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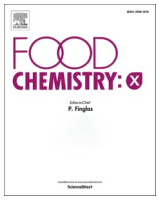

Investigation on the pro-aroma generation effects of fatty acids in beef via thermal oxidative models

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ABSTRACT

Fatty acids (FAs) in lipids are important precursors for the formation of meat aroma compounds. However, the specific roles of individual FAs and related reactions in beef aroma formation remain unclear. This study established thermal oxidation models to investigate the impact of different FAs on the formation of beef aroma compounds. The results revealed that thermal oxidation of seven FAs with different saturation produced 42 aroma compounds. Among them, unsaturated fatty acids (UFAs) participating in thermal oxidation degradation is the primary pathway for aroma compound formation, and the types of aroma compounds produced by C18:2n6 and C20:4n6 are similar. The addition of UFAs to lipid-free beef induces lipid oxidation–Maillard reaction interactions, producing more thiophenes, thiazoles, and pyridines, such as 2-acetyl-3-methylthiophene and 2-pentylpyridine, etc. The key aroma compounds in beef with odor characteristics such as fruity, green, fatty or milky are mainly produced by C18:1n9, C18:2n6 and C20:4n6.

1. Introduction

All foods are complex mixtures consisting of various components such as amino acids, carbohydrates, and lipids. During the cooking process, the interactions and reactions among these components typically determine the flavor of the food through the production of both volatile and non-volatile compounds. Flavor is a critical sensory characteristic of meat, influencing its quality and acceptability. The aroma of meat is primarily determined by volatile compounds generated through lipid oxidation and degradation, the Maillard reaction between amino acids and reducing sugars, as well as Maillard–lipid interaction reactions (Man et al., 2023). The Maillard reaction tends to occur under high-temperature conditions, producing S-containing compounds, and N- or O-containing heterocyclic compounds, which are abundant in grilled and fried foods (Ajandouz et al., 2008; Liu et al., 2022). Lipids can oxidize to form hydroperoxides under heat, which then undergo a series of reactions to produce aldehydes, ketones, alcohols, acids, esters, and alkyl furans (Mottram, 1998). Additionally, numerous studies have found that the products of lipid oxidation and degradation can influence meat aroma by interacting with amino acids or intermediate products of the Maillard reaction (Elmore & Mottram, 2000; Xia et al., 2021). Wei et al. (2020) discovered that adding chicken fat to the Maillard reaction can yield more fatty aldehydes and alcohols. Among them, lipid oxidative and degradation is considered to be responsible for more than half of the aroma compounds in cooked meat, and these aroma compounds are generally regarded as contributing to the production of species - specific meat aroma (Sohail et al., 2022; Wu et al., 2024).

The fatty acid (FA) profile in lipids serves as the foundation for the generation of aroma compounds. Particularly, unsaturated fatty acids (UFAs), because of their greater number of oxidation breakpoints and lower melting points, exhibit higher oxidative degradation activity compared to saturated fatty acids (SFAs), thus being more prone to oxidation and degradation to form aroma compounds. Wang et al. (2023) reported that the double bonds in UFAs can undergo oxidation

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through three mechanisms: autoxidation, photooxidation, or enzymatic oxidation, with radical chain oxidation reactions being the primary pathway for the formation of aroma compounds during the thermal processing of meat (Abeyrathne et al., 2021; Huang & Ahn, 2019). Although extensive literature has explored the relationship between lipid/FAs in meat and aroma compounds (Liu et al., 2024; Man et al., 2023; Zhou et al., 2023), due to the complexity of meat matrix, most of the current studies are indirect studies through correlation, and the studies on the specific role and mechanism of UFAs in the formation of aroma compounds are still insufficient.

In the previous study on the relationship between beef lipid and aroma, we noted that the thermal oxidative degradation of FAs is closely related to the generation of beef aroma (Zhou et al., 2024). Based on previous research findings, this study chose the beef matrix as the subject to investigate and simulate the simple thermal processing procedure for meat. By simplifying the complex matrix, three groups of thermal oxidation reaction models were constructed to study the generated aroma compounds: single FA, lipid-free beef (LFB) + FA, and beef + FA, with FAs selected being the main SFAs, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) found in beef. The objectives of this study are (i) to identify the aroma compounds that each FA can produce and their mechanisms and (ii) to explore the specific roles of each FA in the generation of aroma compounds in beef matrix. The results of this study will contribute to a deeper understanding of the formation mechanisms of meat flavor substances, providing a scientific basis for food processing and flavor regulation.

2. Materials and methods

2.1. Materials

All animal procedures conducted in this study were approved by the Inspection Form of Experimental Animal Welfare and Ethical of Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China), with permit number IAS 2024–104. Ten male Huaxi cattle aged 20–24 months were raised at the experimental cattle facility and subsequently transported to the designated slaughterhouse. They were slaughtered according to the standards required by the China Council on Animal Care, that is, the animals were stunned, bled, their internal organs were removed, and they were cut along the midline of the carcass. Afterward, cattle carcasses were drained of acid at 0–4 °C for 72 h. The samples of longissimus dorsi muscle of Huaxi cattle were collected and stored at −80 °C.

2.2. Chemicals

The FA standards, which comprised palmitic acid (C16:0), stearic acid (C18:0), (7*Z*)-palmitoleic acid (C16:1n7), (9*Z*)-oleic acid (C18:1n9), (9*Z*, 12*Z*)-linoleic acid (C18:2n6), (9*Z*, 12*Z*, 15*Z*)-linolenic acid (C18:3n3), (5*Z*, 8*Z*, 11*Z*, 14*Z*)-arachidonic acid (C20:4n6), methyl undecanoate, and fatty acid methyl esters (FAME) standard mixture were procured from Sigma-Aldrich (Shanghai, China). For the analysis of aroma compounds, the standards including pentanal, hexanal, nonanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*, *E*)-2,4-decadienal, benzaldehyde, hexanol, 1-octen-3-ol, 1-octen-3-one, acetoin, 2-acetyl-2-thiazoline, and 3-methylthiopropanal were obtained from Aladdin Biochemical Technology Co., Ltd. Furthermore, (*E*)-2-undecenal, 2-butylfuran, and 2-pentylfuran were sourced from Macklin Biochemical Technology Co., Ltd. Octanal, 2-methyl-3-heptanone, and *n*-alkanes (C7–C40) were supplied by Sigma-Aldrich (Shanghai, China). Methyl-*tert*-butyl ether was purchased from Aladdin Biochemical Technology Co., Ltd. Lastly, methanol and dichloromethane were acquired from Merck (Darmstadt, Germany).

2.3. Preparation of samples

Ten beef samples were uniformly mixed under liquid-nitrogen freezing conditions for subsequent experiments. The preparation of LFB matrix was as follows: Step (1): Referring to the method of extracting lipids with dichloromethane used by Li et al. (2020), appropriate adjustments were made. Weigh a certain mass of beef samples, add an appropriate amount of methanol, and homogenize the beef samples in methanol using a tissue pulverizer (SCIENTZ−48, Ningbo Scientz Biotechnology Co., Ltd., China). Add dichloromethane to the homogenized samples and then vortex for 5 min. Add an appropriate amount of water, vortex again for 5 min, and then perform suction filtration on the mixture using a high-pressure vacuum machine to collect the meat samples. The ratio of each substance is sample: methanol: dichloromethane: water = 4:30:60:25 (*w*/*v*/*v*/v). The above operation needs to be repeated once. Step (2): Extract the remaining lipids from the beef samples collected in step (1) with methyl *tert*-butyl ether. Add the corresponding reagents according to the ratio of sample/methanol/methyl *tert*-butyl ether/water = 4:30:100:25 (w/v/v/v). The operation method is the same as that in step (1). This operation needs to be repeated three times. The collected meat samples were freeze-dried and stored in a −80 °C refrigerator. The meat samples were rehydrated before use. As referenced in Zhou et al. (2024), the method of high-performance liquid chromatography was employed to analyze the lipid content in LFB and beef, with the defatting effect illustrated in Fig. S1.

2.4. FA analysis

The FA composition of beef samples was analyzed using a gas chromatograph (GC) with a CP-Sil 88 column (100 m × 0.25 mm i.d., 0.2 μm film thickness) and a flame ionization detector (Agilent 7890 A, Santa Clara, CA, USA). Following a modified method from Li et al. (2023), a 0.5 g beef sample was weighed using methyl undecanoate as an internal standard. The sample was then treated with 2 mL of 0.5 mol/L sodium hydroxide in methanol and heated at 95 °C for 15 min to conduct the saponification reaction. After cooling, 2 mL of 15 % boron trifluoride in methanol was added, heated again at 95 °C for 15 min, and then cooled to carry out the methylation reaction. The mixture was then treated with 4 mL of saturated NaCl solution and centrifuged, and the supernatant was filtered through a 0.22 μm membrane for GC analysis. The instrument settings were configured as follows: the injector temperature was set to 270 °C, and the detector temperature was set to 280 °C. The temperature program involved an initial temperature of 100 °C, maintained for 13 min, followed by a ramp to 180 °C at a rate of 10 °C/min and a hold for 6 min. Subsequently, the temperature was increased to 200 °C at a rate of 1 °C /min and held for 20 min. Finally, the temperature was further increased to 230 °C at a rate of 4 °C/min and held for 10.5 min. Nitrogen was the carrier gas, split ratio was 100:1, and injection volume was 1.0 μL. FAs were identified by matching retention times to FAME standard mixtures.

2.5. Model reactions

Based on the FA analysis results in beef, as seen in section 3.1, the main SFAs, C16:0 and C18:0, MUFAs C16:1n7 and C18:1n9, as well as PUFAs C18:2n6, C18:3n3, and C20:4n6, were selected. Three groups of thermal oxidation models were constructed to simulate the simple thermal processing process of beef. The model groups were as follows. Group (1): 0.015 g of single FAs were thermally oxidized, including C16:0, C18:0, C16:1n7, C18:1n9, C18:2n6, C18:3n3, C20:4n6. Group (2): 3 g of LFB was added with 0.015 g of FAs for thermal oxidation; the specific combinations were LFB, LFB + C16:0, LFB + C18:0, LFB + C16:1n7, LFB + C18:1n9, LFB + C18:2n6, LFB + C18:3n3, and LFB + C20:4n6. Group (3): 3 g of beef was added with 0.015 g of FAs for thermal oxidation, with the main FAs affecting the production of aroma compounds selected, as seen in section 3.3; the specific combinations

were beef, beef + C16:1n7, beef + C18:1n9, beef + C18:2n6, beef + C18:3n3, and beef + C20:4n6. Each sample was placed in a 20 mL headspace vial and heated in a 100 °C water bath for 30 min. After heating, the samples were cooled to room temperature with running water and then subjected to instrumental testing.

2.6. Gas chromatography–olfactometry–mass spectrometry (GC–O–MS) analysis

The GC–O–MS analysis was performed using a Q–Exactive GC–Orbitrap–MS (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an olfactory detection port (ODP) 3 sniffing device and a VF WAX capillary column (60 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, CA, USA) to determine the aroma compounds in each sample across three model groups. The testing method and instrument conditions were adapted from the study by Li et al. (2022) with minor modifications. Prior to heating, 10 μL of an internal standard solution (2-methyl-3-heptanone, 0.01 μg/μL in methanol) was added to groups (2) and (3), followed by sealing the vials and heating them in a water bath at 100 °C for 30 min. At post-heating, the samples were incubated at 55 °C for 20 min. Headspace solid–phase microextraction (HS–SPME) using a DVB/CAR/PDMS fiber (divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA, USA) was performed at 55 °C for 40 min. The extracted substances were desorbed at 250 °C in the injector for 3 min, and the desorbed compounds were split 1:1 between the mass spectrometer and ODP 3 for analysis. The mass spectrometer was configured to operate in full scan mode, covering a mass-to-charge (m/z) ratio from 30 to 400. The temperature of the ODP 3 was adjusted to 90 °C.

The identification of aroma compounds was carried out using the NIST mass spectrometric database, linear retention indices (LRI), sensory olfactometric determination, and flavor standards. The LRI of aroma compounds was calculated based on the retention times of the homologous series of *n*-alkanes (C7–C40). GC–O analysis involved selecting sensory members trained with pre-processed samples and standard aromatic compounds to analyze the effluents at ODP 3. When the aroma was detected, the sniffing members were required to record the odor characteristics, intensity, and duration, with the odor intensity being scored on a 4-point scale where "1" indicated very weak and "4" indicated very strong, and matched with the retention times of flavor compounds during data analysis. The same GC–O–MS method was used to analyze the standard flavor compounds, and their LRI and ion fragments were compared with those of the aroma compounds in the sample to accurately verify the aroma compounds. Semi-quantification was carried out using the internal standard solution, and the accurate quantification of aroma compounds identified by GC–O was carried out by the external standard method using the flavorless matrix and flavor standards with reference to the method of (Zhou et al., 2024). The odor activity value (OAV) was calculated by dividing the concentration of accurately quantified aroma compounds by the odor perception threshold of these compounds in water. The thresholds of aroma compounds in water were referred to Gemert (2011).

2.7. Data analysis

All experiments were performed in triplicate, with the experimental data presented as "mean ± standard deviation". Statistical analysis was conducted using one-way ANOVA via IBM SPSS 20 (IBM Corp., Armonk, NY, USA), with $p < 0.05$ adopted as the threshold for significant differences. Heatmaps were generated using Hiplot Pro (https://hiplot.com.cn/), bar charts were produced with GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA), and bubble plots and link fill bars were created using Bioinformatics (https://www.bioinformatics.com.cn/).

3. Results and discussion

3.1. FA composition of beef

In this study, the FA composition of Huaxi cattle muscle was analyzed using GC. The results indicated the following (Fig. 1A–C). The total content of SFAs was 0.690 ± 0.004 g/100 g, with predominant C16:0 (0.366 ± 0.001 g/100 g) and C18:0 (0.264 ± 0.003 g/100 g). The total content of MUFAs was 0.546 ± 0.004 g/100 g, mainly comprising C18:1n9c (0.474 ± 0.004 g/100 g) and C16:1n7 (0.040 ± 0.000 g/100 g). The total content of PUFAs was 0.103 ± 0.000 g/100 g, with the contents in descending order being C18:2n6c (0.064 ± 0.000 g/100 g), C20:4n6 (0.023 ± 0.001 g/100 g), and C18:3n3 (0.004 ± 0.000 g/100 g), among others. These research results are similar to those of Silva et al. (2016) and Zhu et al. (2017) on the FA composition of beef.

3.2. Aroma compound profile from single FA thermal oxidation

Comprehensive analysis of aroma compounds generated by the thermal oxidation of FAs was conducted using the combined technique of HS–SPME and GC–O–MS. The chromatogram showed that the spectra of each FA after thermal oxidation were different, and that the spectra and peaks produced by UFAs were more abundant (Fig. 2A). A total of 42 aroma compounds, including aldehydes, alcohols, ketones, acids, esters, and furans, were identified as being produced by the thermal oxidation of seven FAs (Table S1). After normalization, a heatmap was constructed (Fig. 2B), indicating that different FAs produced different types of aroma compounds. These differences are not only due to the varying positions of double bonds in UFAs but are also related to the process in which UFAs, under the influence of reactive oxygen species such as hydroxyl radicals and hydroperoxyl radicals, undergo dehydrogenation and oxidation at the α-methylene of their double bonds to produce primary metabolites—hydroperoxides (Aikens & Dix, 1991; Frijhoff et al., 2015). During this process, allylic radicals form, and the electrons on these radicals can delocalize across three carbon atoms, leading to the generation of various allylic hydroperoxides (Huang & Ahn, 2019). Therefore, the oxidation of different FAs can produce a variety of hydroperoxides. This notion is also mentioned in the report by Ahmad et al. (2023). During thermal processing, various hydroperoxides undergo homolytic cleavage to produce alkoxyl and hydroxyl groups, with further breaking of the carbon–carbon bond in the alkoxyl group being the main pathway for the formation of aroma compounds (Frankel et al., 1981). Additionally, the α-scission and β-scission of the alkoxyl group result in different aroma compounds (Zhou et al., 2015) (Fig. 2E). Statistical analysis demonstrated that UFAs produced a greater number of aroma compounds compared to SFAs (Fig. 2D). The contents of aroma compounds produced by C20:4n6, C18:2n6, and C18:3n3 in PUFAs were significantly higher than those produced by C16:1n7 and C18:1n6c in MUFAs ($p < 0.05$), while SFAs C16:0 and C18:0 essentially did not produce aroma compounds (Fig. 2C). This aligns with the content mentioned in the review on meat lipid oxidation by Huang and Ahn (2019), which indicated that the sensitivity of lipids to oxidation depends on the degree of unsaturation of FAs; the more double bonds there are in FAs, the more methylene in double bonds and the more oxidation products are produced.

Statistical analysis was conducted on various compounds generated from the thermal oxidation of single FAs (Fig. 3A–G). Among aldehydes (Fig. 3A and B), C18:1n9 can produce compounds such as heptanal, octanal, nonanal, (*E*)-2-decenal, and (*E*)-2-undecenal. These compounds are mainly formed by the homolytic degradation of four intermediate products, namely 8, 9, 10, and 11-hydroperoxide (Frankel, 1984). Specifically, 8-hydroperoxide can generate octanal and (*E*)-2-undecenal; 9-hydroperoxide or 10-hydroperoxide can produce nonanal or (*E*)-2-decenal; and 11-hydroperoxide can yield heptanal and octanal (Al-Dalali et al., 2022; Varlet et al., 2007; Zhang et al., 2015). The types of compounds produced by C18:2n6 and C20:4n6 are similar, such as

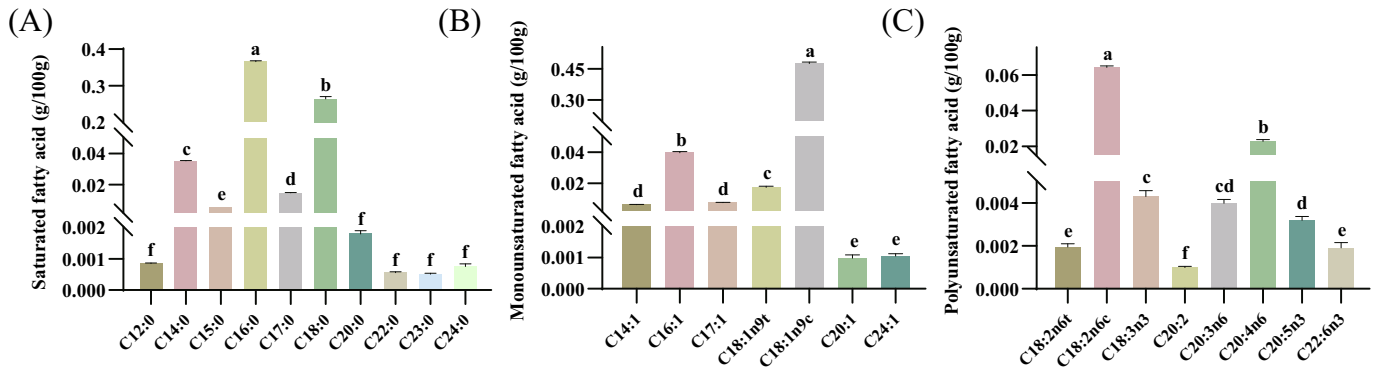

Fig. 1. The composition of fatty acids in beef ($n = 3$). The contents of (A) saturated fatty acids, (B) monounsaturated fatty acids and (C) polyunsaturated fatty acids in beef. Different letters indicate significant differences ($p < 0.05$).

pentanal, hexanal, and 2,4-decadienal. Pentanal and hexanal are mainly derived from the fragmentation of 13-hydroperoxide (from C18:2n6) and 15-hydroperoxide (from C20:4n6) alkyl radicals, while 2,4-decadienal is produced from 9-hydroperoxide (from C18:2n6) and 11-hydroperoxide (from C20:4n6) (Al-Dalali et al., 2022; Varlet et al., 2007; Wu et al., 2021). C18:3n3 mainly produces compounds such as (*E*)-2-pentenal, (*E*, *E*)-2,4-heptadienal, and benzaldehyde. Among them, (*E*, *E*)-2,4-heptadienal can be formed by the degradation of the intermediate 12-hydroperoxide generated from C18:3n3 (Hsieh & Kinsella, 1989). Among the alcohols, pentanol and 1-octen-3-ol were mainly produced by C20:4n6 and C18:2n6, while heptanol was produced by C18:1n9 (Fig. 3C). In terms of the reaction mechanism, alcohols can be produced by the hydroxyl-induced formation of alkyl radicals resulting from the cleavage of carbon-carbon bonds in alkoxyl hydroperoxides. These alcohols can also undergo further oxidation to yield aldehydes or ketones (Fig. 2E). Previous studies have shown that C18:2n6 and C20:4n6 can degrade to produce 1-octen-3-ol (Frankel, 1984; Wu et al., 2021), which is consistent with the results of this study. In the ketones, 2-heptanone, 3-octanone, 3-octen-2-one, and 1-octen-3-one were predominantly produced by C20:4n6 and C18:2n6 (Fig. 3D). Most of the acids, esters, and furans could be produced by C20:4n6, C18:2n6, and C18:3n3, with C20:4n6 and C18:2n6 generating higher amounts of hexanoic acid, 2-butylfuran, 2-pentylfuran, and γ-caprolactone through thermal oxidation; C18:3n3 produced octanoic acid and 2-ethylfuran (Fig. 3E–G). Acids are formed by the retention of the carboxylic acid end upon the cleavage of hydroperoxide alkoxyls (Fig. 2E). Esters are formed by the dehydration condensation of the hydroxyl group (-OH) from alcohols and the carboxyl group (-COOH) from acids, while lactones are heterocyclic compounds produced by the dehydration condensation of the -OH and -COOH within the same molecule, such as hydroxy FAs (Kang et al., 2016) (Fig. 2E). In the study by Ueda et al. (2022), it was found that 4-hydroxyhexanoic acid, which is produced by the cleavage and oxidation degradation of the intermediates 9-hydroperoxide or 13-hydroperoxide generated from C18:2n9, can cyclize to form γ-caprolactone. Furans may be formed by the further internal cyclization reaction of unsaturated aldehydes. Previous research only indirectly suggested that 2-pentylfuran might be produced by C18:2n6 (Benet et al., 2015; Li et al., 2022).

3.3. Aroma compound profiles from LFB with added FAs

Through the addition of FAs with different degrees of saturation to LFB and subsequent thermal processing, a heatmap of the formed aroma compounds was constructed, with the results shown in Fig. 4A. Differences were observed in the enriched aroma compounds among the groups. Compared to the LFB group, the content of aroma compounds produced by thermal oxidation significantly increased ($p < 0.05$) after the addition of C18:2n6, C18:3n3, and C20:4n6, while the content of aroma compounds produced after the addition of C16:0 and C18:0 showed no significant differences ($p > 0.05$) (Fig. 4B). Meanwhile, we also found that the content of aroma compounds produced by beef after defatting decreased significantly (Fig. S2). Gao et al. (2024) obtained similar results, finding that the defatting process may lead to the loss of some volatile substances, and that the addition of fat heating treatment can increase the volatile components. Fig. 4C further reveals that, compared to the LFB group, the LFB + C18:2n6, LFB + C18:3n3, and LFB + C20:4n6 groups had 45, 51, and 73 significantly different aroma compounds ($p < 0.05$) (Table S2). Further statistical analysis revealed that the trend of aroma compound changes produced after FAs were added to LFB was consistent with those generated by the thermal oxidation of individual FAs. Most aroma compounds produced by the thermal oxidation of individual FAs were also generated when the FAs were added to LFB. For example, the LFB + C20:4n6 and LFB + C18:2n6 groups produced increased levels of pentanal, hexanal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*, *Z*)-2,4-decadienal, (*E*, *E*)-2,4-decadienal, 1-octen-3-ol, 2-heptanone, 3-octanone, 2-butylfuran, 2-pentylfuran, and other compounds compared to the LFB group; the LFB + C18:3n3 group produced higher levels of (*E*, *E*)-2,4-heptadienal and 2-ethylfuran, among other compounds (Fig. 4D–G).

Additionally, the thermal processing of some FAs added to LFB also produced certain S-containing compounds such as thiophenes and thiazoles, as well as pyridines. For instance, the LFB + C18:2n6 and LFB + C20:4n6 groups produced significantly higher levels of 2-acetyl-3-methylthiophene (76.62 ± 11.63 μg/kg, 120.53 ± 18.40 μg/kg), 2-pentylpyridine (3.14 ± 1.53 μg/kg, 9.24 ± 0.80 μg/kg), 2-ethylthiophene (8.30 ± 1.27 μg/kg, 9.85 ± 1.83 μg/kg), and other compounds compared to the LFB group (16.62 ± 2.72 μg/kg, 0.15 ± 0.07 μg/kg, 0.82 ± 0.08 μg/kg); the LFB + C18:3n3 group generated notably higher levels of 2-pentylthiophene (32.98 ± 7.63 μg/kg) relative to the LFB group (0.83 ± 0.12 μg/kg), and the LFB + C18:1n9 group exhibited significantly higher levels of 2-hexanoylthiophene (13.32 ± 1.97 μg/kg) compared to the LFB group (4.52 ± 0.91 μg/kg). It is speculated that the addition of different fatty acids may affect the Maillard reaction in the complex environment of the LFB matrix containing various amino acids and sugars. Some products of lipid oxidation degradation and the Maillard reaction can interact to undergo a series of reactions and form more complex meaty compounds, characterized by having one or more nitrogen or sulfur atoms and a long-chain alkyl group with four or more carbon atoms (Sun et al., 2022). Farmer et al. (1989) reported that a mixture of cysteine and ribose, similar to the Maillard reaction system, produced more “meaty and beefy” odor after the addition of lipid interactions. Zhao et al. (2019) mentioned that aldehydes, especially the most reactive lipid degradation products, unsatusrated aldehydes, are prone to react with amino acids and some Maillard reaction products (such as H_2S and NH_3). We found that more thiophene compounds are produced in the interactive reactions. This is consistent with the reports of Farmer et al. (1989) and Tian et al. (2014), where thiophene compounds dominate in this interaction. Regarding alkyl chain compounds,

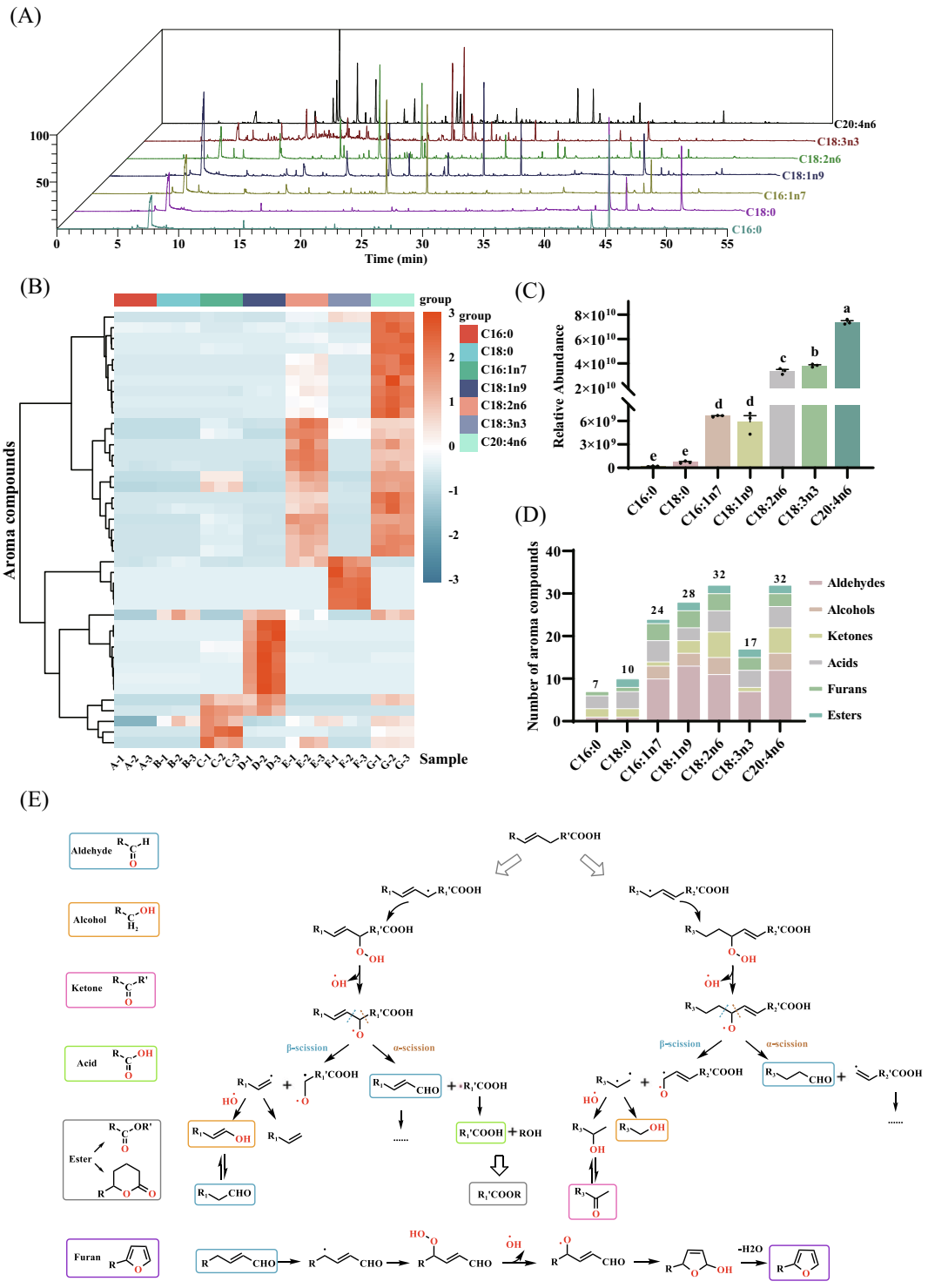

Fig. 2. Identified aroma compounds from thermal oxidative degradation of seven fatty acids ($n = 3$) and the thermal oxidation degradation mechanism of unsaturated fatty acids. (A) Chromatogram, (B) Heatmap and (C) Relative abundance of aroma compounds generated by seven fatty acids. The blue and orange cells in the heatmap represent aroma compounds. (D) Distribution of classes and quantities of the aroma compounds produced. Different letters indicate significant differences ($p < 0.05$). (E) Mechanism of thermal oxidative degradation of UFAs that produces aldehyde, alcohol, ketone, acid, ester, and furan. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2-alkylthiophenes can be formed by the reaction of 2,4-dienals with amino acids or H_2S; for example, (*E*, *E*)-2,4-heptadienal or (*E*, *E*)-2,4-nonadienal can react with H_2S to produce 2-pentylthiophene, and 2-ethylthiophene may come from the reaction of 2,4-hexadienal with H_2S (Elmore & Mottram, 2000; Mottram, 1998; Zhao et al., 2019). Zhang and Ho (1989) also showed that 2-alkylthiophene can be formed when 2,4-decadienal and cysteine are heated in an aqueous solution. 2-Pentylpyridine is found in the reaction of mixtures of cysteine, ribose, and methyl linoleate (Whitfield & Mottram, 1992), which can be produced by the reaction of 2,4-decadienal with amino acids or peptides (Ho et al., 1989). The exact origin of 2-acylthiophene compounds has not been found in the literature, but Zhao et al. (2019) mentioned that 2-

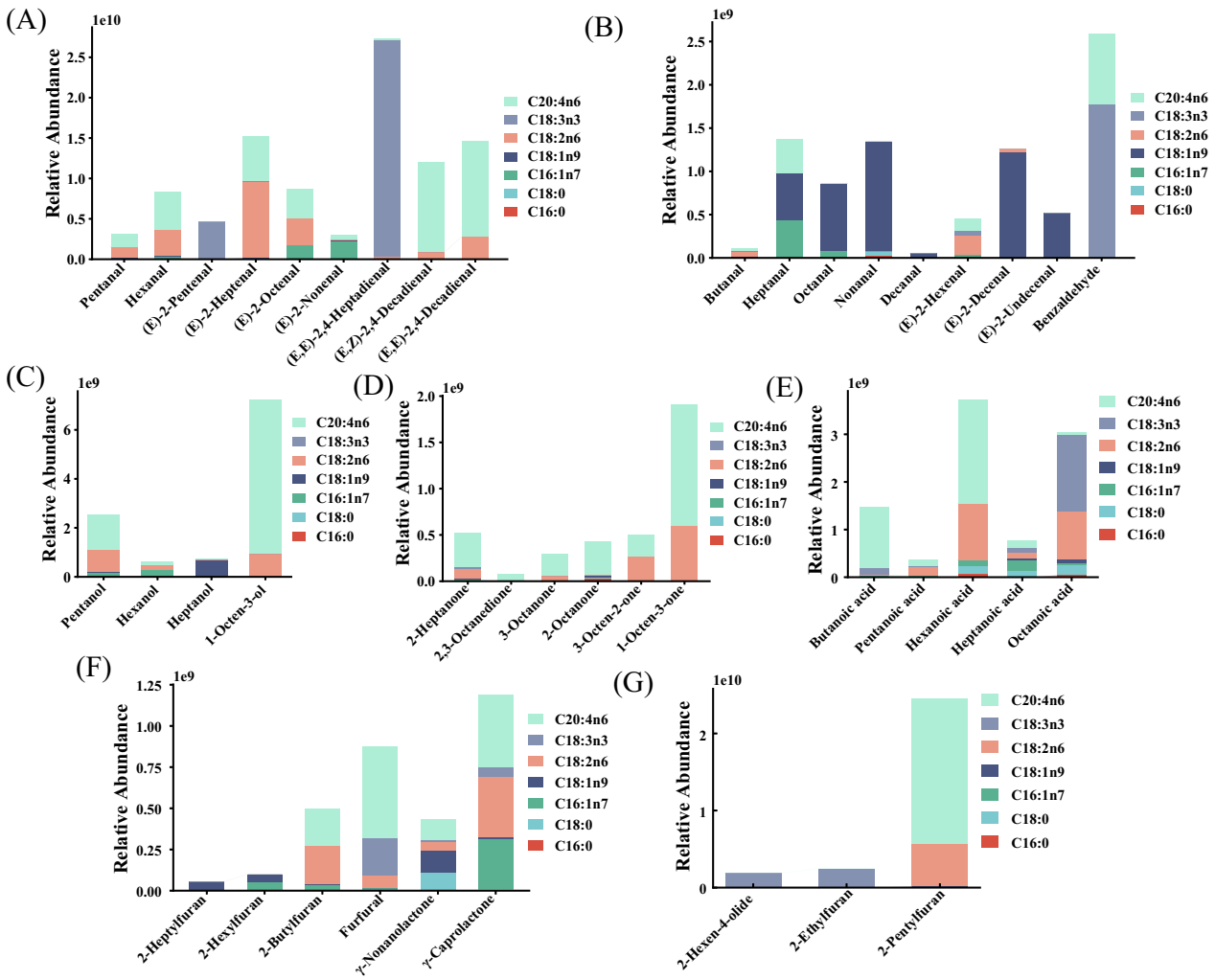

Fig. 3. Aroma compounds identified from the production of each fatty acid, (A-B) aldehydes, (C) alcohols, (D) ketones, (E) acids and (F-G) esters and furans ($n = 3$).

acylthiophenes may form by the cyclization of 2,4-dienals followed by reaction with H_2S and then undergo cyclization, dehydration, and oxidation reactions. However, in this study, no in-depth investigation was conducted on the amino acids and sugars in the matrix. Therefore, the specific mechanism of the interaction between the products of lipid oxidative degradation and the Maillard reaction needs to be further explored in subsequent studies.

3.4. Aroma compound profiles from beef with added FAs

Based on the aforementioned results, we have come to understand that the aroma compounds produced by FA oxidation primarily originate from UFAs. Consequently, we constructed an in vitro thermal oxidation model with beef supplemented with UFAs to validate the impact of various UFAs on the formation of beef aroma compounds. Fig. 5A depicts a heatmap of the aroma compounds produced by each group, showing differences in the levels of aroma compounds between groups. Compared to the beef group, the beef + C18:2n6 and beef + C20:4n6 groups showed significantly increased levels of aroma compounds ($p < 0.05$) (Fig. 5B), with 32 and 62 significantly different aroma compounds, which are mainly aldehydes and ketones (Fig. 5C, Table S3). The aroma compounds identified by GC–O and with OAV > 1 are considered as important aroma compounds in beef. In this study, 18 aroma compounds in beef were identified by GC–O analysis, comprising 10 aldehydes, 2 ketones, 2 alcohols, 2 furans, and 2 S-containing compounds. Except for 2-butylfuran, the OAV of all the other compounds was greater than 1 (Table S4). The identification results, odor intensity and aroma characteristics were shown in Fig. 5D. The primary aroma characteristics perceived were fruity, green, fatty, and milky, with hexanal and 3-methylthiopropanal exhibiting higher odor intensities. Yang et al. (2023) studied volatile compounds in beef fat and found that higher levels of UFAs produce more aroma compounds, especially saturated/unsaturated aldehydes, which also have a lower odor threshold and can contribute significantly to the aroma of beef. The production of fatty aldehydes contributes to the fatty flavor of cooked meat (Jayasena et al., 2013).

Further statistical analysis of these important aroma compounds (Fig. 5E and F, Table S3) showed that the beef + C18:2n6 and beef + C20:4n6 groups produced significantly higher levels of pentanal, hexanal, (*E*)-2-nonenal, (*E*, *E*)-2,4-decadienal, hexanol, 1-octen-3-ol, 1-octen-3-one, acetoin, and 2-butylfuran compared to the beef group ($p < 0.05$). Additionally, the beef + C20:4n6 group had significantly higher levels of (*E*)-2-octenal and 2-pentylfuran compared to the beef group ($p < 0.05$). The beef + C18:1n9 group also showed significantly increased levels of octanal, nonanal, (*E*)-2-decenal, (*E*)-2-undecenal, and acetoin ($p < 0.05$). According to Elmore et al. (1999), aldehydes, 2-enals, and alcohols in cooked beef originate from the autoxidation of C18:1n9 and C18:2n6, and PUFAs in beef may accelerate their thermal degradation. The decomposition of FAs propagates through chain reaction, and the initial PUFAs easily form free radicals, which lead to chain reaction. Once the chain reaction is initiated, the dependency on the properties of PUFAs decreases, leading to increased decomposition of C18:1n9 and

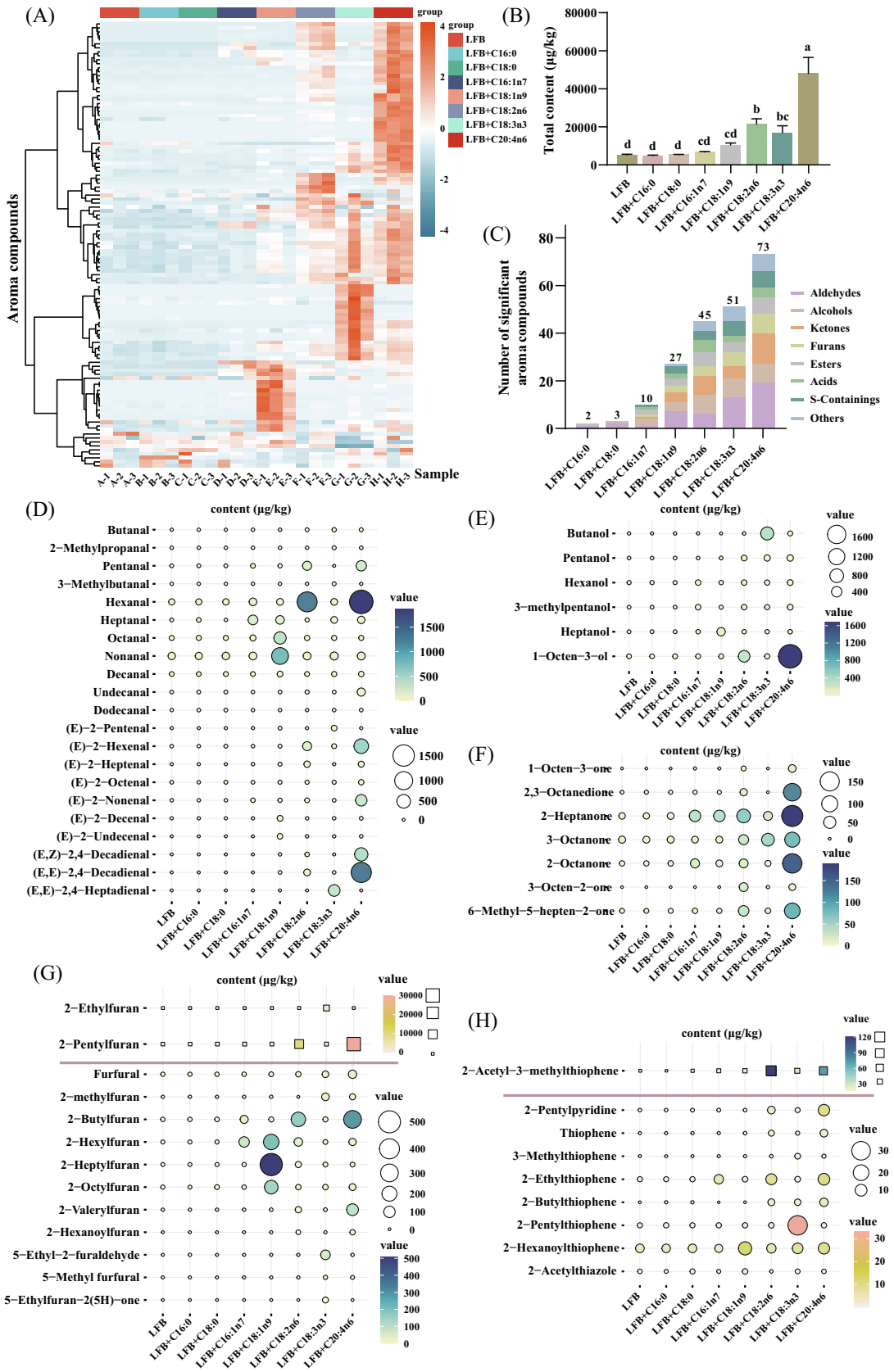

Fig. 4. Aroma compounds identified from the addition of fatty acids to the lipid-free beef ($n = 3$). (A) Heatmap and (B) Content of produced aroma compounds. (C) Number of significantly different aroma compounds compared to the lipid-free beef group. Content of the identified compounds, including (D) aldehydes, (E) alcohols, (F) ketones, (G) furans and (H) S-containing compounds and others. Circles and squares represent different concentration ranges of aroma compounds; the larger the shape and the deeper the color, the higher the concentration. Different letters indicate significant differences ($p < 0.05$).

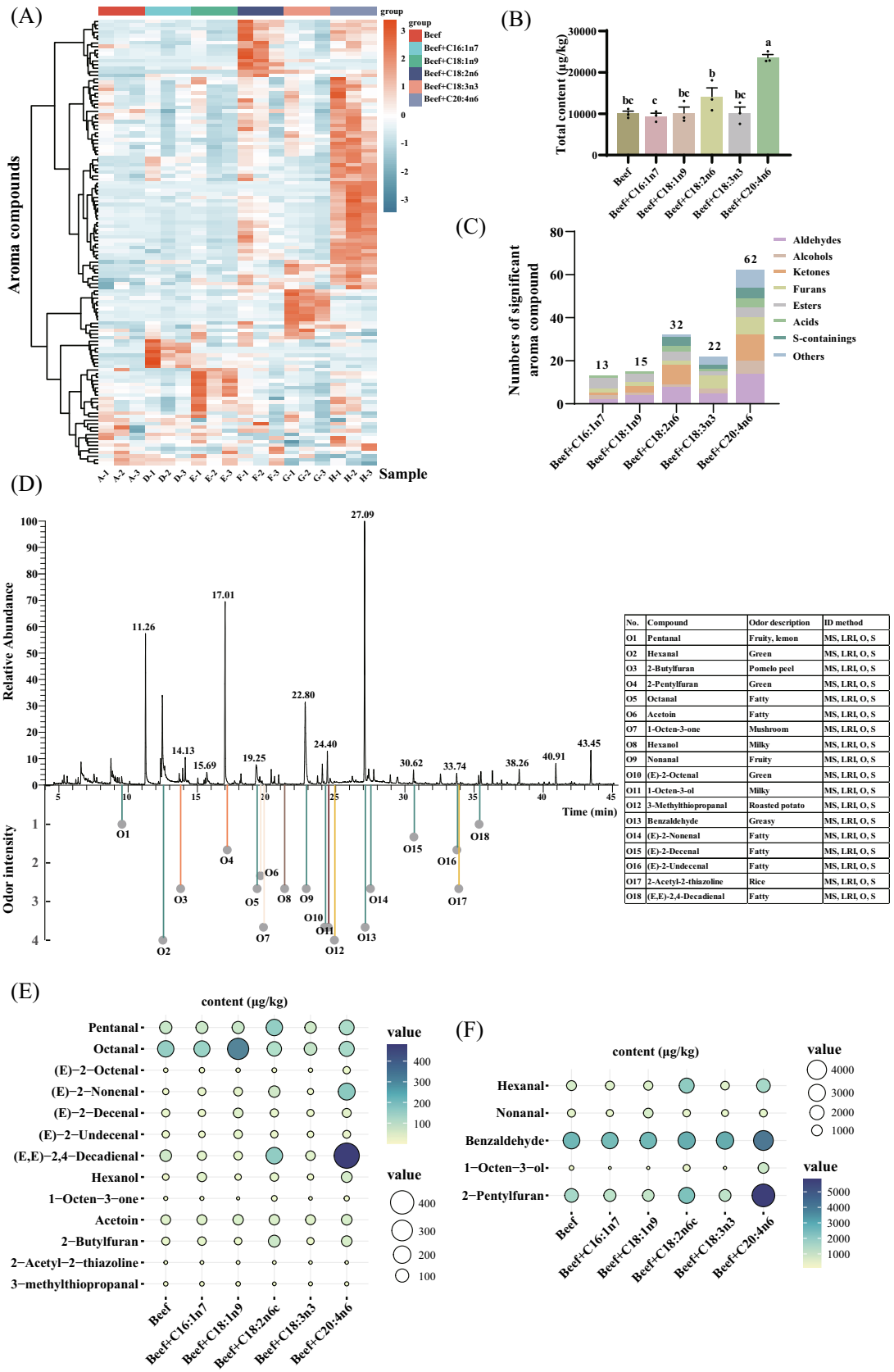

Fig. 5. Aroma compounds identified from the addition of fatty acids to beef ($n = 3$). (A) Heatmap and (B) Content of produced aroma compounds. (C) Number of significantly different aroma compounds compared to the beef group. (D) GC–O–MS chromatogram and odor intensity values of beef aroma compounds. (E–F) Content of important aroma compounds in beef among groups. Different letters indicate significant differences ($p < 0.05$).

C18:2n6 in beef (Elmore et al., 1999; Frankel, 1980). We also found that there were no significant differences in the contents of the S-containing compounds 2-acetyl-2-thiazoline and 3-methylthiopropanal among the groups ($p > 0.05$). Among them, 2-Acetyl-2-thiazoline is produced by the Maillard reaction (Sohail et al., 2022), while 3-methylthiopropanal is generated by the Strecker degradation of amino acids promoted by lipid oxidation products (Hidalgo & Zamora, 2016). We speculate that the formation of 3-methylthiopropanal may be influenced by other lipid products not investigated in this study. Based on the above results, it was found that 13 out of the 18 important aroma compounds in beef could be produced by C18:1n9, C18:2n6, or C20:4n6.

4. Conclusion

This study simulated beef thermal processing conditions and established three thermal oxidative degradation models: single FA, LFB + FA, and beef + FA, to investigate the impact of different FAs on the formation of aroma compounds. The results indicate that the unsaturation of FAs is positively correlated with the content of aroma compounds produced, especially that PUFAs generate higher content of aroma compounds, such as aldehydes. Additionally, the oxidative degradation products of UFAs can react with amino acids and sugars in LFB, generating various heterocyclic compounds, particularly thiophenes. Among FAs, C18:1n9, C18:2n6, and C20:4n6 are important precursors for the generation of major beef aroma compounds, such as hexanal, octanal, (*E*)-2-nonenal, (*E*, *E*)-2,4-decadienal, and 1-octen-3-ol. This study provides a new perspective on understanding the role of FAs in beef aroma formation and offers scientific basis for food processing and flavor regulation. Future research should further optimize the model, consider a broader range of FA types, structures, and combinations, and conduct in vivo validation in practical application scenarios.

CRediT authorship contribution statement

Longzhu Zhou: Writing – original draft, Visualization, Investigation, Formal analysis. **Yimeng Ren:** Investigation. **Yujie Shi:** Visualization. **Liyuan Zhao:** Data curation. **Huihui Tian:** Investigation. **Xiaohui Feng:** Methodology. **Jing Li:** Validation. **Youyou Yang:** Validation. **Weihai Xing:** Validation. **Yanan Yu:** Data curation. **Qingyu Zhao:** Project administration. **Junmin Zhang:** Supervision, Funding acquisition. **Chaohua Tang:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102291.

Data availability

Data will be made available on request.

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