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Accurate evaluation of the lipid characteristics and flavor of abdominal muscle resulting from different cooking processes using lipidomics and GC-IMS analysis

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ABSTRACT

This study employed UPLC-MS/MS and GC-IMS techniques to compare and analyze the lipid metabolites and volatile flavor compounds in raw abdominal muscle (CK), sour video abdominal muscle (SV), steamed abdominal muscle (ST), and oven-cooked abdominal muscle (OC). A total of 42 subclasses and 1230 lipids were identified. Among these, lysophosphatidylethanolamine (LPE) 18:2/0:0, lysophosphatidyletholine (LPC) 18:2/0:0, and triacylglycerol (TG) 16:0_18:1 and phosphatidylethanolamine (PE) P-18:18:18:2 influenced the aroma retention of roasted abdominal muscle, whereas phosphatidylcholine (PC) 16:0_18:2 and phosphatidylethanolamine (PE) P-18:18:18:2 influenced the aroma retention of roasted abdominal muscle. Additionally, 250 differentially abundant metabolites were identified as potential markers for differentiating various cooking methods. Seven compounds were recognized as potential indicators for distinguishing cooking methods: propanal-D, n-pentanal-M, n-pentanal-D, butanal-D, 3-methylbutanal, 1-hexanal-M, and 1-hexanal D. Correlation analysis results indicated a significant positive correlation between aldehydes and phospholipid molecules, including PC, PE, LPC, and LPE.

1. Introduction

Meat and its products are popular among consumers because of their unique flavor and significant nutritional benefits. The taste of meat strongly affects acceptance among buyers (Han, Zhang, Fauconnier, & Mi, 2020). Uncooked meat primarily contributes a flavor reminiscent of blood and metal (Fu, Cao, Yang, & Li, 2022). Approximately 90 % of the aroma compounds found in cooked meat originate from the breakdown of lipids, whereas the other 10 % are produced through various other chemical reactions (Amjad et al., 2022). Lipids are vital for the development of flavor in meat products, and this process is influenced by numerous factors, such as the type of meat, genetic background, feeding practices, and methods of food processing.

A variety of cooking methods exist for abdominal muscle, a widely favored type of meat, which can influence its nutritional value by causing the loss of lipid components while enhancing flavor and color (Ángel-Rendón et al., 2020). The cooking process enhances the creation of new characteristic flavors. Compared with other cooking methods,

baking is characterized by longer durations and higher temperatures, leading to increased oxidation and volatile compounds. Consequently, the Maillard reaction and lipid oxidation contribute to flavor enhancement. Vacuum steaming, in particular, minimizes the production of harmful byproducts, thereby promoting a healthier cooking outcome (Jiang et al., 2022). Lipids, which constitute 38 % of the dry weight of meat, serve as the primary substrates for flavor production in roasted meat products (Liu et al., 2020).

Lipids have the capacity to generate a wide variety of aromatic compounds via autooxidation or thermal oxidation processes. The generation of these aromatic compounds during thermal treatment is intricately linked to the particular lipid types and fatty acids present (Li Zhou, Zhao, Bindler, & Marchioni, 2014). Liu et al. (2024) reported that phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) play crucial roles in the production of key aroma substances in roasted pork. Liu et al. (2021) reported that PC and PE may play major roles in the formation of aromatic compounds. Lipid molecules, such as LPE (22:6), LPE (20:4), LPE (20:5), LPE (18:3), LPE (16:1),

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and LPE (18:2), are likely key lipids responsible for generating distinctive flavor compounds during the hot processing of crayfish (Zhou et al., 2023). Unsaturated fatty acids (UFAs) are recognized as the primary flavor components in meat (Longzhu Zhou et al., 2024).

In recent years, advancements in metabolomics technology have led to high reliability and accuracy in measuring volatile flavor compounds and lipid components. Gas chromatography-ion mobility spectrometry (GC-IMS) is a technique that offers high sensitivity, resolution, and rapid analysis, making it advantageous for various applications. This approach is especially useful for detecting functional groups, including amino, thiol, aldehyde, ketone, and ether groups, present in food flavorings (Wang, Chen, & Sun, 2020). Currently, some scholars have investigated the effects of various cooking methods on the flavor components of beef (Watanabe et al., 2015), mutton (Roldán, Ruiz, Del Pulgar, Pérez-Palacios, & Antequera, 2015), and fish (Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2017). However, there is a scarcity of research reports focusing on pork, and a comprehensive comparison of the influence of common Chinese cooking methods on pork remains lacking. Lipidomics, which uses mass spectrometry, has become a crucial method for studying the lipid makeup of food products (Song et al., 2022). This approach offers detailed insights into the complete lipid profiles of samples without the need for prior screening. Currently, lipidomics is extensively applied to detect lipid components and potential biomarkers in both raw and processed meats (Li, Al-Dalali, Wang, Xu, & Zhou, 2022). Research has indicated that 97 distinct lipids exist among donkey, beef, and lamb, with 3 phospholipids proposed as potential markers for differentiating between these meats and 13 characteristic VOCs exhibiting negative correlations with 21 phospholipid markers (Man et al., 2023). Jia, Shi, and Shi (2021) employed fragmentation mechanisms along with UHPLC-Q-Orbitrap MS/MS to investigate the transformations of lipids and their molecular mechanisms, revealing alterations in the lipid profile and quality of Tannan sheep after refrigeration periods of 12 and 24 days.

To date, few studies have combined lipidomics with volatile flavor compounds to examine differences in the cooking of abdominal muscle. The Nanyang black pig, a highly esteemed breed from China, is celebrated by consumers for its flavorful meat, high fatty acid content, and diverse nutrient profile. Unsaturated fatty acids are prevalent in black pork and are prone to oxidation and decomposition, resulting in the formation of aldehydes, ketones, alcohols, and other small volatile flavor compounds during processing. Consequently, this study aims to employ UPLC-MS/MS and GC-IMS techniques to (i) delineate the lipidomic and volatile profiles of raw abdominal muscle, sous vide abdominal muscle, steamed abdominal muscle, and oven-cooked abdominal muscle; (ii) identify key lipids and volatile compounds responsible for these differences; and (iii) explore the relationships between significant lipid molecules and volatile compounds. The findings of this study will enhance our understanding of lipid profile alterations during the processing of abdominal muscle products and serve as a reference for advancing related production technologies.

2. Materials and methods

2.1. Chemicals and reagent

HPLC-grade acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), dichloromethane (CH2Cl2), and tert-butyl methyl ether (MTBE) were procured from Merck (Darmstadt, Germany). HPLC-grade formic acid (FA) and ammonium formate (AmFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained via a Milli-Q system (Millipore, Billerica, MA). Lipid standards were acquired from either Sigma-Aldrich or Avanti Polar Lipids (Alabaster, AL).

2.2. Cooking processing of abdominal muscle

The abdominal muscle of black pigs (n = 3, 100–110 kg live weight,

about 6 months old) was sourced from a farm in Nanzhao County, Nanyang City, Henan Province, China. The fresh abdominal muscle (1 kg) was refrigerated and transported to the laboratory within one hour of the pigs were slaughtered and dissected. Divide one kilogram of abdominal muscle into four equal portions, each weighing 250 g. Prior to cooking, the skin of the abdominal muscle was removed, and each sample was cut to dimensions of 0.5 \times 1 \times 2 cm.

Four cooking methods were employed in this study: raw abdominal muscle, steam cooking, sous vide, and oven cooking. These widely used techniques were selected because of their significant variations in terms of food heating mechanisms.

2.2.1. Raw abdominal muscle (CK)

Unprocessed raw abdominal muscle.

2.2.2. Sous vide (SV)

The samples were pretreated and then vacuum-sealed using a Lavezzini Univac vacuum sealer (Lavezzini Univac, Fiorenzuola d'Arda, PC, Italy) in OPA/PP 15/65 bags (Orved, Musile di Piave, Italy). These sealed samples were subsequently boiled at 75 $^{\circ}\text{C}$ in a water bath for 45 min

2.2.3. Steaming (ST) (100 % steam)

The samples were subjected to steaming at an atmospheric pressure of 100 $^{\circ}$ C for 180 min in a Combo-Steam SL oven (V-Zug, Zurich, Switzerland).

2.2.4. Oven cooking (OC)

An electric oven (T7-L3840, Guangdong Midea Kitchen Appliance Manufacturing Co., Ltd.) was preheated to 180 $^{\circ}$ C. After 15 min, the samples were removed and allowed to cool. During the baking process, the samples were turned every 5 min.

Each of the four groups of experimental samples was repeated five times in for a total of 20 samples. Once cooked, the samples were placed at room temperature, minced using a meat grinder, vacuum-packed, and stored at $-80\,^{\circ}\text{C}$ until further analysis.

2.3. Lipid oxidation indices

2.3.1. Determination of the peroxide value (POV)

The POVs of the abdominal muscle samples were determined via the titration method according to GB/T5009.227–2016.

2.3.2. Determination of thiobarbituric acid reactive substances (TBARS)

Based on the research method reported by (Domínguez, Gómez, Fonseca and Lorenzo, 2014a), the TBARS of various samples were determined. Six grams of chopped and mixed abdominal muscle were combined with 20 mL of a 10 % trichloroacetic acid solution containing 0.1 % ethylene diamine tetraacetic acid (EDTA) and filtered through Whatman filter paper. Subsequently, 5 mL of the filtrate was combined with 5 mL of a 0.02 mol/L TBA solution, swirled for 10 s, and incubated at 90 °C for 40 min. After cooling to room temperature, the absorbance was measured at 532 nm.

2.4. Lipid extraction procedure

About 20 mg of sample was allowed to thaw and then transferred into a correctly labeled 2 mL centrifuge tube, and the weights were recorded. To this end, 1 mL of extraction solvent (methyl tert-butyl ether/methanol: 3/1, V/V) was introduced. The resulting mixture was vortexed for 15 min. Following this step, $200~\mu L$ of water was added, and the mixture was vortexed for 15 min. Afterward, the mixture was centrifuged at 4 °C (12,000 rpm) for 10 min via a 5424 R centrifuge from Eppendorf GmbH. Subsequently, $200~\mu L$ of the supernatant was subsequently transferred to a 1.5 mL centrifuge tube for concentration. Once concentrated, the lipid extract was reconstituted in $200~\mu L$ of

isopropanol (1:1, v/v), vortexed for 3 min, and then centrifuged again at 12,000 rpm for 3 min. The supernatant was then collected for analysis via LC-MS/MS. To create a quality control sample, twenty microliters of supernatant from each sample were pooled together.

2.5. HPLC conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD, https://sciex.com.cn/; MS, QTRAP® 6500+ System, https://sciex.com/). The column temperature was 45 °C. Flow rate: 0.35 mL/min. Mobile phase A was composed of acetonitrile (Merck, Darmstadt, Germany) and water at a ratio of 60:40, whereas mobile phase B was composed of acetonitrile/isopropanol (Merck, Darmstadt, Germany) (95/5). The program for the chromatographic gradient elution was designed as follows: at 0 min: A/B (80:20, V/V), at 2.0 min: 70:30 (V/V), at 4 min: 40:60 (V/V), at 9 min: 15:85 (V/V), at 14 min: 10:90 (V/V), at 15.5 min: 5:95 (V/V), at 17.3 min: 5:95 (V/V), then returning to 80:20 (V/V) at 17.3 min, and finally at 20 min: 80:20 (V/V).

2.6. ESI-MS/MS conditions

The experimental parameters for ESI-MS/MS were established with ionization voltages of (+) 5.5 kV and (–) 4.5 kV. The temperatures for the ion source (TEM), as well as the settings for nebulizer gas (GC1), auxiliary gas (GC2), and curtain gas (CUR), were controlled at 500 $^{\circ}\text{C},$ 45 psi, 55 psi, and 35 psi, respectively. Instrument tuning and mass calibration were performed with 10 and 100 $\mu\text{mol/L}$ polypropylene glycol solutions in QQQ and LIT modes, respectively.

2.7. Volatile compound analysis

Volatile compound assessment was performed utilizing a GC-IMS device (FlavourSpec®, G.A.S., Dortmund, Germany). A 2 g sample of abdominal muscle was introduced into a 20 mL headspace vial and incubated at 60 °C for 15 min. Next, 500 μ L of the sample was injected, with the injector temperature set to 85 °C and the incubation speed adjusted to 500 rpm. The MXT5 (15 m \times 0.53 mm ID) capillary column was used, and the temperature of the column was held constant at 60 °C throughout the entire procedure. Nitrogen gas, with a purity of \geq 99.999 %, was utilized as the carrier gas. The flow rate commenced at 2.0 mL/min for the initial 2 min, subsequently ramped linearly to 10.0 mL/min within an 8-min timeframe, and further increased linearly to 100.0 mL/min over the course of 10 min, which was then maintained at 100.0 mL/min for an additional 10 min. Ionization of analytes occurred within the IMS chamber before being directed into the drift tube (150 mL/min).

2.8. Relative odor activity value (ROAV)

The ROAV method was employed to assess how particular aroma components influence the overall flavor profile. The component that had the greatest impact on the flavor of the sample was designated $ROAV_x = 100$. The ROAV values for the other components (x) were derived via the formula outlined below:

$$ROAV_X \approx 100 \times \frac{C\%_X}{C\%stan} \times \frac{T_{stan}}{T_X}$$

In the formula, C%X signifies the percentage content relative to the volatile component, whereas TX denotes the sensory threshold associated with it. Additionally, C% reflects the percentage of the component that has the most significant impact on the sample's overall flavor, and T is the sensory threshold of that particular flavor-contributing component.

2.9. Statistical analysis

Data on lipids were analyzed via Analyst 1.6.3 software, whereas R

software (available at www.r-project.org) was used for multivariate statistical analyses. Flavor data analysis was carried out utilizing the instrumental data processing software VOCal. Analysis of variance (ANOVA) and Duncan's multipole difference test (P < 0.05) were used to evaluate the differences between samples. Both PCA (utilizing UV scaling) and OPLS-DA (employing Par scaling) analyses were executed with Simca 14.1 to differentiate the smell and taste characteristics of abdominal muscle samples.

3. Results and discussion

3.1. Lipid oxidation

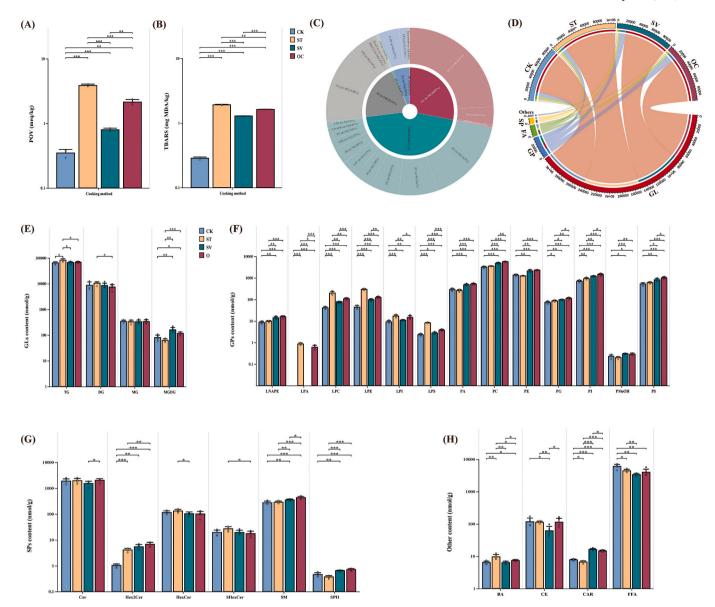
The effects of various cooking methods on abdominal muscle POV and TBARS are illustrated in Fig. 1A and Fig. 1B, respectively. Compared with those in the control group (CK), the POV and TBARS levels in the SV, ST, and OC groups tended to increase. Specifically, the CK group presented POV and TBARS values of 0.28 meq/kg and 0.35 mg MDA/kg, respectively, whereas the ST group presented values of 1.91 meg/kg and 3.85 mg MDA/kg, indicating increases of approximately 7 and 11 times, respectively, compared with those of the CK group. Although the POV and TBARS values in both the SV and OC groups were lower than those in the ST group were, they remained elevated compared with those in fresh abdominal muscle. This finding is consistent with Broncano et al. (Broncano, Petrón, Parra, & Timón, 2009), who demonstrated that cooking significantly increases physicochemical indicators of pork, such as the TBARS value and cholesterol oxide content, thereby reflecting the extent of fat oxidation. These cooking methods can considerably increase the degree of oxidation of pork fat, potentially contributing to the development of flavor compounds.

3.2. Lipid changes in abdominal muscle under different cooking methods

Abdominal muscle samples treated with four cooking methods were analyzed by UPLC-MS/MS to assess changes in lipid components resulting from these techniques. A mixed solution was utilized as the QC sample. As depicted in Fig. S1, the curves representing the total ion flow for lipid detection showed considerable overlap, with consistent retention times and peak intensities. These findings indicate the robust stability of signals when identical samples are analyzed across various time points, which suggests high reproducibility and dependability of the data. The lipid composition of the abdominal muscle processed by the four cooking methods was shown in Fig. 1C, and a total of 1230 lipids were identified. These included 553 glycerophospholipids (GP), 248 sphingolipids (SP), 345 glycerolipids (GL), 74 fatty acids (FAs), and 10 other lipid compounds. Among these lipid subclasses, GP were the most prevalent, followed by GL and SP. This observation aligns with earlier studies on pork lipids, which also indicated that the GL, GP, and SP subclasses constituted more than 90 % of the total lipid content (H. Liu et al., 2024). These lipid compounds can be further categorized into 42 subclasses, predominantly consisting of triglycerides (TG), phosphatidylethanolamines (PE), phosphatidylcholines (PC), and ceramides (Cer). Triglycerides are the primary form of lipids found in both animals and plants, and their degradation into free fatty acids is crucial for flavor development. This finding aligns with previous research on pork lipids (Wu, He, Yang, & Li, 2024).

The alterations in the lipid content of the abdominal muscle following various heat treatments are illustrated in Fig. 1D. GLs were the most abundant lipids both before and after heat treatment, followed by GP, FA, and SP. The contents of various lipid subclasses were further analyzed, among which the lipid contents of DG and TG were the highest in ST, significantly higher than those of the other three samples (Fig. 1E). Under heat treatment conditions, DGs and TGs are susceptible to thermal degradation and hydrolysis, yielding free fatty acids (FFA). These FFAs can subsequently undergo β -homolysis, ketone-enol tautomerization, or isomerization at carbon-carbon double-bond reaction

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sites, forming various types of hydroperoxides (Yin, Xu, & Porter, 2011). These compounds are then decomposed into smaller molecular species, including hydrocarbons, ketones, aldehydes, and alcohols (Zhang, Qin, Lin, Shen, & Saleh, 2015). These findings indicate that the FA content in the abdominal muscle decreased following different heat treatments, likely due to the oxidation of FAs into smaller molecular weight compounds (Fig. 1H). Compared to CK, the levels of LPC, LPE, and LPS were significantly elevated in both ST SV and OC, with the highest concentration observed in ST (Fig. 1F). This increase may be attributed to the hydrolysis of PC and PE into LPC and LPE under aerobic heating conditions. Cooking temperature is a crucial factor influencing phospholipase activity, which in turn regulates the conversion of PC and LPC in abdominal muscles (Jia, Li, Wu, Liu, & Shi, 2021). Notably, CK and SV do not contain LPA. The contents of PE, PG, PI, PS, PC, and PA were highest in OC, followed by SV (Fig. 1F). This finding may be associated with the alteration and degradation of lipid molecules during oxidation (Tu et al., 2022). Additionally, the content of SM increased significantly following heat treatment, particularly during baking (Fig. 1G).

The contents of TG, LPC, and LPE in the ST group were significantly greater than those in the other treatment groups. The highest levels of LPE, LPC, and TG were observed for LPE (18:2/0:0), LPC (18:2/0:0), and TG (16:0_18:1_18:1), respectively. Moreover, the concentrations of PE and PC in the OC group significantly increased relative to those in the other groups. The lipid molecules found in the highest abundance within PC and PE were identified as PC (16:0_18:2) and PE (P-18:0_18:2), respectively. It has been reported that lipid content has a greater influence on aroma than does lipid type (M. Zhou et al., 2023). Additionally, the findings revealed that the aroma retention of steamed abdominal muscle was influenced by LPE (18:2/0:0), LPC (18:2/0:0), and TG (16:0_18:1_18:1), whereas PC (16:0_18:2) and PE (P-18:0_18:2) could significantly contribute to the aroma retention of roasted abdominal muscle.

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With respect to double bond equivalents (DBE), triglycerides (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), with DBE values of 2, dominate the abdominal muscle subjected to various cooking methods. The most frequently observed DBE value for free fatty acids (FFA) is 1 (Fig 2ABC). An analysis of lipid compounds with different chain lengths revealed that TG (chain lengths: 46, 48, 50, 52, and 54) presented the highest content in the abdominal muscle.

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Additionally, PE (chain lengths: 36, 38), PC (chain lengths: 34, 36, 38), and FFA (chain length: 18) were among the most abundant (Fig. S2). The concentration of TG (chain lengths: 46, 48, 50, 52, and 54) in the ST sample was significantly greater than that in the other three samples, whereas the concentration of PE (chain lengths: 36 and 38) was significantly lower than that in the other samples (Fig 2EF). Compared with that in the control (CK), the concentration of PC (chain lengths: 34, 36,

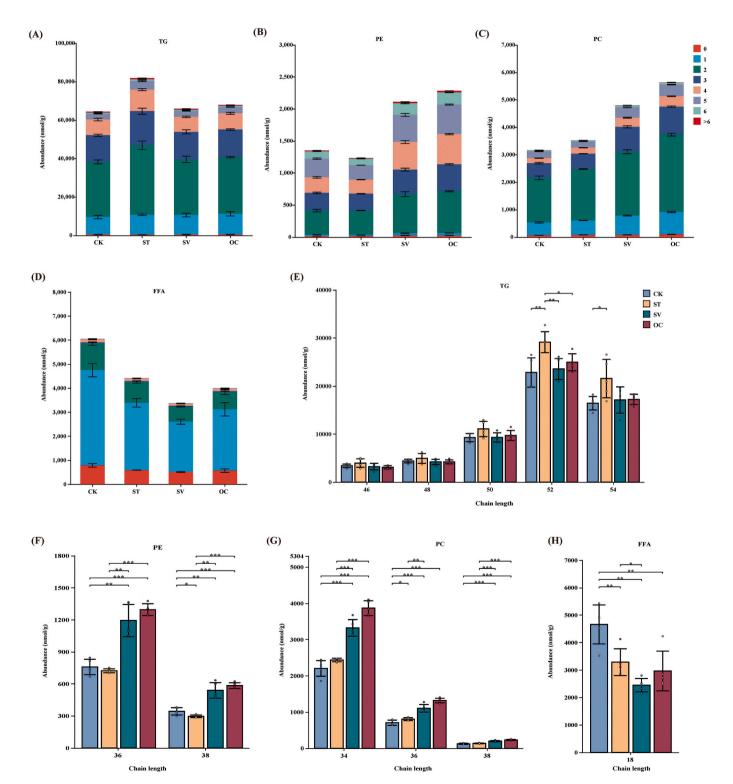


Fig. 2. Content of different double bond equivalents (DBEs) in abdominal muscle (A) TG, (B) PE, (C) PC, (D) FFA. Content of lipid compounds with different chain lengths in abdominal muscle (E) TG, (F) PE, (G) PC, (H) FFA. *P < 0.05, **P < 0.01, *** P < 0.001. CK: raw abdominal muscle, SV: sous vide abdominal muscle, ST: steamed abdominal muscle, OC: oven-cooked abdominal muscle.

38) increased significantly following heat treatment, whereas the concentration of FFA (chain length: 18) decreased significantly (Fig 2GH).

3.3. Differential lipidomics analysis in cooking abdominal muscle

Principal component analysis (PCA) allows for the visual interpretation of complex datasets, revealing groupings, trends, and outliers within the data (Guo et al., 2022). Orthogonal partial least squares discriminant analysis (OPLS-DA) is a supervised pattern recognition multivariate statistical analysis method that effectively removes unrelated influences to identify differentially abundant metabolites. Utilizing a dataset comprising 1230 lipids, PCA and OPLS-DA analyses were conducted on the metabolite data of cooked abdominal muscle to discern differences among samples subjected to various cooking treatments (Fig 3AB). The PCA results showed that PC 1 and PC 2 account for 36.19 % and 20.1 % of the total model, respectively, highlighting differences among the four sample groups. Notably, the difference between SV and OC was minimal, whereas the differences between CK and ST,

and OC were substantial. The classification metrics R2Y and Q2 were 0.995 and 0.785, respectively, demonstrating that the OPLS-DA model is reliable and has good fitting and predictive capabilities (Bi et al., 2023). These findings indicate that OPLS-DA is effective for differentiation applications. Furthermore, the results demonstrate that the metabolic profile of abdominal muscle undergoes significant changes after cooking, aligning with previous studies.

To identify the distinctive lipid markers in the abdominal muscle samples subjected to the four different cooking methods, we analyzed the key differentially abundant metabolites within each group. Notably, the metabolites in the SV, ST, and OC groups were significantly different from those in the CK group. The Variable Importance in the Projection (VIP) method was employed to identify significant variables. Typically, variables with VIP scores greater than 1.0 are regarded as significant for differentiating between samples. By applying the screening criteria of variable importance in projection (VIP > 1), a false discovery rate (FDR < 0.05), and a fold change (FC) greater than 2 or less than 0.5, we identified a total of 250 metabolites that exhibited significant

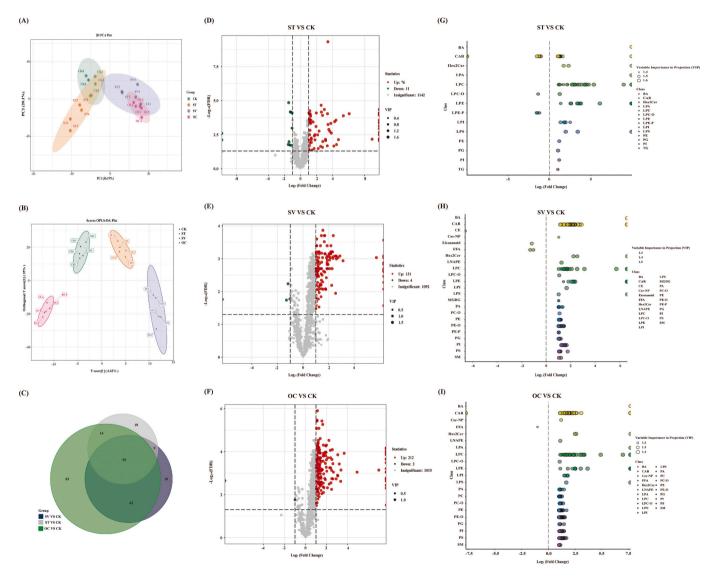


Fig. 3. PCA score plot (A), orthogonal partial least squares discriminant analysis (OPLS-DA) score plots (B). Venn diagram of the number of differential lipid molecules in abdominal muscle between different cooking methods (C). Volcano plot of differential lipid molecules in ST vs CK (D), SV vs CK (E), OC vs CK (F). Each point in the volcano plot represents a metabolite, with the x-axis reflecting the fold change in lipid differences between groups (log₂FoldChange) and the y-axis indicating the level of significance (-log₁₀FDR). Significantly upregulated lipids are represented as red points, significantly downregulated lipids as green points, and gray points indicate metabolites detected but not significantly different. The size of the points corresponds to the VIP value. Effect of heat treatment on the amount of differentially changed lipids in abdominal muscle: ST vs CK (G), SV vs CK (H), OC vs CK (I). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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differences. Approximately 135 metabolites were detected when the SV and CK groups were compared, with 131 being upregulated and 4 being downregulated (Fig. 3D). In the comparison between the ST and CK groups, approximately 87 metabolites were identified, including 76 that were upregulated and 11 that were downregulated (Fig. 3E). The analysis of the OC and CK groups revealed approximately 214 metabolites,

with 212 upregulated and 2 downregulated (Fig. 3F). Fig 3GHI illustrates the distribution of these differential lipids, revealing a notable trend of upward regulation among most lipid molecules. Venn diagram analysis revealed 55 lipid molecules as shared differentially abundant metabolites (Fig. 3C), which could serve as potential biomarkers. This group comprises 36 glycerophospholipids, 15 fatty acyl groups, and 4

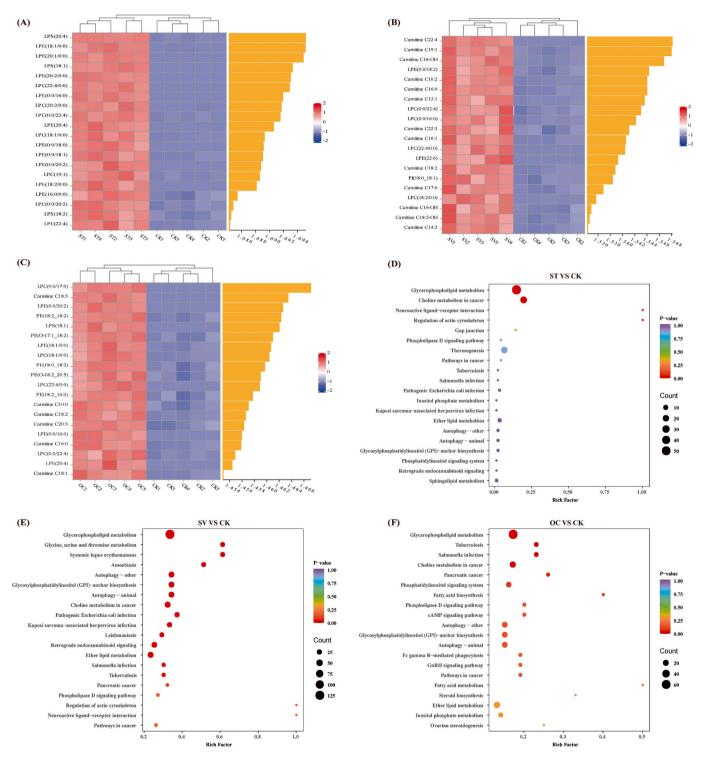


Fig. 4. VIP value analysis of differential metabolites between groups: ST vs CK (A), SV vs CK (B), OC vs CK (C). Potential metabolic pathway analysis based on significantly differential metabolites in abdominal muscle between different groups: ST vs CK (D), SV vs CK (E), OC vs CK (F). The Rich Factor, defined as the ratio of the number of differential lipids in the corresponding pathway to the total number of lipids annotated in that pathway, indicates a higher degree of enrichment with increasing values. Additionally, a P-value closer to 0 signifies greater significance in enrichment. The size of the dots in the analysis represents the number of significantly different lipids enriched in each corresponding pathway.

other lipids. A total of 8 lipid subclass variables (CAR, LPE, LPC, LPS, PI, LPI, BA, and Hex2Cer) had significant effects on the observed differences in cooked abdominal muscle (P < 0.05) (Table S1).

To further elucidate the metabolic differences in abdominal muscle attributable to various cooking methods, we compared and analyzed the metabolites from differently cooked abdominal muscle samples. By utilizing the OPLS-DA results, we identified the top 20 metabolites that exhibited significant differences. Compared with those in raw abdominal muscle, the differentially abundant metabolites in steamed abdominal muscle were glycerophospholipids, with LPS (20:4), LPE (18:1/0:0), and LPE (20:1/0:0) identified as the most significant

(Fig. 4A). For the sous vide abdominal muscle, the primary differentially abundant metabolites included fatty acyls and glycerophospholipids, particularly Carnitine C22:4 and Carnitine C19:1, which were the most significant (Fig. 4B). In an oven-cooked abdominal muscle, the differentially abundant metabolites were also predominantly fatty acyls and glycerophospholipids, with LPC (0:0/17:0), Carnitine C18:3, PE (0:0/20:2), and PE (18:2:2) displaying the most pronounced differences (Fig. 4C).

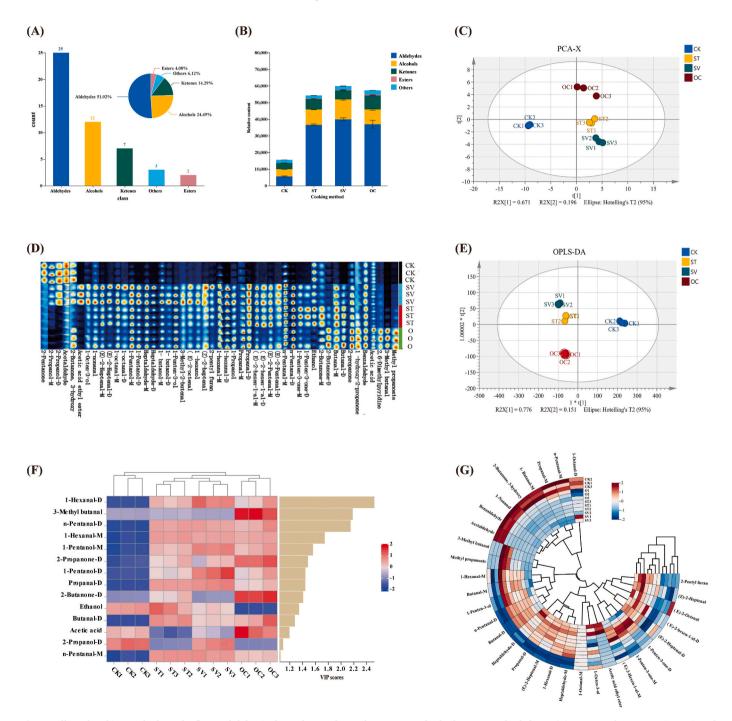


Fig. 5. Effect of cooking methods on the flavor of abdominal muscle. Number and percentage of volatile compound subclasses (A). Types and concentration ratios of volatile compounds in abdominal muscle from different heat treatments (B). PCA score plot(C). Fingerprint spectrum of sample gallery plot (D). M, monomer; D, dimer. OPLS-DA score(E). VIP score plot of different flavor compounds in abdominal muscle with different cooking methods, with screening conditions of VIP >1 (F). Circular heat map of important flavor compounds(G).

3.4. Analysis of metabolic pathways of different metabolites in abdominal muscle treated by different cooking methods

The KEGG database serves as an effective tool for analyzing metabolic pathways and their interrelationships. Consequently, we utilized the KEGG database to enrich and analyze the differentially abundant metabolites in samples subjected to various cooking methods. We compared the SV, ST, and OC groups with the CK group and performed KEGG pathway enrichment analysis. We selected the top 20 pathways ranked by *P* value, displaying them from smallest to largest (Fig. 4 DEF). The results indicated that the pathways involved in glycerophospholipid metabolism and ether lipid metabolism were highly enriched across all comparison groups. These findings suggest that lipid metabolism in abdominal muscle following heat treatment primarily involves glycerophospholipid metabolism.

3.5. Analysis of volatile components in abdominal muscle processed by different cooking methods

3.5.1. Qualitative analysis of volatile components

The analysis of the volatile components across the four sample groups was conducted using GC-IMS techniques. Compared with CK, the presence of a larger area and a more pronounced red hue in the twodimensional spectrum implies an increased concentration of the related volatile substance, whereas a deeper blue shade signifies a reduced concentration (Fig. S3A and B). The results showed that the concentration of volatile substances in heat treated abdominal muscle was significantly higher than that in raw abdominal muscle. This observation aligns with previous research findings (Cheng et al., 2023). To facilitate a clearer comparison of the differences in volatile compounds present in the abdominal muscle between the CK group and the treated group, n-ketones C4-C9 (China National Pharmaceutical Chemical Reagents, Beijing, China) were employed as external standards for determining the retention index (RI) of these volatile compounds and for assessing their changes. The characterization of the volatile flavor compounds found in the abdominal muscle samples was conducted using built-in software (NIST 2020) in conjunction with the IMS database. Comprehensive details regarding the volatile organic compounds identified in each sample are provided in Table S2. A total of 49 distinct volatile flavor compounds were detected, which included 25 aldehydes, 12 alcohols, 7 ketones, 2 esters, 1 furan, 1 acid, and 1 pyridine. Aldehydes were found to have the highest content, accounting for 51.02 %, followed by alcohols and ketones, which accounted for 24.49 % and 14.29 %, respectively (Fig. 5A).

The cooking method significantly influences the content of volatile flavor compounds. Compared with those in the CK group, the contents of various compounds, particularly aldehydes, alcohols, and ketones, in the heat-treated abdominal muscle increased notably (Fig. 5B). The total content of aldehydes and alcohols was highest in the sous vide abdominal muscle, whereas the total content of ketones was highest in the oven-cooked abdominal muscle. The fingerprints indicate more pronounced differences among the four sample groups (Fig. 5D). The results of this study demonstrated that the volatile compounds produced by different cooking methods in abdominal muscle primarily consisted of ketones, alcohols, and aldehydes. Compounds such as 2-pentanone, 2propanol, and acetaldehyde are most abundant in CK; 1-octen-3-ol, nonanal, octanal, pentanol, E-2-heptenal, heptanal, and butanol are most abundant in SV; 1-penten-3-one, 2-butanone, and ethanol are most abundant in ST; and acetone, hydroxyacetone, benzaldehyde, acetic acid, and 3-methylbutanal are most abundant in OC. Aldehydes exhibit strong volatility and high concentrations, rendering them important flavor compounds in cooked meat (Liu et al., 2019). Alcohol is primarily generated from the oxidative decomposition of fats (Estévez, Morcuende, Ventanas, & Cava, 2003). The production of some ketones is influenced by factors such as composition, temperature, duration, and cooking method, thereby significantly enhancing the flavor of pork

(Song Ze et al., 2019).

3.5.2. Analysis of OPLS-DA model

PCA revealed that the principal components of different samples were distinctly separate, with evident clustering for each group and significant differences observed among the other sample groups (Fig. 5C). The OPLS-DA model employs the R2 and Q2 to distinguish between abdominal muscle samples that display notable variations. The parameters for the R2X, R2Y, and Q2 models were 0.989, 0.993, and 0.98, respectively (Fig. 5E). Additionally, the regression line for Q2 crosses the horizontal axis at a negative intercept, which lends support to the robustness of the model, as illustrated in Fig. S3C. The analysis revealed a notable disparity in the distance between the CK group and the other samples, underscoring the significant impact that the cooking treatments had on the flavor profile of the abdominal muscle. The results for the SV and ST samples are comparable, although a certain distance is noted between them; both are situated in the first quadrant, suggesting that the flavors of the Sous vide and Steaming abdominal muscles are similar. The OC group was positioned in the third quadrant, at a significant distance from the other sample groups, indicating that the flavor of oven-cooked abdominal muscle was markedly different from that of the other samples. To obtain the key differences in the abdominal muscle samples across various cooking methods via the OPLS-DA model, we calculated the variable importance in projection (VIP) to evaluate the influence of each variable on the samples, as well as its explanatory ability. A higher VIP value signifies a more substantial difference; specifically, a VIP greater than 1 indicates that the volatile substance is of considerable importance to the sample (Martín-Gómez et al., 2023). As illustrated in Fig. 5F, a total of 14 key compounds (monomers and dimers) were identified: 1-hexanal-D, 3-methylbutanal, n-pentanal-D, 1hexanal-M, 1-pentanol-M, 2-propanone-D, 1-pentanol-D, propanal-D, 2-butanone-D, ethanol, butanal-D, acetic acid, 2-propanol-D, and npentanal-M. These compounds comprise 7 aldehydes, 2 ketones, 4 alcohols, and 1 acid, all of which play a significant role in the flavor profile of abdominal muscle. The results indicate that aldehydes and alcohols are the primary contributors to the flavor of cooked abdominal muscle.

3.5.3. Relative odor activity value (ROAV)

The development of distinctive flavors is not solely the result of a straightforward accumulation of volatile compounds (VCs) but rather emerges from the interactions among these VCs. Factors such as concentration, flavor characteristics, and threshold (OT) also play significant roles. The ROAV is calculated to determine if a compound acts as a primary odor-active substance. In this study, the ROAVs for the compounds were assessed by normalization to those of n-octanal. An elevated ROAV signifies that the compound contributes more significantly to the overall flavor. A substance is classified as a key flavor compound when it has an ROAV of ≥ 1 , whereas a compound with an ROAV between 0.1 and 1 is considered to have an impact on the overall flavor of the sample (Bi et al., 2024). About 31 characteristic volatile flavor compounds of Nanyang black abdominal muscle were identified when the ROAV exceeded 0.1. As presented in Table 1, 22 and 25 key flavor compounds were identified in CK and SV, respectively, whereas 23 and 24 key flavor compounds were found in ST and O, respectively. 1-Octanal-M has a waxy, citrus, orange, fruity, and fatty aroma and exhibited a high relative content and low odor threshold in this study, thereby contributing significantly to the overall flavor. To effectively illustrate the effects of various cooking methods on the composition of volatile flavor compounds present in the samples, a comprehensive clustering heatmap analysis was conducted. This analysis focused on 31 specific volatile flavor compounds whose relative odor activity value (ROAV) was greater than 0.1, as demonstrated in Fig. 5G. The contents of benzaldehyde, 1-nonanal, n-pentanal-M, acetaldehyde, propanal-M, 1-octanal-D, 1-butanol-M, 2-butanone, and 3-hydroxy were significantly greater in CK than in the other three cooking methods. The contents of heptaldehyde-D, (E)-2-octenal, (Z)-2-heptenal, 1-octen-3-ol,

Table 1Volatile flavor compounds and ROAV values.

Chemical compound	Flavor description ^a	Odor Threshold value(µg/kg) b	ROAV			
			CK	SV	ST	OC
benzaldehyde	bitter almond, cherry, nutty	350	0.25 ± 0.02^a	$0.06 \pm 0.01^{\mathrm{b}}$	$0.07\pm0.00^{\text{b}}$	$0.09\pm0.01^{\rm b}$
1-nonanal	rose, citrus, strong oily	1	59.44 ± 4.85^a	27.45 ± 1.36^{b}	22.14 ± 0.60^c	30.76 ± 1.37^{b}
(E)-2-heptenal-M	spicy, green vegetables, fresh, fatty	13	2.35 ± 0.39^c	6.81 ± 0.79^a	6.84 ± 0.55^a	5.42 ± 0.50^{b}
(E)-2-heptenal-D	spicy, green vegetables, fresh, fatty	13	0.84 ± 0.11^a	1.02 ± 0.03^a	0.94 ± 0.11^a	$0.53\pm0.16^{\mathrm{b}}$
1-octanal-M	aldehyde, waxy, citrus, orange, fruity, fatty	0.7	100.00	100.00	100.00	100.00
(E)-2-hexen-1-al-M	green, banana, fat	17	$2.30\pm0.37^{\rm b}$	2.64 ± 0.30^{a}	3.06 ± 0.28^a	$2.34\pm0.26^{\mathrm{b}}$
heptaldehyde-M	fresh, aldehyde, fatty, green herbs, wine, fruity	3	25.54 ± 1.34^{c}	$39.68 \pm 3.21^{\mathrm{b}}$	37.30 ± 0.36^{b}	$41.80 \pm 2.36^{\circ}$
heptaldehyde-D	fresh, aldehyde, fatty, green herbs, wine, fruity	3	7.23 ± 1.74^c	21.04 ± 2.62^a	$14.94 \pm 0.77^{\mathrm{b}}$	15.89 ± 2.26^{l}
(E)-2-octenal	fresh cucumber, fatty, green herbal, banana, green leaf	3	3.33 ± 0.47^a	3.71 ± 0.27^a	$3.42\pm0.13^{\text{a}}$	3.05 ± 0.13^b
(Z)-2-heptenal	null	13	$1.15\pm0.31^{\rm c}$	2.31 ± 0.28^a	1.81 ± 0.14^a	$1.73\pm0.27^{\rm b}$
1-hexanal-M	fresh, green, fat, fruity	4.5	$72.00 \pm \\ 17.89^{\rm b}$	79.46 ± 12.44^{a}	92.12 ± 1.72^a	$102.63 \pm \\14.38^{a}$
1-hexanal-D	fresh, green, fat, fruity	4.5	11.14 ± 2.96^{c}	$151.39 \pm \\ 17.96^a$	$\begin{array}{l} 126.14 \pm \\ 5.04^{b} \end{array}$	140.86 ± 9.9
n-pentanal-M	green grassy, faint banana, pungent	12	25.00 ± 4.71^{a}	$14.33\pm2.03^{\mathrm{b}}$	$16.34\pm0.57^{\mathrm{b}}$	16.56 ± 2.73^{l}
n-pentanal-D	green grassy, faint banana, pungent	12	8.65 ± 2.99^{c}	$39.09 \pm 5.56^{\rm b}$	$44.45\pm0.34^{\mathrm{b}}$	$50.05 \pm 3.21^{\circ}$
3-methyl butanal	chocolate, fat	5.4	$2.15\pm0.29^{\mathrm{b}}$	$1.02\pm0.15^{\mathrm{b}}$	4.32 ± 0.41^{b}	55.40 ± 13.6
butanal-D	pungent, fruity, green leaf	9	0.90 ± 0.11^{c}	9.89 ± 1.75^{b}	15.48 ± 0.32^{a}	17.19 ± 1.09
propanal-D	pungent, green grassy	9.5	$4.40\pm1.48^{\rm b}$	23.40 ± 3.85^{a}	24.20 ± 0.67^{a}	21.82 ± 1.24
acetaldehyde	green, slight fruity	15	10.17 ± 0.49^{a}	$1.35\pm0.18^{\mathrm{b}}$	$1.32\pm0.10^{\rm b}$	1.46 ± 0.23^{b}
butanal-M	pungent, fruity, green leaf	9	4.93 ± 0.90^{b}	$5.28 \pm 0.77^{\mathrm{b}}$	7.38 ± 0.14^a	7.52 ± 1.19^a
propanal-M	pungent, green grassy	9.5	14.32 ± 3.19^a	$6.20\pm1.05^{\mathrm{b}}$	7.35 ± 0.36^{b}	9.40 ± 2.08^{b}
1-octanal-D	aldehyde, waxy, citrus, orange, fruity, fatty	0.7	10.75 ± 0.46^{a}	9.76 ± 1.77^{a}	8.94 ± 0.09^{b}	$7.47\pm1.23^{\mathrm{b}}$
(E)-2-hexen-1-al-D	green, banana, fat	17	0.24 ± 0.02^{b}	0.34 ± 0.05^a	0.34 ± 0.05^a	$0.19\pm0.07^{\mathrm{b}}$
1-octen-3-ol	mushroom, lavender, rose, hay	1	$18.31 \pm 2.47^{\mathrm{b}}$	22.60 ± 2.64^{a}	$16.11 \pm 2.37^{\mathrm{b}}$	20.01 ± 1.76
1- butanol-M	wine	500	0.25 ± 0.03^a	$0.10\pm0.02^{\mathrm{b}}$	$0.09\pm0.01^{\mathrm{b}}$	$0.12\pm0.01^{\rm b}$
1-penten-3-ol	ethereal, green, tropical fruity	400	0.14 ± 0.02^c	0.18 ± 0.03^{b}	0.19 ± 0.01^{b}	0.22 ± 0.02^a
2-butanone, 3- hydroxy	butter, cream	800	0.25 ± 0.03^a	0.05 ± 0.01^{c}	0.03 ± 0.00^{c}	0.07 ± 0.01^b
1-penten-3-one-M	strong pungent odors	1	7.24 ± 1.68^{c}	$12.64\pm4.24^{\mathrm{b}}$	21.83 ± 1.06^a	13.21 ± 1.97
1-penten-3-one-D	strong pungent odors	1	$3.13\pm0.37^{\rm b}$	3.36 ± 1.36^{b}	6.07 ± 1.00^a	$3.47\pm1.27^{\mathrm{b}}$
2-pentyl furan	bean, fruity, earthy, green, vegetable	6	$3.80\pm0.10^{\rm b}$	6.54 ± 0.97^a	5.77 ± 0.53^a	4.04 ± 0.96^{b}
acetic acid ethyl ester	fresh, fruity, sweet, grassy	5	$0.72\pm0.06^{\rm b}$	4.64 ± 0.79^a	$0.46\pm0.08^{\rm b}$	$0.51\pm0.06^{\rm b}$
methyl propanoate	Fruit, Rum	20	0.53 ± 0.04^{c}	0.39 ± 0.06^{c}	0.87 ± 0.10^{b}	2.43 ± 0.37^a

a: Odor descriptions are obtained from the technology of food flavoring (3rd ed. ed.) (Sun, 2017). and http://www.odor.org.uk.

2-pentyl furan, and acetic acid ethyl ester were significantly greater in SV than in the other three sample groups. The contents of (E)-2-hexen-1-al-M, 1-Penten-3-one-M, and 1-Penten-3-one-D are significantly high in the ST. The contents of 1-Hexanal-M, n-Pentanal-D, 3-Methyl butanal, and Butanal are significantly high in the OC.

After benzaldehyde is subjected to heat treatment, its content significantly decreases, resulting in a bitter almond scent that may be linked to the breakdown of α -linolenic acid (C18:3) (Cheng et al., 2023). 1-Octene-3-ol is generated primarily through the oxidation of arachidonic acid and other unsaturated fats, imparting a mushroom flavor to meat products (Zang et al., 2020). As the most common alcoholic compound, 1-octene-3-ol plays a critical role in enhancing the flavor of prepared Nanyang black pork because of its lower odor detection threshold. The levels of 1-octene-3-ol were notably increased through roasting and slow cooking techniques. 3-Hydroxy-2-butanone, which is a prevalent methyl ketone in meat, originates from triglyceride β-keto acids and adds a fruity and buttery taste (Domínguez et al., 2014a, 2014b), thus contributing to an enjoyable fatty flavor in meat products. In this study, a significant reduction in the concentration of 3-hydroxy-2-butanone was observed post-cooking, with the OC group exhibiting the lowest amount of 3-hydroxy-2-butanone, whereas the St and SV groups gradually increased. These findings indicate that the levels of 3hydroxy-2-butanone generally decrease as the cooking temperature or duration increases, which is consistent with results from earlier studies (Domínguez et al., 2014a, 2014b). Additionally, while 2-pentylfuran has been detected consistently in cooked meat, it was not classified as a significant flavor compound in this analysis. We identified seven key volatile compounds that can distinguish abdominal muscle prepared via

different cooking techniques. This identification was accomplished by analyzing the ROAV and VIP values of these volatile flavor compounds.

3.6. Correlation analysis between differential lipids and characteristic volatile compounds

To analyze the relationships between lipids and aroma compounds, the Pearson method was employed for correlation analysis to examine the associations between 250 differential lipid molecules and 7 key aromatic compounds in abdominal muscle subjected to various cooking methods (Fig. 6). Notably, n-pentanal-D, 1-hexanal-M, 1-hexanal-D, propanal-D, and n-pentanal-M were significantly negatively correlated with FFA (16:1). With the exception of FFA (16:1), all other lipid molecules demonstrated a positive correlation with the key flavor compounds. However, the levels of anal-D, n-pentanal-D, 1-hexanal-M, 1-hexanal-D, propanal-D and n-Pentanal-M were significantly positively correlated with the levels of the lipid molecules LPC, LPE and CAR. Additionally, a significant positive correlation was observed between 3-methyl butanal and the lipid molecules PC, PE, and PS (P < 0.05). Furthermore, butanal was positively correlated with the lipid molecules LPC, LPE and LPI.

3.7. Discussion

The four cooking methods can increase lipid oxidation in the abdominal muscle. Compared with those in raw abdominal muscle, the values of POV and TBARS in cooked pork are significantly elevated. The aroma of pork is influenced by the type and concentration of aroma

b: The aroma threshold of flavor substances is mainly derived from the technology of food flavoring (Sun, 2017).

Fig. 6. Correlation heatmap of key differential metabolites and key volatile compounds selected from abdominal muscle under different cooking methods. The red and green boxes represent positive and negative correlations, respectively (|r| > 0.8, P < 0.05). *P < 0.05; **P < 0.01; ***P < 0.001. The darker the color, the stronger the correlation, and vice versa. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compounds, their odor thresholds, and the interactions among them. In this study, via GC-IMS analysis, we detected 49 volatile flavor compounds in raw, sous vide, steamed, and oven-cooked abdominal muscle; among these, 25 were identified as aldehydes, which are the primary components contributing to the aroma of the abdominal muscle. This finding is consistent with previously reported results (Bi et al., 2022). Aldehydes are characterized by their high volatility, low detection thresholds, and enhanced aroma detectability. Their presence contributes distinctive aroma and flavor characteristics to cooked meat Through statistical analysis, seven key flavor markers were identified on the basis of VIP > 1 and ROAV >1: propanal-D (pungent, green grassy), n-pentanal-M (green grassy, faint banana, pungent), n-pentanal-D, butanal-D (pungent, fruity, green leaf), 3-methyl butanal (chocolate, fatty), 1-hexanal-M (fresh, green, fatty, fruity), and 1-hexanal-D. Hexanal is a primary degradation product of lipid β-oxidation (Gruffat, Bauchart, Thomas, Parafita, & Durand, 2021) and significantly contributes to meat flavor due to its characteristic grass and mushroom aroma. Valeraldehyde is a type of saturated fatty acid aldehyde. (Li, Al-Dalali, et al., 2022), in their study of the characteristic volatile compounds of boiled duck meat, reported that lipid oxidation typically occurs at unsaturated bonds, resulting in the production of peroxides such as valeraldehyde and ethyl acetate.

A total of 250 key differential lipids were identified through absolute quantitative analysis of lipids derived from various cooking methods. Among these, phospholipids, including PC, PE, LPC, and LPE, constituted 78.4 % of all identified differential lipids. Correlation analysis revealed that most phospholipids exhibited significant positive correlations with key volatile flavor compounds. Phospholipids serve as crucial precursors for the production of volatile organic compounds in meat (Calkins & Hodgen, 2007). C18:2 can be degraded into hexanal (Gruffat et al., 2021). Abdominal muscle is rich in PUFAs, and phospholipids rich in PUFAs are closely linked to characteristic VOCs (Man et al., 2023).

However, butanal-D was significantly positively correlated with LPC (0:0/16:0), whereas n-Pentanal-M, 1-hexanal-D, and propanal-D were significantly positively correlated with PI (18:0:1). Additionally, propanal-D, n-pentanal-M, and n-pentanal-D 1-hexanal-M demonstrated a significant positive correlation with LPC (O-18:2), and 3-methylbutanal, along with PS (18:1_20:3), also showed a significant positive correlation. These findings suggest that these compounds may play crucial roles in the production of key aroma compounds. Furthermore, after heat treatment, the content of lipid molecules in LPC and LPE increased significantly, and these molecules incorporated C18:1, C18:2, C20:4, and other unsaturated fatty acid (UFA) chains, potentially influencing the aroma retention of the abdominal muscle. Triglycerides containing C18:1 or C18:2 fatty acid chains, in addition to various phosphatidylcholine (PC) species such as PC (16:1e_20:4), PC (16:0e_20:4), and PC (18:2e 18:2), as well as phosphatidylethanolamine (PE) (16:1e20:4), were found to play a substantial role in generating vital aroma compounds in beef.(Zhou et al., 2024).

4. Conclusion

In this study, lipidomics and GC-IMS were employed to characterize the lipid and volatile flavor compounds of raw abdominal muscle, sous vide abdominal muscle, steamed abdominal muscle, and oven-cooked abdominal muscle. The analysis of the POV and TBARS results revealed that cooked abdominal muscle had a greater lipid content than raw abdominal muscle. A total of 49 flavor substance were identified through GC-IMS. Aldehydes and alcohols are the primary contributors to the flavor of cooked abdominal muscle. A total of 1230 lipids were identified, including 553 glycerophospholipids (GP), 345 (GL), 248 sphingolipids (SP), 74 fatty acids (FA), and 10 other lipid compounds. There were 250 differentially abundant metabolites and 7 distinct flavor compounds were identified as potential markers to differentiate abdominal muscle samples processed by various cooking methods. Correlation analysis results indicated that butanal-D was significantly positively correlated with LPC (0:0/16:0), whereas n-Pentanal-M, 1hexanal-D, and propanal-D were significantly positively correlated with PI (18:0:1). Additionally, propanal-D, n-pentanal-M, and n-pentanal-D 1-hexanal-M demonstrated a significant positive correlation with LPC (O-18:2), and 3-methylbutanal, along with PS (18:1_20:3), also showed a significant positive correlation. Phospholipid molecules (LPC, LPE, PC, and PE) containing unsaturated fatty acid (UFA) chains were major contributors to the formation of key aroma compounds in abdominal muscle. This study provides a reference for analyzing the effects of different heat treatment methods on the lipid composition and aroma components of abdominal muscle.

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CRediT authorship contribution statement

Jicai Bi: Writing – original draft, Methodology, Funding acquisition. Bian Li: Writing – review & editing, Writing – original draft, Validation, Formal analysis. Zhuo Chen: Data curation. Chunyuan Ping: Investigation. Junyang Zhang: Software. Qiong Luo: Investigation. Yunbo Li: Project administration. Hongju He: Writing – review & editing.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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