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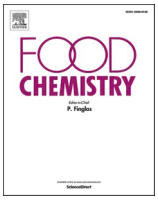

Qualitative and quantitative assessment of key aroma compounds, advanced glycation end products and heterocyclic amines in different varieties of commercially roasted meat products

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ABSTRACT

Studies on the interactions and links between aroma and hazardous compounds were inadequately investigated. A complete analysis was conducted on the key aroma compounds, typical hazardous compounds and their precursors in 25 samples of roasted meats. Forty-nine aroma compounds were identified as essential odorants with odor-activity values exceeds 1. N^{ε}-carboxymethyl lysine (CML, 11.78–49.32 μg/g) and N^{ε}-carboxyethyl lysine (CEL, 8.48–171.00 μg/g) were identified as representative advanced glycation end products (AGEs) of meats with high concentrations. Harman and Norharman were typical heterocyclic aromatic amines. Meanwhile, correlation analysis indicated that aldehyde and alcohols showed a negative correlation with AGEs ($p < 0.01$), while pyrazines might affect the formation of Harman and Norharman. The furaldehyde, 1-hexanol, 2, 4-Decadienal, AGEs, and creatine were regarded as potential biomarkers that distinguished different roasted meat products. Therefore, the study could provide new insights for synergistic regulation of aroma and hazardous compounds in roasted meat products.

1. Introduction

Roasted meat is widely favored by consumers for its attractive flavor, texture and colour (Nawaz et al., 2023). The thermal processing of meat unavoidably induces heat treatments that stimulate the occurrence of the Maillard reaction, protein denaturation, and lipid oxidation, and these chemical pathways are recognized as the primary mechanisms responsible for meat flavor formation (Liu et al., 2020). Nevertheless, the intricate composition of food matrices and chemical reactions involved in flavor development often give rise to the presence of several hazardous compounds that pose possible health risks, such as heterocyclic aromatic amines (HAAs), advanced glycation end products (AGEs) and acrylamide. The presence of these deleterious substances elevates the susceptibility to malignancies, cardiovascular ailments, and neurodegenerative conditions such as Alzheimer's disease (Chen & Smith, 2015; Gibis Monika, 2016; Li et al., 2021).

Numerous investigations have been conducted to examine the quantification of aroma compounds and hazardous compounds, as well as their formation pathways, in meat products subjected to various roasting conditions (Chen & Smith, 2015; Liu et al., 2019; Li et al., 2021; Nawaz et al., 2023). However, there is a paucity of research on the synergistic formation of these two compounds in meat products. Over one thousand aromatic compounds have been found in meat products that have undergone thermal processing. According to a recent review conducted by Sohail et al. (2022), it was found that (*E*)-2-octenal, (*E*,*Z*)-2,6-nonadienal, octanal, (*E*)-2-nonenal, and nonanal were identified as key odorants in grilled mutton shashlik (Sohail et al., 2022). The presence of nitrogen-containing compounds, such as 2-ethyl-3,5-dimethylpyrazine, is typically observed in meat that has been grilled or roasted (Liu et al., 2019).

AGEs are known to be formed in various organisms and foods (Poulsen et al., 2013). These AGEs have been specifically identified as N^{ε}-carboxymethyl lysine (CML), N^{ε}-carboxyethyl lysine (CEL), and methylglyoxal-derived hydroimidazolinone (MG-H1). HAAs are a class of chemical compounds that have been identified as both carcinogenic and mutagenic. These compounds are known to be created as a result of

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Table 1
Information on traditional roasted meat products.

Breeds	Name	Origin	Roasting method	Abbreviation	Breeds	Name	Origin	Roasting method	Abbreviation
Pork	Roasted pork 1	Chongqing City, China	Oven roasting	RP1	Lamb	Roasted lamb 7	Inner Mongolia Province, China	Charcoal roasting	RL7
Pork	Roasted pork 2	Chongqing City, China	Oven roasting	RP2	Chicken	Roasted chicken 1	Shandong Province, China	Oven roasting	RC1
Pork	Roasted pork 3	Guangdong Province, China	Charcoal roasting	RP3	Chicken	Roasted chicken 2	Shandong Province, China	Oven roasting	RC2
Pork	Roasted pork 4	Shandong Province, China	Charcoal roasting	RP4	Chicken	Roasted chicken 3	Heilongjiang Province, China	Fruit wood roasting	RC3
Beef	Roasted beef 1	Inner Mongolia Province, China	Charcoal roasting	RB1	Duck	Roasted duck 1	Beijing City, China	Fruit wood roasting	RD1
Beef	Roasted beef 2	Inner Mongolia Province, China	Charcoal roasting	RB2	Duck	Roasted duck 2	Beijing City, China	Oven roasting	RD2
Beef	Roasted beef 3	Inner Mongolia Province, China	Charcoal roasting	RB3	Duck	Roasted duck 3	Beijing City, China	Charcoal roasting	RD3
Lamb	Roasted lamb 1	Inner Mongolia Province, China	Charcoal roasting	RL1	Duck	Roasted duck 4	Jiangsu Province, China	Oven roasting	RD4
Lamb	Roasted lamb 2	Shanxi Province, China	Charcoal roasting	RL2	Duck	Roasted duck 5	Guangdong Province, China	Oven roasting	RD5
Lamb	Roasted lamb 3	Inner Mongolia Province, China	Charcoal roasting	RL3	Goose	Roasted goose 1	Heilongjiang Province, China	Wood roasting	RG1
Lamb	Roasted lamb 4	Xinjiang Province, China	Charcoal roasting	RL4	Goose	Roasted goose 2	Heilongjiang Province, China	Oven roasting	RG2
Lamb	Roasted lamb 5	Xinjiang Province, China	Charcoal roasting	RL5	Goose	Roasted goose 3	Guangdong Province, China	Oven roasting	RG3
Lamb	Roasted lamb 6	Xinjiang Province, China	Fruit wood roasting	RL6					

the thermal processing of meat products, as highlighted in a study conducted by Yao et al. in 2023. Dong et al. (2020) have detected over 30 heterocyclic aromatic amines (HAAs) in beef products subjected to thermal processing. Among these HAAs, only a limited number, such as IQ-type and PhIP, have formation pathways that are relatively well understood. The Maillard reaction serves as the main pathway for the formation of both aroma and hazardous compounds. The composition of meat, including amino acids and glucose, as well as the types of condiments and the specific heating circumstances such as temperature, time, and moisture, during the roasting process, contribute to the variability in meat flavor, AGEs and HAAs (Uribarri et al., 2010; Liu et al., 2020).

Previous studies have explored the aroma compounds or hazardous compounds in roasted meat products, respectively. Insufficient research has been conducted about the interplay and connections between aroma and hazardous compounds. Nevertheless, it is worth noting that in real food products, the reactive precursors of both of these molecules are found to coexist. Moreover, there is a dearth of research on the shared aroma and potentially hazardous compounds found in many types of roasted meats. Therefore, this study aimed to (i) qualitative and quantitative the aroma compounds, hazardous compounds and precursors in different kinds of roasted meat products and (ii) to investigate the qualitative and quantitative relationships between the common aroma compounds, typical hazardous compounds and main precusors in roasted meat products through PLS-DA analysis and correlation analysis. It was anticipated to gain a comprehensive understanding of the prevalent scent compounds, potentially harmful compounds, and primary precursors found in various types of roasted meat products. Additionally, this research aimed to establish a theoretical basis for synergistic regulation of aroma and hazardous compounds in roasted meat products.

2. Materials and methods

2.1. Samples and materials

Twenty-five categories of commercially roasted meat products with regional specialities, including pork, beef, lamb, chicken, duck and goose, were purchased from online stores. All samples were transported via cold chain to the lab and stored at -30 °C before analysis. The leg meats were collected and specific information about each sample was shown in Table 1. AGEs standards (99.9%): CML, CEL and MG-H1 were obtained from Toronto Research Chemicals Inc. (Toronto, Canada). HAAs standards (99.9%): Harman, Norharman, Glu-*P*-1, Glu-P-2, Trp-*P*-1, Trp-P-2, IQ, IQx, MeIQ, 8-MeIQx, 7,8-DiMeIQx, 4,8-DiMeIQx, PhIP, AαC, MeAαC, purchased from Toronto Research Chemicals Inc. (Toronto, Canada). The 2-methyl-3-heptanone (99%) was purchased from Dr. Ehrenstorfer (Beijing, China). The methanol (99.8%) and acetonitrile (99.9%) were purchased from ThermoFisher Scientific (Beijing, China). The ammonium acetate was purchased from Sigma-Aldrich (St Louis, Mo, USA). Magnesium sulfate ($MgSO_4$), Sodium acetate (CH_3COONa), Primary and Secondary Amine (PSA) and End-Capped-C-18EC Solid phase extraction packing were purchased from Agilent Technologies (USA). Other chemicals and reagents were analytical grades purchased from Sigma-Aldrich (St Louis, Mo, USA) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Aroma compounds analysis

The SPME method used to extract and quantitate the aroma compounds was determined according to Liu et al. (2019), with some modifications. Briefly, 3 g of minced sample was accurately weighed into a 20 mL headspace vial. A 1.0 μL of internal standard (2-methyl-3-heptanone, 1.36 μg/μL in methanol) was added immediately and the vial was sealed with a PTFE-silicon stopper.

Aroma compounds in different varieties of roasted meat products were separated and identified by Thermo Scientific TM ISQTM LT Single Quadrupole Gas-Mass System equipped with a DB-WAX (60 m × 0.25 mm × 0.25 mm) column at a helium flow rate of 1 mL/min. DB-WAX chromatographic column procedure: initial temperature 40 °C, maintained for 3 min, increased to 70 °C at 2 °C/min, increased to 130 °C at 3 °C/min, and then increased to 230 °C at 10 °C/min, maintained for 10 min. Electron bombardment voltage 70 eV, ionisation source temperature 230 °C, quadrupole temperature 150 °C, full scan mode, mass scan range 30–450 amu. Aroma compounds were identified by comparing their mass spectra with the NIST 20 mass spectral library and standard alkanes' retention index (RI) (C_7-C_{32}).

The detection procedure of GC-O-MS was the same as that described

above for roasted meat products. The splitting ratio between the sniffer and mass spectrometer was 1:1 to ensure that the mass spectral peak of the substance appeared on the mass spectrometer at the same time as the smell. The retention time, odor description and flavor intensity (weak, middle, strong) of each flavor component were recorded for each sample.

2.3. Odor Activity value (OAV) analysis

Odor Activity Value (OAV) is calculated by the ratio of the content of an odor substance to its odor threshold in water. The odor threshold of substances in water is obtained by references (Liu et al., 2019; Li et al., 2023).

2.4. AGEs analysis

2.4.1. Sample preparation for AGEs

AGEs were extracted and purified according to methods reported previously by Tavares et al. (2017) and Shi et al. (2021) with slight modifications. Briefly, the freeze-dried minced samples (100 mg) were reduced with 0.4 mL $NaBH_4$ in 2 mL 0.2 M borate buffer (pH = 9.2) for 8 h. The samples were acidolysis at 110 °C for 24 h, and the acid hydrolysates were filtered through a 0.22 μm nylon membrane (Tianjin Jinteng Experimental Equipment Co., Ltd., Tianjin, China). The Poly-Sery MCX solid phase extraction column (Shanghai Ampu Experimental Technology Co., Ltd) was activated with 3 mL of methanol, equilibrated with 3 mL of the aqueous solution containing 2% formic acid, and the sample solution was passed through the MCX solid phase extraction column, then removed with 3 mL of aqueous solution containing 2% formic acid and 3 mL of methanol in turn. The eluate was collected under a vacuum, concentrated to near dryness by nitrogen blowing, and then dissolved in 1 mL of 20% acetonitrile water. The solution was filtrated through a 0.22 μm nylon membrane.

2.4.2. UPLC-MS/MS analysis of AGEs

The identification and quantification of AGEs were conducted with a UPLC-MS/MS (6470, Agilent, USA) equipped with a triple quadrupole following the method of Yu et al. (2018). 3 μL sample was injected into a BEH Amide (100 mm × 2.1 mm; 1.7 μm) (Waters, USA) under 35 °C column temperature. The mobile phase included 5 mM ammonium acetate and 0.1% formic acid as solvent A and 100% acetonitrile as solvent B. The Mass spectrometer was operated in positive electrospray ionisation (ESI) mode with a capillary voltage of 4.00 kV. The dryer temperature was 350 °C. Nitrogen (over 99.9%) is used as desolvation and cone gas. The flow rate of gas is 10 L/min. The results were analysed with MassHunter software provided by the system, and CML and CEL contents were expressed using μg/g sample (on a dry weight basis).

2.5. HAAs analysis

2.5.1. Sample preparation for HAAs

The extraction of HAAs refers to QuECHERS (quick, easy, cheap, effective, rugged, safe) method (Suleman et al., 2019; Ding et al., 2021). Briefly, 2 g of minced roasted meat samples were mixed with 1 homogenised ceramic stone and 10 mL deionised water and shaken for 20 min at room temperature. 10 mL of acetonitrile solution containing 1% acetic acid was added. Furthermore, mix the solution with 4 g of $MgSO_4$ and 1 g of CH_3COONa, and shake it for 1 min. Centrifuged at 4 °C and 3200 g for 10 min; added 6 mL of supernatant to the centrifuge tube (containing 900 mg of anhydrous magnesium sulphate, 300 mg of propyl ethylenediamine and 300 mg of octadecyl siloxane, end-capped), homogenised at 1000 rpm for 1 min; centrifuged at 4 °C and 3200 g for 5 min. The supernatant was collected and concentrated to near dryness by nitrogen blowing. 0.50 mL of methanol solution was added, and the resolved solution was filtered through a 0.22 μm PVDF membrane.

2.5.2. UPLC-MS/MS analysis of HAAs

The identification and quantification of AGEs were conducted with a UPLC-MS/MS (6470, Agilent, USA) equipped with a triple quadrupole. HAAs contents were expressed using ng/g.

2.6. Moisture and lipids analysis

The official AOAC methods 950.46 and 960.39 were used to determine the moisture and lipid contents of roasted meat products, respectively (AOAC, 2004).

2.7. Possible precursors analysis

2.7.1. Reducing sugar analysis

Minced roasted meat samples (5 g) were mixed with 5 mL of ultrapure water, homogenised at 4 °C, sonicated for 10 min, and then centrifuged at 5000 r/min for 15 min and collected all the supernatant and constant to 15 mL. The supernatant (1 mL) was mixed with 8% sulphosalicylic acid (1 mL) and centrifuged at 10,000 r/min for 15 min. Then, 1 mL of the supernatant was dried with nitrogen gas and resolved in 10 mL ultrapure water. Quantification of reducing sugar was measured using an ICS-3000 ion chromatograph equipped with Dionex CarboPacTM PA10 (3 mm × 150 mm) according to the methods of Liu et al. (2020).

2.7.2. Amino acids analysis

The extraction of amino acids was determined by the method of Ding et al. (2021). Quantification of amino acids was carried out using ninhydrin column-ion exchange chromatography (Hitachi 8900).

2.7.3. Creatine and creatinine analysis

The creatine and creatinine contents were determined following the method described by Burns and Ke (1985) and Mora et al. (2010) with minor changes. Briefly, 2 g of minced roasted meat products were homogenised with 10 mL of pre-coded 0.4 mol/L perchloric acid, centrifuged at 12,000 r/min for 15 min (below 4 °C) and then collected the supernatant. The solution was neutralised (pH 6.5–7.0) by 0.67 mol/L disodium hydrogen phosphate solution by volume 1:1. Finally, the sample was centrifuged for 10 min at 4 °C, the supernatant (1 mL) was filtered through a 0.22 μm PVDF membrane and quantification of creatine and creatinine was measured using an HPLC (Agilent, USA).

2.8. Statistical analysis

The experimental results of aroma compounds were based on six replicates; other experiments were performed in triplicate. The statistical analysis was performed using SPSS 22.0 (SPSS, International Business Machines Corporation, Armonk, NY, USA). Heatmaps and PLS-DA analysis were performed using MetaboAnalyst 5.0. The relationship between key aroma compounds, typical hazardous compounds and precursors was studied using Origin 2023 (Origin Lab Corporation, Northampton, MA, USA), and $p < 0.05$ or $p < 0.01$ or $p < 0.001$ represented different statistically significant levels. Aroma characteristics of volatile compounds were described according to the Flavornet database (https://www.thegoodscentscompany.com).

3. Results and discussion

3.1. Identification, quantitation, and OAV analysis of aroma compounds in roasted meat products

In this study, a comprehensive analysis was conducted using HS-SPME/GC–MS in conjunction with HS-SPME/GC–MS/O to identify a total of 25 roasted meat products derived from 6 distinct breeds. The types of aroma compounds that were identified and detected in the roasted meat products varied between 68 and 114. Considerable

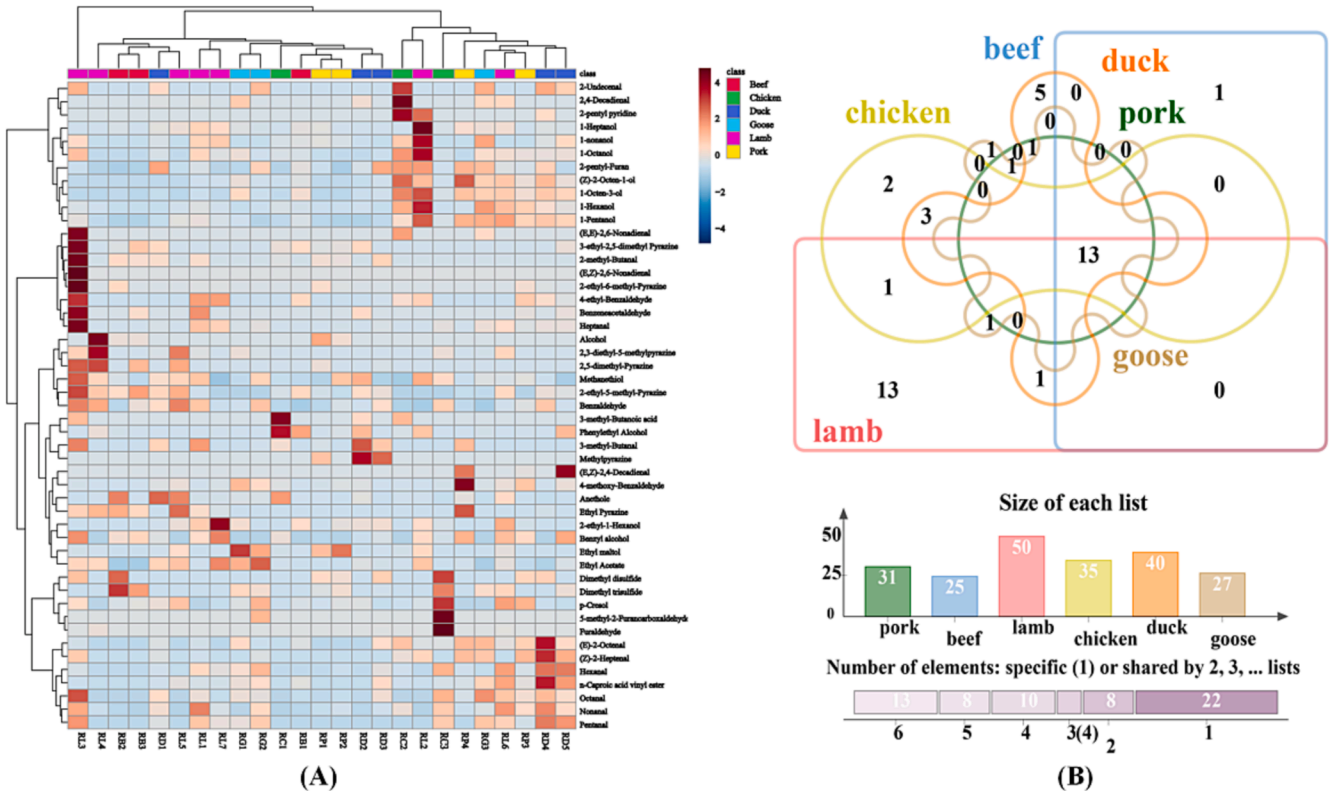

Fig. 1. Aroma compounds in roasted meat products. (A) Heatmap clustering of 49 aroma compounds in 25 kinds of roasted meat products based on HS-SPME-GC–MS and HS-SPME-GC-O-MS; (B) Venn diagram of aroma compounds.

variation was seen in the composition and amount of aroma compounds across different roasted meat products. However, it is important to note that not all aroma compounds had a significant contribution to the overall odor. The aroma compounds identified in roasted meat products can be categorized into ten major groups: aldehydes, ketones, alcohols, esters, nitrogenous compounds and sulphur-containing compounds. Roasted meat products exhibited a relatively greater concentration of aldehydes and alcohols, followed by nitrogenous compounds such as 2, 5-dimethyl-pyrazine and 2-pentyl-furan.

The assessment of the contribution of specific scent compounds was conducted by determining the Odor Activity Value (OAV), which takes into account both the concentration of aroma compounds and their corresponding odor thresholds. The results indicated that a higher OAV was positively correlated with a more pronounced aroma (Li et al., 2023). OAV values were calculated for the aroma compounds that were quantified in the roasted meats. A total of 49 aroma compounds were identified with OAVs greater than 1. In order to visualise the amount and classification of aroma compounds with OAV > 1 in different varieties of roast meat, a flavor heat map was generated, as depicted in Fig. 1-A. Depending on the number of aroma compounds, aldehydes and alcohols were predominant in roasted meat products while sulphur-containing and pyrazine compounds were also important aroma compounds in roasted meat products, which were consistent with the review by Sohail et al. (2022). For example, (*E*)-2-octenal, (*E*, *Z*)-2, 4-decadienal, (*Z*)-2-octan-1-ol and 1-octen-3-ol exhibited higher concentrations in roasted pork products. Conversely, vinyl hexanoate was found to be much more abundant in poultry samples (specifically chicken, duck, and goose) compared to livestock samples (pork, beef, and lamb). In addition, it was observed that roasted lamb products exhibited a significantly wider range of aroma compounds with OAV greater than 1, compared to roasted beef products which had a comparatively limited variety. As shown in Fig. 1-B, this diagram visualised the different varieties of roasted meat aroma compounds and their interrelationships. (*E, E*)-2, 6-nonadienal and (*E*, *Z*)-2, 6-nonadienal were identified as distinctive components in roasted chicken and roasted lamb products, respectively.

Aldehydes play a significant role in the formation of aroma compounds in roasted meat products. Benzaldehyde was found to be present in all roasted meats, with concentrations ranging from 12.98 to 598.78 ng/g. This compound is known to contribute to the development of tastes characterized by woody, nutty, and almondy notes. 2-pentyl furan, which had a soy and fruit flavor, was also one of the common aroma compounds in roasted meats and played an important role in the development of distinctive flavors in roasted meats. According to Xia et al. (2022), pyrazines are responsible for imparting a distinct roasted and nutty flavor in roasted meat products. The analysis of roasted lamb sample RL3 revealed a diverse array of nitrogenous chemicals, including 2, 3-diethyl-5-methylpyrazine, 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-5-methylpyrazine, and 3-ethyl-2, 5-dimethylpyrazine. The process of lipid degradation typically results in the formation of aliphatic aldehydes, ketones, alcohols, and esters. These compounds contribute to the development of distinct meat flavor that are characteristic of different animal breeds (Bassam et al., 2022), such as (*E*)-2-octenal and hexanal. The analysis of the heatmap revealed distinct variations in the richness of aroma compounds among various roasted meat products. This was due to the different combinations of aroma compounds and the different contributions of each compound to the distinctive odor. Based on the OAV evaluation, the aroma compounds in roasted meat products identified as significant contributors included nonanal, hexanal, octanal, benzaldehyde, 1-octen-3-ol, methyl mercaptan, ethyl acetate, dimethyl trisulphide, 2, 5-dimethyl pyrazine, 2-ethyl-5-methylpyrazine, 3-ethyl-2, 5-dimethylpyrazine, and 2-pentyl furan.

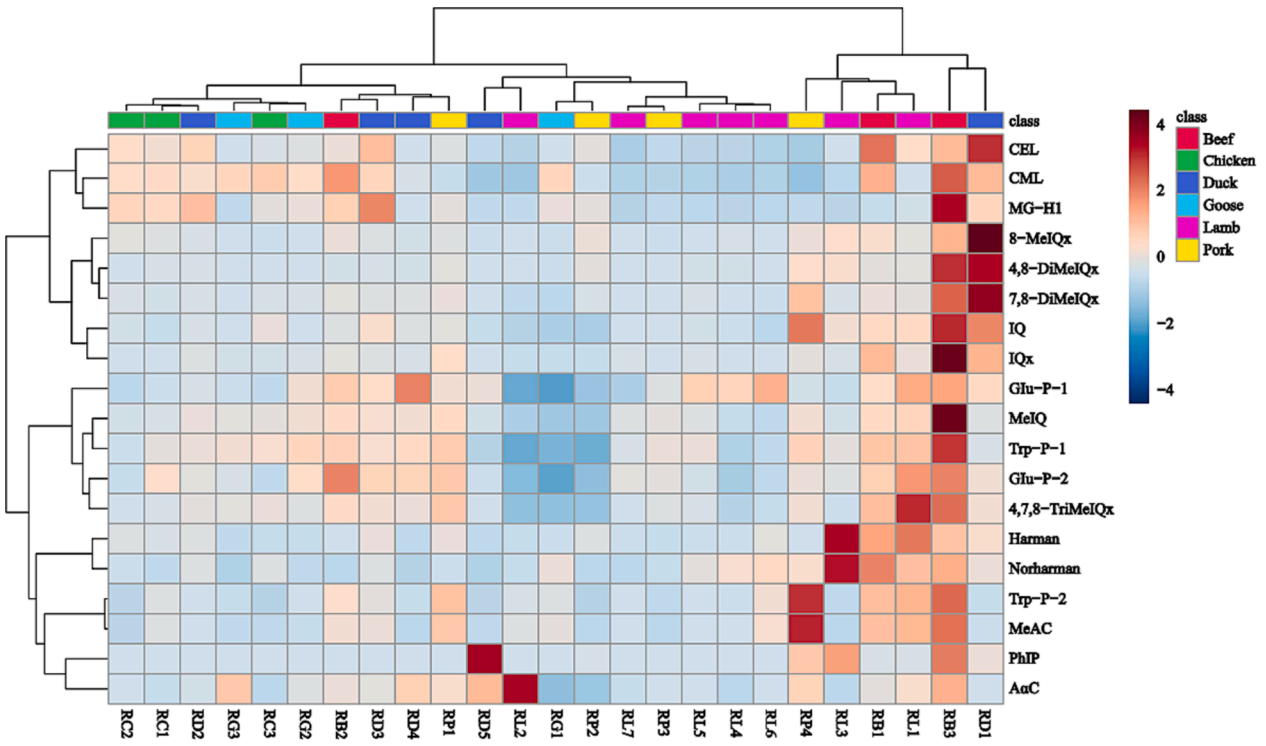

Fig. 2. Heatmap clustering of 19 hazardous compounds in 25 kinds of roasted meat products based on UPLC-MS/MS.

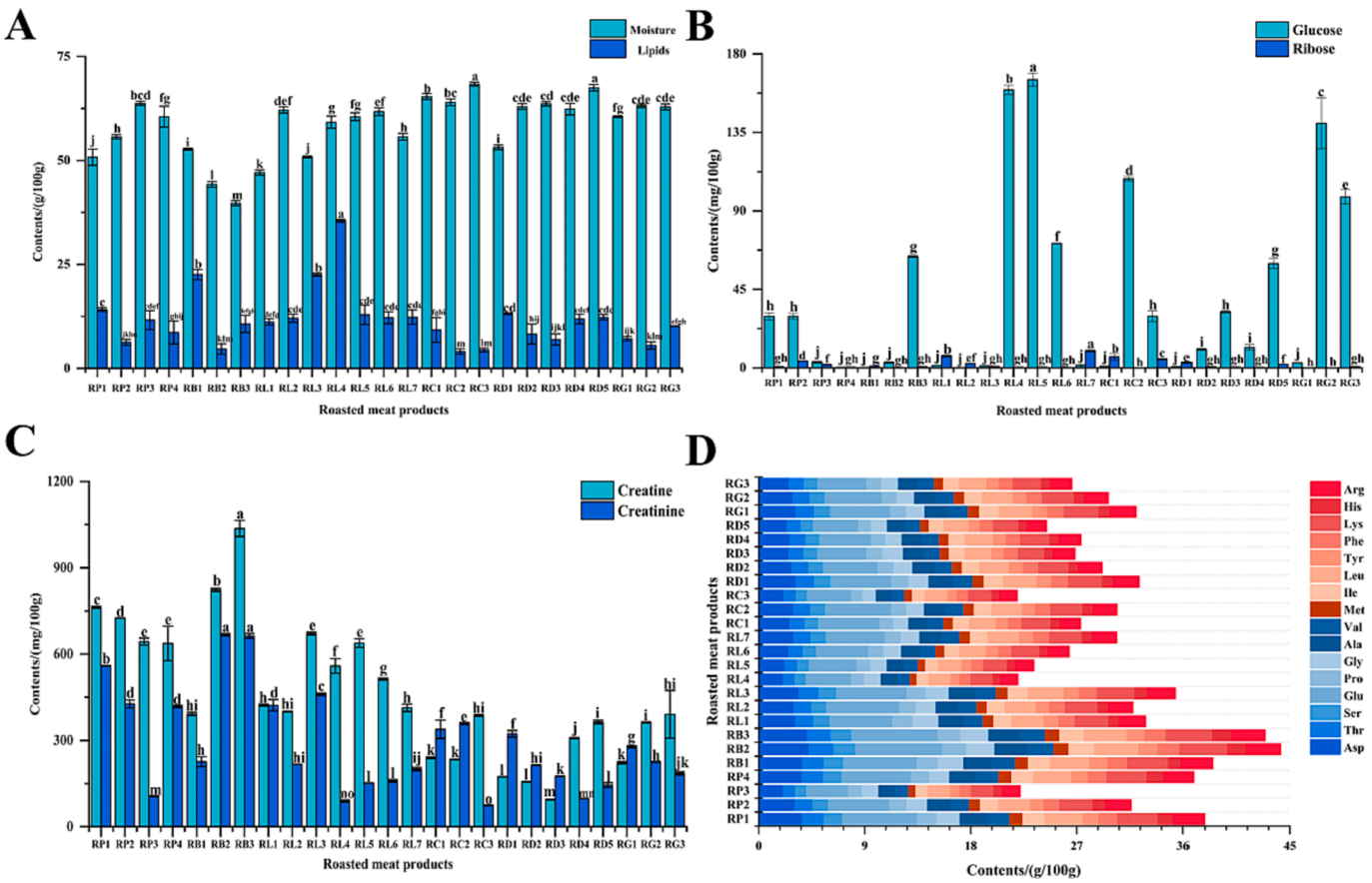

Fig. 3. Moisture, lipids, reducing sugars, creatine, creatinine and amino acids contents in roasted meat products.

3.2. Comprehensive analysis of typical hazardous compounds in roasted meat products

The roasting process greatly enhanced the flavor and quality of the meat. However, the formation of attractive flavors is frequently accompanied with a variety of hazardous compounds with potential risks to human health, such as AGEs and HAAs. (Hamzalıoglu & Gokmen, 2020). The accumulation of these hazardous compounds augments people's susceptibility to the onset of ailments such as cancer, cardiovascular illness, and Alzheimer's disease (Chen & Smith, 2015; Monika Gibis, 2016).

3.2.1. Harman and Norharman were typical HAAs in different roasted meat products

Fig. 2 showed the types and contents of HAAs in roasted meat products. Quinoline, quinoxaline and carboline HAAs were the main HAAs in various types of roasted meats. The levels of the β-carbolines HAAs, namely Harman and Norharman, were found to be significantly elevated compared to the other heterocyclic amines. The observed concentrations ranged from 0.98–26.46 ng/g for Harman and 1.64–31.49 ng/g for Norharman. The presence of Pyridine HAAs, specifically PhIP, was observed exclusively in a limited number of roasted meat products, including RP2, RP4, RB1, RL1, RL3, RD1, and RD5. The roasted duck meat products exhibited the greatest concentration of PhIP in RD5, with a recorded value of 5.48 ng/g. The findings of this study indicated that the formation of HAAs in meat was influenced by the type of meat used in thermal processing, which aligns with the conclusion drawn by Monika Gibis (2016). The contents of γ-carboline HAAs (Trp-*P*-1, trp-2) and ζ-carboline HAAs (Glu-*P*-1 and Glu-P-2) were not significantly different between varieties of roasted meat products. The concentration of Harman and Norharman compounds in the RL3 sample of roasted lamb products exhibited a statistically significant increase compared to other roasted meat products. Furthermore, notable variations in the composition of HAAs were observed among various roasted meat products of the identical variety. This observation provided additional evidence that distinct pre-treatment techniques and diverse roasting conditions have an impact on the formation of hazardous compounds within the same variety of roasted meat.

3.2.2. CML and CEL were typical AGEs in different roasted meat products

The main representative compounds of AGEs formed by the Maillard reaction are CML, CEL and MG-H1. All AGEs were detected in 25 kinds of roasted meat products, and the contents ranged from 11.78–49.32 μg/g dry sample, 8.48–171.00 μg/g dry sample and 8.41–45.76 μg/g dry sample, respectively. The roasted pork sample RP4 exhibited lower levels of CML, CEL, and MG-H1, but the roasted beef products displayed considerably higher concentrations of AGEs. This finding suggested a potential association between the formation of AGEs and different types of meat, which aligns with the findings reported by Huang et al. (2021). The poultry samples (chicken, duck, and goose) exhibited higher levels of AGEs, whereas the livestock samples (pork, beef, and lamb) displayed lower levels of AGEs. The amounts of CML, CEL, and MG-H1 exhibited variations within the same species of roasted meats, with the former two being comparatively higher than MG-H1. It has been indicated that common AGEs found in roasted meat product include CML and CEL, with lysine serving as the precursor amino acid.

3.3. Comprehensive analysis of possible precusors of aroma compounds and hazardous compounds in roasted meat products

3.3.1. Moisture and lipids analysis

Fig. 3 shows the content of basic components of 25 kinds of roast meat. Results showed that the moisture content of all analysed roasted meat products of different varieties ranged between 39.73 and 68.39 g/100 g. The moisture content of poultry samples had a greater value compared to the other roasted samples. The roasted beef sample RB3 exhibited the lowest moisture content, whereas the roast chicken sample RC3 displayed the highest moisture level. Studies have shown that an increased moisture content in food has a negative impact on the rate of the Maillard reaction, thereby influencing the formation of Maillard reaction products (Jiao et al., 2019). The lipid contents of all roasted meat products varied from 4.06 to 35.52 g/100 g. It can be observed that roasted chicken sample RC2 had the lowest lipid content, but roasted lamb sample RL4 demonstrated the greatest lipid content. The lipid content of roasted meats from livestock was found to be greater compared to that of roasted meats from poultry-roasted meats.

3.3.2. Reducing sugars analysis

Results showed that the main reducing sugars in meat products were glucose and ribose (Fig. 3-B). Both can react with amino acids and peptides to form aroma compounds, HAAs and AGEs, which played a crucial part in the development of both desirable aroma compounds and potentially hazardous compounds (Cui et al., 2023; Zhang et al., 2023). Glucose served as the main precursor to the HAAs and AGEs formation. As shown in Fig. 3-B, there were significant differences in glucose and ribose contents in roasted meats of the same variety of different meat products. The glucose levels in roasted lamb samples RL4 and RL5 were found to be substantially higher than in the other samples ($p < 0.05$), with concentrations of 159.46 ± 2.66 and 165.29 ± 3.76 mg/100 g, respectively. The roasted beef sample RB1 had the lowest levels of glucose content, measuring at 0.32 ± 0.08 mg/100 g. The decreased glucose content observed could potentially be attributed to the involvement of glucose in the Maillard reaction and its subsequent thermal degradation that occurs during the heating process. The maximum level of ribose content for analyzed roasted meat products was roasted lamb sample RL7 (9.78 ± 0.72 mg/100 g). Nevertheless, the presence of ribose was not observed in the analyzed roasted goose products. Researches had demonstrated that ribose had the potential to undergo reactions with sulfur-containing amino acids presented in roasted meats, resulting in the formation of sulfur-containing aroma compound, including dimethyltrisulfur and 3-methylthiopropanal.

3.3.3. Creatine and creatinine analysis

Creatine and creatinine are important precursors for forming HAAs, and creatinine can be formed by the breakdown of creatine. The concentration of creatine is known to vary among different meat species, as demonstrated by Mora et al. (2008). The contents of creatine and creatinine were shown in Fig. 3-C. Results showed that roasted livestock meats contained higher creatine levels than those roasted poultry meats. Creatine content was generally higher than creatinine in roasted livestock samples, while most poultry meat products, such as RC1, RC2, RD1, RD2, RD3 and RG1, did the opposite. Therefore, it was hypothesized that the rate of creatine degradation during the roasting process of these meat products exceeded the rate of formation of HAAs, thereby facilitating the accumulation of creatinine content.

3.3.4. Free amino acid analysis

A free amino acid refers to an individual amino acid molecule that exists independently, without being bound to other molecules. These free amino acids have the ability to undergo thermal breakdown, leading to the formation of appealing aroma compounds. Meanwhile, it is also an important precursor to the hazardous compounds formation in roasted meat products. Results showed that the contents of aspartate, glutamate, lysine and leucine in roasted meat products were significantly higher than other free amino acids ($p < 0.05$) (Fig. 3-D). The lysine concentration, which was the free amino acid precursor associated with the formation of CML and CEL, ranged from 1.87 to 3.97 g/100 g. The methionine content observed in various types of roast meat exhibited a statistically significant decrease compared to the levels of other amino acids ($p < 0.05$). According to the findings of Liu et al. (2020), it was observed that the interaction between methionine and ribose resulted in the formation of dimethyltrisulfide, leading to a

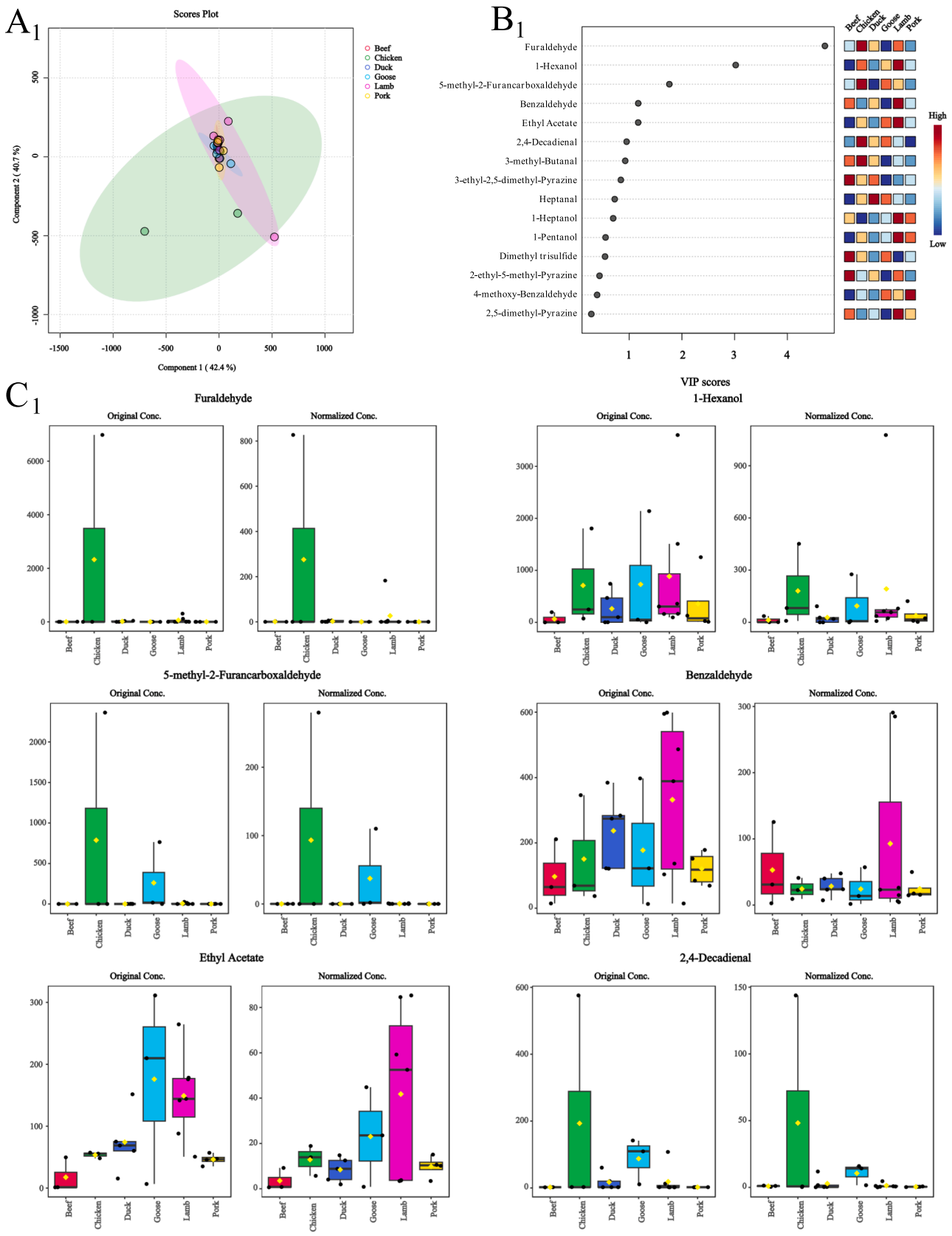

Fig. 4.1. Partial least squares-discriminant analysis (PLS-DA) of aroma compounds.

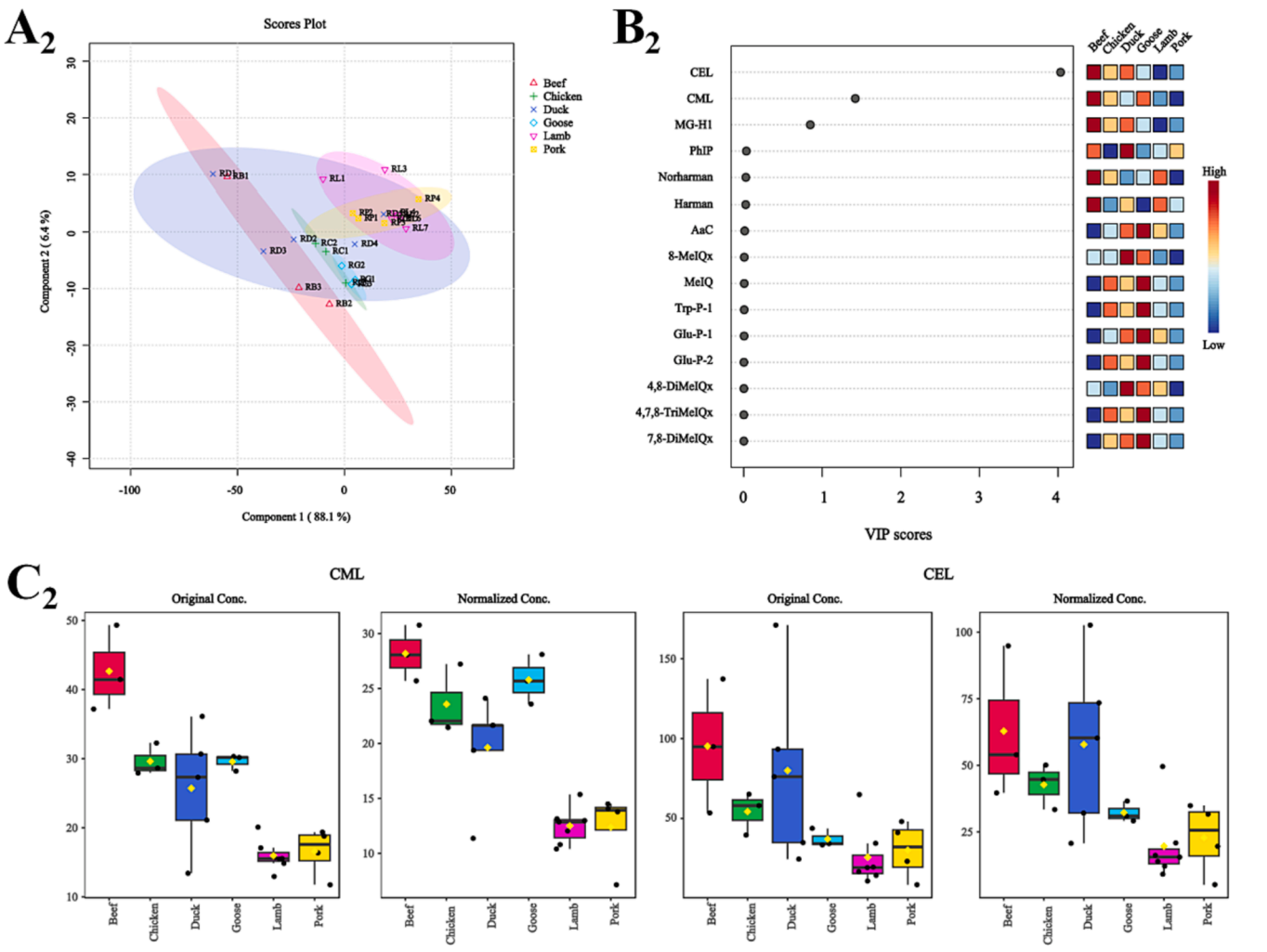

Fig. 4.2. Partial least squares-discriminant analysis (PLS-DA) of hazardous compounds.

decrease in methionine levels. The Maillard reaction Strecker degradation processes can result in the formation of aroma compounds from amino acids, including phenylalanine and leucine (Sohail et al., 2022). Furthermore, it was observed that all amino acids that imparted a distinct taste to the roasted meats were perceptible, exhibiting elevated concentrations of sweet and bitter amino acids compared to their fresh ones.

Maillard reaction is affected by many factors, such as precursors, temperature and moisture content (Jin et al., 2013). Roasting and other cooking methods can accelerate the formation of hazardous compounds in food due to higher temperatures (Uribarri et al., 2010). Furthermore, the dicarbonyl compounds produced by lipid oxidation can also accelerate the formation of AGEs (Goldberg et al., 2004; Jacobsen et al., 2010; Hu et al., 2017; Zhu et al., 2019). Hence, the moisture and lipid contents of roasted meats exert an influence on the formation of potentially hazardous compounds. However, further analysis is required to examine the correlation between the basic components, aroma compounds and hazardous compounds in roasted meat.

3.4. Critical aroma compounds, hazardous compounds, and their precursors exploration from the perspective of chemometrics methods

In order to conduct a more comprehensive analysis of the distinctions among various roasted meat products, the PLS-DA model was used to investigate the composition of aroma compounds, potentially hazardous compounds, and their respective precursors. Furthermore, in order to ascertain the aroma compounds, hazardous compounds, and precursors that may account for the variations observed across various types of roasted meat products, the variable importance value (VIP) was computed in the predictions.The VIP was used to explain the weight of the independent variable in explaining the dependent variable. There is a positive correlation between the VIP value and the magnitude of the component difference between groups. Additionally, the classification for identifying component types becomes more crucial as the VIP value increases (Gao et al., 2021; Scavarda et al., 2021; Xu et al., 2021). When the VIP value exceeds 1, it is considered vital in the PLS-DA discrimination process. The sample size in the model was 25. Y variables were pork, beef, lamb chicken, duck and goose. X-variables were 49 aroma compounds, 19 hazardous compounds and 22 precursors. The discriminant effect of the PLS-DA model for three types of compounds is shown in Fig. 4.

3.4.1. Aroma compounds modelling with PLS-DA approaches

In the PLS score chart of aroma compounds, the total variance of interpretation was 83.1%, with 42.4% from component 1 and 40.7% from component 2. The further the distance between different varieties of roasted meat products, the greater their difference and vice versa (Wang et al., 2020). In Fig. 4-1, there is a considerable overlap observed among the samples. It indicated they had common key aroma compounds between different breeds of roasted meat products. It showed that detecting aroma compounds had a good classification effect on roasted lamb sample RL4 and pork samples RP1 and RP2. The PLS-DA

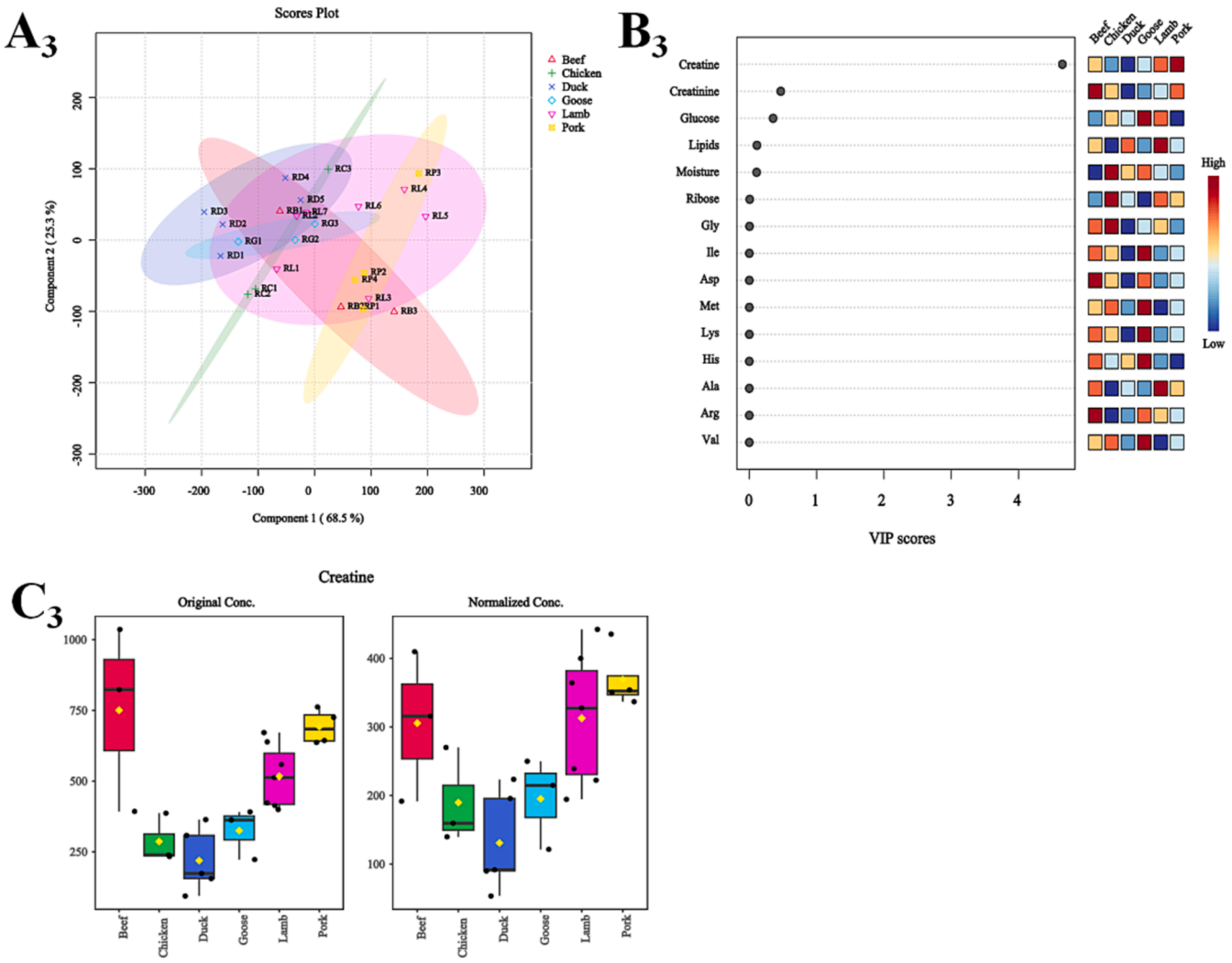

Fig. 4.3. Partial least squares-discriminant analysis (PLS-DA) of precursors.

score plot (Fig. 4-1B) demonstrated the significance of six aroma compounds with VIP scores greater than 1 in discerning between roasted samples of pork, beef, lamb, chicken, duck, and goose. The six aroma compounds identified in the study were furaldehyde, 1-hexanol, 5-methyl-2-Furancarboxaldehyde, benzaldehyde, ethyl acetate, and 2,4-Decadienal. Fig. 4-1C displays the box plots of the six aroma compounds that possess VIP values greater than 1, as derived from the PLS-DA model. There were considerable variations observed in the six key biomarkers across various breeds of roasted meats. Furaldehyde (odor: sweet, woody, almond, fragrant, baked, bread) showed the maximum levels in chicken samples. 1-Hexanol (odor: banana, flower, grass, herb) was higher in chicken, goose and lamb samples. The contents of 5-methyl-2-Furancarboxaldehyde (odor: sweet, spicy, coffee, caramel-like odor) were higher in chicken and goose samples. Benzaldehyde (odor: bitter almond, burnt sugar, cherry, malt, roasted pepper) was detected in all samples, with higher levels in lamb samples. Ethyl acetate (odor: aromatic, brandy, contact glue, grape) had relatively high contents in lamb and goose samples. 2,4-Decadienal (odor: coriander, deep fried, fat, oil, oxidised) was found in higher levels in chicken samples.

3.4.2. *HAAs and AGEs modelling with PLS-DA approaches*

In the PLS score chart of hazardous compounds, the total variance of interpretation variables was 94.5%, of which 88.1% came from component 1 and 16.4% from component 2 (Fig. 4-2A). The results indicated that the majority of the sample information was concentrated in components 1 and 2. The roasted beef samples exhibited clear discernibility in comparison to the pork, chicken, lamb, and goose samples, however the duck samples did not demonstrate a statistically significant distinction from the other types. In addition, there was no significant difference between the roasted chicken and goose samples. In the PLS-DA score chart of hazardous compounds, there were two hazardous compounds with VIP > 1, indicating their importance in distinguishing pork, beef, lamb, chicken, duck and goose roasted meat products by hazardous compounds. The two hazardous compounds, namely CML and CEL, were typical AGEs formed in roasted meats (Fig. 4-2B). The levels of CML and CEL in the roasted beef samples were found to be considerably elevated compared to the levels observed in the other samples. The sample of roasted duck exhibited significantly high levels of CEL concentration.

3.4.3. *Moisture, lipids, reducing sugars, amino acids, creatine and creatinine modelling with PLS-DA approaches*

In the PLS score chart of precursors, the total variance of interpretation variables was 93.8%, with 68.5% from component 1 and 25.3% from component 2 (Fig. 4-3A), indicating that component 1 and component 2 contained most of the sample information. Roasted pork samples were well distinguished from chicken and duck samples. There was only one precursor with VIP > 1 In the PLS-DA score chart of precursors. The study revealed that creatine serves as the primary distinguishing compound in roasted meats derived from pork, beef, lamb,

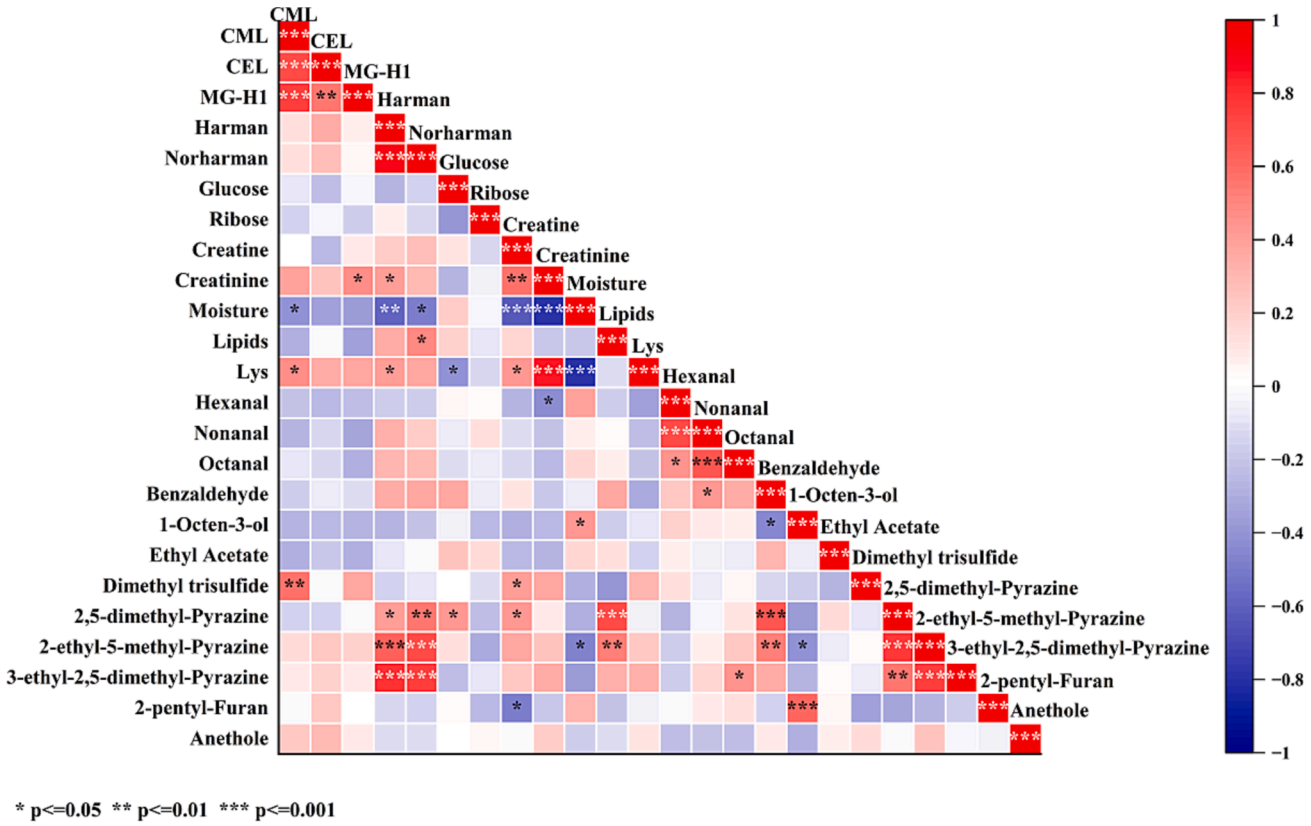

Fig. 5.1. Correlation between key aroma compounds, typical hazardous compounds and precursors.

chicken, duck and goose, as evidenced by its impact on aroma and hazardous precursors. The concentration of creatine in the livestock samples was found to be greater in comparison to the poultry samples.

It was noteworthy that various types of roasted meats had a significant overlap in terms of their aroma compounds. In this manner, a comprehensive analysis of the interplay of aroma compounds, hazardous compounds and precursors in roasted meat products can be conducted.

3.5. Correlation analysis of aroma compounds, hazardous compounds, and main precursors of roasted meat products

The correlation between common key aroma compounds, AGEs, HAAs and other components in roasted meat products is shown in Fig. 5-1. The levels of aldehydes and alcohols, including hexanal, nonanal, octanal, benzaldehyde, and 1-octen-3-ol, in roasted meat products exhibited a negative correlation with the contents of AGEs. Conversely, the levels of nonanal, octanal, and benzaldehyde showed a positive correlation with the presence of Harman and Norharman. There was a significant positive correlation between dimethyltrisulfide and CML contents ($p < 0.01$). The levels of 2, 5-dimethyl pyrazine and 2-ethyl-5-methylpyrazine in roasted meats had a significantly positive correlation with lipid contents ($p < 0.01$). Both saturated and unsaturated fatty acids can be thermally decomposed to produce hydroperoxides under aerobic conditions, forming aldehydes, alcohols and ketones. Furthermore, these compounds can further interact with each other, ultimately resulting in the formation of pyrazines (Tamura et al., 2022). Pyrazines, specially 2, 5-dimethyl pyrazine, 2-ethyl-5-methylpyrazine and 3-methyl-2, 5-dimethyl pyrazine, exhibited a significant positive correlation with Harman and Norharman ($p < 0.01$). The study conducted by Zhang et al. (2022) supported the notion that the development of a desirable roasted meat flavor is accompanied by an elevated presence of potentially hazardous compounds. Therefore, the feasibility of enhancing flavor appeal by the extension of roasting duration is limited. In addition, 2, 5-dimethyl pyrazine and 2-ethyl-5-methylpyrazine showed a significantly positive correlation with benzaldehyde and lipid content ($p < 0.01$). There was a notable positive connection ($p < 0.01$) seen between the concentration of 2, 5-dimethyl pyrazine and glucose. ($p < 0.01$). Glucose, one of the vital flavor precursors in meat, can be involved in the Maillard reaction at room temperature. The reaction becomes more intense with the increasing temperature and result in the formation of furan, pyrazine and other meat aroma compounds (Sun et al., 2023). The content of 1-Octen-3-ol exhibited a significantly positive correlation with 2-amyl furan and moisture content ($p < 0.01$, $p < 0.05$). Conversely, the presence of aldehyde demonstrated a significant negative correlation with the content of 2, 5-dimethyl pyrazine and benzaldehyde ($p < 0.05$).

CML was a representative substance of typical AGEs in roasted meats, and its content was significantly positively correlated with lysine content ($p < 0.05$). The contents of CML, Harman and Norharman showed a negative correlation with moisture content ($p < 0.05$). There existed an inverse relationship between the moisture content and the levels of hazardous compounds, whereby an increase in moisture content led to a decrease in the concentrations of those compounds. The observed phenomenon was likely attributable to the elevated moisture levels present, which might impede the occurrence of the Maillard reaction and the breakdown of amino acids. Results showed that amino acids, reducing sugars, creatinine and creatinine played a significant role as precursors in the formation of aroma and hazardous compounds in roasted meats, which was consistent with the study of Poulsen et al. (2013). The lysine content in roasted meats had a significantly positive correlation with CML, Harman, creatine and creatinine content. In contrast, it had a significantly negative correlation with the glucose and moisture content ($p < 0.05$). Moreover, lipid content showed a significantly positive correlation with Norharman content, and Harman content was significantly positively correlated with creatine content ($p <$

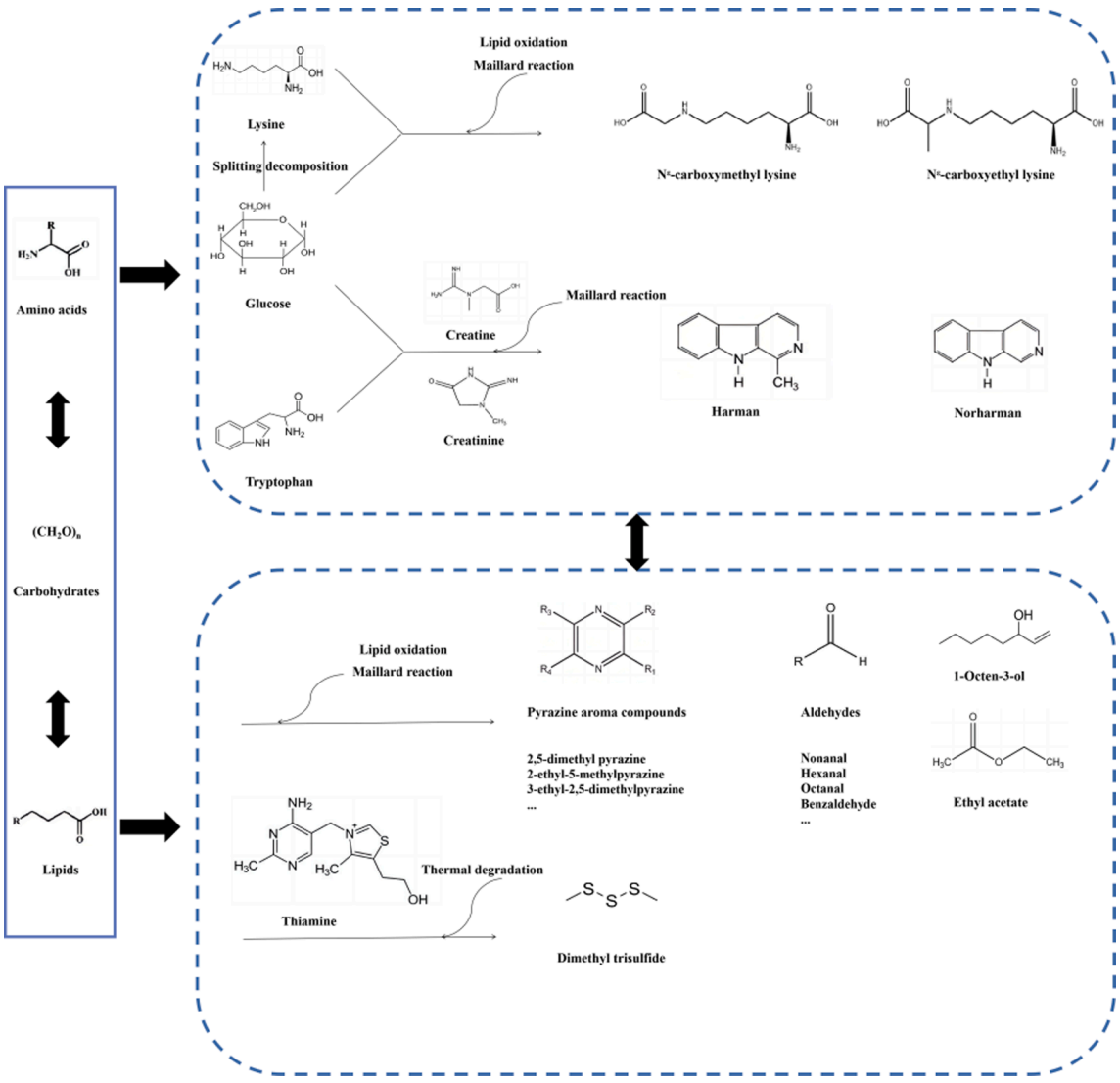

Fig. 5.2. Possible formation pathway between key aroma compounds, typical hazardous compounds and precursors.

0.05).

The formation of CML, CEL and MG-H1 showed a significant positive correlation with each other, Harman and Norharman also showed a similar correlation ($p < 0.001$). Meanwhile, it was observed that 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 3-methyl-2, 5-dimethylpyrazine had a significant positive correlation among themselves. Possible formation pathways between key aroma compounds, typical hazardous compounds and precursors were shown in Fig. 5-2. These results indicated that the formation of Maillard reaction products was interrelated.

4. Conclusion

In this study, HS-SPME-GC–MS combined with HS-SPME-GC–MS/O identified the aroma compounds in different varieties of roasted meat products. In conjunction with OAV greater than 1, it was found that nonanal, octanal, hexanal, benzaldehyde, 1-octene-3-ol, methyl mercaptan, ethyl acetate, dimethyltrisulfur, 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 3-ethyl-2, 5-dimethylpyrazine and 2-amyl furan were the main aroma compounds in roasted meat products. The hazardous compounds in roasted meats were analyzed using UPLC-MS/MS. The typical AGEs in roasted meat were CML and CEL, whereas the typical HAAs were Harman and Norharman. Further multivariate statistical analysis indicated that aldehyde and alcohols, such as hexanal, nonanal, octanal, benzaldehyde, and 1-octen-3-ol, in roasted meat showed a negative correlation with CML, CEL, and MG-H1 ($p < 0.01$). Nonanal, octanal and benzaldehyde were positively correlated with Harman and Norharman. The formation of appealing aroma compounds was accompanied by the presence of hazardous compounds. The formation of CML was found to be enhanced by the dimethyltrisulfide, whereas the pyrazine compounds, specifically 2, 5-dimethyl pyrazine, 2-ethyl-5-methylpyrazine, and 3-methyl-2, 5-dimethyl pyrazine, encouraged the formation of Harman and Norharman. Nine key biomarkers (furaldehyde, 1-hexanol, 5-methyl-2-furancarboxaldehyde, benzaldehyde, ethyl acetate, 2, 4-Decadienal, CML, CEL, and creatine) could be used to distinguish between roasted meat products. It provides a data basis for further research on the synergistic formation and regulation between aroma and hazardous compounds in roasted meats. Summarily, these results provide comprehensive data on the various types and compositions of aroma compounds, hazardous compounds, and precursors found in roasted meat products. These results contributed to a more profound comprehension on the interconnections among the common aroma compounds, typical hazardous compounds and main precusors. It is of great importance for researching and developing nutritious and healthy meat products for consumers.

CRediT authorship contribution statement

Haonan Shi: Conceptualization, Methodology, Visualization, Writing – original draft, Formal analysis. **Rongmei Gao:** Investigation, Data curation, Visualization. **Huan Liu:** Visualization, Methodology, Writing – review & editing. **Zhenyu Wang:** Project administration. **Chunjiang Zhang:** Writing – review & editing, Project administration. **Dequan Zhang:** Supervision, Resources, Funding acquisition, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137742.

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