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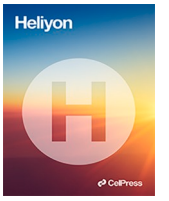

Research article

Effect of alterations in phospholipids and free fatty acids on aroma-active compounds in instant-boiled chuck tender, sirloin and silverside beef

Yimeng Ren [a,b,1], Longzhu Zhou [b,1], Yujie Shi [a,b], Yanan Yu [b], Weihai Xing [b], Qingyu Zhao [b], Junmin Zhang [b], Yueyu Bai [a], Jing Li [a,b,**], Chaohua Tang [b,*]

[a] School of Agricultural Sciences, Zhengzhou University, Zhengzhou, 450001, China
[b] State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Sciences of Chinese Academy of Agricultural Sciences, Beijing, 100193, China

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ABSTRACT

Beef flavor profiles are strongly influenced by cooking methods and lipid composition. However, the effect of instant-boiling on the aroma of different beef slices was unclear. This study investigated the lipid profiles and instant-boiling volatile profiles of chuck tender (M. *Supraspinatus*), sirloin (M. *Longissimus dorsi*) and silverside (M. *Biceps femoris*). Quantitative lipidomics identified 336 lipid molecular species, of which 84–112 were quantitatively different among the three beef slices. Sirloin had lower phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol and free fatty acids than chuck tender and silverside. The unsaturated fatty acid acyl chains in phosphatidylethanolamine differed significantly. Solid phase microextraction-gas chromatography-olfactometry-mass spectrometry (SPME-GC-O-MS) identified hexanal, octanal, nonanal, decanal, (E)-2-octenal, (E)-2-nonenal, (E)-2-undecenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, 1-octen-3-ol, 2-pentylfuran and acetoin as the aroma-active compounds of instant-boiled beef. Unsaturated free fatty acids and phosphatidylglycerols with unsaturated fatty acid residues positively correlated with the aroma-active compounds and might be crucial in flavor differences among the three beef slices. These findings provide greater understanding of the lipid and instant-boiling aroma-active compound profiles in chuck tender, sirloin and silverside, and reflect the suitability of different beef slices for instant-boiling from the aroma perspective.

1. Introduction

Flavor preference is an intuitive reflection of consumer acceptability for beef [1]. Lipids are the precursors of aroma compounds in beef, and during thermal processing, the unsaturated fatty acids in the lipids generate hydroperoxides that are cleaved to form the volatile compounds [2]. Therefore, the formation of aroma-active compounds in beef is influenced by lipid profiling. Intramuscular fat

* Corresponding author. Institute of Animal Sciences of Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Beijing, 100193, China.
** Corresponding author. School of Agricultural Sciences, Zhengzhou University, No. 157 Kexue Avenue, Zhengzhou, 450001, China.
E-mail addresses: chnlijing@zzu.edu.cn (J. Li), tangchaohua@caas.cn (C. Tang).
[1] These authors contributed equally to this work and should be considered co-first.

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content influences consumer flavor preference of the roasted beef and a previous study showed that fat deposition varies among 33 different cuts of Canadian AA beef depending on the location of the cuts [1,3]. Effect of phospholipids on aroma formation is also important because the fatty acid acyl chains of phospholipids in beef are more unsaturated than those of neutral lipids, and unsaturated fatty acids such as oleic acid and linoleic acid are actively involved in the oxidation and aroma formation [4,5]. In addition, the increased antioxidant capacity in red oxidized fiber beef partially inhibits the formation of lipid-derived volatile compounds [6]; the reactivity and extent of interactions between flavor precursors vary depending on the heating method, temperature and duration of cooking [7,8]. Flavor precursors of different beef slices have different degrees of interactions in cooking, resulting in different adaptability to cooking methods and then flavor differences [9]. As such, an exploration of the effect of instant-boiling on the aroma of different beef slices could provide a reference for improving consumer acceptance of beef.

Instant-boiling is a traditional Chinese cooking method in which meat is cut into thin slices, quickly heated in boiling water for less than a minute, then consumed immediately [10]. In instant-boiled beef, the content of hexanal was found to increase compared with boiled, steamed and roasted beef [7]. To our knowledge, however, little is known about the aroma-active compounds in instant-boiled beef from different slices. SPME-GC-O-MS combined with odor-activity values (OAVs) of characteristic aroma compounds is an effective method to determine the relative aroma contribution of each volatile compound. Gas chromatography-olfactometry (GC-O) describes the characteristic odors of the aroma-active compounds by human sensory evaluation and ranks the contribution of different odors in combination with calculation of OAVs. For example, aroma-active compounds such as dimethyl trisulfide and 2-methyl-3--furanethiol in soy sauced beef, had higher OAVs and contributed its characteristic flavor [11]. 1-Octen-3-ol was perceived as a strong mushroom odor and linalool was perceived as a floral odor in beef meatballs by GC-O [12].

Huaxi is a beef cattle breed formed by taking Simmental beef cattle as the sire and crossbreeding with the hybrid progeny from Mongolian, Sanhe, Simmental and Charolais combinations as the dam [13]. Huaxi cattle have high-quality economic traits and are increasingly popular in the north-central provinces of China, so they were selected as the test breed for this study. The aims of this study were (1) to identify and quantify the lipids in chuck tender, sirloin and silverside beef by quantitative lipidomics; (2) to identify the volatile compounds and quantify the aroma-active compounds in instant-boiled beef by SPME-GC-O-MS; and (3) to analyze the correlation between lipids and aroma-active compounds in instant-boiled beef. The overall aim was to compare lipid compositional changes in chuck tender, sirloin and silverside beef, to gain insights into their contributions to flavor after instant-boiling.

2. Materials and methods

2.1. Chemicals

The lipid analytical standards: TG (18:1_18:1_18:1), Cer (d18:1_18:0), SM (d18:1_18:0), PC (16:0_18:1), PE (16:0_18:1), PI (16:0_18:1), PG (16:0_18:1), PS (18:1_18:1), LPC (18:0), LPE (18:0), stearic acid (18:0); lipid isotope analytical standards: TG (15:0(d_5)_18:1_15:0), Cer (18:1(d_7)_15:0), SM (16:0(d_{31})), PC (16:0(d_{31})_18:1), PE (16:0(d_{31})_18:1), PI (16:0(d_{31})_18:1), PG (16:0(d_{31})_18:1), PS (16:0(d_{31})_18:1), LPC (16:0(d_{49})), LPE (17:0(d_5)), palmitic acid (16:0(d31)); volatile analytical standards 2-methyl-3-heptanone and C7–C40 n-alkanes were from Sigma-Aldrich (USA). The volatile analytical standards: pentanal, hexanal, heptanal, (Z)-4-heptenal, octanal, (E)-2-heptenal, nonanal, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, undecanal, (E)-2-decenal, benzeneacetaldehyde, 2-butyl-2-octenal, dodecanal, 4-ethylbenzaldehyde, (E)-2-undecenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, hexadecanal, 2-heptanone, 6-methyl-2-heptanone, 3-octanone, 2-octanone, acetoin, 1-octen-3-one, 2,3-octanedione, 6-methyl-5-hepten-2-one, 2-nonanone, 3-octen-2-one, 2-decanone, benzene, alpha-pinene, beta-pinene, o-xylene, limonene, gamma-terpinene, styrene, p-cymene, 1-pentanol, 1-hexanol, 1-octen-3-ol, (Z)-5-octen-1-ol, 1-dodecanol, hexanoic acid, decanoic acid, octyl formate, gamma-butyrolactone, citronellyl acetate, gamma-caprolactone, methyl palmitate, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfone, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-hexylfuran, 2-pentylpyridine, 2-pentylthiophene and 2-acetyl-2-thiazoline were from Aladdin (CN). Chromatographic grade ethanol, ether, hexane, methyl *tert*-butyl ether (MTBE), chloroform and ammonium acetate were from Sinopharm (CN), isopropanol, methanol and acetonitrile were from Merck KGaA (GER).

2.2. Animals and sample collection

All experimental animal procedures were approved by the Animal Care and Use Committee of the Institute of Animal Sciences of the Chinese Academy of Agricultural Sciences (CN) with permission number No. IAS 2024–104. All cattle in this study were uncastrated bulls. The Huaxi cattle were fed until 18 months old at the experiment base. A total of 14 animals of similar weight were selected for the experiment. According to the China council on Animal care [14], the animals were electrocuted and slaughtered after one night of fasting and free water. Animal diets composition is shown in Table S1. After aging for 3 days, the chuck tender, sirloin and silverside were cut from the left side of each carcass. Muscle strips were fixed in paraformaldehyde for muscle fiber property analysis. After meat quality trait analysis, the samples were frozen at −80 °C for lipid and volatile profiling.

2.3. Physicochemical property analysis

Meat quality traits in chuck tender, sirloin and silverside were determined. Meat pH at both 24 and 72 h after slaughter was determined with a pH meter (HANNA Instruments, USA).

After 30 min of bloom time on the inner surface of the meat to oxidize and keep the meat color relatively stable [15], CEILAB color was determined with a colorimeter (Konica Minolta Sensing, JP). L* (brightness), a* (redness) and b* (yellowness) were determined

and the chroma and hue angles were then calculated.

Cooking loss calculating equation was as follows:

$$Cooking\ loss = (M1 - M2)/M1 \times 100\%.$$

Fresh meat was weighed (M1), then heated in water at 80 °C and removed when the center of the meat reached 70 °C. It was then stored at 4 °C for 12 h and reweighed (M2). The cooked meat was cut into 5 × 1 × 1 cm^3 strips along the muscle fibers for the shear force test and 1 cm^3 cubes for the texture profile test. Shear force and texture properties were determined with a texture analyser from Stable Micro Systems (UK). Test speed 1.0 mm/s. Texture profile test parameters: 75 % strain; trigger force 5.0 g.

Paraformaldehyde-fixed samples were dried, dehydrated, paraffin-embedded, sectioned, baked, dewaxed, hydrated, HE-stained and sealed. Sample images were taken with an OLYMPUS BX43 microscope at 200x magnification (Olympus Corporation, JP). Image Pro-Plus 7.2 was used to measured skeletal muscle fiber diameter, perimeter, area, and density (Media Cybernetics, USA). The intramuscular fat content of beef was determined with anhydrous ether using the Soxhlet method [16].

2.4. Quantitative lipidomics analysis

Based on a UPLC-Q-Exactive Orbitrap MS, lipids were identified and analyzed (Thermo Fisher, USA). Frozen muscle tissue (40 mg) and methanol (300 μL) were homogenized with a steel ball mill and vortexed with MTBE (1 mL) containing lipid isotope standards, i.e., TG (15:0(d_5)_18:1_15:0), Cer (18:1(d_7)_15:0), SM (16:0(d_{31})), PC (16:0(d_{31})_18:1), PE (16:0(d_{31})_18:1), PI (16:0(d_{31})_18:1), PG (16:0(d_{31})_18:1), PS (16:0(d_{31})_18:1), LPC (16:0(d_{49})), LPE (17:0(d_5)), palmitic acid (16:0(d31)). Water (250 μL) was added into the mixture and vortexed again. After centrifugation (4 °C, 5 min, 2400×*g*), the upper organic phase was retained, blown dry with nitrogen, then frozen at −80 °C. Lipids were redissolved in chloroform/methanol (2:1, v/v) before analysis.

Reversed-phase chromatographic analysis was performed on 2.1 mm × 100 mm Cortecs C18 column and XSelect CSH C18 column, respectively, for positive and negative ion modes (Waters, USA). Linear gradient elution program parameters: 0−23 min, 30 %–98 % B; 23−30 min, 98 % B; 30−35 min, 98−30 % B. Mobile phase compositions: A: acetonitrile in water (3:2, v/v), with 10 mM ammonium acetate; B: acetonitrile in isopropanol (1:9, v/v).

Mass spectrometry parameters: spray voltage; positive mode 3200 V; negative mode 2800 V; capillary temperature 320 °C; carrier gas flow rate 35 Arb; auxiliary gas flow rate 10 Arb.

The Lipidsearch database was searched to compare precursor and fragment mass information of the lipids (Thermo Fisher, USA). Calibration equations were established using lipid standards and isotope standards, and the muscle lipid content was calculated using the external standard method.

2.5. Preparation of instant-boiled beef

The instant-boiled beef was prepared based on the method of Wang [7] with some modifications. The meat was cut into thin slices (5 cm × 5 cm × 2 mm) in the direction of the vertical muscle fibers using a slicer (Hualing, CN). The sliced meat was dipped into boiling water and removed after 30 s. The instant-boiled beef was snap-frozen, and then chopped and frozen at −80 °C. Volatile analysis was performed as soon as possible.

2.6. Identification of volatile compounds in instant-boiled beef

Based on a Q Exactive GC Orbitrap-MS (Thermo Fisher, USA) instrument equipped with an olfactory port (GERSTEL, GER), volatile compounds were identified and analyzed. Instant-boiled beef (3 g) was mixed with 0.01 μg/μL 2-methyl-3-heptanone in methanol (10 μL). After incubation (55 °C, 20 min) of instant-boiled beef, the volatile compounds were extracted for 40 min at the same temperature using a SPME fiber tip (divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm) from Sigma Aldrich (USA). Desorption was then carried out at the inlet of the GC injector (250 °C, 3 min). The volatile compounds were separated on a 60 m × 0.25 mm × 0.25 μm VF-WAXms GC column (Agilent, USA), followed by a 1:1 split between the mass spectrometer and sniffer. Column temperature program: initial temperature 40 °C; heating rate 4 °C/min; heat to 230 °C for 5 min. Carrier gas: high-purity helium (2.0 mL/min).

Mass spectrometry parameters: full scan mode; electron impact ion source; electron energy 70 eV; mass range: 30−400 *m/z*; ion source 280 °C; transmission line 250 °C. Sniffing port parameters: sniffing port 90 °C; sniffing transmission line 250 °C.

The NIST 2.0 and Wiley databases were searched to compare the mass spectral information of the volatile compounds. Linear retention indices (LRIs) were compared with literature values. Authentic standards were used to confirm the identities and LRIs and olfactometry was used to identify the aroma of compounds. The LRI of the volatile compounds was calculated from the retention time of the n-alkane standard mixture (C7–C40):

$$LRI = 100n + 100(t_x - t_n) \ / \ (t_{n+1} - t_n)$$

where n and n + 1 represent the number of carbon atoms of the anterior and posterior n-alkanes with the closest retention indices to each compound, t_n and t_{n+1} represent the retention times of the n-alkanes, t_x represent the retention times of volatile compounds.

All panelists had experience in food sensory evaluation and were trained on volatile standards prior to the experiment until the participants were able to accurately differentiate and describe volatile compounds. The selected panelists consisted of two females and one male, between the ages of 22 and 25. The testing environment was conducted at a room temperature of 24 °C and all samples were

sniffed to ensure that the compounds could be reproducibly characterized. During the experiment, if two or three of the panelists smelled an odor at a similar time and with a similar characterization, the corresponding compound odor was recorded.

2.7. Quantification of aroma-active compounds in instant-boiled beef

All volatile compounds were semi-quantified using internal 2-methyl-3-heptanone standard. The odorless matrix was prepared for the quantification of aroma-active compounds based on the method of Liu [17] with some modifications. Instant-boiled beef samples were extracted by vortexing sequentially with water (1:4, w/v), ethanol (1:4, w/v) and ether-hexane mixture (1:2:1, w/v/v). The beef samples were lyophilized for 96 h to prepare odorless matrix. The analytical standard mixture was added to the odorless matrix along with the 2-methyl-3-heptanone, then extracted as for the original samples. Calibration equations were established based on the peak area ratio and concentration ratio of the aroma-active compounds to 2-methyl-3-heptanone. Concentrations of aroma-active compounds were calculated by the external standard method, and the OAV was calculated.

2.8. Statistical analysis

Data were presented as mean ± standard error. Student's t-test and one-way ANOVA were performed using SPSS (IBM, USA) with $p < 0.05$ as the limit for statistically significant differences. Partial least squares discriminant analysis (PLS-DA) was performed using MetaboAnalyst 5.0 (www.metaboanalyst.ca). Pearson correlation analysis was performed using Origin 2021b (OriginLab, USA). TBtools [18] and GraphPad Prism 9.5 (GraphPad, USA) were used to draw heatmaps and bar graphs.

3. Results

3.1. Changes in physicochemical characteristics of chuck tender, sirloin and silverside

Meat quality traits and intramuscular fat contents of chuck tender, sirloin and silverside are shown in Table 1. Sirloin had a lower a* value than chuck tender and silverside ($p < 0.05$). The chroma value of sirloin was significantly less than that of silverside, the hue angle of sirloin was significantly less than chuck tender ($p < 0.05$). Sirloin had a lower cooking loss and a higher shear force than chuck tender and silverside ($p < 0.05$). Texture profile analysis determined that the hardness, gumminess and chewiness of sirloin were significantly lower than those of chuck tender ($p < 0.05$). There was no significant difference in chroma, hue angle, cooking loss, shear force, hardness, gumminess and chewiness between chuck tender and silverside (Table 1). Moreover, there was no significant difference in intramuscular fat among chuck tender, sirloin and silverside (Table 1). Sirloin had a lower muscle fiber density than chuck tender and silverside ($p < 0.05$, Fig. 1D).

3.2. Comparison of lipid profiles among chuck tender, sirloin and silverside

Quantitative lipidomics was performed to compare the lipid profiles of the three beef slices. False-positive ions were manually eliminated as described previously [19]. Eleven lipid classes containing 336 lipid molecular species were identified, 101 from positive ion mode and 235 from negative ion mode (Fig. 2A and B, Table S2). Based on available molecular structure information and noise levels in positive and negative ion modes [20], triglyceride (TG) was selected for further data analysis from positive ion data. Similarly, ceramide (Cer), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) and free fatty

Table 1
Meat quality traits and intramuscular fat contents of chuck tender, sirloin and silverside beef. (n = 14).

	chuck tender	sirloin	silverside	*P*-Value
pH_{24h}	5.35 ± 0.04	5.26 ± 0.05	5.23 ± 0.03	0.119
pH_{72h}	5.72 ± 0.07	5.64 ± 0.12	5.53 ± 0.07	0.372
L*	39.42 ± 0.58	38.08 ± 0.74	40.22 ± 0.58	0.070
a*	20.88 ± 0.64^{a}	18.27 ± 0.76^{b}	21.12 ± 0.60^{a}	0.008
b*	12.53 ± 0.42	11.79 ± 0.42	13.21 ± 0.36	0.058
Chroma	24.36 ± 0.74^{ab}	21.76 ± 0.86^{b}	24.91 ± 0.68^{a}	0.013
Hue angle	59.04 ± 0.45^{a}	57.05 ± 0.47^{b}	57.96 ± 0.37^{ab}	0.010
Cooking loss (%)	35.07 ± 0.99^{a}	31.31 ± 1.12^{b}	33.58 ± 1.03^{ab}	0.046
Shear force (N)	113.4 ± 5.98^{b}	148.8 ± 9.70^{a}	105.0 ± 7.43^{b}	0.000
Hardness (g)	14406 ± 699.3^{a}	11657 ± 492.8^{b}	13129 ± 521.6^{ab}	0.007
Springiness (ratio)	0.59 ± 0.01	0.56 ± 0.01	0.58 ± 0.01	0.170
Cohesiveness (ratio)	0.56 ± 0.01	0.56 ± 0.01	0.56 ± 0.01	0.939
Gumminess (g)	8140 ± 501.9^{a}	6514 ± 349.7^{b}	7389 ± 344.1^{ab}	0.025
Chewiness (g)	4842 ± 347.9^{a}	3639 ± 204.3^{b}	4320 ± 268.4^{ab}	0.015
Resilience (ratio)	0.21 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.044
Intramuscular fat (%)	1.96 ± 0.18	2.40 ± 0.25	2.17 ± 0.20	0.356

Means ± standard error followed by different lowercase letters in the same row are significantly different at $p < 0.05$ after one-way ANOVA.

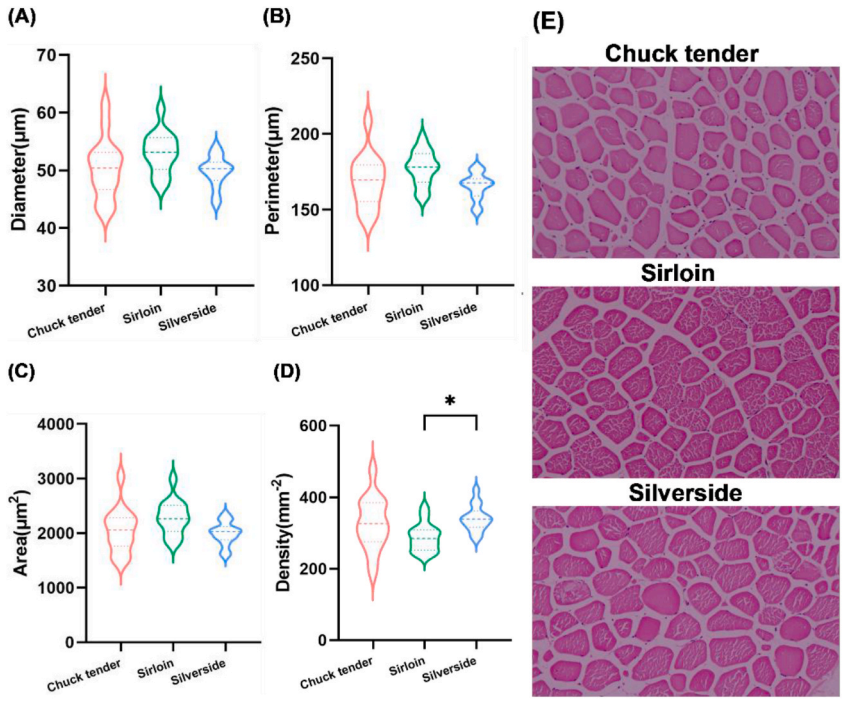

Fig. 1. Comparisons of muscle fiber characteristics of chuck tender, sirloin and silverside beef. Violin plots of: (A) Muscle fiber diameter, (B) Muscle fiber perimeter, (C) Muscle fiber area, (D) Muscle fiber density. (E) Microscopic images of chuck tender, sirloin and silverside at 200x magnification. * Represents $p < 0.05$. (n = 14).

acid (FFA) were selected for further analysis from negative ion data. The lipid content was determined from calibration curves established by lipid standards and isotope standards (Table S3). PLS-DA score plots revealed a clear separation among chuck tender, sirloin, and silverside (Fig. 2C, F and I). Variable importance in projection (VIP) > 1 and $p < 0.05$ were used as the thresholds to select the most significantly differential lipids to discriminate among the different beef slices. There were 101 differential lipids between chuck tender and sirloin, which are presented in a heatmap, showing changes in the relative levels of differentially expressed lipids, including 15 PCs, 15 PEs, 39 Cers, 9 FFAs, and 9 PIs (Fig. 2D and E). There were 84 differential lipids between chuck tender and silverside, including 18 Cers, 17 FFAs, and 12 PEs (Fig. 2G and H). There were 112 differential lipids between sirloin and silverside, including 52 Cers, and 20 FFAs (Fig. 2J and K). Therefore, Cers, PCs, PEs and FFAs were the main differential lipid molecular species that distinguished the three beef slices.

3.3. Phospholipids and free fatty acids varied among chuck tender, sirloin and silverside

The content of each lipid class was calculated. No significant difference in total lipid content was found among chuck tender, sirloin and silverside, consistent with their relative intramuscular fat contents (Fig. 3A). PC (19.51%–22.94 %), PE (11.47%–14.27 %) and TG (53.07%–60.57 %) were the main lipids in chuck tender, sirloin and silverside (Fig. 3B–D). There was no significant difference in TG and PS content among the groups. The contents of PC, PI, and Cer in sirloin were significantly lower than in chuck tender and silverside ($p < 0.05$), and were similar in chuck tender and silverside. Chuck tender had the highest PE content ($p < 0.05$), and those of sirloin and silverside were similar. The LPC content of silverside was 150.30 % and 162.51 % higher than chuck tender and sirloin, respectively ($p < 0.05$). The LPE content of silverside was 131.62 % and 123.96 % higher than chuck tender and sirloin, respectively ($p < 0.05$). Silverside had a higher PG content than chuck tender and sirloin ($p < 0.05$, Fig. 3E and F). Therefore, the various phospholipid classes varied differently among the three different beef slices and sirloin had a lower total phospholipid content than chuck tender and silverside.

The fatty acid composition of the various lipids strongly influences the concentration and composition of volatile compounds during cooking of meat. The fatty acid residue composition of PC and PE was analyzed (Fig. 3G–L). Oleic acid (18:1) and linoleic acid (18:2) were the main fatty acid residues in PC and PE in all three beef slices (Fig. 3G and J). The relative proportions of the different fatty acids in PC were similar among chuck tender, sirloin and silverside, whereas those of PE varied significantly among the three beef slices. In PE, sirloin had less oleic acid (37.76 %) than chuck tender (39.52 %) and silverside (39.92 %, $p < 0.05$). Arachidonic acid (20:4) in sirloin (18.89 %) was higher than in chuck tender (16.33 %) and silverside (16.56 %, $p < 0.05$, Fig. 3J), and docosapentaenoic acid (22:5) in sirloin was higher than in chuck tender and silverside ($p < 0.05$, Fig. 3K).

The FFA profiles in the three beef slices were compared (Fig. 4). A total of 34 FFAs were identified: 14 saturated fatty acids (SFA), 8 monounsaturated fatty acids (MUFA) and 12 polyunsaturated fatty acids (PUFA). Besides, the combined contents of the (16:0), (18:0),

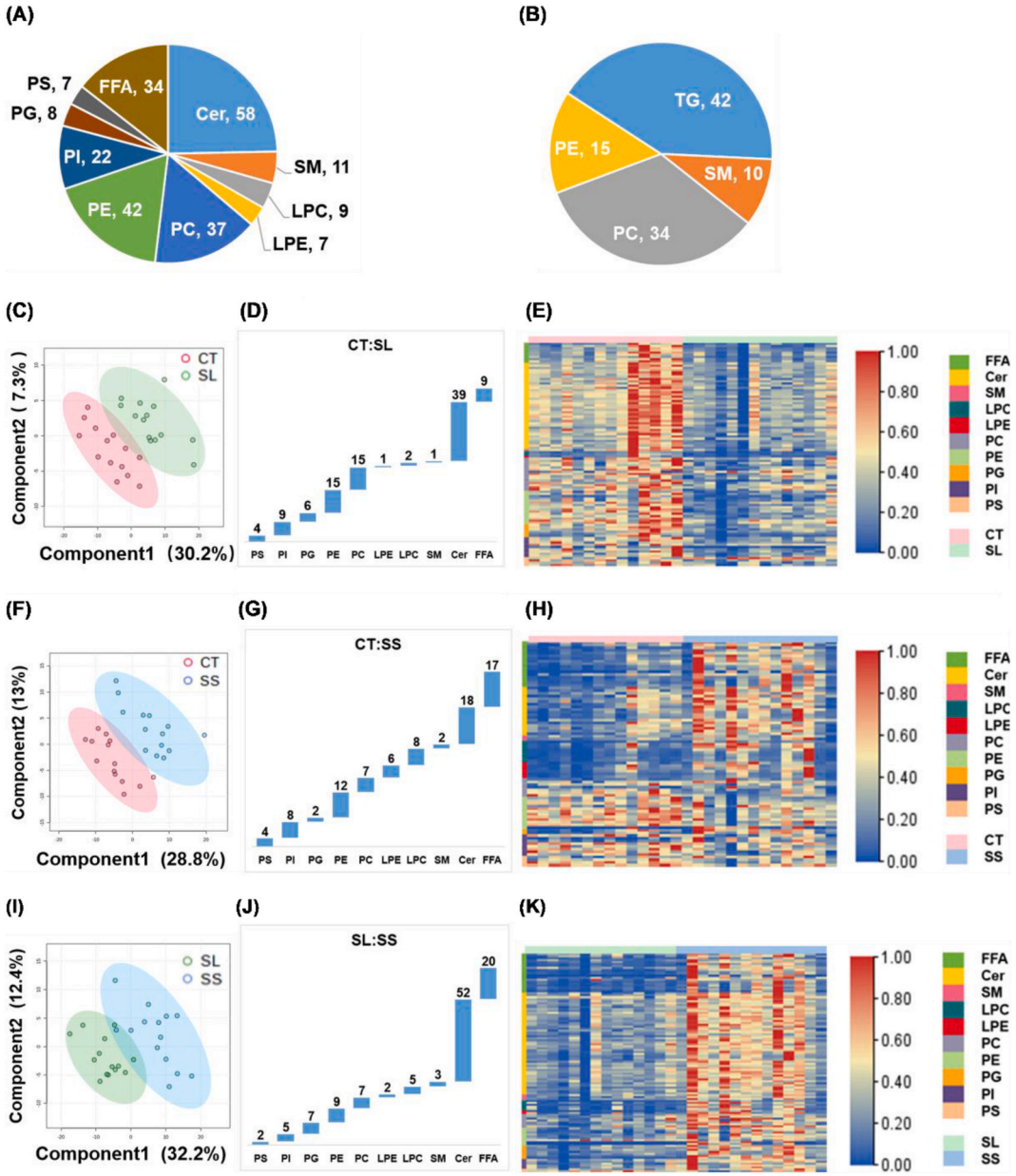

Fig. 2. Comparisons of chuck tender (CT), sirloin (SL) and silverside (SS) beef lipid profiles. Total lipid molecular species detected in: (A) negative ion mode, (B) positive ion mode. (C) PLS-DA score plot comparing CT and SL. (D) Number of significantly different lipid molecular species in lipid classes between CT and SL. (E) Heatmap of significantly different lipid molecules between CT and SL. (F) PLS-DA score plot comparing CT and SS. (G) Number of significantly different lipid molecular species in lipid classes between CT and SS. (H) Heatmap of significantly different lipid molecules between CT and SS. (I) PLS-DA score plot comparing SL and SS. (J) Number of significantly different lipid molecular species in lipid classes between SL and SS. (K) Heatmap of significantly different lipid molecules between SL and SS. (n = 14). FFA: free fatty acid, TG: triglyceride, Cer: ceramide, SM: sphingomyelin, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PG: phosphatidylglycerol, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine.

(18:1), (18:2) and (20:4) FFAs accounted for 88.18 %–90.57 % of the total FFA in the three beef slices; these fatty acids are the main free fatty acids. The total MUFA and oleic acid contents differed among the three beef slices, and sirloin had the lowest total MUFA and oleic acid content ($p < 0.05$, Fig. 4A and B). The total PUFA content, and those of the (18:2), (18:3), (20:2), (20:3), (20:4), (20:5), (22:4), (22:5) and (22:6) FFAs in chuck tender and sirloin, were similar, and lower than silverside ($p < 0.05$, Fig. 4A–E). The total PUFA contents in chuck tender, sirloin and silverside were 21.05 %, 16.09 % and 29.10 %, respectively. That is, the free fatty acid content in silverside was relatively high.

Overall, the phospholipid and FFA contents, especially those of unsaturated fatty acids, differed significantly among chuck tender,

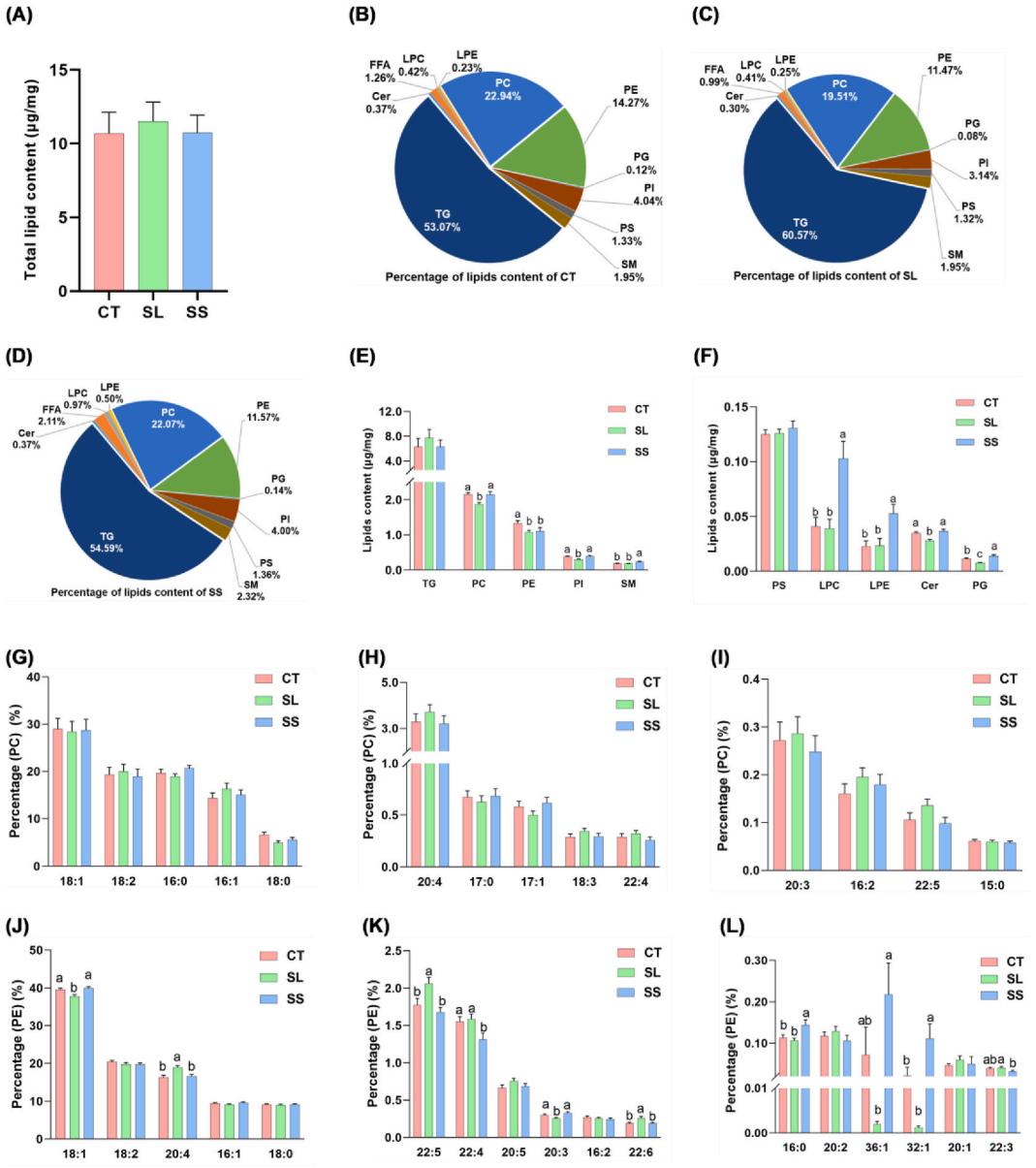

Fig. 3. Quantitative comparisons of lipid classes in chuck tender (CT), sirloin (SL) and silverside (SS) beef. (A) Total lipid content, (B–D) Percentage of each lipid class. (E–F) Lipid class content. Percentage of each fatty acid in: (G–I) PC, (J–L) PE. Letters represent significant differences from one-way ANOVA with $p < 0.05$. (n = 14).
FFA: free fatty acid, TG: triglyceride, Cer: ceramide, SM: sphingomyelin, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PG: phosphatidylglycerol, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine.

sirloin and silverside, indicating that these differences could affect their instant-boiling volatile profiles.

3.4. Aroma-active compound profiles of instant-boiled beef differed among chuck tender, sirloin and silverside

To elucidate the differences in volatile compound composition among the three beef slices, volatilomics, based on SPME-GC-O-MS was performed. Eight compound classes, including 22 aldehydes, 11 ketones, 12 alkanes, 8 heterocyclic compounds, 6 alcohols, 4 acids, 7 esters, and 4 sulfur compounds were identified (Fig. 5A, Table S4). PLS-DA clearly distinguished the volatile profiles of the three beef slices, indicating differences in volatile profiles (Fig. 5B–D). GC-O analysis showed that 12 of the 74 volatile compounds had significant aroma activity (Table S4). The concentrations of these 12 compounds were determined from calibration curves of authentic standards, which had good linearity ($R^2 > 0.99$; Table 2). Of these compounds, hexanal had the highest concentration in the three beef slices, followed by acetoin, octanal, nonanal and 1-octen-3-ol. The concentrations of hexanal, octanal, nonanal, 1-octen-3-ol, (E)-2-

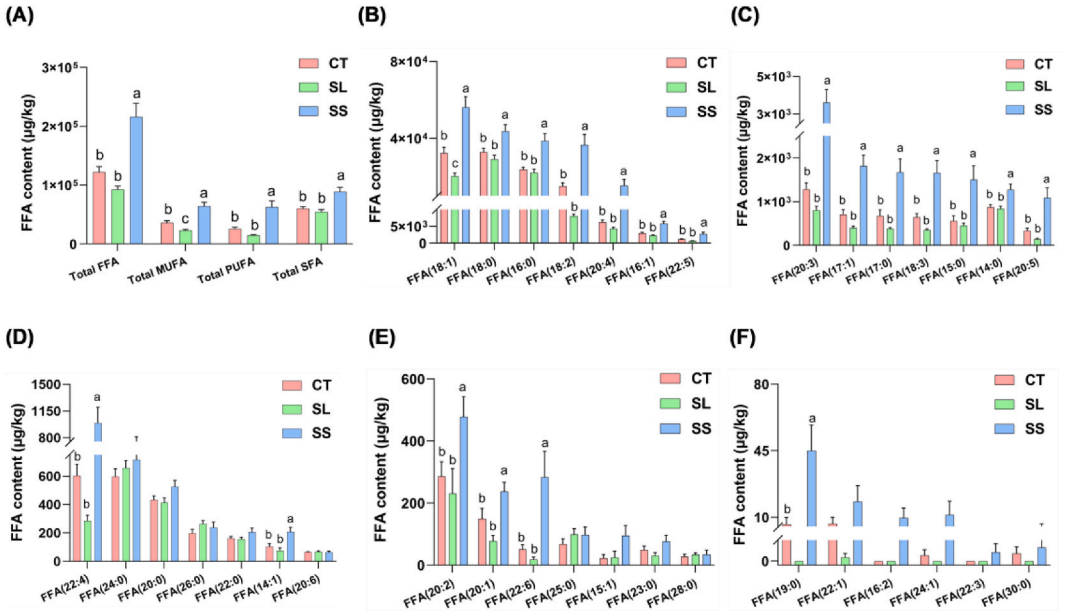

Fig. 4. Comparisons of free fatty acid (FFA) contents in chuck tender (CT), sirloin (SL) and silverside (SS) beef. (A) Contents of total FFA, total MUFA, total PUFA and total SFA in CT, SL and SS. (B–F) Contents of FFA in CT, SL and SS. Letters represent significant differences from one-way ANOVA with $p < 0.05$. (n = 14) MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, SFA, saturated fatty acid.

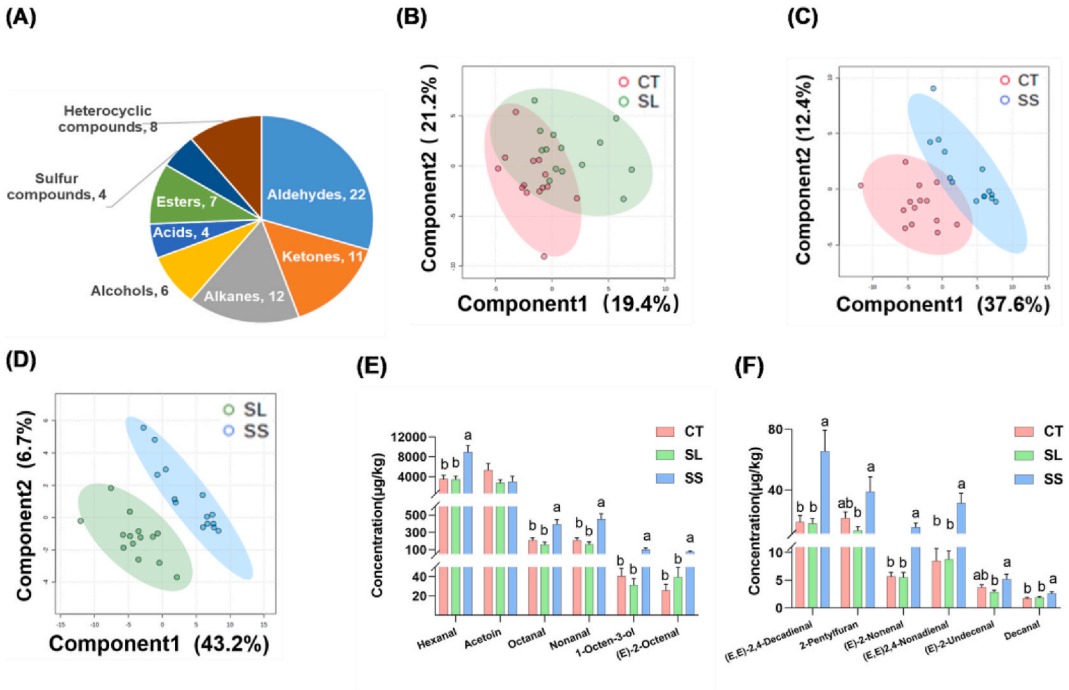

Fig. 5. Comparisons of volatile aroma compound profiles of instant-boiled chuck tender (CT), sirloin (SL) and silverside (SS) beef. (A) Numbers of volatile compounds identified. PLS-DA score plots between: (B) CT and SL, (C) CT and SS, (D) SL and SS. (E–F) Concentrations of aroma-active compounds from instant-boiled chuck tender (CT), sirloin (SL) and silverside (SS) beef. Letters represent significant differences from one-way ANOVA with $p < 0.05$. (n = 14).

octenal, (E,E)-2,4-decadienal, (E)-2-nonenal, (E,E)-2,4-nonadienal and decanal in chuck tender and sirloin were similar, and significantly lower than in silverside ($p < 0.05$; Fig. 5E and F). The contents of 2-pentylfuran and (E)-2-undencenal were significantly lower in sirloin than in silverside ($p < 0.05$; Fig. 5E and F).

Table 2
Calibration equations of aroma-active compounds in instant-boiled chuck tender, sirloin and silverside beef. (n = 14).

Compounds[a]	Ions[b](m/z)				Calibration equations[c]	R^2	Validation range (μg/kg)
Hexanal	67.0542	44.0256	57.0334	82.0777	y = 16.024x-22.477	0.9920	196.44–13550.91
Octanal	81.0699	69.0698	67.0542	95.0855	y = 6.3422x+1.3749	0.9908	18.89–3254.76
Nonanal	81.0699	95.0855	67.0542	70.0777	y = 1.4308x+0.0147	0.9977	10.96–1606.67
Decanal	81.0699	95.0855	67.0542	109.1011	y = 0.5425x+0.1195	0.9951	1.11–155.47
(E)-2-Octenal	83.0492	67.0542	39.0229	93.0698	y = 12.65x+0.1242	0.9924	3.78–623.56
(E)-2-Nonenal	83.0492	55.0542	93.0698	111.0804	y = 3.5166x+0.1186	0.9901	1.97–305.17
(E)-2-Undecenal	83.0492	67.0542	55.0542	82.0777	y = 3.5562x+0.2896	0.9902	0.76–318.27
(E,E)-2,4-Nonadienal	81.0336	41.0385	79.0542	95.0491	y = 14.813x+0.2578	0.9949	3.11–310.94
(E,E)-2,4-Decadienal	81.0336	67.0542	95.0491	123.0804	y = 7.5677x+0.3492	0.9961	0.77–307.48
1-Octen-3-ol	57.0334	81.0698	85.0647	99.0804	y = 3.1267x+0.1227	0.9970	1.67–620.93
2-Pentylfuran	81.0336	94.0777	55.0385	138.1039	y = 0.1547x-0.0486	0.9950	1.59–404.41
Acetoin	45.0335	42.0100	43.0178	88.0518	y = 131.71x+4.5995	0.9950	158.08–12928.43

[a] Aroma-active compounds detected in instant-boiled beef.
[b] Monitored ions used for quantification.
[c] The variables are represented as the ratio of the peak area (x) or the concentration (y) of the compounds to 2-methyl-3-heptanone.

To evaluate the contributions of these 12 compounds to the overall aroma, the OAVs were calculated, i.e., the ratio of the concentration of each compound to its odor threshold in water (Gemert, L.J. van, 2011). The relative OAV contributions are summarized in Table 3. The highest OAV in chuck tender was for hexanal (1458), followed by (E,E)-2,4-decadienal (696), acetoin (382) and octanal (357). The highest OAV in sirloin was for hexanal (1453), followed by (E,E)-2,4-decadienal (664). The OAVs in silverside of hexanal (3737), (E,E)-2,4-decadienal (2425) and octanal (672) were the highest of the beef slices. Of the 12 aroma compounds, hexanal (42.73%–46.95 %), (E,E)-2,4-decadienal (20.41%–28.64 %) and octanal (7.94%–10.45 %) accounted for 73.59%–80.72 % of the OAV contributions, making them the major contributors to the aroma of instant-boiled beef. The OAVs of aroma-active compounds varied among the three beef slices and the concentrations differed significantly ($p < 0.05$), which may lead to changes in the characteristic flavor of instant-boiled beef.

3.5. *Unsaturated FFAs and PGs might be crucial in flavor differences of instant-boiled chuck tender, sirloin and silverside beef*

Lipids generally make strong contributions to aroma formation, so their effects on aroma differences among the three beef slices were evaluated (Fig. 6). Pearson correlation analysis was performed to identify the lipid classes associated with the 12 aroma compounds with the highest OAVs. The FFA and PG classes significantly positively correlated hexanal, octanal, nonanal, decanal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, and 1-octen-3-ol ($p < 0.05$, Fig. 6A). The molecules of PC, PE, PG and FFA were further analyzed as major phospholipid classes or classes with higher correlation coefficients (Fig. 6B–J). PCs containing the fatty acids including (15:0, 18:2), (16:1, 20:4), (18:0, 20:3), (18:0, 20:4), (18:2, 18:2) and (18:2, 22:6) positively correlated with hexanal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, 1-octen-3-ol and 2-pentylfuran when compared between chuck tender and sirloin ($p < 0.05$, Fig. 6B). In comparison between chuck tender and silverside, and sirloin and silverside, PCs containing the fatty acids including (16:0, 16:0), (16:1, 18:1), (17:0, 18:2) and (18:0, 16:0) positively correlated with hexanal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal and 1-octen-3-ol ($p < 0.05$, Fig. 6C and D). PEs containing the fatty acids including (16:1e, 20:4), (16:1e, 22:6), (18:1e, 20:2), (18:1e, 20:4),

Table 3
OAVs (Odor active values) of aroma-active compounds in instant-boiled chuck tender, sirloin and silverside beef. (n = 14).

Compounds[a]	Thresholds[b] (μg/L)	odors[c]	OAVs[d]			Contribution rates[e] (%)		
			chuck tender	sirloin	silverside	chuck tender	sirloin	silverside
Hexanal	2.4	grassy, green	1458	1453	3737	42.73 %	46.95 %	44.14 %
Octanal	0.59	fruity, fresh, green	357	270	672	10.45 %	8.74 %	7.94 %
Nonanal	1.1	citrus	190	148	410	5.57 %	4.78 %	4.84 %
Decanal	0.19	citrus, fresh	9	10	13	0.28 %	0.32 %	0.16 %
(E)-2-Octenal	0.34	meaty, fatty	75	116	217	2.21 %	3.76 %	2.57 %
(E)-2-Nonenal	0.08	green, fresh	71	69	192	2.09 %	2.24 %	2.27 %
(E)-2-Undecenal	0.78	green, grassy, smoky	5	4	7	0.14 %	0.12 %	0.08 %
(E,E)-2,4-Nonadienal	0.062	fatty, nutty, almond	138	141	504	4.03 %	4.56 %	5.95 %
(E,E)-2,4-Decadienal	0.027	fatty, deep fried	696	664	2425	20.41 %	21.45 %	28.64 %
1-Octen-3-ol	1.5	milky, mushroom	27	21	66	0.79 %	0.67 %	0.78 %
2-Pentylfuran	5.8	fruity	4	2	7	0.11 %	0.07 %	0.08 %
Acetoin	14	butter	382	196	216	11.19 %	6.34 %	2.55 %

[a] Aroma-active compounds detected in instant-boiled beef.
[b] Odor thresholds in water from Ref. [21].
[c] Evaluator's odor descriptions.
[d] Odor active values.
[e] Contribution rates of OAVs.

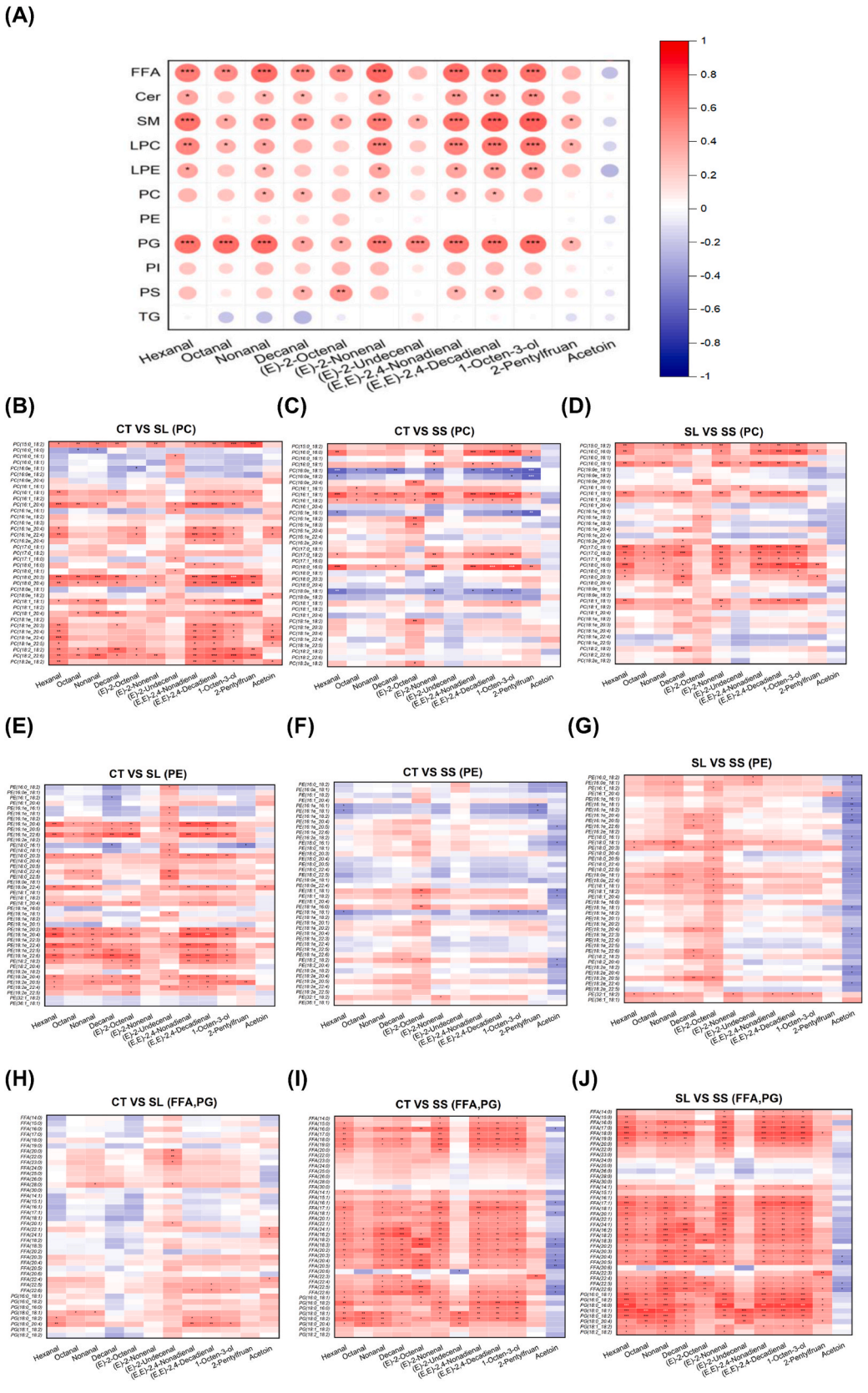

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Fig. 6. Pearson correlation analysis between muscle lipid content and aroma-active compounds from instant-boiled chuck tender (CT), sirloin (SL) and silverside (SS) beef. Correlation coefficient heatmaps between: (A) lipid class contents and aroma-active compound concentrations from CT, SL and SS; PC and aroma-active compound concentrations from (B) CT and SL, (C) CT and SS, (D) SL and SS; PE and aroma-active compound concentrations from (E) CT and SL, (F) CT and SS, (G) SL and SS; FFA and PG with aroma-active compound concentrations from (H) CT and SL, (I) CT and SS, (J) SL and SS. *, ** and *** denote $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. (n = 14).
FFA: free fatty acid, TG: triglyceride, Cer: ceramide, SM: sphingomyelin, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PG: phosphatidylglycerol, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine.

(18:1e, 22:4) and (18:1e, 22:6) positively correlated with hexanal, decanal, (E)-2-octenal, (E,E)-2,4-nonadienal and (E,E)-2,4-decadienal when compared between chuck tender and sirloin ($p < 0.05$, Fig. 6E). PGs containing the fatty acids (16:0, 18:1), (16:0, 18:2), (18:0, 16:0), (18:0, 18:1), (18:0, 18:2) and (18:0, 20:4) positively correlated with hexanal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal and 1-octen-3-ol, in comparisons between chuck tender and silverside, and sirloin and silverside ($p < 0.05$, Fig. 6I and J). Unsaturated FFAs positively correlated with hexanal, nonanal, decanal, (E)-2-octenal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal and 1-octen-3-ol, when compared between chuck tender and silverside, and sirloin and silverside ($p < 0.05$, Fig. 6I and J). The contents of unsaturated FFAs, and PGs with unsaturated fatty acid residues, correlate strongly with those of aroma-active compounds, and might have a strong influence on the flavor differences among instant-boiled chuck tender, sirloin and silverside.

4. Discussion

Meat, especially beef, is differentiated by the various commercial cuts sold to consumers at a wide range of prices. Differences in the meat quality traits of beef cuts are related to their muscle fiber composition, motility and enzyme activity [22]. After slaughter, meat undergoes a period of aging, during which muscle fibers break down and adenosine triphosphate (ATP) is degraded, resulting in production of the taste compound inosine monophosphate (IMP) [23]. The hemoglobin concentration is the main contributor to the color differences among the various beef cuts after slaughter [24]. If the a* (red/green balance) value is > 14.5 (high redness), there is a 95 % probability that consumers will consider beef to be acceptable [25]. The chuck tender, sirloin and silverside cuts from Huaxi cattle all have high redness, so they should have high consumer acceptance (Table 1). The higher meat muscle fiber density could improve tenderness and appears to be associated with decreased shear force [22,26]. Therefore, the highest shear force of sirloin, of the three beef cuts, is affected with its lowest muscle density.

Sirloin (*M. Longissimus dorsi*) had a lower phospholipid content than chuck tender (*M. Supraspinatus*) and silverside (*M. Biceps femoris*). Beef is an important source of dietary phospholipids, and increasing the dietary intake of beef phospholipids may be beneficial for preventing diseases such as obesity and diabetes [27]. A comparison of the lipid contents of different beef cattle muscles [28] found that the total phospholipid content of buffalo *Longissimus dorsi* was lower than that of *Triceps brachii* or *Biceps femoris.* The ratio of polyunsaturated to saturated fatty acids, which reflects the phospholipid levels, was higher in *Supraspinatus* than in *Bicep femoris* and *Longissimus thoracis* in Thai cattle [29]. The *Psoas major* had higher levels of PC and total phospholipid than *Longissimus dorsi* and *Longissimus thoracis* from various breeds of pasture-fed cattle [30]. Differences in the phospholipid content of muscle fibers are related to fiber diameter, fiber density, and the abundance of organelles [31]. Higher muscle fiber density of the red-oxidative muscle type results in a higher membrane content and therefore, phospholipid content than the white-glycolytic type [31]. In our study, sirloin had a lower a* and less fiber density than chuck tender and silverside, which is consistent with the above reports.

Phospholipid levels are relatively suppressed when the proportion of neutral lipid increases. As the cattle grow, the enlargement and proliferation of adipocytes increases the intramuscular triglyceride and total lipid content, whereas the phospholipid, as a structural component of the cell, makes a relatively diluted contribution to intramuscular fat [32]. Intramuscular triglyceride and total lipid contents in *Longissimus, Semitendinosus, Triceps brachii* and *Rectus abdominis* were also been found increased linearly with carcass fat content, whereas phospholipids did not increase [33]. Therefore, the highest percentage of TG (60.57 %, Fig. 3C) in sirloin could have influenced the phospholipid levels. PC and PE are the main source of unsaturated fatty acids in beef [34,35]. The PC profiles of skeletal muscles appear to be related to muscle location [36]. In chuck tender, sirloin and silverside, we found the unsaturated fatty acid composition of PE changed significantly, suggesting that, the contribution of PE fatty acids to lipid oxidation and flavor production in beef slices are influenced by variations in fatty acid composition. In addition, sirloin had the highest percentage of arachidonic acid (20:4), which is much more susceptible to oxidation than oleic (18:1) and linoleic (18:2) acid [5]. The highest FFA content of silverside is associated with lipase activity. Muscle can obtain energy by degrading lipids into FFAs, which are then metabolized by β-oxidation in the mitochondria [37]. Differences in lipase activity result in variations of FFA content with muscle type [37,38]. Sirloin had the lowest phospholipid content and free polyunsaturated fatty acid proportion, suggesting that the FFA profiles might be associated with the composition of total lipid. Therefore, the differences in phospholipid and FFA content in chuck tender, sirloin and silverside might be associated with the differences in muscle fibers, adipocytes and lipase activity.

The volatile profile of beef is composed of perceptible aroma-active components and imperceptible aroma-inactive components. The aldehydes in instant-boiled beef have low olfactory thresholds and made a large flavor contribution, consistent with previous reports on cooked beef [39–41], providing green, fruity and fatty aromas [42–44]. The aldehydes in instant-boiled beef are the most numerous of the aroma-active compounds and are all lipid-derived compounds [5,45]. In the presence of active conditions (e.g. high temperature), oxygen attacks the methylene sites near the unsaturated bonds of fatty acids, cleaving them to form hydroperoxides, which are susceptible to cleavage to form stable non-radical products such as aldehydes, alcohols and alkanes [46]. In our study, chuck tender, sirloin and silverside phospholipids contained a high percentage of oleic (18:1) and linoleic acid (18:2). Oxidation of oleic acid produces C8 to C11 hydroperoxides that are susceptible to cleavage, generating saturated aldehydes, whereas linoleic acid produces C9

and C13 hydroperoxides that generate enals, dienals, and short-chain saturated aldehydes [5,47]. In our study, hexanal, (E,E)-2, 4-decadienal and octanal were the key volatile compounds responsible for the aroma of instant-boiled beef. Hexanal and (E,E)-2, 4-decadienal are considered to be characteristic of the flavor of cooked beef [41]. An increase in n-3 fatty acids increased the total content of saturated and unsaturated aldehydes in a meat-like model system, and the hexanal, (E,E)-2,4-decadienal and (E)-2-octenal content increased with increased n-6 fatty acid content [48]. Similar methods found that the contents of octanal and nonanal increase with increasing content of n-9 fatty acids [49]. 1-Octen-3-ol provides a mushroom flavor to instant-boiled beef, in agreement with previous studies, whereas 2-pentylfuran, with a lower OAV, was perceived only as a fruity flavor, rather than the beany flavor found by previous researches [11,50]. Previous studies have shown that the contents of 1-octen-3-ol and 2-pentylfuran were significantly promoted ($p < 0.05$) by increased n-6 fatty acids in beef, which are probably produced from n-6 fatty acids, especially linoleic acid (18:2) [48,51]. Acetoin provides a butter flavor [52,53]. It is thought to be present in fruits, cheese and cooked meat [54,55]. Although studies have shown that animal synthesis is a natural source of acetoin, the underlying mechanisms in mammal are not clear [54]. The effect of different slices on acetoin in the present study was not significant, and its precursor and interactions with other precursors of volatile flavor compounds in beef slices require further studies to determine.

As mentioned above, phospholipids are the main source of unsaturated fatty acids, so the fatty acids from phospholipids should contribute to beef flavor. Furthermore, the percentage of unsaturated fatty acid in beef phospholipids changes after cooking [56]. Removing triglycerides from beef before cooking has less of an effect on volatile flavor compound generation than removing both phospholipids and triglycerides [57]. It has been suggested that the abundance of PC, PE, PG and PI decreased with increasing temperature in cooked chicken, suggesting that phospholipids are hydrolyzed during cooking [58]. Similarly, PC and PE molecules were reported to be important substrates for the formation of volatile flavor compounds and were significantly correlated with the key aroma compounds (OAV>1) in grilled lamb [59]. PG (18:1_18:1) was reported to show the most significant changes with roasting time in chicken meat and could be a potential biomarker for chicken meat with different roasting times [60]. Hexanal is regarded as a measure of lipid peroxidation [61]. Previously, in a study based on phospholipidomics and GC-O-MS, PG (18:1_18:2) and PG (18:2_18:2) were found to be strongly correlated with hexanal in different cuts of pork [62]. These studies suggest that phospholipids might be involved in oxidation reactions during instant-boiling, resulting in their strong contribution to generation of lipid-derived aroma-active compounds. FFAs contribute strongly to flavor production in beef [37]. It has been reported that during the freeze-drying process, free fatty acids in the beef may promote the formation of aldehydes such as hexanal, octanal and nonanal from the beef jerky [63].

Overall, these findings indicate that the phospholipid and FFA content of beef slices change much more extensively than the content of other lipid species during instant-boiling, and that PGs with unsaturated fatty acid residues and unsaturated FFAs might be crucial in flavor differences of three beef slices. However, little is known about the molecular mechanisms of these changes. In addition, the effect of changes in the aroma-active compound profile on sensory evaluation has not been determined. Further research will be needed to elucidate these last two issues.

5. Conclusions

In this study, the physicochemical characteristics, lipid profiles and instant-boiling volatile profiles of chuck tender, sirloin and silverside beef were investigated. Sirloin had a lower cooking loss and a higher shear force than chuck tender and silverside. There was no difference in intramuscular fat among chuck tender, sirloin and silverside. Lipidomic analysis identified 336 lipid molecular species, and clear separations were obtained among chuck tender, sirloin and silverside using PLS-DA analysis of their lipid profiles. Sirloin had lower PC, PI, PG and Cer content than chuck tender and silverside. Chuck tender had the highest PE content, and there was no difference in PE between the sirloin and silverside. The free fatty acid contents differed among the three different beef slices. Volatilomics showed that 12 of the 74 volatile compounds identified were aroma-active compounds. Of these, OAVs indicated that hexanal, (E,E)-2,4-decadienal and octanal were the most aroma-active compounds. The concentrations of these three compounds were the highest in silverside. Unsaturated free fatty acids and PGs with unsaturated fatty acid residues positively correlated with most of the aroma-active compounds, so the former appear to have a strong influence on the flavor differences among beef slices. Overall, these findings advance our understanding of the relationship among the flavor of instant-boiled chuck tender, sirloin and silverside beef, their endogenous lipids and the aroma-active compounds derived from them. This deeper understanding should help to promote the development of beef processing from the perspective of aroma.

Ethical statement

All experimental animal procedures were approved by the Animal Care and Use Committee of the Institute of Animal Sciences of the Chinese Academy of Agricultural Sciences (CN) with permission number No. IAS 2024–104. All participants gave informed written consent for questionnaire data to be used for collection, analysis and publication. Furthermore, the studies were conducted in accordance with the Declaration of Helsinki.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Yimeng Ren: Writing – original draft, Investigation, Formal analysis, Data curation. **Longzhu Zhou:** Investigation, Data curation. **Yujie Shi:** Investigation. **Yanan Yu:** Methodology. **Weihai Xing:** Validation, Methodology. **Qingyu Zhao:** Project administration. **Junmin Zhang:** Supervision. **Yueyu Bai:** Supervision. **Jing Li:** Writing – review & editing, Formal analysis. **Chaohua Tang:** Writing – review & editing, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36382.

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