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Investigating the impact of lipid oxidation on the duck odorous smell during storage and reheating based on lipidomics

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ABSTRACT

In the actual production of duck meat, the cooking time for marinated and sliced duck products is around 30 min. Before consumption, it is recommended to use a water bath for reheating at 75 °C for 10 min. However, many consumers reflect that after reheating, there will be an unpleasant smell, affecting the quality of food. Therefore, to investigate the effects of lipid oxidation on the duck odorous smell during heat treatment and reheating, lipid oxidation, sensory evaluation and lipidomics were performed on raw duck meat, 90 °C processed, and reheated cooked duck meat stored at 4 ± 1 °C for 3 and 7 days, respectively. The results showed that the duck odorous smell increased after heat treatment and reheating. A total of 26 lipid subclasses and 519 lipid molecules were identified in duck meat based on lipidomics. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in duck meat phospholipids played an important role in the production of duck odorous smell. These findings may contribute in reducing duck odorous smell by targeted inhibition of lipid oxidation.

1. Introduction

China is recognized as the world's largest producer, consumer, and exporter of duck meat, placing this product in a crucial position within the nation's meat consumption framework due to its nutritional value, short growth cycle, and low cost. Duck meat, a high-quality poultry product, is widely acknowledged as a nutritious food source by consumers for its high protein content, abundance of vitamins, and low-calorie profile (Lung et al., 2022). In Chinese cuisine, duck meat is typically prepared using traditional methods such as boiling or steaming. Alternatively, it can be processed at higher temperatures for shorter durations in an industrial setting, although this generally results in products with a less robust flavor (Garcia-Segovia et al., 2007).

Lipids are a fundamental class of biological molecules that play crucial roles in numerous biological processes and are widely distributed in both animals and plants (Li et al., 2021). They primarily exist in the forms of triglycerides, phospholipids, and free fatty acids (Shibata et al., 2015). Generally, triglycerides consist of a higher proportion of saturated fatty acids and are less susceptible to oxidation. In contrast, phospholipids are characterized by a predominance of unsaturated fatty acids, which lead to the production of numerous volatile components. Although free fatty acids are highly prone to oxidative degradation reactions, they are typically present at low levels within living organisms (Igene and Pearson, 2006). Duck meat is rich in unsaturated fatty acids, making it susceptible to oxidation during thermal processing and storage reheating. This oxidation process leads to the production of free radicals, hydroperoxides, and free fatty acids, which further react to generate aldehydes, ketones, alcohols, and other volatile components, resulting in an unpleasant odor and reduced quality of the duck meat. Previous studies have demonstrated that lipids significantly influence the production of odorous compounds in duck meat (Zhao et al., 2024). Lipidomics, an increasingly utilized analytical approach, has facilitated large-scale and comprehensive studies of lipids in various food matrices. However, there remains a lack of extensive understanding of lipids, particularly their fingerprinting and dynamic alterations, which continues to be an unexplored and ambiguous area (Bourlieu et al., 2018). Several literature reports related to lipidomics in food and nutrition describe the workflow of lipidome analysis, the application of lipid characterization, quality control and evaluation of food raw materials, as well as lipid metabolism in healthy diets. These literatures provide a valuable reference for research in food science and lipidomics (Sun et al., 2020). Lipidomics based on HPLC-MS methodology has been

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successfully utilized for lipid characterization in pork (Mi et al., 2019), duck (C. Li et al., 2020), and lamb (J. Li et al., 2020).

Significant research efforts have been dedicated to investigating the impact of various factors on the odor of duck meat, including thawing methods, selection of antioxidants, meat conditions, storage methods, and processing methods. Some studies have examined the changes in the flavor of chicken (Nie et al., 2024) and duck meat (Zhang et al., 2022) during processing and storage using sensory evaluation, gas chromatography, and odor activity values (OAVs). Factors such as low-temperature storage, high-temperature heating, and vacuum packaging have been found to influence the flavor of duck meat. In particular, lipid oxidation resulting from high temperatures and alterations in glutathione enzyme activity are major contributors to flavor deterioration (Hoac et al., 2006; Zhang et al., 2022). Additionally, numerous studies have explored various methods for deodorizing duck meat, including the use of 400W ultrasonic waves and pulsed electric field-assisted defrosting, both of which have been found to be effective in achieving deodorization (Sun et al., 2023; Lung et al., 2022). While significant progress has been made in this field, few studies have specifically targeted the removal of odorous substances. Therefore, it is necessary to identify the key odorous compounds and control them to reduce unpleasant odors.

In this study, we conducted a comprehensive investigation of lipid metabolites in duck meat under various treatments using UPLC-MS, focusing specifically on Cherry Valley duck breast meat. The screening, identification, and qualitative and quantitative analysis of differential lipid metabolites were performed in conjunction with relevant bioinformatics tools. Furthermore, changes in key lipid metabolites were analyzed. Additionally, we explored the impact of lipid oxidation on the odor of duck meat to establish a theoretical foundation for investigating odor precursors and their causes in duck meat.

2. Material and methods

2.1. Material and chemical

Cherry Valley duck breasts and abdominal fat were obtained from New Hope Liuhe Co., Ltd. (Guantao, Shandong, China) for this study. The raw duck breasts and abdominal fat were transported under cold chain conditions and stored at −40 °C until use. The chemical reagents, methanol and acetonitrile, were chromatographically pure and obtained from Thermo Fisher Scientific Co. (Waltham, Massachusetts, USA). Ammonium formate, also chromatographically pure, was sourced from Honeywell Fluka Corporation (Charlotte, North Carolina, USA). Formic acid, also chromatographically pure, was provided by DIMKA Co. (Beijing, China). All other chemicals used in this study were of analytical grade and were supplied by Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

After the removal of excess skin, fat, and connective tissue from the surface of the duck breasts using a scalpel, the breasts were cut into uniform pieces (2 × 2 × 2 cm). The duck meat samples were then boiled in water at 90 °C for 30 min, maintaining a meat-to-water weight ratio of 1:2 in all treatments. After cooling, the samples were quickly vacuum-sealed and labeled. The samples were stored at 4 ± 1 °C for 0, 3, and 7 days, respectively. Based on actual production practices for similar products, the cooking time for marinated and sliced poultry is approximately 30 min. Before consumption, reheating in a water bath at 75 °C for 10 min is recommended. In this study, the samples were reheated for 10 min at 75 °C after 3 and 7 days of storage. The unheated duck meat samples served as the control group. The relevant physico-chemical experiments were conducted within 3 days.

2.3. Sensory evaluation of odorous smell

After training the postgraduate students in the meat specialization laboratory, a team of 10 members (5 male, 5 female, aged 20–26 years) was selected for the sensory evaluation of duck meat samples. After cooking, the samples were cooled to room temperature and cut into cubes (2 × 2 × 2 cm^3). Before presenting the samples to the panelists, they were reheated in a microwave for 10 s to release the aroma and flavor of the meat. The samples were randomly assigned 3-digit codes, placed in 10 mL volumetric cups (n = 10), and presented for rapid sniffing. The intensity of odor was evaluated using a 10-point scale divided into five levels: absent (0 < score ≤2), very weak (2 < score ≤4), moderate (4 < score ≤6), strong (6 < score ≤8), and very strong (8 < score ≤10).

2.4. Lipid extraction

Lipid extraction was performed following the method described by Folch et al. (1957) with minor modifications. Briefly, the duck samples were processed, weighed (5.0 g), and mixed with 60 mL of chloroform-methanol (2:1, v/v). The mixture was allowed to stand for 1 h, then extracted and filtered, with the process repeated. The resulting filtrate was combined with saline solution at 0.2 times its volume (7.3 g/L NaCl, 0.5 g/L $CaCl_2$). The solution was centrifuged at 3000 r/min for 15 min using an Allegra 64R centrifuge (Beckman Coulter Trading, China). The lower layer of liquid obtained after centrifugation was evaporated to dryness using a vacuum rotary evaporator (N-1100, Ditto Biotechnology, Shanghai, China) by nitrogen blowing at 44 °C. The pure lipids were stored at −20 °C and used within two weeks of extraction.

2.5. Peroxide value (POV) determination

The peroxide value (POV) is primarily used to evaluate the extent of lipid oxidation by measuring the formation of the primary oxidation product, hydroperoxide (-HPOD). A 2.0 g sample was accurately weighed and thoroughly mixed with 30 mL of chloroform-methanol solution. Then, 1 mL of saturated potassium iodide solution was added to the mixture and allowed to stand in the dark for 3 min. After adding 100 mL of deionized water and mixing well, the solution was titrated with sodium thiosulfate.

Calculated according to equation (1):

$$\mathrm{POV(g/100g)} = \frac{(v - v_0) \times c \times 0.1269}{m} \times 100 \quad (1)$$

V and V_0 are the consumed volumes of sodium thiosulfate standard solution in the specimen test and the blank test respectively, with the unit of mL; m is the mass of the sample with the unit of g. c is the concentration of sodium thiosulfate solution with the unit of mol/L. 0.1269 is the mass of iodine equivalent to 1 mL of standard titration solution of sodium thiosulfate with the unit of g.

2.6. Acid value (AV) determination

The acid value measures the quantity of free carboxylic acid groups present in a compound or mixture. It specifically refers to the number of milligrams of standard potassium hydroxide or sodium hydroxide required to neutralize 1 g of free fatty acids in fat. This value indicates the level of free fatty acids in the fat and the extent of rancidity in the food. Briefly, 5.0 g of the sample were weighed and added to 100 mL of an ethyl ether-isopropanol mixture, along with three drops of phenolphthalein indicator solution. The mixture was then titrated with a standard NaOH solution.

Calculated according to equation (2):

$$\mathrm{AV(mg/g)} = \frac{(v - v_0) \times c \times 56.1}{m} \quad (2)$$

V and V_0 are the consumed volumes of standard titration solution in the specimen test and the blank test respectively, with the unit of mL; c is the molar concentration of the standard titration solution with the unit of mol/L; m is the mass of the sample with the unit of g. 56.1 is the molar mass of potassium hydroxide with the unit of g/mol.

2.7. Thiobarbituric acid value (TBARS) determination

TBARS values are used to evaluate the extent of secondary lipid oxidation, indicating the presence of malondialdehyde (MDA). A 10 g sample of grated duck meat was weighed into a conical flask, and 50 mL of 7.5% trichloroacetic acid (containing 0.1% EDTA) was added, followed by shaking for 30 min to ensure dissolution. After filtering the mixture twice using double filter paper, 5 mL of the supernatant was transferred to a test tube. Then, 5 mL of 2-thiobarbituric acid (0.02 mol/L) solution was added to the test tube and heated in a water bath at 90 °C for 40 min. After cooling, the mixture was centrifuged at 1600 rpm for 5 min. After stratification and standing, an additional 5 mL of trichloromethane was added to the supernatant. The mixture was thoroughly shaken and left to stratify again before measuring its absorbance at 532 nm and 600 nm.

Calculated according to equation (3):

$$\mathrm{TBARS}\left(10^{-2}\mathrm{mg}/\mathrm{g}\right) = \frac{(A_{532} - A_{600})}{155} \times 72.06 \times 10 \quad (3)$$

A_{532} and A_{600} are the absorbance values measured at wavelengths of 532 nm and 600 nm respectively. 155 is the absorbance coefficient. 72.06 is the molecular weight of malondialdehyde in grams per mole (g/mol).

2.8. Conjugated diene (CD) determination

The determination of Conjugated Dienes (CD) was carried out with minor modifications to the method described by Koh and Surh (2015). Briefly, a 0.25 g sample was weighed into a 25 mL volumetric flask, diluted with isooctane, and brought to a constant volume. The absorbance (A) was measured at 234 nm, with isooctane serving as the blank control.

Calculation according to equation (4):

$$\mathrm{CD} = \frac{A}{4 \times m \times 1} \quad (4)$$

A is the absorbance of the sample at 234 nm; m is the sample mass/g; 1 is the optical diameter of the cuvette/cm.

2.9. Extraction of lipid metabolites

The extraction of lipid metabolites was performed using the method described by Narvaez-Rivas and Zhang (2016), with minor modifications. A 25 mg sample of duck meat was weighed into a 2 mL centrifuge tube, and 800 μL of pre-cooled dichloromethane/methanol (3:1, v/v) was added. The mixture was then homogenized using a TissueLyser grinder (JXFSTPRP, Shanghai Jingxin Industrial Development Co., Ltd., Shanghai, China) for 5 min. Subsequently, it was sonicated (KQ2200, Kunshan Shumei Ultrasonic Instrument Co., Ltd., Kunshan, China) in an ice bath for 10 min and then left overnight at −20 °C. After removal of proteins and other debris, the samples were centrifuged at 4 °C for 15 min at 25,000 rpm. Subsequently, 600 μL of the supernatant was extracted and concentrated using a Freezing Vacuum Concentrator (Maxi Vabeta, GENE Co., Ltd., Copenhagen, Denmark). The resulting residue was dissolved in a mixture of isopropanol/acetonitrile/ultrapure water (2:1:1, v/v/v), vortexed for 10 min (QL-901, Limber Instrument Manufacturing Co., Ltd., Jiangsu, China), and then sonicated in an ice bath for an additional 10 min. Finally, the sample for analysis was obtained by recentrifugation under the same conditions.

2.10. Chromatographic conditions

The analysis was performed using a CSH C18 column (1.7 μm, 2.1 × 100 mm, Waters, Milford, Massachusetts, USA). The column temperature was maintained at 55 °C with a flow rate of 0.4 mL/min and an injection volume of 5 μL. The gradient elution procedure was as follows: from 0 to 2 min, the composition of liquid B increased from 40% to 43%; from 2 to 2.1 min, it rose from 43% to 50%; from 2.1 to 7 min, it further increased from 50% to 54%; then, it sharply rose from 54% to 70% between 7 and 7.1 min; from 7.1 to 13 min, it gradually increased from 70% to 99%, and then rapidly decreased to 40% over the final 0.9 min (13–13.9 min). Finally, the composition of liquid B was maintained at 40% for the remaining time until 15 min. The mobile phases were: in positive ion mode, 60% acetonitrile-water with 10 mM ammonium formate and 0.1% formic acid (liquid A), and 90% isopropanol with 10% acetonitrile and 10 mM ammonium formate (liquid B); in negative ion mode, 60% acetonitrile-water with 10 mM ammonium formate (liquid A), and 90% isopropanol with 10% acetonitrile and 10 mM ammonium formate (liquid B).

2.11. Mass spectrometry conditions

A Q Exactive HF mass spectrometer (Thermo Fisher Scientific Co., Waltham, Massachusetts, USA) was employed for both primary and secondary mass spectrometry data acquisition. The mass spectrometry scan covered a mass-to-charge ratio range of 200–2000, with a primary resolution of 120,000, an AGC target of 3e6, and a maximum injection time (IT) of 100 ms. The top three ions were selected for fragmentation based on parent ion intensity, and secondary data was acquired with a secondary resolution of 30,000, an AGC target of 1e5, and an IT of 50 ms. Fragmentation energy levels were set at 15 eV, 30 eV, and 45 eV. The Electrospray Ion Source (ESI) parameters were configured as follows: sheath gas flow rate of 40 psi, auxiliary gas flow rate of 10 psi, spray voltage of 3.80 kV in positive ion mode and 3.20 kV in negative ion mode, ion transport tube temperature maintained at 320 °C, and auxiliary gas heater temperature set to 350 °C.

2.12. Data processing and statistical analysis

One-way analysis of variance (ANOVA) was performed to assess the significance ($p < 0.05$) of the data for relevant physical and chemical indicators of the samples using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). Data were visualized using Origin 2023 software (OriginLab Corp., Northampton, MA, USA). Lipidomics data were processed and analyzed with LipidSearch v4.1 software (Thermo Fisher Scientific, Wilmington, MA, USA) for pre-processing and peak extraction. Subsequently, the pre-processed data were subjected to multivariate statistical analysis and plotting using SIMCA 14.1 software (Umetrics, Malmö, Scania, Sweden).

3. Results and discussion

3.1. Sensory score for odorous smell of duck meat

Fig. 1 showed the changes in the odor of duck meat following heat treatment and reheating during storage, as determined by sensory evaluation. Compared to untreated duck meat, the sensory score for odor significantly increased ($p < 0.05$) in duck meat treated at 90 °C. The sensory score for the odor of duck meat reheated after 3 days of storage did not show a significant difference from that on day 0, but it was notably higher than that of untreated duck meat. Furthermore, the highest sensory score for odor was observed in duck meat reheated after 7 days of storage. These results indicated an increase in the sensory perception of odor in duck meat following heat treatment and subsequent reheating after storage.

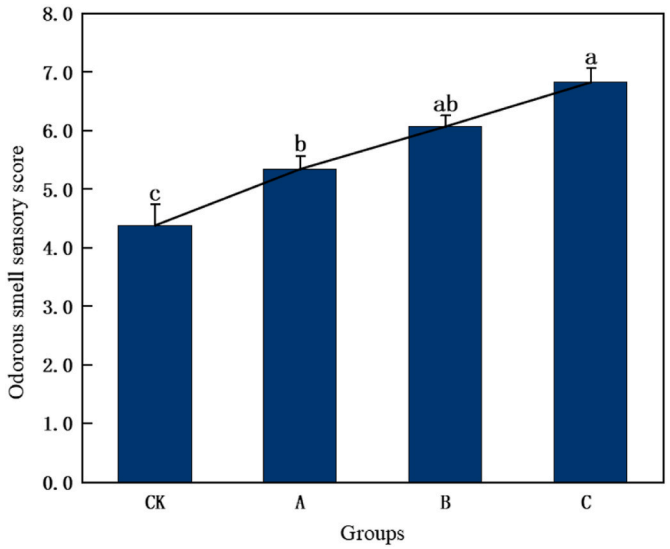

Fig. 1. Sensory score of the duck odorous smell after heat treatment and reheating after storage.
Notes: CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

3.2. Analysis of indicators related to lipid oxidation

Fig. 2-A illustrated the trend in peroxide value (POV) during heat treatment and reheating after storage of duck meat. The POV of duck meat samples showed a significant increase ($p < 0.05$) after heat treatment, as well as during the 3-day and 7-day reheating stages following storage. Lipids continued to produce primary oxidation products during this period, leading to further oxidation. The increase in POV observed in this study may be attributed to shorter storage periods and higher levels of lipid oxidation substrates. As lipid oxidation is still in its primary stage, the rate of hydroperoxide formation exceeds the decomposition rate, resulting in an upward trend in POV values.

The raw duck meat initially had a low TBARS value of 0.37 mg/kg, which increased significantly after heat treatment ($p < 0.05$) (Fig. 2-B). The metabolites generated during the early stages of lipid oxidation are highly unstable and prone to further oxidation. This leads to the formation of malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) at higher temperatures, resulting in the production of a pink substance. There are two reasons for this phenomenon: first, as the meat sample is heated, it loses water, leading to an increased proportion of fat in the sample and causing a notable rise in the TBARS value; second, MDA forms a compound when it reacts with amino acids, proteins, glycogen, and other substances. However, heating causes this compound to break down and release MDA, thereby contributing to an increase in TBARS value. Furthermore, the TBARS values of the duck meat samples continued to increase after reheating following 3 days of storage due to ongoing lipid oxidation and the reaction of more oxidation intermediates to produce MDA. Even after 7 days of storage, lipid oxidation persisted in reheated duck meat samples but remained at a similar level to those reheated after 3 days of storage.

The acid value (AV) serves as a crucial indicator of the free fatty acid content in a sample's lipids. The levels of free fatty acids are subject to dynamic changes, with phospholipids, triglycerides, and other substances undergoing hydrolysis to generate free fatty acids, thereby increasing their content. Conversely, free fatty acids undergo oxidation to form smaller molecules such as aldehydes, ketones, and acids, leading to a decrease in their content. As depicted in Fig. 2-C, the AV of heat-treated duck meat samples exhibited a significant increase ($p < 0.05$) compared to the unheated group. This may be attributed to the diminished stability of lipids under heat treatment and the rapid rate of lipid pyrolysis, which facilitates an increase in the production of free fatty acids. Furthermore, the AV of reheated samples displayed a notable increase ($p < 0.05$) after 7 days of storage.

The conjugated diene (CD) value reflects the number of conjugated double bond structures that are formed within the unsaturated fatty acid molecule during lipid oxidation. As shown in Fig. 2-D, the CD values of heat-treated and storage-reheated duck meat were significantly higher than those of the untreated group ($p < 0.05$). However, the CD values of duck meat samples reheated after 3 days of storage were not

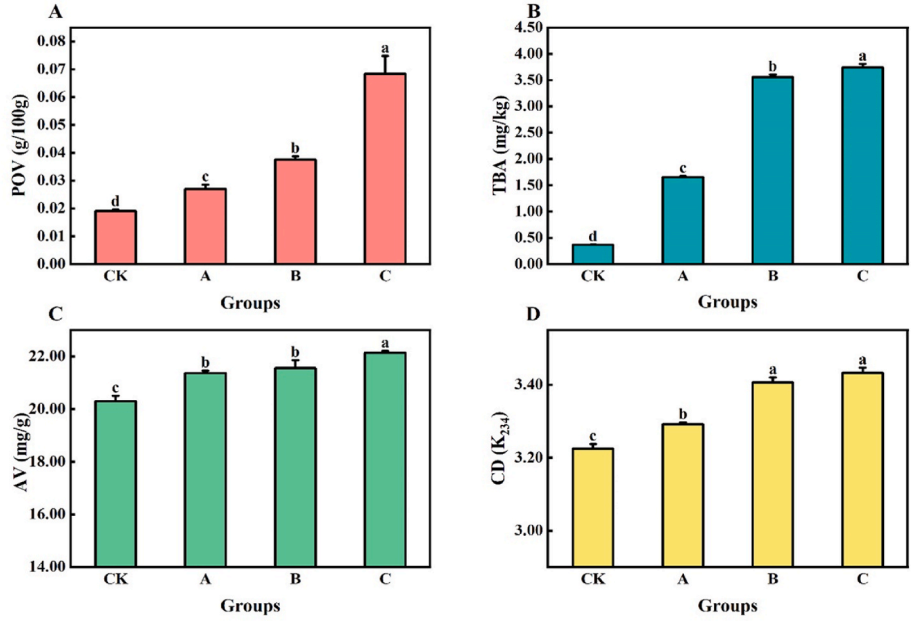

Fig. 2. Analysis chart of lipid oxidation in duck meat after heat treatment and reheat storage. The relevant indicators are (A) POV; (B) TBA; (C) AV; (D) CD.
Notes: The groups CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

significantly different from those of the samples reheated after 7 days of storage ($p > 0.05$). During the early stages of oxidation, unsaturated fatty acids undergo conjugation, and peroxide formation is the dominant reaction. In the middle and late stages, peroxide decomposition predominates, leading to a slowdown or decrease in CD values.

The aroma of duck meat is more pronounced after heat treatment and reheating, as lipid oxidation increases during these processes. This suggests a close relationship between lipid oxidation in duck meat and the production of its characteristic odor. Combined with previous sensory evaluation, it was found that the aroma of duck meat was more obvious after heat treatment and reheating, and the lipid oxidation degree in duck meat was also deepened. This further indicates that lipid oxidation in duck meat is closely related to the production of duck odor smell.

3.3. Lipidomic analysis

3.3.1. Lipid composition of duck meat and Hierarchical clustering analysis

According to the current classification, lipids are categorized as fatty acyls (FA), glycerol esters (GL), glycerophospholipids (GP), sphingolipids (SP), sterolipids (ST), glycolipids (SL), pregnenolone lipids (PR), and polyethylene (PK). Each type is further divided into different subclasses based on variations in polarity head (Fahy et al., 2009). Lipidomic analysis was performed on duck meat samples undergoing various treatments. Fig. 3(a) showed that a total of 519 lipid molecules and 26 lipid subclasses were identified within the duck meat. Among the phospholipids, PC species constituted 30.06% of the total lipid molecules, PE for 20.23%, LPC for 6.74%, PI for 4.82%, PS for 4.62%, and PG for 2.31%. Among the glycerolipids, TG species constituted 8.86% of the total lipid molecules, while DG for 2.31%. For sphingolipids, SM species constituted 7.9% of the total, while Cer for 2.12%. The sum of PC and PE species constituted 50.29% of the total lipid molecules. The complexity and diversity of lipid species and structures endow lipid molecules with a variety of physiological functions. For example, SM and its metabolites constitute a class of biologically important signaling molecules that regulate cell growth, differentiation, and many other critical signaling processes; Cer acts not only as a precursor to SM synthesis but also as a catabolic product of SM (Bienias et al., 2016); TG serves as a major energy storage compound within cells, while LPC and PI act as signaling messengers in cellular biosynthetic pathways (Gross and Han, 2011). Additionally, lipid composition is closely linked with the physicochemical properties of cell membranes, and certain highly biologically active lipids are susceptible to oxidation caused by free radical attack (Li et al., 2019).

As shown in Fig. 3(b), by tracking the changes in 26 lipid subclasses, it was observed that some of the lipids in the duck meat samples degraded after heating and reheating treatment. The increase in odor of the duck meat samples after heat treatment and subsequent storage is likely due to the degradation of these lipid subclasses.

3.3.2. Partial least squares-discriminant analysis of lipid subclasses

To further investigate and extract key information, a partial least squares discrimination analysis (PLS-DA) was conducted. The results were displayed in Fig. 4-A. The model demonstrated a total explained variance R^2X of 0.823, R^2Y of 0.936, and a predictive power Q^2 of 0.644, enabling better differentiation between the various treatment groups of duck meat samples. Additionally, to avoid over-fitting of the PLS-DA model, validation through 200 permutations was conducted. As depicted in Fig. 4-B, the rightmost R^2 and Q^2 values are higher than those on the left, and the regression line for Q intersects with a negative intercept on the horizontal axis, indicating the robustness of the model. The results indicated that the current PLS-DA model effectively explains and predicts differences between lipid subclasses in duck meat after heating and reheating. Consequently, further exploration into these distinct lipid subclasses can be pursued.

The variable importance projection (VIP) value in the PLS-DA model is used to assess the degree of contribution of significantly different lipid subclasses. A VIP value greater than 1 for a lipid subclass indicates that it significantly influences the model. The significantly different lipid subclasses (VIP >1) were DG, Cholesteryl Ester (ChE), Monoglyceride (MG), Lyso-phosphatidylethanolamine (LPE), SM, PC, LPC, Dimethylphosphatidylethanolamine (dMEPE), Lyso-phosphatidylglycerol (LPG), PI, Phosphatidylinositol (PIP), Lyso-phosphatidylinositol (LPI), Cer, and Lyso-phosphatidylserine (LPS).

3.3.3. Partial least squares-discriminant analysis and screening of differential lipid molecules

After analyzing the lipid subclasses of duck meat after heat treatment and reheating after storage, it is also necessary to analyze the lipid molecules. As shown in Fig. 4-C, the total explained variance of the lipid molecule model is that R^2X was 0.848, R^2Y was 0.987 and the predictive power Q^2 was 0.761. The regression line of Q in the permutation test

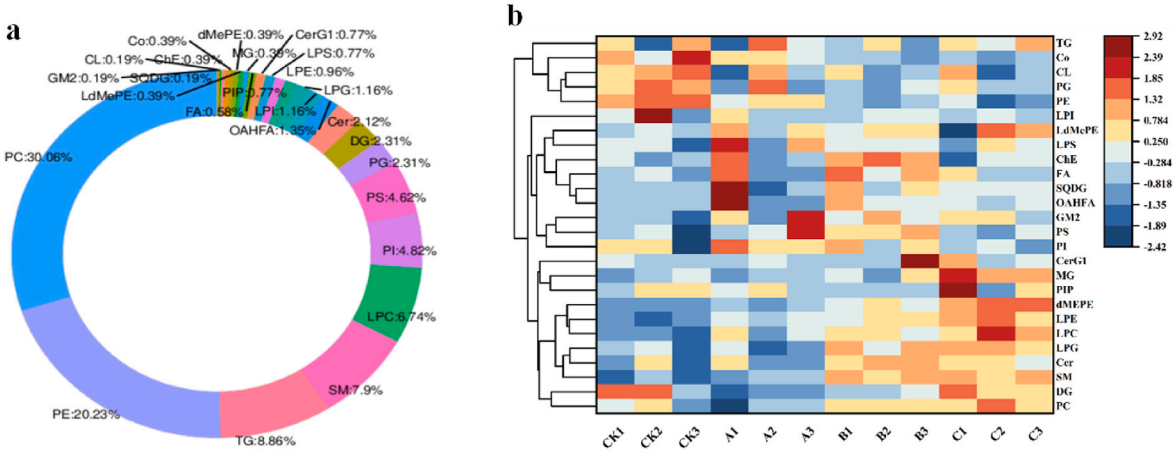

Fig. 3. (a) Fat composition of duck breast meat. **(b)** Heat map of lipid subclasses in duck meat treated by heating and reheating during storage.
Notes: The groups CK1, CK2, and CK3 represent three sets of parallel samples without heat treatment. A1, A2, and A3 represent three sets of parallel samples treated with 90 °C heating. B1, B2, and B3 represent three parallel sets of samples stored for 3 d after heat treatment. C1, C2, and C3 represent three parallel sets of samples stored for 7 d after heat treatment. Differences in the relative content of lipid subclasses in duck meat are indicated by different colors, where dark red indicates a high relative content of that component and dark blue the opposite. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

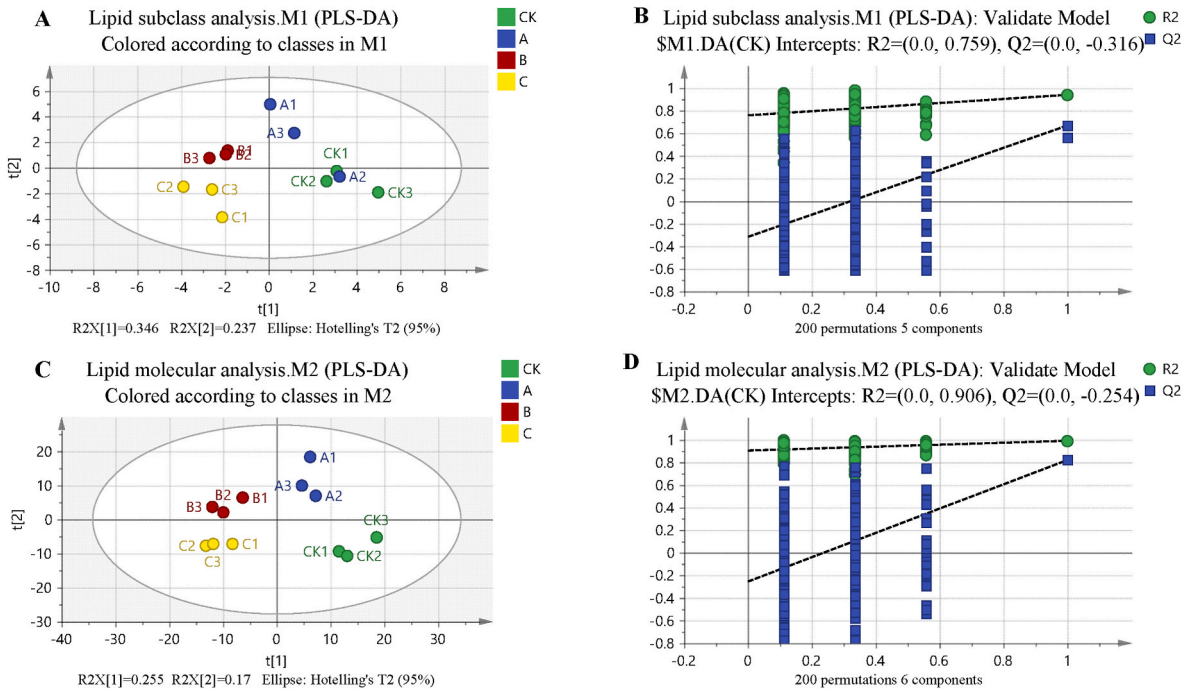

Fig. 4. PLS-DA (A) and Permutation Test (B) of lipid subclasses and PLS-DA (C) and Permutation Test (D) of lipid molecules in duck meat treated by heating and reheating during storage.
Notes: CK1, CK2, and CK3 represent three sets of parallel samples without heat treatment. A1, A2, and A3 represent three sets of parallel samples treated with 90 °C heating. B1, B2, and B3 represent three parallel sets of samples stored for 3 d after heat treatment. C1, C2, and C3 represent three parallel sets of samples stored for 7 d after heat treatment. R2 and Q2 denote the explanatory and predictive power of the model. The closer R2 and Q2 are to each other, the better the explanatory and predictive power of the model is.

model crossed the horizontal axis with a negative intercept, effectively distinguishing samples from different treatment groups. Additionally, the model did not exhibit overfitting (Fig. 4-D). PLS-DA (VIP >1) and ANOVA ($p < 0.05$) serve as criteria for identifying significantly different lipid molecules in different treatment groups of duck meat. Lipids with significant differences following heat treatment and reheating after storage were then identified and categorized. As shown in Supplement Table 1 showed that a total of 161 differential lipids were screened, including 47 PC, 34 PE, 6 PI, 14 PS, 12 LPC, 4 TG, 7 DG, and others.

3.3.4. *Molecular correlation analysis of differential lipids*

To further investigate the potential association between lipid species and to analyze variables with significant differences, 49 differential lipid molecules were selected using PLS-DA (VIP >1.2) and ANOVA ($p < 0.05$). The data were analyzed using the Pearson correlation coefficient (Zhang et al., 2023), and the results are presented in Fig. 5-A.

3.3.5. *Molecular chord diagram analysis of differential lipids*

Chord diagrams are effective tools for illustrating the proportions of lipid molecules and the potential associations within a sample. In this study, 49 differential lipids were analyzed, and the results were shown in Fig. 5-B. The graph showed that PC, LPC and TG levels were higher among the lipid species compared to SM, DG, LPE and PE. This suggested that phospholipids and triglycerides may play an important role in the

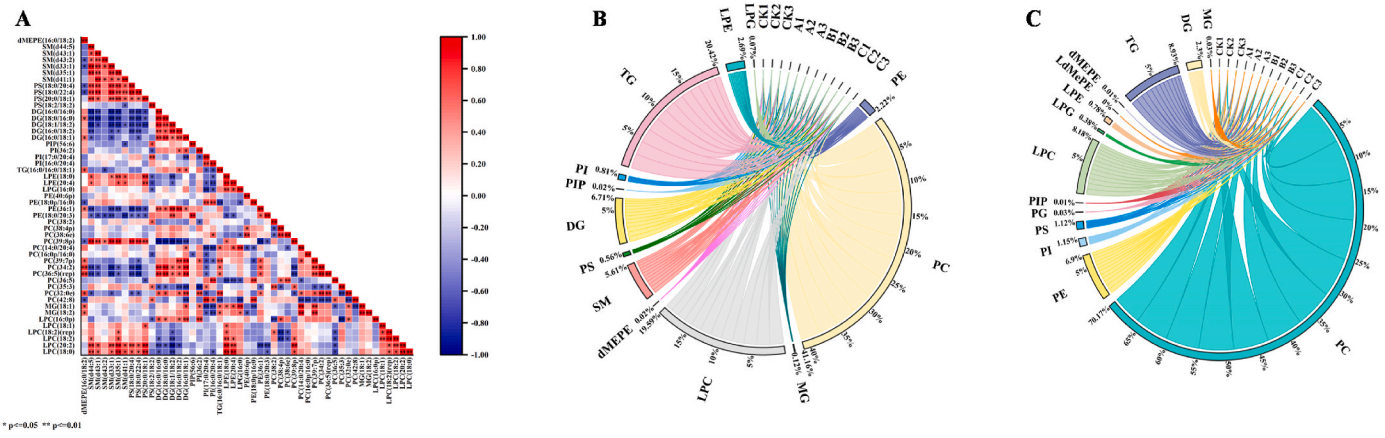

Fig. 5. A. Correlation analysis of differential lipid molecules in heated and reheated duck meat. **B.** Chord analysis of differential lipid molecules in heated and reheated duck meat. **C.** Chord analysis of phospholipids and glycerides in heated and reheated duck meat.
Notes: Rep refers to the repeated occurrence of the lipid in the identification results, which is due to the undetermined fatty acid chain, the uncertainty of the fatty acid chain and double bond positions, or being repeatedly detected in different ion modes.

heat treatment and reheating processes after storage of duck meat. Fig. 5-C further illustrated that PC, PE, LPC, and TG collectively constitute a high proportion of each lipid. Notably, PC, PE, and LPC were all classified as phospholipids. This finding reinforced the notion that phospholipids played a major role in the heat treatment and reheating process following storage.

3.3.6. *Molecular analysis of differential lipids*

Phospholipids and triglycerides are the predominant components of intramuscular and subcutaneous lipids, which influence meat flavor. In contrast, free fatty acids, cholesterol, and other lipids have lesser impact (Dominguez et al., 2019). This is primarily due to the high content of unsaturated fatty acids in phospholipids and triglycerides, which makes them susceptible to oxidation during heat treatment and reheating after storage (Li et al., 2021). Fig. 6 clearly demonstrated that molecules such as PC (36:4) and PC (34:2), which contain unsaturated fatty acid chains, experienced a notable decrease after heat treatment. Although the content of PC (38:4), PC (36; 1) also decreased post-heating, their levels increased after 3 days of storage reheating compared to day 0. Additionally, their content increased significantly after reheating for 7 days. In contrast, the contents of molecules such as PC (42:8), PC (40:6), PC (32:2), PC (34:4), PC (18:2/18:2) and PC (38:6) increased after heat treatment but decreased after 3 and 7 days of reheating. Interestingly, molecules like PC (18:2/20:4) and PC (20:4/20:4) showed a decrease during heating and reheating after storage. However, the contents of molecules including PC (30:0), PC (18:0/13:0) and PC (33:0), which contain saturated fatty acid chains, increased significantly after heat treatment and then decreased after 3 and 7 days of reheating. The key factors in fat oxidation are the fatty acid chains, especially the phospholipid chains, therefore phospholipids are highly susceptible to oxidation and oxidize at a rapid rate, resulting in fluctuating relative contents of different phosphatidylcholine molecules.

Fig. 7 showed that phosphatidylethanolamine (PE) exhibited a decreasing trend after heat treatment and reheating following 3 and 7 days of storage. However, the levels of PE (36:1), PE (18:0/18:1), PE (20:0/18:2), PE (38:2), PE (18:1/22:0), and PE (18:0/22:6) increased after 7 days of reheating following storage. The decrease in the content of these phospholipid molecules is primarily attributed to oxidation during heat treatment and reheating following storage. This is due to the destruction of cell structures, the inactivation of antioxidant enzymes, and the release of oxygen and iron ions by ferritin, which promote lipid oxidation (Xiong et al., 2020). Additionally, meat phospholipids contain more unsaturated fatty acids than triglycerides, making them more susceptible to oxidation, which in turn affects the meat's flavor quality (Li et al., 2021). It has been noted that oxidation of essential phospholipids disrupts cell membranes, leading to internal component oxidation and thereby accelerating the overall oxidation process within cell membranes (Jia et al., 2021).

Fig. 8 showed that LPC molecules containing both unsaturated and saturated fatty acid chains exhibited an increasing trend after heat treatment and reheating following 3 days of storage, but a decreasing trend after reheating following 7 days of storage. Previous studies have demonstrated that heat treatment can increase the levels of lysophosphatidylcholine (Shi et al., 2019). Phospholipids may degrade into other lipids or substances through enzymatic or non-enzymatic reactions during heat treatment and reheating following storage. Changes in phospholipid and haemolytic phospholipid content during this process may be linked to the degradation of phospholipids (Jia et al., 2021; Kurtovic et al., 2009). Phospholipases play a crucial role in the transportation, digestion, and processing of lipids in living organisms and possess a certain degree of heat tolerance (Liu et al., 2018; Verlotta and Trono, 2014). Currently, phospholipase A (PLA1 and PLA2),

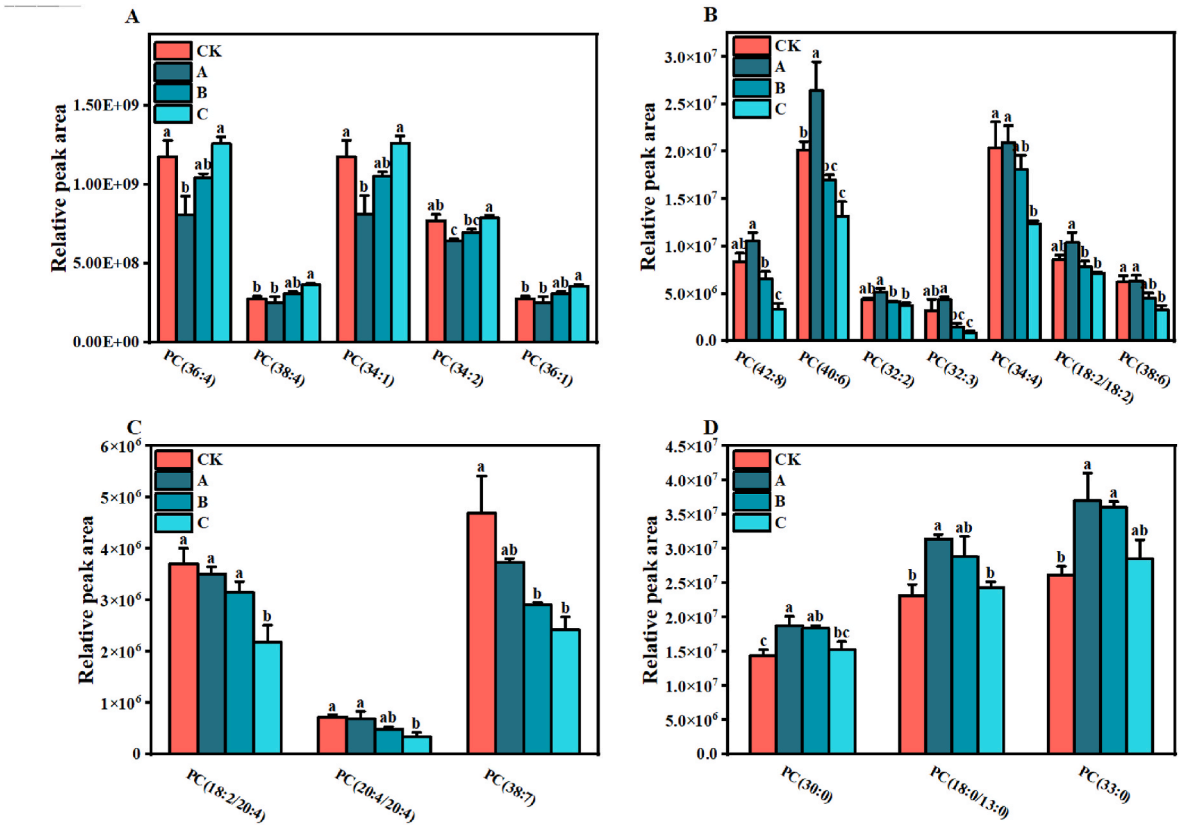

Fig. 6. Analysis of phosphatidylcholine (PC) in heated and reheated duck meat. Notes: CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

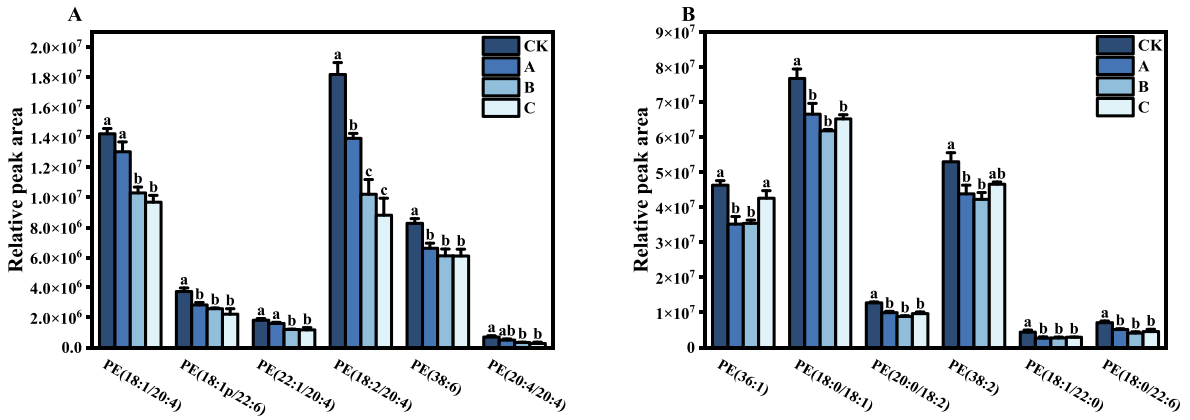

Fig. 7. Analysis diagram of phosphatidylethanolamine (PE) in heated and reheated duck meat.
Notes: CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

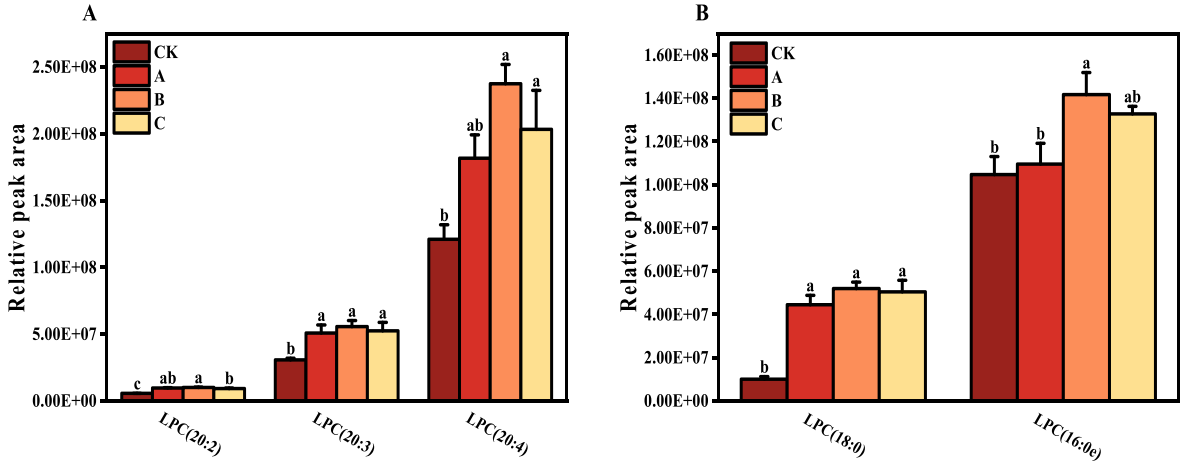

Fig. 8. Analysis of lysophosphatidylcholine (LPC) in heated and reheated duck meat.
Notes: CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

phospholipase C (PLC), and phospholipase D (PLD) are the primary enzymes responsible for hydrolyzing phospholipids. For instance, PLA hydrolyzed PC (36:4) and PC (16:0/20:4) to produce FA (16:0) with LPC (20:4) or FA (20:4) with LPC (20:4), indicating that the increase in lysophosphatidylcholine and the decrease in phospholipids during heat treatment and storage reheating may be related to enzymatic digestion

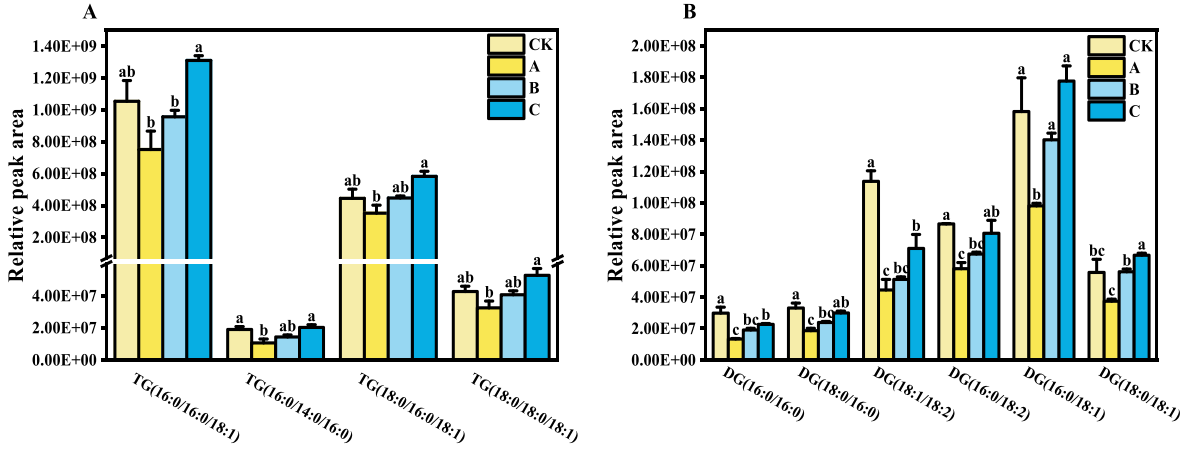

Fig. 9. Analysis of triglycerides (A) and Diglyceride (B) in duck meat by heating and storage reheat treatment.
Notes: CK, A, B and C represent untreated duck meat, duck meat treated at 90 °C, duck meat stored for 3 and 7 days after treatment, respectively. The lowercase letters a, b, and c represent the significant differences among the four groups of samples under different treatments (Duncan's test, $p < 0.05$).

of phospholipids.

As can be seen from Fig. 9, both triglycerides (TG) and diglycerides (DG) molecules showed a downward trend after heat treatment, followed by an upward trend after reheating at 3 and 7 days of storage. Structurally, members of the glycerol ester (TG, DG) and phospholipid (PC, PE) families share a common glycerol backbone; however, they possess distinct chemical properties—neutral and polar. Polyunsaturated fatty acids in both triglycerides and phospholipids tend to be located at the Sn-2 position. Triglycerides are typically stored in lipid droplets within fat cells, where they are better protected from oxidation due to being surrounded by the cell membrane. As a result, they exhibit a lower rate of oxidation compared to phospholipids (Parchem et al., 2019). DG is predominantly found in cell membranes, where it functions as an intracellular messenger (Hannun and Obeid, 2008). The decrease in TG and DG after heat treatment may result from temperature influence or oxidation induced by other lipids. During storage reheating, other lipids react, while the saturated fatty acid chains in TG and DG remain stable and less susceptible to oxidation, leading to an increase in their content.

4. Conclusions

The odorous smell of duck meat intensified following heat treatment and reheating after storage, which was accompanied by an increase in lipid oxidation. A total of 26 lipid subclasses and 519 lipid molecules were identified in the duck meat samples. Among these, phosphatidylcholine (PC), phosphatidylethanolamine (PE), triglycerides (TG), sphingomyelin (SM), and lysophosphatidylcholine (LPC) were the predominant lipid subclasses, accounting for 156, 105, 46, 41 and 35 types respectively. These figures represent approximately 30.06%, 20.23%, 8.86%, 7.9%, and 6.74% of the total lipid molecules detected in the samples.

Of the 49 selected lipid molecules, PC (32:0e), PC (36:5) (rep), LPC (18:0), LPC (20:2), DG (16:0/18:1), MG (18:1), SM (d44:5), and SM (d35:1) were found to have more associations with other lipid-like species. Phospholipids in duck meat play a major role in heat treatment and reheating after storage, with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) being the most significant. In practical production processes, targeted prevention and control measures can be implemented to address these odor precursor components for deodorization purposes.

CRediT authorship contribution statement

Gaiming Zhao: Conceptualization, Investigation, Methodology, Supervision, Formal analysis. **Ying Zhang:** Writing – review & editing, Software, Data curation, Writing – original draft. **Jiali Zhang:** Software, Supervision. **Sen Wang:** Software, Data curation, Supervision. **Ke Wang:** Investigation, Supervision. **Long Xu:** Investigation, Supervision. **Qiuhui Zhang:** Investigation, Supervision. **Chaozhi Zhu:** Investigation, Methodology, Writing – review & editing, Funding acquisition.

Written informed consent for sensory evaluation

I declare that I have voluntarily participated in the sensory evaluation study of the effect of heating and reheating after storage on the odorous smell of duck meat. I understand that I can choose to withdraw from the study at any time without the need to provide a reason, and I will not face any negative consequences.

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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Ethics Declaration

All experiments involving human sensory perception were conducted according to the protocol approved by the Research Ethics Committee of Henan Agricultural University (authorization No. IACUC-henau-20210830). Before conducting the sensory evaluation, written informed consent was obtained from all participants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100884.

Data availability

Data will be made available on request.

References

Bienias, K., Fiedorowicz, A., Sadowska, A., Prokopiuk, S., Car, H., 2016. Regulation of sphingomyelin metabolism. Pharmacol. Rep. 68 (3), 570–581. https://doi.org/10.1016/j.pharep.2015.12.008.

Bourlieu, C., Cheillan, D., Blot, M., Daira, P., Trauchessec, M., Ruet, S., Gassi, J.Y., Beaucher, E., Robert, B., Leconte, N., Bouhallab, S., Gaucheron, F., Gésan-Guiziou, G., Michalski, M.C., 2018. Polar lipid composition of bioactive dairy co-products buttermilk and butterserum: emphasis on sphingolipid and ceramide isoforms. Food Chem. 240 (3), 67–74. https://doi.org/10.1016/j.foodchem.2017.07.091.

Dominguez, R., Pateiro, M., Gagaoua, M., Barba, F.J., Zhang, W., Lorenzo, J.M., 2019. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants 8 (10), 429–460. https://doi.org/10.3390/antiox8100429.

Fahy, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R., Shimizu, T., Spener, F., Van, M.G., Wakelam, M.J., Dennis, E.A., 2009. Update of the LIPID MAPS comprehensive classification system for lipids. JLR (J. Lipid Res.) 50 (Suppl. l), S9–S14. https://doi.org/10.1194/jlr.R800095-JLR200.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226 (1), 497–509. https://doi.org/10.1016/s0021-9258(18)64849-5.

Garcia-Segovia, P., Andres-Bello, A., Martinez-Monzo, J., 2007. Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). J. Food Eng. 80 (3), 813–821. https://doi.org/10.1016/j.jfoodeng.2006.07.010.

Gross, R.W., Han, X., 2011. Lipidomics at the interface of structure and function in systems biology. Chemistry Biology 18 (3), 284–291. https://doi.org/10.1016/j.chembiol.2011.01.014.

Hannun, Y.A., Obeid, L.M., 2008. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat. Rev. Mol. Cell Biol. 9 (2), 139–150. https://doi.org/10.1038/nrm2329.

Hoac, T., Daun, C., Trafikowska, U., Zackrisson, J., Akesson, B., 2006. Influence of heat treatment on lipid oxidation and glutathione peroxidase activity in chicken and duck meat. Innovat. Food Sci. Emerg. Technol. 7 (1–2), 88–93. https://doi.org/10.1016/j.ifset.2005.10.001.

Igene, J.O., Pearson, A.M., 2006. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. J. Food Sci. 44 (5), 1285–1290. https://doi.org/10.1111/j.1365-2621.1979.tb06420.x.

Jia, W., Li, R., Wu, X., Liu, S., Shi, L., 2021. UHPLC-Q-Orbitrap HRMS-based quantitative lipidomics reveals the chemical changes of phospholipids during thermal processing methods of Tan sheep meat. Food Chem. 360 (27), 130153. https://doi.org/10.1016/j.foodchem.2021.130153.

Koh, E., Surh, J., 2015. Food types and frying frequency affect the lipid oxidation of deep frying oil for the preparation of school meals in Korea. Food Chem. 174 (9), 467–472. https://doi.org/10.1016/j.foodchem.2014.11.087.

Kurtovic, I., Marshall, S.N., Zhao, X., Simpson, B.K., 2009. Lipases from mammals and fishes. Rev. Fish. Sci. 17 (1), 18–40. https://doi.org/10.1080/10641260802031322.

Li, C., Al-Dalali, S., Zhou, H., Wang, Z., Xu, B., 2021. Influence of mixture of spices on phospholipid molecules during water-boiled salted duck processing based on shotgun lipidomics. Food Res. Int. 149 (11), 110651. https://doi.org/10.1016/j.foodres.2021.110651.

Li, C., Li, X., Huang, Q., Zhuo, Y., Xu, B., Wang, Z., 2020a. Changes in the phospholipid molecular species in water-boiled salted duck during processing based on shotgun lipidomics. Food Res. Int. 132 (6), 109064. https://doi.org/10.1016/j.foodres.2020.109064.

Li, J., Tang, C., Zhao, Q., Yang, Y., Li, F., Qin, Y., Liu, X., Yue, X., Zhang, J., 2020b. Integrated lipidomics and targeted metabolomics analyses reveal changes in flavor precursors in psoas major muscle of castrated lambs. Food Chem. 333 (32), 127451. https://doi.org/10.1016/j.foodchem.2020.127451.

Li, L., Zhong, S., Shen, X., Li, Q., Xu, W., Tao, Y., Yin, H., 2019. Recent development on liquid chromatography-mass spectrometry analysis of oxidized lipids. Free Radical Biology And Medicine 144 (15), 16–34. https://doi.org/10.1016/j.freeradbiomed.2019.06.006.

Liu, Y., Tian, Y., Cong, P., Chen, Q., Li, H., Fan, Y., Xu, J., Wang, J., Wang, Y., Xue, C., 2018. Lipid degradation during salt-fermented antarctic krill paste processing and their relationship with lipase and phospholipase activities. Eur. J. Lipid Sci. Technol. 120 (4), 443–451. https://doi.org/10.1002/ejlt.201700443.

Lung, C.T., Chang, C.K., Cheng, F.C., Hou, C.Y., Chen, M.H., Santoso, S.P., Yudhistira, B., Hsieh, C.W., 2022. Effects of pulsed electric field-assisted thawing on the characteristics and quality of Pekin duck meat. Food Chem. 390 (25), 133137. https://doi.org/10.1016/j.foodchem.2022.133137.

Mi, S., Shang, K., Li, X., Zhang, C.H., Liu, J.Q., Huang, D.Q., 2019. Characterization and discrimination of selected China's domestic pork using an LC-MS-based lipidomics approach. Food Control 100 (6), 305–314. https://doi.org/10.1016/j.foodcont.2019.02.001.

Narvaez-Rivas, M., Zhang, Q., 2016. Comprehensive untargeted lipidomic analysis using core-shell C30 particle column and high field orbitrap mass spectrometer. J. Chromatogr., A 1440 (14), 123–134. https://doi.org/10.1016/j.chroma.2016.02.054.

Nie, R., Zhang, C., Liu, H., Wei, X., Gao, R., Shi, H., Wang, Z., 2024. Characterization of key aroma compounds in roasted chicken using SPME, SAFE, GC-O, GC–MS, AEDA, OAV, recombination-omission tests, and sensory evaluation. Food Chem. X 21, 101167. https://doi.org/10.1016/j.fochx.2024.101167.

Parchem, K., Sasson, S., Ferreri, C., Bartoszek, A., 2019. Qualitative analysis of phospholipids and their oxidised derivatives – used techniques and examples of their applications related to lipidomic research and food analysis. Free Radic. Res. 53 (Suppl. 1), 1068–1100. https://doi.org/10.1080/10715762.2019.1657573.

Shi, C., Guo, H., Wu, T., Tao, N., Wang, X., Zhong, J., 2019. Effect of three types of thermal processing methods on the lipidomics profile of tilapia fillets by UPLC-Q-Extractive Orbitrap mass spectrometry. Food Chem. 298 (29), 125029. https://doi.org/10.1016/j.foodchem.2019.125029.

Shibata, A., Uemura, M., Hosokawa, M., Miyashita, K., 2015. Formation of acrolein in the autoxidation of triacylglycerols with different fatty acid compositions. J. Am. Oil Chem. Soc. 92 (11–12), 1661–1670. https://doi.org/10.1007/s11746-015-2732-2.

Sun, H.L., Zhao, Y.Q., Zhao, J., Sun, J.X., 2023. Ultrasound thawing for improving the eating quality and off flavor of frozen duck meat and its possible mechanisms. Food Sci. Technol. 187, 115314. https://doi.org/10.1016/j.lwt.2023.115314.

Sun, T., Wang, X., Cong, P., Xu, J., Xue, C., 2020. Mass spectrometry-based lipidomics in food science and nutritional health: a comprehensive review. Compr. Rev. Food Sci. Food Saf. 19 (5), 2530–2558. https://doi.org/10.1111/1541-4337.12603.

Verlotta, A., Trono, D., 2014. Expression, purification and refolding of active durum wheat (Triticum durum Desf.) secretory phospholipase A2 from inclusion bodies of Escherichia coli. Protein Expr. Purif. 101 (9), 28–36. https://doi.org/10.1016/j.pep.2014.05.009.

Xiong, Q., Zhang, M., Wang, T., Wang, D., Sun, C., Bian, H., Li, P., Zou, Y., Xu, W., 2020. Lipid oxidation induced by heating in chicken meat and the relationship with oxidants and antioxidant enzymes activities. Poultry Sci. 99 (3), 1761–1767. https://doi.org/10.1016/j.psj.2019.11.013.

Zhao, G.M., Zhang, J.L., Wang, S., Yu, X.L., Zhang, Q.H., Zhu, C.Z., 2024. Influence of heating temperatures and storage on the odor of duck meat. Food Chem. X 21 (1), 101242. https://doi.org/10.1016/j.fochx.2024.101242.

Zhang, M., Chen, M., Fang, F., Fu, C., Xing, S., Qian, C., Liu, J., Kan, J., Jin, C., 2022. Effect of sous vide cooking treatment on the quality, structural properties and flavor profile of duck meat. Int. J. Gastron. Food Sci. 29 (3), 100565. https://doi.org/10.1016/j.ijgfs.2022.100565.

Zhang, M., Xie, D., Wang, D., Xu, W., Zhang, C., Li, P., Sun, C., 2023. Lipidomic profile changes of yellow-feathered chicken meat during thermal processing based on UPLC-ESI-MS approach. Food Chem. 399 (2), 133977. https://doi.org/10.1016/j.foodchem.2022.133977.