid aroma), whereas neutral lipids do not (Chen, Balagiannis, & Parker, 2019). Volatile aldehydes produced by the hydrolysis of lipids in DHA-rich egg yolks to free fatty acids and further oxidation contribute to the egg yolks' 'fishy' aroma (Wang et al., 2023). In addition, GP metabolism is an essential factor contributing to changes in the lipid composition of dry-cured lambs and ham during processing (Guo et al., 2022). Previous studies have shown that phospholipids contribute significantly to forming volatile compounds, and their content in lambs is relatively high. Therefore, this study aimed to characterize phospholipids and volatile compounds in grilled lambs during the traditional charcoal process.

In 2003, Han and Gross proposed lipidomics as a subfield of metabolomics. A lipidomic analysis is usually performed using liquid chromatography-mass spectrometry (LC-MS) and the "shotgun lipidomics" methods. These two methods are currently the most commonly used techniques for characterizing and quantifying GP. The "shotgun lipidomics" method is a mass spectrometry technique based on electrospray ionization mass spectrometry, which is based on the principle of in-source separation and can be directly injected into the sample without chromatographic separation. This method avoids the residual problems associated with liquid chromatography while effectively identifying and characterizing approximately 90 % of phospholipids (Parchem, Sasson, Ferreri, & Bartoszek, 2019). Lipidomics has become increasingly popular in food science over the previous two decades. Ali et al. (2017) analyzed phospholipid molecules in five milk powders using ultra-performance liquid chromatography-electrospray ionization-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF-MS). Their study revealed that phosphatidylcholine and phosphatidylethanolamine are the two most abundant phospholipids in milk powders. In a separate study, Shen et al. (2012) developed and optimized a shotgun lipidomics method without chromatographic separation for rapid phospholipid analysis in the viscera of three fish species: perch, grass carp, and crucian carp. This novel birdshot lipidomics method is more than ten times faster than the conventional method.

This study aimed to (a) obtain comprehensive information about the lipid fingerprints and primary lipids responsible for producing volatile compounds in grilled lambs; (b) employ multivariate data analysis to identify differential phospholipid molecules that contribute to the formation of volatile compounds in grilled lambs; (c) elucidate the relationship between different phospholipid molecules and volatile compounds in grilled lambs; and (d) explore the underlying mechanism for the formation of grilled lamb flavor.

2. Materials and methods

2.1. Chemicals and solvents

The phospholipid standards, including 1,2-dimyristoyl-*sn-glycero*-3-phosphocholine [PC (14:0/14:0)], 1,2-dipalmitoyl-*sn-glycero*-3-phospho-L-serine (sodium salt) [PS (14:0/14:0)], 1,2-dipentadecanoyl-*sn-glycero*-3-phosphoethanolamine [PE (15:0/15:0)], 1,2-dipalmitoyl-*sn-glycero*-3-phospho-(1′-myo-inositol) (ammonium salt) [PI (16:0/16:0)], 1,2-dipentadecanoyl-*sn-glycero*-3-phospho-(1′-*rac*-glycerol) (sodium salt) [PG (15:0/15:0)], and 1,2-dimyristoyl-*sn-glycero*-3-phosphate (sodium salt) [PA (14:0/14:0)], were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Chloroform and methanol of chromatographic grade were procured from Sigma-Aldrich (St. Louis, MO, USA). High-purity water was obtained using a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

A total of 150 Sunit lambs, a Chinese sheep breed, were reared collectively in Xilinguole grassland with an altitude of 1000 m in Inner Mongolia Province, China. Fifteen lambs (average live weight 30.0 ± 2.5 kg) were randomly chosen at the age of six months, sharing comparable genetic lineage and dietary regimen. The dietary composition is delineated in Table S1. The lambs were evenly partitioned into five groups, each comprising three lambs. Ethical approval for all animal procedures in this study was obtained from the Animal Care and Use Committee of the College of Food Science and Engineering, Bohai University (BHU-2021-326). All animal procedures strictly adhered to international standards. Preceding lamb slaughter, the Sunit lambs were transported to a slaughter facility within 1 h and subjected to a 24 h fast. Slaughter was conducted on a single day according to the standard procedures of a commercial slaughterhouse (Xilingol Sheep Herding Co., Ltd.), following international standards, and the longissimus thoracis muscle was collected. The longissimus thoracis was then matured at 4 °C for 72 h and rapidly frozen to −35 °C before being transported to the laboratory using cold chain logistics. The samples were stored at −20 °C and grilled within a week to ensure minimal changes.

Before experimentation, the longissimus thoracis muscle was thawed (MIR-154-PC, Panasonic, Japan) at 4 ± 1 °C until the core temperature reached −3 and −5 °C. After removing the fat and connective tissue, the longissimus thoracis muscle was cut into approximately 2 cm × 1.5 cm × 1 cm cuboids. Four cuboids from the longissimus thoracis muscle of the same lamb were threaded onto a cluster using 30 cm-length iron sticks, each treatment group needed 2 clusters, that was, 8 cuboids of lamb, and were grilled in 2 batches, each batch consisted of three replicates of each grilling level (0 min, 3 min, 6 min, 9 min, and 12 min). A total of 120 pieces of lamb were used to analyze changes in volatile compounds and phospholipids during grilling. The meat clusters were placed 10 cm away from the charcoal fire, where the grilling temperature in two batches was about 180 ± 5 °C measured by a digital thermocouple (PT100, Jiangsu Chuangwei Automation Instrument Co., Ltd., China), with the clusters turned over every 30 s. After grilling, the meat samples of each grilling level were separately crushed using a grinder, vacuum packed and stored at −20 °C for further analysis. 9 min and 12 min treatment groups required an additional 72 clusters of 288 pieces of lamb each to be grilled and were used for sensory evaluation.

2.3. Sensory evaluation

The sensory evaluation was performed by modifying according to the literature (Xiao et al., 2020). Forty sensory panelists were screened according to the GB/T 16291.1-2012 standard. All panelists were trained in ISO 4121:2003 and GB/T 29604-2013 guidelines. After a high level of agreement among panelists, 12 assessors were selected to perform sensory evaluation of lamb that had been grilled for 9 min and 12 min (meat samples with a core temperature of 75 °C or higher that were safe for consumption) on a 0–7 points scale (in increments of 1 point). A total of 6 sessions were performed, each evaluated with 2 clusters grilled lamb (9 min and 12 min). A total of 576 blocks of grilled lamb from 6 lambs (3 lambs each at 9 min and 12 min), of which 96 blocks from 1 lamb were divided among 12 panelists.

First, the color of the surface of the grilled lamb was observed. Then the samples were mixed well before serving, and that were placed in white plates coded with 4-digit numbers arranged randomly. A mandatory 5 min break was given between different sample types, where panelists were required to cleanse their palates with dried water crackers and rinse with water. Char-grilled flavor (7 = intense, 0 = absent/non-existent), fatty aroma (7 = intense, 0 = absent/non-existent), meaty flavor (7 = intense, 0 = absent/non-existent) and overall acceptability (7 = like very much, 0 = dislike very much).

2.4. Headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME/GC-MS) analysis

The Solid Phase Microextraction (SPME) method was employed according to and with slight modifications to extract the volatile

compounds (Qi, Liu, Zhou, & Xu, 2017). First, the frozen samples are thawed at 4 ± 1 °C to −3 ~ −5 °C. A minced grilled lamb sample weighing 4.5 ± 0.20 g was placed in a 20 mL headspace vial. Subsequently, 7 μL of an internal standard (cyclohexanone) at a concentration of 1.11 μg/μL was injected into the vial, which was then quickly sealed with a spiral lid containing a Teflon spacer. Before extraction, vials were placed in a constant-temperature water bath at 50 °C for 10 min. The volatile compounds were then extracted at 50 °C for 45 min using a 75 μm SPME fiber (Carboxen/polydimethylsiloxane; Supelco, Bellefonte). Following extraction, the loaded SPME fiber was swiftly inserted into the injection port of the GC-MS instrument and desorbed at 250 °C for 4 min.

GC-MS (7890 B GC System, 5975A MSD; Santa Clara, CA, USA) was employed, and samples were analyzed on an HP-5 MS capillary column (30 m × 250 μm × 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). The helium carrier gas (purity of 99.99 %) flowed at a constant 1 mL/min flow rate in the splitless injection mode. The injector temperature was set at 250 °C. The temperature program was as follows: the initial column temperature was 35 °C for 5 min, then increased to 130 °C at 4 °C/min for 3 min, increased to 200 °C at 8 °C/min for 3 min, and finally increased to 250 °C at 12 °C/min for 5 min. The mass spectral detection conditions were as follows: GC and MS interface temperature, 280 °C; quadrupole temperature, 150 °C; ion source temperature, 230 °C; electron impact (EI) ionization energy, 70 eV; and scanning range, 50 ~ 550 *m/z*.

2.5. Identification and quantitation of volatile compounds

Using cyclohexanone as the internal standard, volatile compounds in the grilled lamb samples were identified based on their mass spectra, using the NIST 14.0/Wiley MS library as a reference. Each compound's match retention index (RI) was determined by comparison with the reference standard. Initially, the RI values were calculated by analyzing the retention times of homologous *n*-alkanes (C7-C30). Subsequently, the concentration of the volatile compounds was determined by calculating the ratio of the peak area of the internal standard compound to that of the internal standard compound.

2.6. Odor-activity values (OAVs)

Odor-activity values (OAVs) were calculated to assess the impact of volatile compounds on the grilled lamb aroma. OAVs represent the ratio of volatile compound concentrations to their perceived threshold values. Aromatic compounds with OAVs > 1 significantly contributed to the overall aroma of the grilled lamb. Conversely, volatile compounds with OAVs < 1 indicated a minor contribution (Schieberle & Hofmann, 2011).

2.7. Phospholipid extraction

The PLs in the grilled lamb were extracted according to a previously described method (Li, Li, Huang, Xu, & Wang, 2020) with some modifications. First, the grilled lamb with different grill times was ground with a meat grinder (180E-Y, Nail, Cixi, China) and freeze-dried in a vacuum freeze-dryer for 12 h, and the freeze-dried samples were ground into powder. Next, 5 g of freeze-dried lamb powder was accurately weighed into a 50 mL polytetrafluoroethylene (PTFE) centrifuge tube, 30 mL of chloroform/methanol (1:2, v/v) was added, and the mixture was homogenized at 0 °C for 10 s. Then, 5 mL of chloroform was added to the mixture, vortexed for 20 s, and static extraction was performed for 15 min. Subsequently, 10 mL of deionized water was added, stirring the mixture for 20 s. After centrifugation at 4 °C for 10 min at 8000 r/min, the solvent was separated into two phases. First, the upper organic phase was transferred to a PTFE tube, and the above steps were repeated to extract the lower aqueous phase. Finally, the organic solvents were extracted three times, evaporated to 1 mL with a gentle nitrogen flow, and stored at −20 °C until analysis.

2.8. UPLC-ESI-MS/MS analysis

The PLs in the grilled lamb were subjected to comprehensive untargeted lipidomic analysis using an ACQUITY UPLC 1-Class system equipped with a Xevo G2-S Q-TOF mass spectrometer (Waters Corp., Manchester, UK) (Mi et al., 2018). The chromatographic conditions were as follows: an ACQUITY UPLC ® BEH C18 1.7 μm (2.1 × 100 mm) chromatographic column was used, and the automatic sampler temperature was set to 8 °C, with a flow rate of 0.25 mL/min, and a column temperature of 50 °C. The injection volume for gradient elution was 2 μL. The mobile phase consisted of acetonitrile: water = 60:40 (0.1 % formic acid + 10 mM ammonium formate) and isopropanol: acetonitrile = 90:10 (0.1 % formic acid + 10 mM ammonium formate). The gradient elution procedure was as follows: 0 ~ 5 min, 70 ~ 57 % C; 5 ~ 5.1 min, 57 %~50 % C; 5.1 ~ 14 min, 50 %~30 % C; 14 ~ 14.1 min, 30 % C; 14.1 ~ 21 min, 30 %~1 % C; 21 ~ 24 min, 1 % C; 24 ~ 24.1 min, 1 % ~70 % C; 24.1 ~ 28 min, 70 % C.

Mass spectrometry conditions: An electric spray ion source (ESI) and positive and negative ion ionization modes were used. The positive-ion spray voltage was 3.50 kV, the negative-ion spray voltage was 2.50 kV, the sheath gas was 30 ARB, and the auxiliary gas was 10 ARB, respectively. The capillary temperature was 325 °C; scanning had a resolution of 35000, scanning range of 150 ~ 2000, secondary cracking with HCD, collision voltage of 30 EV, and unnecessary MS/MS information was removed using dynamic exclusion.

Quantification of phospholipid molecules: The phospholipid internal standard stock solution was diluted with a chloroform/methanol (1:2, V/V) solution to 5, 10, 50, 100, 500, 1000, 2000, and 5000 ng/mL. The linear regression equation used the phospholipid concentration and excimer ion response values.

2.9. Statistical analysis

All statistical data were analyzed using SPSS (version 19.0; SPSS Inc., Chicago, IL, USA), and significant differences were determined using one-way analysis of variance (ANOVA) (Duncan's test, $P < 0.05$). The results are expressed as the mean ± standard error (n = 6). Carbon-grilling time were fixed effects, temperature as a covariate, whereas the lamb longissimus thoracis and grill batch was random effects. A mixed model analysis of the differences in sensory attributes between different grilling times (9 min and 12 min) was conducted in the sensory evaluation. In each mixed model, session, grill batch and panelist were random effects and grilling time was a fixed effect. Microsoft Excel (2019) was used to calculate the linear regression equation for phospholipid molecular quantification. Data from lamb grilled for different durations were subjected to statistical analysis using principal component analysis (PCA) and partial least squares analysis (PLSR). The variable importance index (VIP) was used to analyze and identify essential phospholipid molecules (VIP > 1). Pearson's correlation analysis was performed on lamb roasted for 9 min using SPSS software (version 19.0). PCA, PLSR, and VIP analyses were performed using the XLSTAT software (Addinsoft, 2015).

3. Results and discussion

3.1. Differences in sensory evaluation of grill lamb

The sensory evaluation data for grilled lamb at 9 min and 12 min are presented in Table 1. These results indicate that the sensory evaluation results of lamb grilled for 9 min were better than those of lamb grilled for 12 min. Notably, the char-grilled flavor of exhibited significant differences ($P < 0.05$) between lamb grilled for 9 min and 12 min. This difference may be attributed to the Maillard reaction occurring during grilling, leading to the production of pyrazine flavor compounds and the

Table 1
Differences in sensory scores between lamb grilled for 9 min and 12 min.

Samples	Char-grilled flavor	Fatty aroma	Meaty odor	Overall acceptability
9 min	5.31 ± 0.07[b]	5.14 ± 0.08[a]	5.04 ± 0.08[a]	5.71 ± 0.8[a]
12 min	6.18 ± 0.08[a]	5.07 ± 0.08[a]	4.83 ± 0.07[a]	4.49 ± 0.11[b]

[a,b] Means within the same column with different letters differ significantly ($P < 0.05$). The sensory attributes were evaluated on a scale from 0 to 7. All data are expressed as the mean ± standard error.

characteristic char-grilled flavor (Tamura, Iwatoh, Miyaura, Asikin, & Kusano, 2022). Fatty aroma and meaty flavor showed no statistically significant differences ($P > 0.05$). However, the overall acceptability of lamb grilled for 9 min was higher than that of lamb grilled for 12 min ($P < 0.05$). This outcome suggests that prolonged heating time and higher temperatures could exacerbate lipid oxidation, resulting in unpleasant odors and negatively impacting the taste of the lamb product (Furuta, Mabuchi, Tanimoto, 2020).

3.2. Volatile compounds in grilled lamb during the heating process

The volatile compounds of grilled lamb for 0 min, 3 min, 6 min, 9 min, and 12 min are shown in Table S2. The grilled lamb contained 52 volatile compounds, including 17 aldehydes, 7 alcohols, 3 ketones, 5 esters, 2 acids, 9 hydrocarbons, 3 benzenes, 2 furans, 2 pyrazines, and 2 others. Table S2 shown the number of volatile compounds in raw lamb were the least (26), followed by grill lamb at 3 min (37), 6 min (42), 9 min (45) and 12 min (51), indicating that the number of volatile compounds increased with longer grilling times. Volatile compounds were detected in varying concentrations in the grilled lamb samples: 1180.59 ng/g for 0 min, 6512.2 ng/g for 3 min, 14243.24 ng/g for 6 min, 12889.44 ng/g for 9 min, and 12637.52 ng/g for 12 min. Aldehydes and alcohols are volatile compounds present at high concentrations in raw and grilled lambs throughout the grilling process.

A heat map of volatile compounds in the grilled lamb process is shown in Fig. 1. The higher the volatile content, the redder the color. In contrast, the lower the content of volatile compounds, the bluer the color. According to the figure, the primary aldehydes and alcohols with high volatile compound content in grilled lamb include hexanal (4320.74 ng/g), 1-octene-3-ol (933.42 ng/g), heptanal (893.78 ng/g), 1-octanol (590.55 ng/g), octanal (478.73 ng/g), 1-pentanol (394.23 ng/g), 1-hexanol (369.51 ng/g), which was consistent with other studies on the grilled lamb (Xiao et al., 2020; Liu et al., 2022). Comparing grilled lamb to raw meat, the concentrations of most aldehydes, alcohols, ketones, and furans increased significantly. The most variable and volatile compounds in grilled lamb were aldehydes, followed by alcohols; 1-

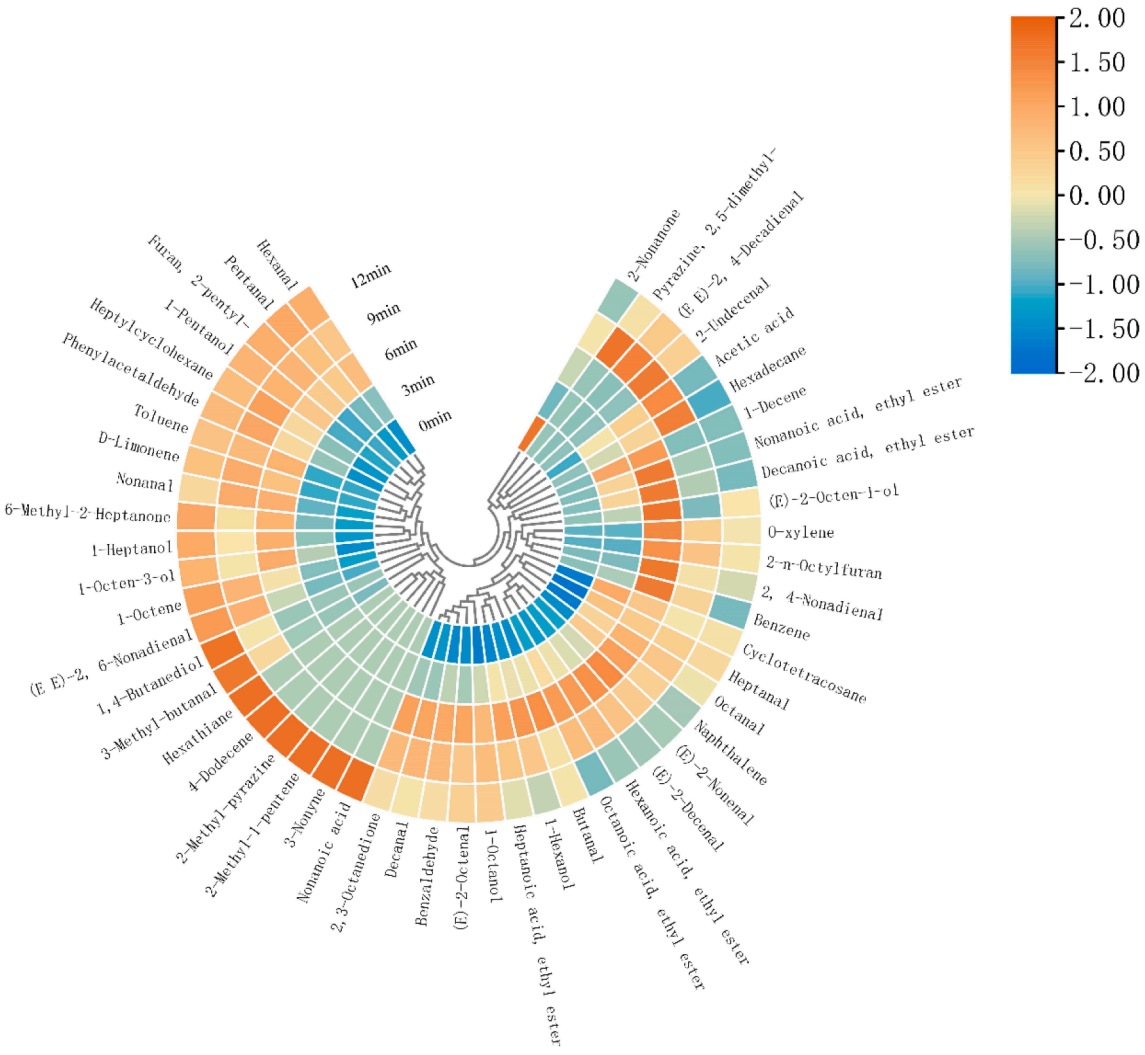


Fig. 1. Volatile compounds in grilled lamb during the heating process. The color represents the content of a volatile component. Blue represents a lower content while red represents a higher content. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

octene-3-alcohol (899.51 ng/g) was the primary alcohol. The alcohol and aldehyde contents increased with increasing grilling time, reaching a maximum of 6 min, indicating that volatile compounds were most abundant at 6 min. Volatile compounds, such as hydrocarbons and esters, reached their maximum concentration when grilled for 6 and 9 min, and the concentration decreased when grilled for 12 min, which might be due to the excessive grilling, partial scorching, and loss of volatile compounds.

Oxidation of unsaturated fatty acids and Strecker degradation produce aldehydes. In grilled lamb, the lipids were hydrolyzed to form free fatty acids, then saturated and unsaturated fatty acids were broken down into hydroperoxides and further reacted to produce aldehydes (Liu et al., 2021). As a result, hexanal content gradually increased in the grilled lamb. For example, the contents of 3-methyl-butanal (95.91 ng/g) and pentanal (687.90 ng/g) in grilled lamb for 12 min were higher than those in other grilled times ($P < 0.05$). In contrast, 2-Undecenal (58.66 ng/g) was the highest at 9 min. Therefore, aldehydes primarily originate from the oxidative degradation of lipids, which is the primary reason for imparting a robust meaty, fatty aroma to grilled lambs.

Alcohol is the second most abundant volatile compound after aldehydes, generated from the oxidative degradation of unsaturated fatty acids (Bassam et al., 2022). The degradation of C18:2n-6 fatty acids (linoleic acid) yielded 1-octen-3-ol, which had the highest concentration in grilled lambs at 12 min (Elmore et al., 2005). The concentration of 1-Octanol increased with the grilling time ($P < 0.05$) and remained balanced after 6 min.

Ketones are formed primarily during high-temperature cooking. The 2, 3-Octanedione (861.92 ng/g) was substantially higher than other ketones. The production of hydrocarbons is closely associated with lipid oxidation and thermal decomposition, primarily resulting from the cleavage of alkoxy groups in fatty acids. D-limonene (11.92 ng/g) had a pleasant lemon aroma and could modify the overall aroma of grilled lamb. Furans are heterocyclic compounds generated through sugar cracking and Maillard reactions (Liu et al., 2021). The oxidation of linoleic acid mainly results in the formation of 2-pentyl furan. It is often regarded as a marker of lipid oxidation, and its presence may play an essential role in the overall aroma (Qi et al., 2017). Nitrogen compounds, such as 2,5-dimethylpyrazine (123.68 ng/g), contribute to the grilled flavor, with the highest concentration observed at 9 min of charcoal grilling, consistent with the sensory evaluation results indicating a higher char-grilled flavor in the 9 min grilled lamb compared to the 12 min lamb (Mortzfeld, Hashem, Vranková, Winkler, & Rudroff, 2020).

3.3. Analysis of OAV values for volatile compounds in grilled lamb

The significance of volatile compounds in grilled lamb was determined not only by their quantity but also by their OAVs. By estimating the OAVs of the volatile compounds in the grilled lamb, 19 volatile compounds (OAV > 1) were identified in the grilled lamb process, including hexanal, 1-octene-3-ol, (E)-2-nonanal, octanal, nonanal, (E)-2-decennial, heptanal, and 2, 3-octadione. The OAVs of the key volatile compounds in raw lamb were the lowest, as shown in Fig. 2. According to one report, the process of grilling lamb results in the oxidation and degradation of nutrients such as fat and protein in the raw meat, leading to the production of more volatile compounds (Dou et al., 2022). The maximum OAVs for 1-octene-3-ol, (E)-2-nonanal, octanal, nonanal, (E)-2-decenal, 2, 3-octadione, ethyl caproate, and 2, 4-nonadienal at 6 min and the concentrations of essential volatile compounds gradually increased, especially hexanal, octanal, nonanal, (E)-2-acetaldehyde, and ethyl caproate.

As the most abundant volatile compounds in grilled lambs, aldehydes play an essential role in the overall aroma of grilled lambs because of their low odor perception threshold (Selli & Cayhan, 2009). Aldehydes are produced by the oxidation of unsaturated fatty acids and the Strecker degradation. As mentioned above, aldehydes have intense volatile and

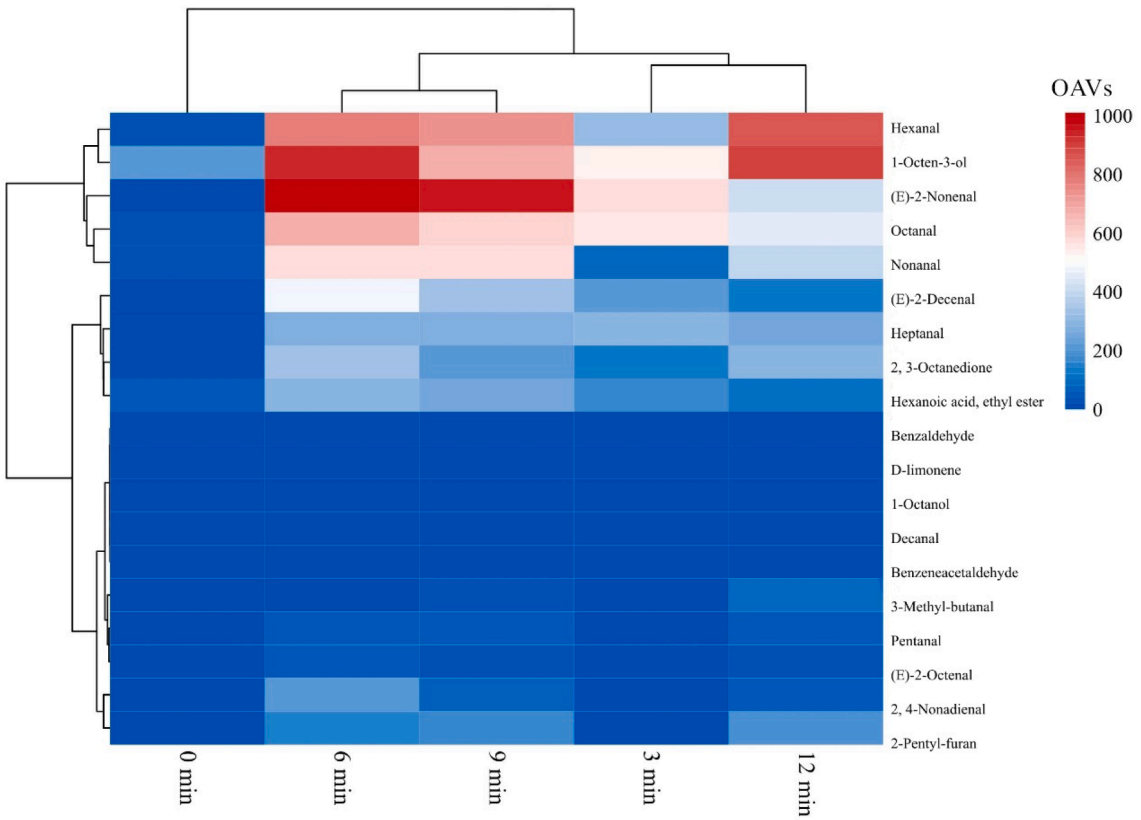


Fig. 2. The formation of predominant volatile compounds (OAV > 1) in the grilled lamb for 0, 3, 6, 9, 12 min.

fatty aromas and are crucial volatile aroma components of grilled lamb and other meats. For example, the aroma of 3-methyl butanal has been described as brothy, meaty, or malty (Li, Han, Frank, McGilchrist, & Warner, 2021). Additionally, long-chain aldehydes, such as (E)-2-nonenal and (E)-2-decenal, contribute a linseed oil odor. Notably, the OAV values of (E)-2-nonenal and (E)-2-decenal were higher in lamb grilled for 9 min compared to 12 min, resulting in a more favorable flavor in the 9 min grilled lamb.

Alcohols have a less substantial effect on meat flavor production than aldehydes (Bassam et al., 2022). The saturated fatty alcohol threshold was too high to make an even minor contribution to the overall aroma of grilled lamb. In contrast, the threshold for unsaturated alcohols was relatively low, and their contribution to the lamb aroma was relatively significant. The unsaturated alcohol with the highest content and OAVs in grilled lamb, 1-octene-3-ol (OAV = 668.20, 9 min), was mainly formed by the self-oxidation of linoleic acid and other polyunsaturated fatty acids, presenting mushroom and soil aroma, and it was commonly found in volatile aroma compounds in grilled meat products (Xi et al., 2018).

Ketones generally have higher odor thresholds than their aldehyde isomers. Among the ketones detected, 6-methyl-2-heptanone (35.62 ng/g) and 2-nonanone (31.6 ng/g) were present at lower concentrations, with OAV values less than 1, suggesting a minimal contribution to the overall aroma of grilled lamb. 2, 3-Octadione had the highest OAVs at 6 min in grilled lamb, with a burnt bitter aroma (Elmore et al., 2005).

In addition to aldehydes, alcohols, and ketones, which are typical fat oxidation products, the hydrocarbon d-limonene has an excellent lemon aroma and improves the overall aroma of grilled lamb; furan 2-pentylfuran has a vegetal aroma and contributes significantly to the overall aroma of grilled lamb owing to its low odor threshold (Frank, Dubois, & Pérez, 2020).

3.4. Identification of phospholipids in grilled lamb

Phospholipid molecules in the grilled lamb were identified using UPLC-ESI-MS/MS. The linear regression equation, correlation coefficient, and linear range of the internal standards for phospholipid molecular quantification are shown in Table S3. PA (14:0_14:0), PG (15:0_15:0), PI (16:0_16:0), and PS (14:0_14:0) showed a good linear relationship in the range of 5–2000 ng/mL, PC (14:0_14:0) and PE (15:0_15:0) had a good linear relationship in the range of 5–5000 ng/mL. In addition, the correlation coefficient is > 0.99. Therefore, these linear regression equations satisfy the analysis requirements in the linear range.

Table S4 shows that 142 phospholipid molecules were identified in positive and negative ion modes. A total of 142 phospholipid molecules were numbered for convenience and simplicity. It is noteworthy that the number of phospholipids remained invariant, whereas the concentrations demonstrated variance. More specifically, phospholipids were detected in the grilled lamb samples exposed for durations of 0 min, 3 min, 6 min, 9 min, and 12 min, presenting concentrations of 12062.24 ng/mg, 7900.97 ng/mg, 9341.95 ng/mg, 9469.39 ng/mg, and 9470.37 ng/mg, correspondingly. The phospholipid types found in the grilled lamb are shown in Fig. 3. There are six types of phospholipids: 52 PC (36.62 %), 3 PA (2.11 %), 17 PS (11.97 %), 19 PI (13.38 %), 6 PG (4.23 %), and 45 PE (31.69 %). Most phospholipids contain polyunsaturated fatty acid chains, and the types and amounts of unsaturated fatty acids in different phospholipid molecules differ significantly. For example, the types and amounts of unsaturated fatty acids in phospholipid molecules comprising PA, PI, and PG differ significantly from those in phospholipid molecules with PC, PE, and PS. As shown in Table S4, the *sn*-1/*sn*-2 fatty acyl chains of phospholipids in grilled lambs mainly were C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:4, and C20:5, which is consistent with the findings of Li et al. (2020).

As can be seen from Table S4, in grilled lamb, the PC content in the detected phospholipid molecules was the highest, followed by PE, PI, PS, PG, and PA, while in raw lamb, the content of PI was the lowest. After processing, the PC content decreased from 8117.51 ng/mg to 6395.11 ng/mg, the PE content decreased from 2800.30 ng/mg to 1791.88 ng/mg, and the content of PS decreased from 349.52 ng/mg to 297.24 ng/mg. The PC and PE levels were significantly higher than those of other phospholipids throughout the grilled lamb process. This result was consistent with the changes in phospholipids observed after processing duck meat (Xu, Xu, Zhou, Wang, & Li, 2008) and dry-cured meat products (Aksu, Dogan, & Sirkecioglu, 2017), where the phospholipid content was significantly reduced. This might be because phospholipids undergo lipid oxidation or enzymatic lipolysis during processing, decreasing phospholipid content. Phospholipases are widely expressed in animals, plants, and microorganisms. It plays a vital role in transporting, digesting, and processing lipids. It is the main hydrolase that hydrolyzes phospholipid molecules (Kurtovic, Marshall, Zhao, & Simpson, 2009). During processing, phospholipids undergo enzymatic hydrolysis and non-enzymatic degradation to produce other small-molecule lipids. Heating directly affects phospholipid stability in lambs.

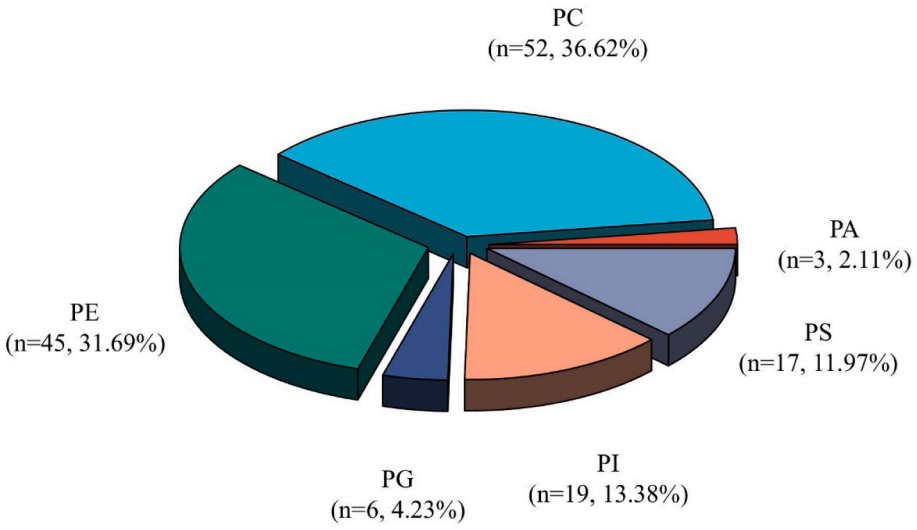


Fig. 3. Phospholipids class composition measured in grilled lamb. (PC, phosphatidylcholine; PE, phosphatidyl ethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; and PG, phosphatidyl glycerol.)

3.5. Multivariate statistical analysis of phospholipids in grilled lamb

PLSR analysis was appropriate for determining the critical phospholipids affecting grilled lamb at different grilling times. As shown in Fig. 4(a), most variables are represented by circles. For example, as shown in the figure, a lamb grilled for 0 min, that is, raw lamb, has a high correlation with most phospholipid molecules, such as PE31, PE20, PE21, PE27, PE22, PE15, PE41, PE33, PC16, PC3, and PG6; the lamb grilled for 3 min was mainly related to phospholipids such as PC36, PE23, PE30, PC40, PE42, PC51, and PC17. The lamb grilled for 6 min mainly correlated with PI2. The lamb grilled for 9 min was mainly related to phospholipids such as PI19, PC26, PC2, PI9, and PI16, and the lamb grilled for 12 min was mainly related to phospholipid molecules such as PI17, PI15, PS1, PC1, PG1, PS7, PA2, and PA3. Grilled lamb had a positive correlation with 9 min and 12 min but was negatively correlated with 0 min. A negative correlation between 3 min and 6 min was observed in grilled lamb. Therefore, thermal decomposition mainly played a leading role in the 6 and 9 min of the grilled lamb. During the pyrolysis process, the lipids in lamb mainly produced volatile compounds, such as aldehydes, alcohols, and ketones, which was also the main reason for the decrease in the content of phospholipid molecules in the grilled lamb. The key volatile compounds in the grilled lamb were highly correlated at 6, 9, and 12 min. The lamb was grilled for 6 min, and the volatile compounds V5, V7, V12, V13, V15, and V28 were highly correlated. Volatile compounds, such as V4, V8, V9, V10, V22, V24, V26, and V39, were highly correlated with grilled lamb for 9 min. V3 showed the highest correlation with lamb grilled for 12 min. Therefore, a grilled lamb for 9 min was the most abundant in volatile compounds.

PLSR analysis was used to identify key phospholipid molecules (VIP $>$ 1.0). Volatile compounds with VIP $>$ 1.0 are considered potential markers for distinguishing different samples, and a higher VIP score indicates better discrimination. Therefore, based on the PLSR analysis of the 142 detected phospholipid molecules, 63 key phospholipids with a VIP $>$ 1 were selected, as illustrated in Fig. 4(b). Among these key differential phospholipids, 30 belonged to PE phospholipid molecules, including PE3, PE4, PE6, PE7, PE8, PE9, and PE10; 12 were PC phospholipid molecules, such as PC3, PC11, PC18, PC26, PC34, PC38, and PC42; 10 were phospholipid PI, including PI1, PI3, PI4, PI7, PI11, and PI12; 8 PS, such as PS3, PS5, PS9, PS12, PS14, and PS15; 3 were PG phospholipid molecules, including PG1, PG4, and PG6. These findings indicate that not all phospholipid molecules are correlated with volatile compounds throughout the entire process of grilling lamb. Additionally, over 50 % of the phospholipid molecules showed low or no involvement in forming volatile compounds.

3.6. Correlation analysis of essential phospholipids and volatile compounds in grilled lamb

Correlation analysis can provide insights into the relationships between variables and help identify the precursor lipids involved in the

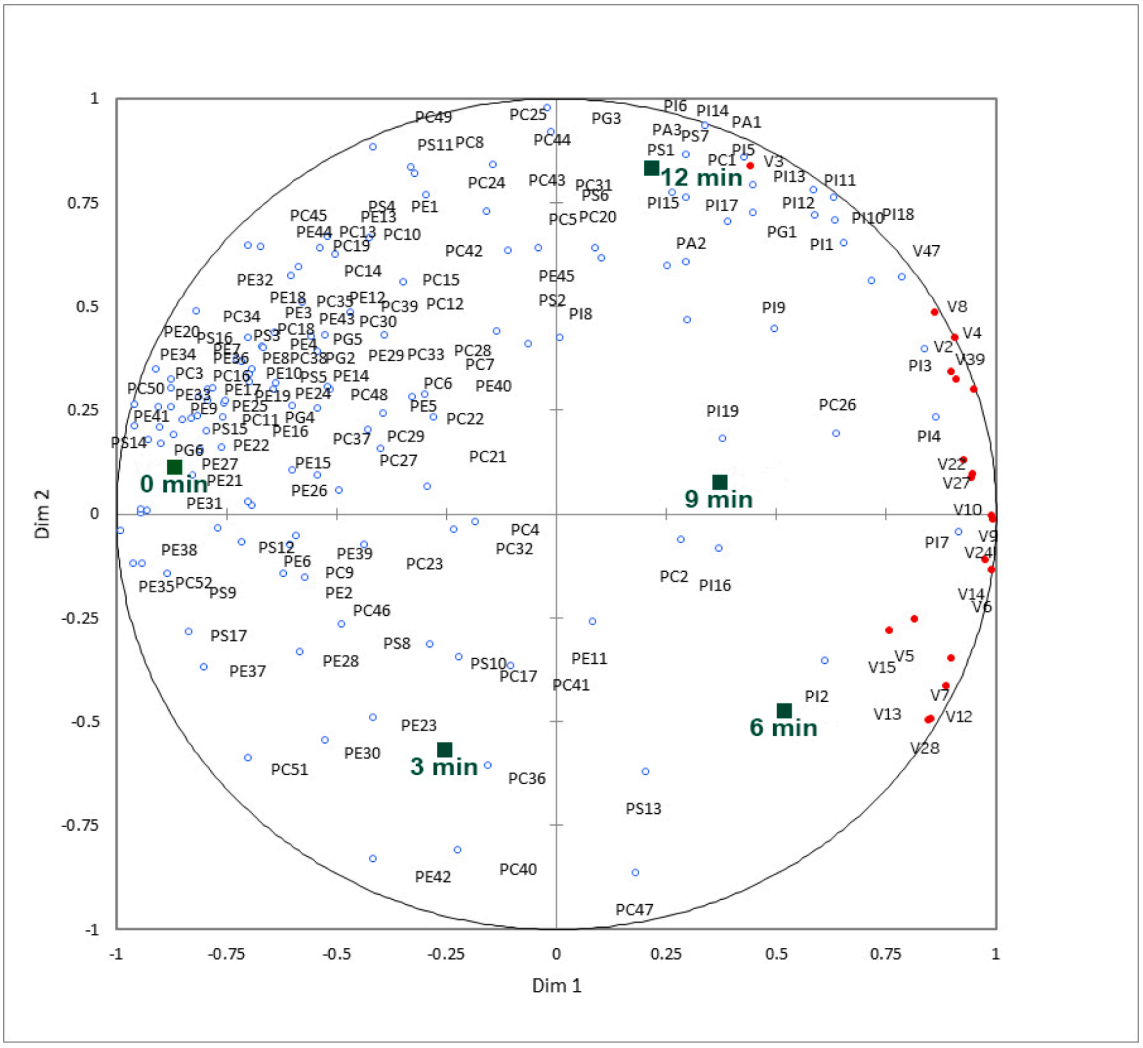


Fig. 4. (a) PLSR related load diagram of phospholipids and key volatile compounds in grilled lamb. (b) VIP scores of phospholipids with VIP $>$ 1.

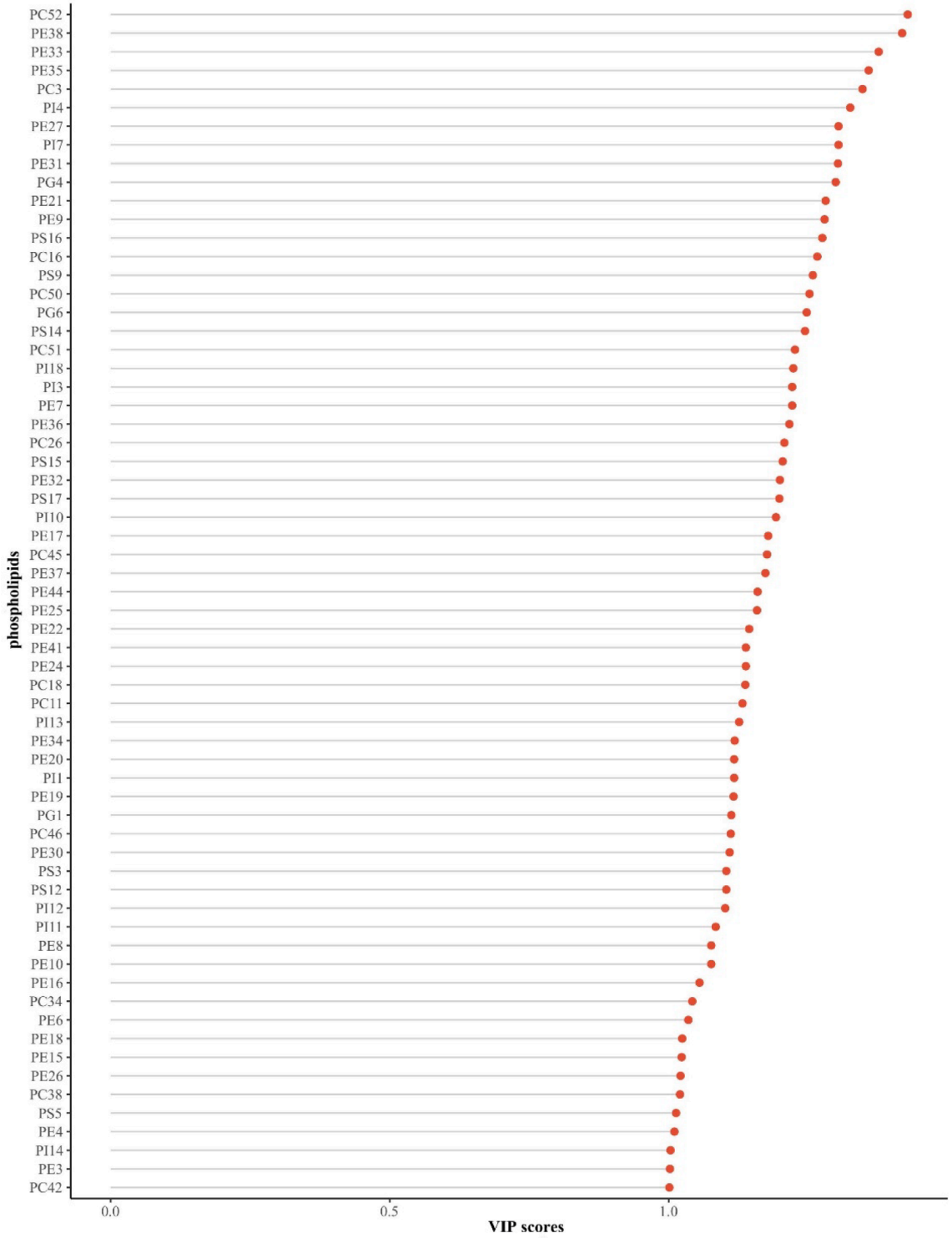


Fig. 4. (*continued*).

formation of flavor components in grilled lambs and their influencing factors. In this study, Pearson's correlation analysis was used to investigate the correlation between phospholipids and volatile compounds in the best grilling time of the longissimus thoracis muscle. This study examined the correlation between 63 key phospholipid molecules and 19 key volatile compounds in grilled lambs. In Fig. 5, the red area indicates a positive correlation and darker shades of red indicate a stronger correlation. The blue area represents a negative correlation, with darker shades indicating a stronger correlation. The results revealed that the volatile compound V3 (3-Methyl-butanal) was positively correlated with phospholipid molecules PI3, PI11, PI14, PI1, PG1, PI12, PI18, and PI10, and negatively correlated with PE37 and PS17 phospholipid molecules. Similarly, PC26 positively correlated with 12 volatile compounds, including V36, V47, V8, V4, V10, and V2. In contrast, PE38 and PC52 were negatively correlated with 15 volatile compounds: V14, V6, V9, and V24. Fig. 5 demonstrates that most phospholipid molecules were negatively correlated with critical volatile compounds in grilled lamb. This outcome signifies that a total of 63

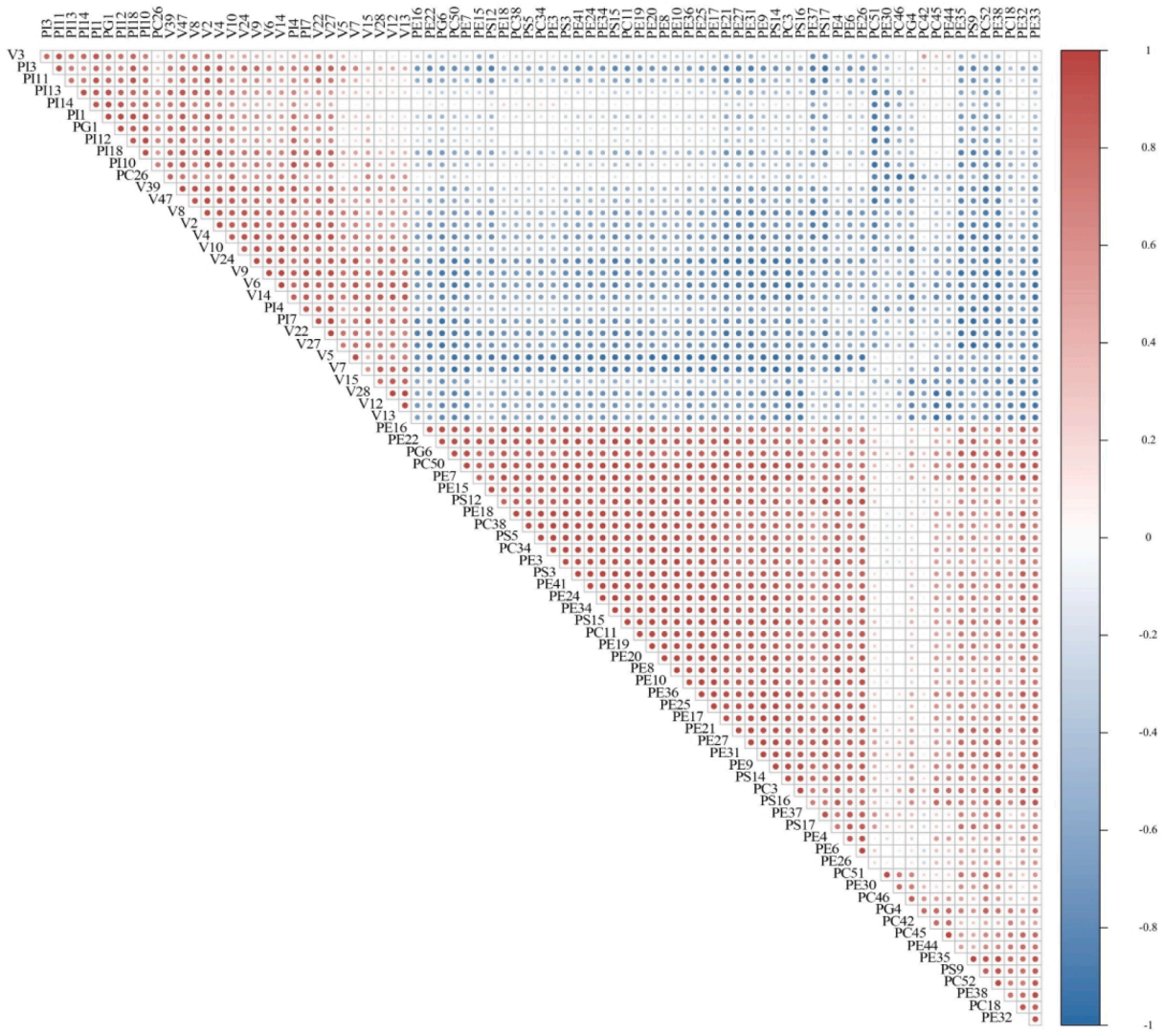


Fig. 5. Correlation analysis of 63 key differential phospholipids with 19 key volatile compounds in the longest thoracic muscle grilled for 9 min. The red unit indicated the positive correlation, in which the redder unit was the greater correlation. Similarity, the blue unit revealed the negative correlation, among which the bluer unit was the greater correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

distinct phospholipids are likely to play a predominant role in the genesis of pivotal volatile compounds within grilled lamb. This observation suggests that 63 phospholipids may contribute to the main aroma components of grilled lambs. Phospholipid molecules containing PC and PE, which are rich in unsaturated fatty acids, such as C16:0, C18:0, C18:2, and C20:4, were found to be critical substrates for the formation of volatile compounds in grilled lamb products.

3.7. Exploration of the formation mechanism of critical volatile flavor substances during the heating process of grilled lamb

A high correlation between key volatile compounds and key phospholipid molecules was shown at 0, 3, 6, 9, and 12 min of lamb grilling ($P < 0.05$). This indicated that the formation of key flavor compounds in grilled lamb during processing was closely related to the hydrolysis and oxidation of key phospholipid molecules during charcoal grilling. Phospholipids are the predominant component of intramuscular fat in meat and are high in unsaturated fatty acids, particularly polyunsaturated fatty acids, making them easily oxidized and degraded. Therefore, the oxidative degradation products directly affected the composition of volatile compounds (Mottram, 1998). Polyunsaturated fatty acids account for 45–55 % of fatty acids in phospholipid molecules. Phospholipid molecules are oxidized and hydrolyzed to form small molecule-free fatty acids, which are then oxidized to form directional compounds.

Degradation of the phospholipid molecule PC7 (PC (16:0_18:1), 2516.42 ng/mg), which has the highest content in raw lamb, usually begins with the unsaturated fatty acid chain C18:1 during grilling. Hydroxyl radical oxidation of the unsaturated fatty acyl chain initially extracts hydrogen atoms from the diallyl methylene position in C18:1 or the allyl methylene, to produce hydroperoxide (Spickett, Reis, & Pitt, 2011). Aldehydes are essential products of phospholipid molecular degradation. Low-molecular-weight aldehydes are characterized by a solid pungent aroma, whereas aldehydes with medium relative molecular weights (5–9 carbon atoms) exhibit an oily and fatty aroma (Stahnke, 1995). The straight-chain aldehydes in this study, hexanal, octanal, heptanal, nonanal, decanal, pentanal, and (E)-2-nonanal, are closely associated with the oxidation of omega-6 unsaturated fatty acids, the primary unsaturated fatty acids found in lambs. In this study, branched-chain aldehydes, such as 3-methyl butyraldehyde, were typically not the product of lipid oxidation. They are generated through the Steckler degradation reaction of amino acids, such as methionine, isoleucine, and lysine, or microbial degradation (Ventanas et al., 1992). However, these branched-chain aldehydes showed a strong negative

correlation with the phospholipid molecules PE37 (PE (18:2e_18:2)) and PS17 (PS (42:8e)) during lamb grilling. This correlation could be attributed to the involvement of these phospholipid molecules in the Strecker degradation of the Maillard reaction that occurs during lamb grilling. The formation route is illustrated in Fig. S1.

Alcohols, acids, ketones, esters, and furans significantly contributed to the overall aroma composition of the grilled lamb. Alkoxy free radicals and lipid molecules reduce aldehydes and ketones under the action of alcohol reductase to produce corresponding alcohols during the degradation of phospholipid molecules. Because of their low threshold, unsaturated fatty alcohols have metal and mushroom aromas, contributing to the overall aroma of grilled lambs. Ketones are also byproducts of lipid oxidation; unsaturated fatty acids produce oxide free radicals through pyrolysis, oxidation, and, finally, ketone compounds. As a result, ketones generally have butter and fruity aromas. Unsaturated ketones were the reason for the formation of critical volatile compounds in grilled lamb. Acids mainly originate from the oxidative hydrolysis of fat. Carboxylic acids containing 1–6 carbon atoms are degradation products of phospholipid molecules.

On the one hand, oxidative degradation of unsaturated fatty acids in phospholipid molecules could form volatile compounds such as aldehydes and alcohol. On the other hand, however, they affect the meat aroma through their interaction in the Maillard reaction (Shahidi & Hossain, 2022). Some studies have shown that in the in vitro Maillard reaction model, the aroma produced by adding phospholipid molecules is better than that produced without phospholipids. Therefore, the typical meat aroma could be attributed to the Maillard reaction, and the formation of distinctive volatile compounds in grilled meat could be attributed to the oxidative degradation of phospholipid molecules. Nonenzymatic browning reactions with a few proteins and amino acids could occur due to fatty acid oxidation degradation products, such as amino groups or aldehydes of phospholipid polar groups—the interaction of several reaction products aided in the synthesis of volatile compounds. Fat can reduce the content of sulfur-containing compounds and other Maillard reaction products and produce volatile compounds, such as carbonyl and alcohol, which positively impact the overall aroma of grilled meat products (Spickett, Reis, & Pitt, 2011).

4. Conclusion

In the present study, the species of phospholipid molecules and volatile compounds in grilled lambs during the heating process by UPLC-ESI-MS/MS and GC-MS. Correlation analysis, PLSR, and PCA allowed us to identify the key phospholipids involved in grill lamb aroma formation. Our findings revealed the presence of 19 key volatile compounds (OAV $>$ 1) throughout the grilling process. UPLC-ESI-MS/MS analysis detected 142 phospholipid molecules in grilled lambs, among which 63 differential phospholipids were screened by PLSR analysis. The remaining phospholipid molecules were minimally involved in forming key volatile compounds in grilled lamb. Moreover, PCA analysis indicated that phosphatidylcholine and phosphatidyl ethanolamine phospholipids are vital substrates for forming volatile compounds in grilled lambs. Understanding the characterization of phospholipid molecular species and volatile compounds in grilled lambs during heating is crucial for elucidating the mechanism underlying flavor formation. Summarily, these results will contribute to a deeper understanding of flavor formation in grilled lamb products, leading to precise flavor modulation and facilitating the development of grilled lamb products.

Funding

This work was supported by the “Science and Technology Project of Unveiling and Commanding” from Liaoning Province [2021JH1/10400033].

CRediT authorship contribution statement

Kexin Cheng: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Teng Liu:** Investigation, Methodology, Writing – review & editing. **Cong Yang:** Methodology, Writing – original draft. **Hui Yang:** Investigation. **Dengyong Liu:** Funding acquisition, Resources, Supervision.

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.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101113.

References

Aksu, M. I., Dogan, M., & Sirkecioglu, A. N. (2017). Changes in the total lipid, neutral lipid, phospholipid, and fatty acid composition of phospholipid fractions during pastirma processing, a dry-cured meat product. *Korean Journal for Food Science of Animal Resources, 37*(1), 18–28. https://doi.org/10.5851/kosfa.2017.37.1.18

Ali, A. H., Zou, X., Huang, J., Abed, S. M., Tao, G., Jin, Q., & Wang, X. (2017). Profiling of phospholipids molecular species from different mammalian milk powders by using ultra-performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry. *Journal of Food Composition and Analysis, 62*, 143–154. https://doi.org/10.1016/j.jfca.2017.05.007

Bassam, S. M., Noleto-Dias, C., & Farag, M. A. (2022). Dissecting grilled red and white meat flavor: Its characteristics, production mechanisms, influencing factors, and chemical hazards. *Food Chemistry, 371*, Article 131139. https://doi.org/10.1016/j.foodchem.2021.131139

Chen, D., Balagiannis, D., & Parker, J. (2019). Use of egg yolk phospholipids to generate chicken meat odorants. *Food Chemistry, 286*, 71–77. https://doi.org/10.1016/j.foodchem.2019.01.184

Dou, L., Liu, C., Yang, Z., Su, R., Chen, X., Hou, Y., Hu, G., Yao, D., Zhao, L., Su, L., & Jin, Y. (2022). Effects of oxidative stability variation on lamb meat quality and flavor during postmortem aging. *Food Science, 87*(6), 2578–2594. https://doi.org/10.1111/1750-3841.16138

Elmore, J. S., Cooper, S. L., Enser, M., Mottram, D. S., Sinclair, L. A., & Wilkinson, R. G. (2005). Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Science., 69*, 233–242. https://doi.org/10.1016/j.meatsci.2004.07.002

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Murphy, R. C., & Dennis, E. A. (2005). A comprehensive classification system for lipids. *Journal of Lipid Research, 46*(5), 839–862. https://doi.org/10.1194/jlr.e400004-jlr200

Frank, D., Kaczmarska, K., Paterson, J., Piyasiri, U., & Warner, R. (2017). Effect of marbling on volatile generation, oral breakdown and in mouth flavor release of grilled beef. *Meat Science, 133*, 61–68. https://doi.org/10.1016/j.meatsci.2017.06.006

Frank, N., Dubois, M., & Pérez, J. F. H. (2020). Detection of furan and five alkylfurans, including 2-pentylfuran, in various food matrices. *Journal of Chromatography A.* , Article 461119. https://doi.org/10.1016/j.chroma.2020.461119

Furuta, A., Mabuchi, R., & Tanimoto, S. (2020). Effects of different heating conditions on the texture and extracts of the meat from each part of the yellowtail Seriola quinqueradiata. *Fisheries Science, 86*, 693–700. https://doi.org/10.1007/s12562-020-01426-0

Guo, X., Shi, D., Liu, C., Huang, Y., Wang, Q., Wang, J., Pei, L., & Lu, S. (2022). UPLC-MS-MS-based lipidomics for the evaluation of changes in lipids during dry-cured mutton ham processing. *Food Chemistry, 377*, Article 131977. https://doi.org/10.1016/j.foodchem.2021.131977

Han, X., & Gross, R. W. (2003). Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry. *Journal of Lipid Research, 44*(6), 1071–1079. https://doi.org/10.1194/jlr.r300004-jlr200

Huang, Y. C., Li, H. J., He, Z. F., Wang, T., & Qin, G. (2010). Study on the flavor contribution of phospholipids and triglycerides to pork. *Food Science and Biotechnology, 19*(5), 1267–1276. https://doi.org/10.1007/s10068-010-0181-0

Kurtovic, I., Marshall, S. N., Zhao, X., & Simpson, B. K. (2009). Lipases from Mammals and Fishes. *Reviews in Fisheries Science, 17*(1), 18–40. https://doi.org/10.1080/10641260802031322

Li, J., Tang, C., Zhao, Q., Yang, Y., Li, F., Qin, Y., Liu, X., Yue, X., & Zhang, J. (2020). Integrated lipidomics and targeted metabolomics analyses reveal changes in flavor

precursors in psoas major muscle of castrated lambs. *Food Chemistry, 333*, Article 127451. https://doi.org/10.1016/j.foodchem.2020.127451

Li, C., Li, X., Huang, Q., Zhuo, Y., Xu, B., & Wang, Z. (2020). Changes in the phospholipid molecular species in water-boiled salted duck during processing based on shotgun lipidomics. *Food Research International, 132*, Article 109064. https://doi.org/10.1016/j.foodres.2020.109064

Li, Z., Ha, M., Frank, D., McGilchrist, P., & Warner, R. D. (2021). Volatile profile of dry and wet aged beef loin and its relationship with consumer flavor liking. *Foods, 10* (12), 3113. https://doi.org/10.3390/foods10123113

Li, X., Xie, W., Bai, F., Wang, J., Zhou, X., Gao, R., Xu, X., & Zhao, Y. (2022). Influence of thermal processing on flavor and sensory profile of sturgeon meat. *Food Chemistry, 374*, Article 131689. https://doi.org/10.1016/j.foodchem.2021.131689

Liu, H., Hui, T., Fang, F., Li, S., Wang, Z., & Zhang, D. (2022). The formation of key aroma compounds in grilled mutton during the traditional charcoal process. *Meat Science, 184*, Article 108689. https://doi.org/10.1016/j.meatsci.2021.108689

Liu, H., Ma, J., Pan, T., Suleman, R., Wang, Z., & Zhang, D. (2021). Effects of grilling by charcoal, electric, microwave and superheated steam methods on (non)volatile compounds in oyster cuts of grilled lamb. *Meat Science, 172*, Article 108324. https://doi.org/10.1016/j.meatsci.2020.108324

Mi, S., Shang, K., Jia, W., Zhang, C., Li, X., Fan, Y., & Wang, H. (2018). Characterization and discrimination of Taihe black-boned silky fowl (Gallus gallus domesticus Brisson) muscles using LC/MS-based lipidomics. *Food Research International, 109*, 187–195. https://doi.org/10.1016/j.foodres.2018.04.038

Mortzfeld, F. B., Hashem, C., Vranková, K., Winkler, M., & Rudroff, F. (2020). Pyrazines: Synthesis and industrial application of these valuable flavor and fragrance compounds. *Biotechnology Journal.* , Article 2000064. https://doi.org/10.1002/biot.202000064

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415–424. https://doi.org/10.1016/s0308-8146(98)00076-4

Parchem, K., Sasson, S., Ferreri, C., & Bartoszek, A. (2019). Qualitative analysis of phospholipids and their oxidized derivatives – used techniques and examples of their applications related to lipidomic research and food analysis. *Free Radical Research*, 1–661. https://doi.org/10.1080/10715762.2019.1657573

Qi, J., Liu, D., Zhou, G., & Xu, X. (2017). Characteristic flavor of traditional soup made by stewing Chinese yellow-feather chickens. *Journal of Food Science, 82*(9), 2031–2040. https://doi.org/10.1111/1750-3841.13801

Schieberle, P., & Hofmann, T. (2011). Mapping the combinatorial code of food flavors by means of molecular sensory science approach. In H. Jelen (Ed.), *Food flavors: Chemical, sensory and technological properties* (pp. 411–437). CRC Press, Taylor and Francis Group.

Selli, S., & Cayhan, G. G. (2009). Analysis of volatile compounds of wild gilthead sea bream (Sparus aurata) by simultaneous distillation–extraction (SDE) and GC–MS. *Microchemical Journal, 93*(2), 232–235. https://doi.org/10.1016/j.microc.2009.07.010

Shahidi, F., & Hossain, A. (2022). Role of lipids in Food Flavor Generation. *Molecules, 27* (15), 5014. https://doi.org/10.3390/molecules27155014

Shen, Q., Wang, Y., Gong, L., Guo, R., Dong, W., & Cheung, H.-Y. (2012). Shotgun lipidomics strategy for fast analysis of phospholipids in fisheries waste and its potential in species differentiation. *Journal of Agricultural and Food Chemistry, 60* (37), 9384–9393. https://doi.org/10.1021/jf303181s

Spickett, C. M., Reis, A., & Pitt, A. R. (2011). Identification of oxidized phospholipids by electrospray ionization mass spectrometry and LC–MS using a QQLIT instrument. *Free Radical Biology and Medicine, 51*(12), 2133–2149. https://doi.org/10.1016/j.freeradbiomed.2011.09.003

Stahnke, L. H. (1995). Dried sausages fermented with Staphylococcus xylosus at different temperatures and with different ingredient levels — Part II. Volatile components. *Meat Science, 41*(2), 193–209. https://doi.org/10.1016/0309-1740(94)00069-j

Tamura, Y., Iwatoh, S., Miyaura, K., Asikin, Y., & Kusano, M. (2022). Metabolomic profiling reveals the relationship between taste-related metabolites and roasted aroma in aged pork. *LWT, 155*, Article 112928. https://doi.org/10.1016/j.lwt.2021.112928

Ventanas, J., Córdoba, J. J., Antequera, T., Garcia, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Food Science, 57*(4), 813–815. https://doi.org/10.1111/j.1365-2621.1992.tb14300.x

Wang, N., Wang, J., Zhang, Y., Wu, Z., Wang, D., Xiao, H., Lyu, X., Chen, H., & Wei, F. (2023). Comprehensive lipidomics and volatile compounds profiling reveals correlation of lipids and flavors in DHA-enriched egg yolk. *Oil Crop Science, 8*(1), 27–34. https://doi.org/10.1016/j.ocsci.2023.03.001

Xi, J., Zhan, P., Tian, H., Wang, P., Geng, Q., Wang, Y., Zhang, F., & Ji, Y. (2018). Comparison of different extraction fibers for analysis of characteristic aroma compounds in Xinjiang grilled lamb by SPME-GC-MS and PCA. *Food Science, 39*(10), 234–241.

Xiao, X., Hou, C., Zhang, D., Li, X., Ren, C., Ijaz, M., Hussain, Z., & Liu, D. (2020). Effect of pre- and post-rigor on texture, flavor, heterocyclic aromatic amines and sensory evaluation of grilled lamb. *Meat Science, 169*, Article 108220. https://doi.org/10.1016/j.meatsci.2020.108220

Xu, W., Xu, X., Zhou, G., Wang, D., & Li, C. (2008). Changes of intramuscular phospholipids and free fatty acids during the processing of Nanjing dry-cured duck. *Food Chemistry, 110*(2), 279–284. https://doi.org/10.1016/j.foodchem.2007.11.044