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Multi-omics analysis reveals flavor differences in Xinjiang brown beef with varying intramuscular fat contents

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ABSTRACT

Beef flavor plays a crucial role in consumer preference, yet research on this trait has been limited by past technological constraints. Intramuscular fat (IMF) is a key determinant of beef quality, influencing taste, marbling, and overall flavor. Xinjiang brown cattle (XBC), an indigenous breed from northern Xinjiang, China, presents significant variation in meat quality, with IMF content ranging from 0.2 % to 4.3 % within the population. This variation suggests strong potential for breeding improvement. In this study, we selected 82 XBC for slaughter and meat quality analysis, categorizing them based on IMF content. Using two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC-TOF MS), we analyzed volatile flavor compounds across different beef cuts (Longissimus dorsi, Semitendinosus, Supraspinatus). Our results showed that beef with higher IMF levels exhibited enhanced flavor profiles, characterized by sweet, green, fruity, and waxy notes, while castrated bulls displayed the weakest flavor intensity. Metabolomic analysis further revealed significant differences in flavor substances between high and low IMF content beef. RNA-Seq analysis identified key genes (AQP4, FZD2, FADS1, BPG1, CEBPD, FABP4) associated with flavor formation, offering valuable insights for breeding strategies aimed at improving XBC meat quality. This comprehensive study provides a robust theoretical foundation for advancing the genetic improvement of XBC.

1. Introduction

Cattle hold a vital position among the world's livestock, and China, with its wide array of beef cattle breeds, stands out (Xia et al., 2023). The Xinjiang brown cattle (XBC), native to northern Xinjiang, China, is renowned for its adaptability, superior grazing, and production performance (X. Wang et al., 2023). Over the past 40 years, selective breeding has substantially increased the XBC population; however, the selection for superior meat quality and taste has not been a primary concern. Previous studies have found that key factors influencing the quality of beef include meat color, muscle cut, intramuscular fat (IMF) content, and marbling distribution (Hocquette et al., 2011). Among these, the IMF is recognized as an essential factor to beef quality. (Boito et al., 2018; Frank, Joo, & Warner, 2016). IMF, which refers to lipid deposits in skeletal muscle, represents the total triglyceride and phospholipid content at a microscopic level (Pethick, Harper, & Oddy, 2004). There is significant variation in IMF content within XBC populations, making the pursuit of high-IMF content a critical target for breeding high-quality meat traits.

Beef flavor is a crucial factor for consumers when choosing beef (Kerth & Miller, 2015). Studies show that consumers prefer flavor over tenderness in ruminant meat because of its distinctive characteristics (Arshad et al., 2018). Proteins, fats and carbohydrates are all significant contributors to meat flavor. Carbohydrates, in particular, transform into important flavor precursors when heated (Fu, Cao, Yang, & Li, 2022). Metabolites generate a variety of flavor substances, which contribute to the diversity of flavors (Ramalingam, Song, & Hwang, 2019). During the heating process, fats act as flavor precursors, generating hydroperoxides and volatile organic compounds (VOCs), such as alcohols, esters, aldehydes, and carbonyl compounds (Pena-Bautista et al., 2019). Although IMF is not a direct sensory indicator of meat quality, it enhances tenderness and juiciness, thereby contributing to the overall meat flavor (Pethick et al., 2004). Several studies suggest that the flavor intensity of beef increases with higher IMF content, but levels off at a certain high

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IMF levels (Frank, Ball, et al., 2016; Hirai, Kawai, Mizuno, Sasaki, & Iida, 2023; Thompson, 2004). However, the BIF-BEEF database indicates a low correlation between IMF content and flavor (r = 0.11), based on sensory evaluations (Hocquette et al., 2011). In summary, the contribution of IMF to beef flavor can be is not clearly studied.

Recent advancements in metabolomics, along with the development of electronic noses and tongues, have significantly deepened our understanding of food flavor. Metabolomics offer an objective standard by identifying volatile compounds that affect meat flavor. Gas chromatography-mass spectrometry (GC–MS) technologies are extensively used for detecting these flavors. Wan et al. used SAFE-GC-O-MS and HS-GC-IMS techniques to identify the flavor characteristics of Tibetan dzo beef (Wan et al., 2023). Yang et al. used GC–MS to identify essential aroma compounds of dry-rendered beef fat (Yang, Pei, Du, & Xie, 2023). Two-dimensional gas chromatography–time-of-flight mass (GC×GC-TOF MS) can identify volatile compounds more sensitively than GC–MS. Wang et al. successfully identified differential volatile compounds in two pig species using GC×GC-TOF MS (X. Y. Wang et al., 2022).

Here, we assessed the meat quality of 82 XBC cattle to identify individuals with superior meat quality (high IMF content) within the same population. The longissimus dorsi, supraspinatus, and semitendinosus muscles were collected and analyzed using GC×GC-TOF MS to determine volatile flavor compounds. RNA-Seq was performed on these muscles, and correlation analysis between differential genes and metabolites helped identify key functional genes linked to flavor compounds in high-quality cattle. This study aimed to reveal variations in flavor substances and genetic correlations among cattle with different IMF contents and identified critical substances impacting XBC meat flavor, offering potential molecular targets for enhancing beef quality.

2. Materials and methods

2.1. Animal

We select 82 Xinjiang Brown Cattle (XBC)for this study, based on their hardiness and quality meat production, under a protocol approved by the Animal Care and Use Committee of the Regional Ethical Review Board (Approval No. 2022-06XJ). The cattle, all seven-month-old males, were sourced from a local farm renowned for its sustainable practices. Upon arrival at the research facility, the animals were given a week to acclimate to their new environment, during which their health was closely monitored to confirm their eligibility for the trial. The cattle were housed under identical conditions in a well-ventilated barn, provided with access to water, and fed a balanced diet tailored to their nutritional needs. The feeding regimen was designed to replicate typical regional practices for this breed, with an emphasis on maximizing meat quality and overall health. Following the acclimation period, the cattle were randomly divided into two groups of 41 and housed in individual pens to accurately monitor their intake. Diets were formulated to meet the metabolic weight requirements of the cattle, calculated as Live Weight 0.75, and provided in two equal portions daily at predetermined times to maintain consistency. Throughout the study, the cattle had unlimited access to fresh water and were kept in well-lit areas with freedom of movement. All cattle were fed the same nutritionally balanced formula, designed with precise macro and micronutrient specifications to ensure scientific rigor in the dietary formulation.

2.2. Sample collection and preparation

The cattle were slaughtered in accordance with humane practices and local regulations. They were fasted overnight with free access to water. The slaughtering process included electrical stunning followed by exsanguination. Post-mortem, the carcasses were dressed, and three muscle samples were collected from the longissimus dorsi, supraspinatus, and semitendinosus muscles. A total of 246 beef samples from 82 cattle were collected, and the meat quality indicators of each sample were measured three times in parallel. These samples were immediately chilled and transported to the laboratory for further analysis. Prior to testing, samples were thawed overnight at 4 °C and brought to room temperature 1 h before analysis to ensure consistency in measurement conditions.

2.3. Meat quality testing

The total lipid content of the muscle samples was determined using the Soxhlet extraction method. A Soxhlet apparatus (Buchi Labortechnik AG, Switzerland) with hexane as the solvent was employed for a continuous 6-hour extraction period. The methodology was adapted from Folch and AOAC standards for lipid extraction in animal tissues (Folch, Lees, & Stanley, 1957; Racette, Lin, Ma, & Ostlund, 2015).

Meat tenderness was quantified using a Warner-Bratzler shear force apparatus (AMETEK, Inc., USA). Cooked meat samples were prepared by broiling to an internal temperature of 71 °C, rested at room temperature for 30 min, and then subjected to shear force measurement. The procedure followed was in line with the American Meat Science Association (AMSA) guidelines for tenderness evaluation. After the IMF content of beef was determined, it was divided into three groups for subsequent analysis, according to the IMF content, those below 1 % were defined as low IMF (n = 23), and those above 1 % were defined as high IMF (n = 48) and castrated bulls (n = 11).

Water-holding capacity (WHC) was assessed using the drip loss method. Samples were suspended and allowed to drip for 24 h at 4 °C. The weight loss during this period, expressed as a percentage of the initial weight, indicates the WHC, with lower values denoting better water retention. This method aligns with the approach described by Honikel for evaluating WHC in meat (Honikel, 1998).

The muscle samples' color parameters (L*, a*, b*) were measured using a Minolta colorimeter (Konica Minolta, Inc., Japan) calibrated against a white standard tile before use. Measurements were taken at three different points on the surface of each sample to account for variability.

The pH of the muscle samples was determined 24 h post-mortem using a digital pH meter (Mettler-Toledo, Switzerland) with a penetration probe. Samples were allowed to equilibrate to room temperature, and the probe was inserted into the center of each sample for measurement. The procedure was based on the standard protocol for post-mortem pH measurement in meat.

2.4. Two-dimensional gas Chromatography–Time-of-Flight mass spectrometry (GC×GC-TOF MS)

GC×GC-TOF MS analysis was performed on a Pegasus 4D instrument (LECO; St. Joseph, MI, USA) equipped with a gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), auxiliary oven, and dualstage quad-jet thermal modulator connected to a time-of-flight mass spectrometer (Zoex Corp., NE, USA). To begin the experiment, 2.5 mL of sample (fresh beef homogenate) was transferred to a 20-mL headspace vial and sealed. The first column was a DB-WAX(dimension: 3 × 107 μm × 0.25 μm × 250 μm), which was used with an injection temperature of 250 ∘C; the initial temperature was retained at 40 ∘C for 3 min, increased to 250 ∘C at a rate of 5 ∘C/min, where it was held for 5 min in the presence of helium (99.9999 %) injected at a rate of 1.0 mL/min without splitting. The second column was DB-17MS (2 × 106 μm × 100 μm × 0.10 μm) at 5∘C. The modem temperature was kept 5°C higher than the second column. The modulation period was 6.0 s during the two-dimensional analysis, and the interface temperature was 270 ∘C. The ion source temperature was 250 ∘C. The electron bombardment source was set at 70 eV with the detector at 1680 V and an acquisition rate of 50 sheets/second. A mass spectrum was acquired from *m/z* 33–500 and cross-checked against the NIST spectral library (https://webbook.nist.gov/chemistry/). The acquisition efficiency was 50

spectra/s. Metabolites obtained by GC×GC-TOF MS were linked to the senses according to their species and contents. Overall, the GC×GCTOF MS analysis was performed using standard parameters (Song et al., 2020). The NIST database was used to annotate flavor substances, and the PubChem database and Classyfire software were used to perform type annotation analysis on the detected flavor substances. The analysis provided data from 82 XBCs and 248 beef samples. Volatile compounds were clustered based on their physicochemical properties such as molecular weight, boiling point, and functional groups. These clusters were then correlated with sensory attributes to define the flavor profiles associated with different IMF levels.

2.5. Liquid Chromatography-Mass spectrometry (LC-MS Analysis)

For metabolite analysis, beef samples from 82 Xinjiang brown cattle (XBC) were prepared by homogenizing 2 g of each sample with 8 mL of methanol, containing internal standards. The mixture was vortexed, sonicated for 10 min, and centrifuged at 12,000 rpm for 10 min. The supernatant was filtered through a 0.22 μm membrane and analyzed using LC-MS. Chromatographic separation was achieved with an Agilent 1290 Infinity LC system equipped with a Waters ACQUITY UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm), employing a gradient elution of 0.1 % formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min. Mass spectrometry was performed with an Agilent 6495B Triple Quadrupole Mass Spectrometer in both positive and negative ionization modes, with a capillary voltage of 3.5 kV, nebulizer pressure of 35 psi, and a drying gas temperature of 350 °C. Data acquisition was conducted over the mass range m/z 50–1000, and the results were analyzed to correlate volatile metabolites with sensory attributes of the beef.

2.6. Rna-seq

Total RNA was extracted from LD muscle tissues using the TRIzol method. Quality and quantity of RNA were assessed via agarose gel electrophoresis and NanoDrop spectrophotometry (Thermo Fisher Scientific), ensuring A260/A280 ratios between 1.8 and 2.1. RNA integrity was verified using the RNA 6000 Nano Assay Kit and the Agilent 2100 Bioanalyzer (Agilent Technologies). Libraries for RNA sequencing were prepared using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, Inc.), following the manufacturer's instructions. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and fragmented into small pieces. First-strand cDNA was synthesized using random hexamer primers and reverse transcriptase, followed by second-strand cDNA synthesis. The cDNA fragments underwent an end repair process, adding a single 'A' base and then ligating the adapters. The products were purified and enriched with PCR to create the final cDNA library. Sequencing was performed on an Illumina HiSeq 2500 platform, generating paired-end reads of 150 bp. Raw reads were processed to remove adapters and low-quality sequences using Trimmomatic. The cleaned reads were aligned to the reference genome using STAR with default settings. Quantification of gene expression was conducted with featureCounts (Liao, Smyth, & Shi, 2014), and differential expression analysis was performed using DESeq2 (Love, Huber, & Anders, 2014).Functional enrichment analysis of gene modules was performed using DAVID (D. W. Huang, Sherman, & Lempicki, 2009).

2.7. Integrative analysis of metabolomics and transcriptomics

For the integrative analysis of metabolomics and transcriptomics, we employed a comprehensive methodology to bridge the data obtained from two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF MS) and RNA sequencing (RNA-Seq). Initially, metabolomic data were processed using ChromaTOF software (https://www.leco.com/products/chromatof/), focusing on peak detection, alignment, and identification. Metabolites were identified by comparing their mass spectral fingerprints and retention times against known NIST mass spectral library standards. For transcriptomic data, after the quantification of gene expression levels, differential expression analysis was conducted to pinpoint genes with significant changes in expression between different experimental groups.

A correlation analysis was carried out using Pearson's correlation coefficient to integrate these datasets to identify significant correlations between metabolite abundances and gene expression levels. This step mapped metabolites to their corresponding biosynthetic or degradation pathways and linked these pathways to specific genes and their expression patterns.

Subsequently, pathway enrichment analysis was performed on both the metabolomic and transcriptomic datasets. For metabolomics, pathways were enriched based on the identified metabolites using tools like MetaboAnalyst (Pang et al., 2022), which references the KEGG database for pathway mapping. For transcriptomics, gene set enrichment analysis (GSEA) was utilized to identify significantly enriched pathways in the gene expression data, shedding light on biological processes and pathways significantly associated with meat quality traits.

2.8. Statistical analysis

Statistical analyses were conducted using SPSS software. The data were tested for normality and homogeneity of variance. Differences between groups were assessed using ANOVA and Tukey's post hoc test for multiple comparisons. Correlation analyses were performed to investigate the relationships between meat quality parameters and molecular data. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. XBC growth and meat quality determination

To explore the differences in growth and meat quality within the XBC population, we selected 71 male XBC and 11 steer XBC with normal appearance and health to conduct growth data recording and slaughter measurement experiments. Bulls were divided into two groups: high intramuscular fat (HIMF, n = 48) content and low intramuscular fat (LIMF, n = 23) content for comparison. The fat content of the HIMF group (1.8 ± 0.801) was significantly higher than that of the LIMF group (0.596 ± 0.242) and higher than that of steers (7.009 ± 3.038). Moreover, the moisture content of HIMF is higher than that of the other two groups of cattle, reflecting the juiciness of the beef of this group. However, the protein content of HIMF was lower than that of the other two groups of cattle (Table 1).

Regarding growth performance, the weight of steers (920.545 ± 91.402) is much higher than that of bulls (674.957 ± 52.681 and 689.563 ± 67.257), and there is a significant difference in body size. The difference between the HIMF and LIMF groups is mainly reflected in the Eye lion area and Thick backfat (Table 2). It can be seen that there is a high-content IMF group in the XBC population, and it is higher than that of castrated bulls.

3.2. XBC beef flavor determination of different parts

To explore the effect of IMF content on beef flavor, we selected beef

Table 1
Descriptive statistics of the nutrient content of the longissimus dorsi.

Item (%)	HIMF	LIMF	Steer	*P* value
Moisture Content	73.252 ± 1.1	70.973 ± 1.886	63.036 ± 3.878	<0.0001
Protein	22.33 ± 4.237	24.325 ± 1.701	22.055 ± 1.853	0.0035
IMF	1.8 ± 0.801	0.596 ± 0.242	7.009 ± 3.038	<0.0001

Table 2
Descriptive statistics of carcass characteristics of cattle.

Items	HIMF	LIMF	Steer	*P* value
Weight	674.957 ± 52.681	689.563 ± 67.257	920.545 ± 91.402	<0.0001
Body height	135.826 ± 3.601	136.042 ± 4.959	135.545 ± 7.16	0.9516
Cross height	138.783 ± 4.056	139.688 ± 5.493	136.364 ± 5.353	0.1541
Chest depth	71.696 ± 3.281	72 ± 3.101	89.364 ± 4.802	<0.0001
Chest width	51.478 ± 4.501	52.25 ± 3.606	57.273 ± 2.054	0.0002
Shiri Naga	51.478 ± 3.058	51.938 ± 3.09	59.455 ± 3.236	<0.0001
Waist angle width	51.913 ± 3.103	53.083 ± 2.835	56.909 ± 4.323	0.0035
Hip width	51.696 ± 3.309	53.458 ± 2.858	65.455 ± 4.48	<0.0001
Ischium width	18.217 ± 1.506	19 ± 1.913	21.273 ± 2.005	<0.0001
Body slope length	180.478 ± 9.204	178.708 ± 9.804	193.545 ± 11.544	0.0008
Circumfere	22.652 ± 1.071	22.896 ± 1.171	26 ± 1.483	<0.0001
Bust	213.522 ± 5.591	216.583 ± 7.322	251.364 ± 7.311	<0.0001
Abdominal Circumference	237.522 ± 12.841	237.563 ± 10.522	281 ± 13.424	<0.0001
Rump Circumference	117.913 ± 6.822	116.854 ± 6.894	138.909 ± 9.093	<0.0001
Thick backfat	0.893 ± 0.163	1.218 ± 0.707	1.134 ± 0.171	0.0812
Eye lion area	84.584 ± 15.5	90.368 ± 17.781	102.646 ± 9.569	0.0438

from three different parts of 82 XBC for testing. The more representative longissimus dorsi (LD), semitendinosus (SM), and supraspinatus (SS) muscles were used for research. First, the meat quality of different parts of cattle muscles with different IMF contents was tested. The IMF content did not significantly impact meat quality, including shear force, water retention capacity, cooking loss, acidity, and meat color. This may be due to the same genetic background and single-factor genetic differences (Additional Table S1). Then, a total of six muscles from two groups of cattle with different IMF contents were tested. Two-dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GC×GC-TOF MS) Detection to obtain volatile compound components. 6,178 compounds were identified in LD (LDH) of HIMF, 5,965 compounds in SM (SMH), 5,889 compounds in SS (SSH), 4,530 compounds in LD (LDL) of LIMF, 4,660 compounds in SM (SML), and 4690 compounds of volatile substances in SS (SSL). It can be concluded that high IMF leads to volatilization in muscles. The overall increase in sexual substances increases the flavor of beef (Fig. 1A). From the perspective of specific species, the compounds that form the flavor of XBC beef are diverse, including alcohols, Aldehydes, Esters, Hydrocarbons, Ketones, and other substances. There is no considerable difference in the species composition of different parts of beef (Fig. 1B). Alcohols and other substances are mainly involved in the contribution of beef flavor (Fig. 1C). Through the sensory analysis radar chart of flavor substances, it can be concluded that there is a massive difference between HIMF and LIMF beef. HIMF contributes more robust flavor components, while there is no noticeable difference in the flavor of different beef parts (Additional Table S2). This illustrates the impact of IMF on beef flavor, which is the main factor (Fig. 1D). ROVA odor activity analysis also proved the slight difference in flavor activity of different beef parts (Fig. 1E).

A partial least squares analysis (PLS-DA) was performed on the flavor compounds of six groups of beef and found that the two main components were not wholly distinguished (Fig. 2A). For the three types of meat, the overall flavor substances in the HIMF group increased significantly compared with the LIMF group, but the flavor substances increased the most in the LD (Fig. 2B). The Venn plot shows a total of 20 common intersectional differential flavor compounds between the HIMF and LIMF groups of the three cuts of beef (Fig. 2C). The differentially expressed flavor substances in the three cuts of meat are shown in the volcano plot in turn (Fig. 2D-F), and the specific substance list is shown in Table 2. From the above results, we conclude that the part of beef is not the critical factor in determining flavor. Still, IMF has a more prominent contribution to flavor, especially in LD.

3.3. Differences in flavor between different intramuscular fat LD and steers

It is generally believed that the beef flavor from steers is significantly reduced due to castration. Given the outstanding contribution of IMF to LD flavor differences, we conducted an in-depth analysis of the LD flavor of 82 XBC and introduced steers as a comparison group. GC×GC-TOF MS results shown there was no apparent difference between the three types of beef in terms of flavor composition (Fig. 3A). The number of differential substances also showed that steers were significantly lower than other uncastrated beef (Fig. 3B). In sensory analysis, the overall flavor of HIMF was greater than that of the other two groups of beef, especially in sweet, green, fruity, and waxy, while the flavor of the steers was lower than that of the LIMF group, which showed that castration led to the loss of beef flavor (Fig. 3C). After comparing the different flavor compounds between each group, it was found that there was not much overlap between the three groups, which reflected the significant difference in flavor compounds between steers and uncastrated cattle (Fig. 3D). After correlating each sensory flavor characteristic with compounds, it was found that sweet is mainly contributed by substances such as Benzyl alcohol, Acetone, Decanal, Pyrrole, etc., green is contributed by Ethyl formate, 2-Hexenal, (E)-, Hexanal, Dodecanal, etc., while fruity is contributed by nonanoic acid, ethyl ester, gamma-Dodecalactone and other compounds (Fig. 3E). All differentially expressed flavor compounds are shown in Additional Table S3.

3.4. Metabolite analysis of bovine LD with different intramuscular fat contents

Given that the volatile flavor metabolites detected in GC XGC are all small molecules, we performed LC-MS metabolome detection on the LD of three XBCs. PLS-DA analysis shows that the overall metabolite differentiation between LIMF and Steer is very large at the metabolite level. There are two quite different groups in HIMF, which the linear distribution of IMF content may cause (Fig. 4A). After conducting KEGG pathway analysis on the highly expressed metabolites detected in the LD of XBC, it can be concluded that metabolic pathways related to meat quality formation such as Linoleic acid metabolism, PPAR signaling pathway, and beta-Alanine metabolism are highly enriched (Fig. 4B). A heat map was performed to display the differentially expressed metabolites. Consistent with the conclusion drawn from PLS-DA analysis, the overall difference in metabolites between LIMF and Steer was the greatest, while HIMF presented two groups connecting the two (Fig. 4C). The most different metabolites between the three types of beef are D-Mannose, Deoxyuridine, 1, Methylhistidine, Hydroxyphenyllactic acid, 1-Hexadecanol, and L-Phenylalanine, which may play an important role in intramuscular fat deposition (Additional Table S4, 5). The LC-MS metabolic panel shows similar differences in the LD of XBC with different intramuscular fat contents.

3.5. Correlation analysis between XBC gene expression and flavor substances

Since the XBC internal population produces individuals with different IMF content distributions and there are considerable differences in the flavor components of meat, we tried to reveal the factors

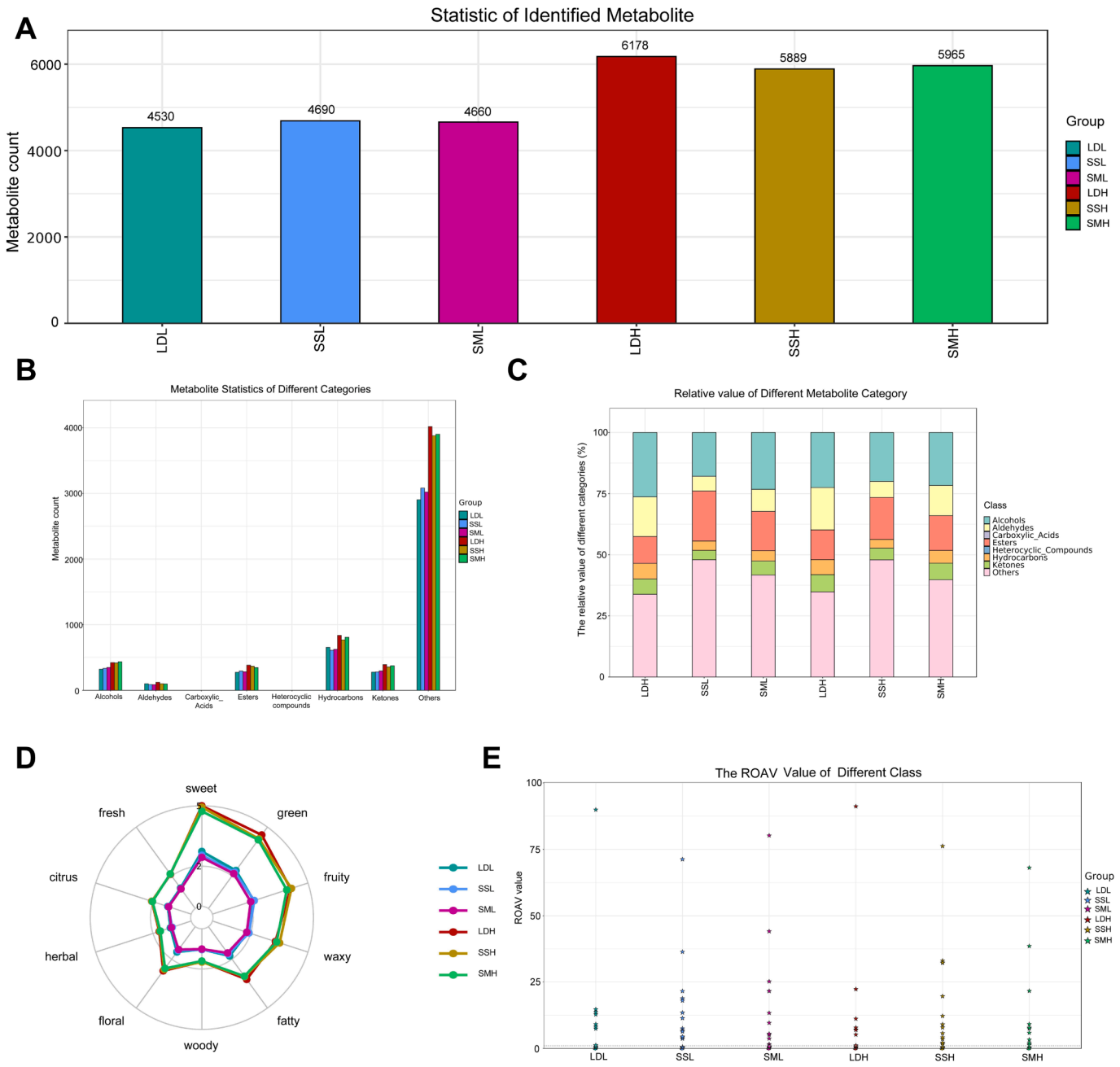

Fig. 1. Analysis of flavor substances in different parts of beef from XBC. A. Statistics of the total number of substances detected in different parts of beef by GC×GC-TOF MS. B. Counts of volatile substances detected in different parts of beef. C. The proportion of volatile substances detected in different parts of beef. D. Cluster radar chart of flavor characteristics of volatile substances detected in different parts of beef. E. ROVA analysis of volatile substances detected in different parts of beef. Longissimus dorsi (LD), semitendinosus (SM) and supraspinatus (SS).

that form the other flavors from a genetic perspective. Transcriptomic sequencing was performed on the LD of a total of 82 XBCs. First, the data of HIMF and LIMF were analyzed. A total of 60 genes were up-regulated and 110 genes were down-regulated (Fig. 5A). KEGG enrichment analysis of differential gene sets found that the classic pathways like PPAR signaling pathway, PI3K-Akt signaling pathway, Adipocytokine signaling pathway, which is closely related to adipogenesis, was highly enriched, reflecting the different characteristics of intramuscular fat content (Fig. 5B). The top 50 genes and flavor substances with the highest degree of difference between HIMF and LIMF groups were selected for Pearson correlation coefficient analysis. It can be seen that AQP4, FZD2, FADS1, and other genes are highly positively correlated with most flavor substances, while BPG1, CEBPD, and FABP4 are highly positively correlated. Negative correlation. They may be the primary genes involved in the formation of beef flavor that is differentially regulated by IMF (Fig. 5C). At the same time, we also conducted the same experiment on LD in steers and LIMF groups. Transcriptomic differential gene analysis found that there are a large number of differential genes between the two, which indicates that their drastic changes in gene expression levels may be caused by IMF differences (Fig. 6A). KEGG enrichment analysis of differential genes found that it was still higher than the PI3K-AKT signaling pathway, indicating that it may have a high-intensity relationship with the formation of IMF differences in XBC (Fig. 6B). The Top50 differential genes and flavor substances were selected for correlation analysis. The results showed that almost all flavor substances except Cinnamaldehyde (E)- were highly correlated.

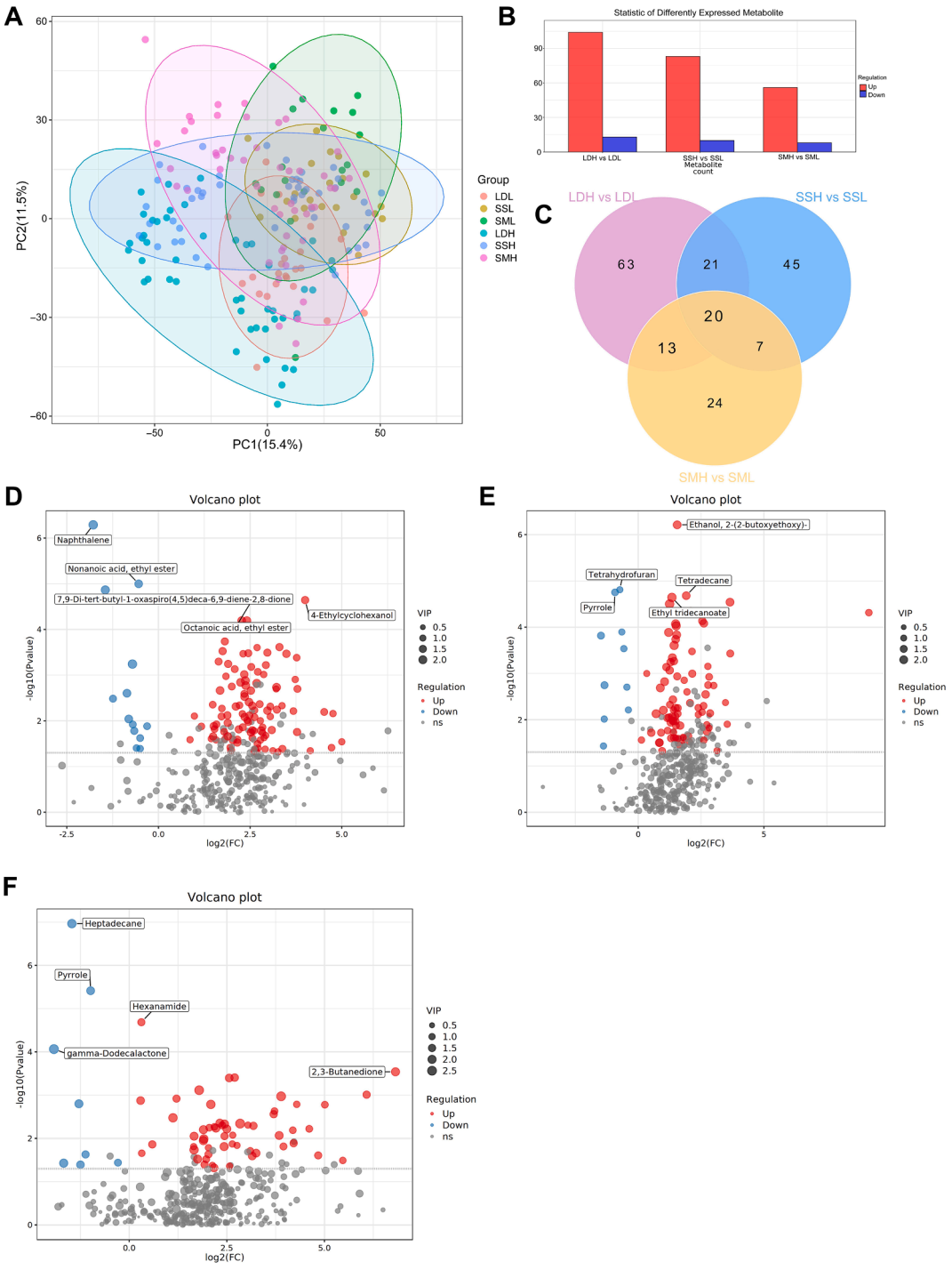

Fig. 2. Difference analysis of volatile flavor compounds in different parts of beef. A. PLS-DA analysis of volatile flavor compounds in different parts of beef. B. Statistics of differential volatile flavor compounds in each part of beef. C. Venn diagram of the differential volatility of different cuts of beef. D. Volcano plot of differentially expressed volatile flavor compounds in LD. E. Volcano plot of differentially expressed volatile flavor compounds in SM. F. Volcano plot of differentially expressed volatile flavor compounds in SS.

This may be caused by the excessive degree of difference. Still, it also showed that Cinnamaldehyde (E)- may be weakly related to the regulation of gene expression (Fig. 6C). It can be seen that there is a specific relationship between the flavor of beef and gene expression, Related signaling pathways may also be involved in regulation. There is a considerable relationship with the content of IMF, even among XBC within the same population.

4. Discussion

XBC has substantial application potential due to its unique genetic traits. Recent advancements in genomic and proteomic technologies have enhanced our understanding of XBC genetics.Wang et al. used whole genome screening to analyze the genetic stability of XBC (X. Wang et al., 2023). The genetic characteristics of milk production traits in XBC were also investigated using a genomic selection approach (Zhang et al., 2022). The differences in non-coding RNA in the

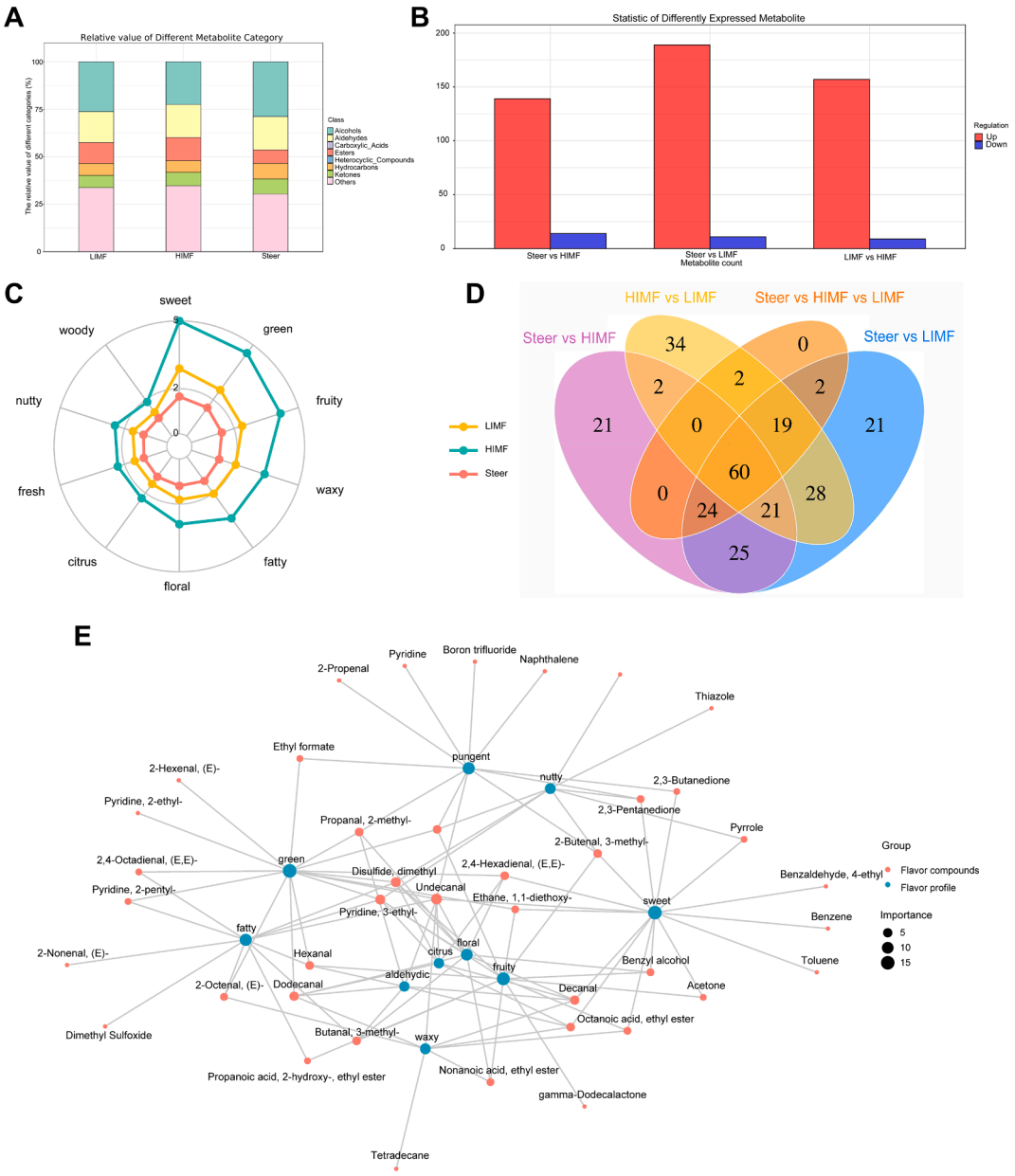

Fig. 3. Analysis of volatile flavor compounds in LD of XBC with different IMF contents. A. Volatile compound components of LD of XBC identified by GC×GC-TOF MS; B. Statistics of differential volatile compounds in comparison of three groups of beef; C. Sensory characteristics analysis of flavor compounds; D. Venn diagram of differential compounds in three groups of beef; E. Correlation analysis between sensory characteristics and flavor compounds.

longissimus dorsi muscle of XBC and Kazakh cattle were also screened using high-throughput RNA-Seq to identify a large number of differentially expressed genes that may be involved in the regulation of muscle development (X. M. Yan et al., 2021; Yan et al., 2020), and the differences were compared at the proteomic level (X. Yan et al., 2021). People have gradually gained a clearer understanding of the genetic effects and gene expression characteristics of XBC but have yet to gain a clearer understanding of IMF and meat quality. The study by Li et al. showed that there are internal populations with significant differences in IMF content in XBC, which proves that IMF is not a stable genetic trait in XBC (Li et al., 2018). Therefore, even XBCs with the same genetic background and population may have different IMF contents, resulting in differences in meat quality, especially beef flavor.

With the development of metabolomics, the capture of tiny molecules has become increasingly refined, and in recent years, we have had a clearer understanding of the unique aroma of meat. In terms of beef flavor recognition, Wan et al. applied SAFE-GC-O-MS and HS-GC-IMS techniques to reveal the flavor characteristics of Tibetan Qiangyao beef. They found that 3-hydroxy-2-butanone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were dominant in it, with buttery and caramel flavors (Wan et al., 2023). A study used GC-O-MS and GC–MS combined techniques to analyze the beef flavor characteristics of Japanese Wagyu and identified 39 odor-active odorants (Ueda, Yamanoue, Sirai, & Iwamoto, 2021). A headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC–MS) method was used to quantify 30 volatile compounds in fresh beef (Bueno, Resconi, Campo, Ferreira, & Escudero, 2019). Our method of GC×GC-TOF MS, with its enhanced sensitivity, we observed that volatile flavor compounds varied significantly with IMF content, independent of beef cuts. Beef with higher IMF content exhibited more pronounced flavors, including sweet, fruity, and green notes. This finding aligns with previous studies indicating that IMF levels strongly correlate with flavor intensity (Li et al., 2018). Metabolomic analysis via LC-MS further supported these results, highlighting significant differences in

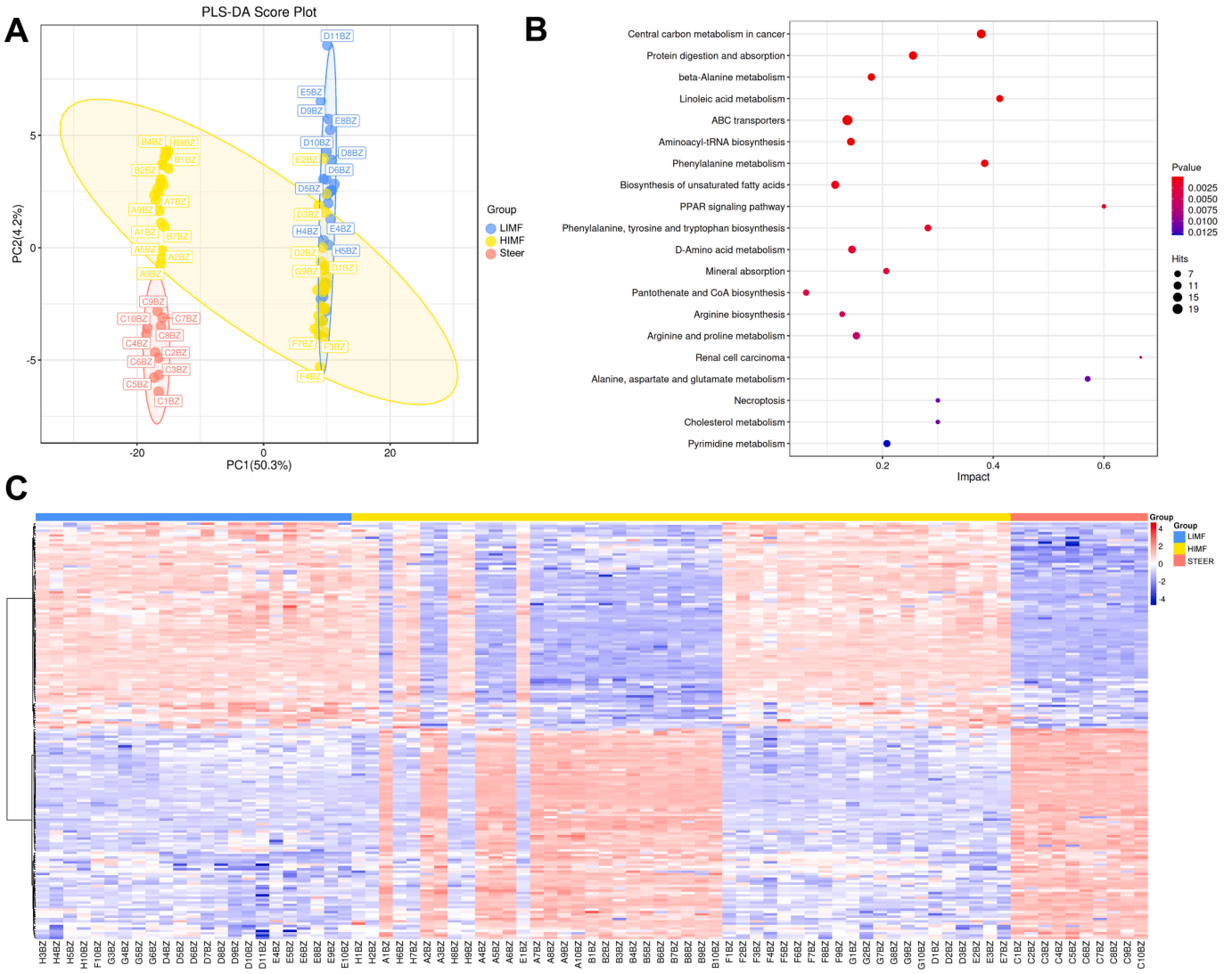

Fig. 4. LD metabolomics analysis of XBC with different IMF contents. A. PLS-DA analysis of LD metabolites of XBC with different IMF contents. B. Metabolic pathway enrichment analysis of metabolites from three groups of beef, showing the top 20 signaling pathways. C. Heatmap showing the differential metabolites of the three groups of beef.

metabolites between high and low IMF XBC beef. At the metabolic level, IMF content significantly affects the distribution of metabolites, impacting texture and taste. Our analysis showed that differential metabolites in XBC are enriched in fat-related signaling pathways, underscoring IMF's role in flavor variation. The substantial differences in IMF content within XBC—up to 8 %—highlight the need for genetic stability to improve meat quality.

Comparatively, castrated bulls, although exhibiting higher IMF and yield, had less flavor intensity, likely due to altered gut microbiota (Shi et al., 2024). This analysis provided a comprehensive view of the flavor characteristics, confirming that high IMF beef possesses richer flavor profiles.

Intramuscular fat is considered one of the most essential traits determining meat quality (Cho et al., 2010; Martin et al., 2022; Mwangi et al., 2019). Especially for beef, beef with rich marble patterns is more likely to be preferred by consumers. It is reported that the composition of IMF is closely related to the flavor of meat, and triglycerides and phospholipids are the flavor-related components, including a large amount of unsaturated fatty acids, and contribute to the formation of meat flavor (Y. C. Huang, Li, He, Wang, & Qin, 2010; Whitfield, 1992). In XBC, the difference in IMF content can be as high as 8 % in the same population, which is surprising. Therefore, obtaining an XBC population with stable inheritance of high IMF content traits is very important to improve its meat quality. Many reports have demonstrated the vast differences in the taste and flavor of meat with different IMF content. For example, the IMF content in the skeletal muscle of Laiwu pigs and Large White pigs is significantly different, which results in the high-quality flavor and taste of Laiwu pigs (Hou et al., 2023). The flavor, creaminess, and sweetness of Angus beef increase as the marbling level increases while the acidity and astringency decrease (Frank, Ball, et al., 2016). The role of specific genetic factors in regulating IMF content and meat quality has been increasingly recognized, with recent studies highlighting the involvement of KLF6 in bovine preadipocyte growth, which is crucial for understanding beef quality at the molecular level (Abbas Raza et al., 2024). Additionally, microRNAs have been identified as key regulators in muscle tissue development, further influencing IMF deposition and overall meat quality (Raza et al., 2020). Our findings identify genes associated with flavor differences in XBC, such as AQP4, FZD2, and FADS1, which are positively correlated with IMF content, and BPG1, CEBPD, and FABP4, which are negatively correlated. These insights could guide future genetic improvement efforts for enhancing XBC meat quality.

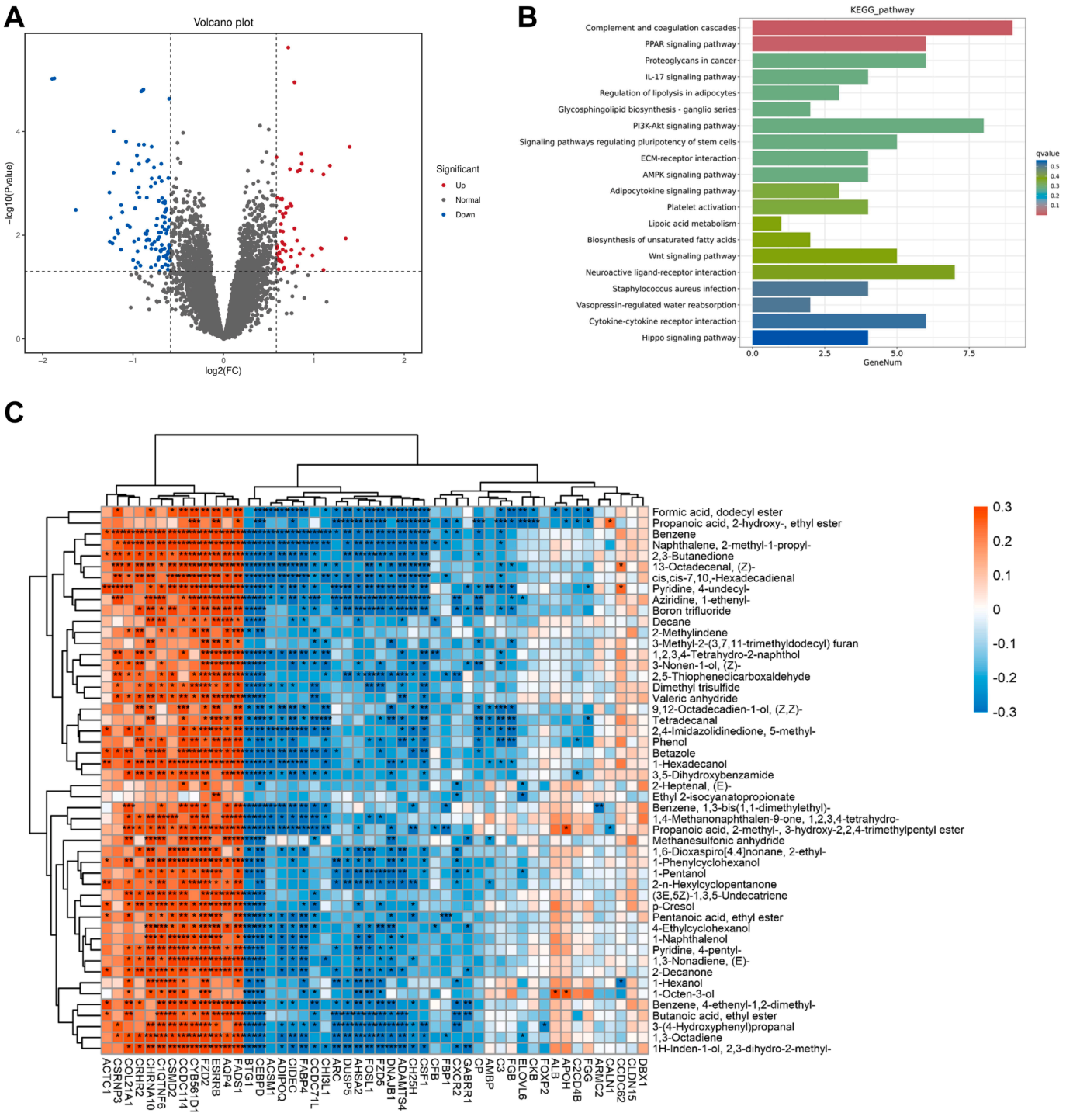

Fig. 5. RNA-Seq analysis of HIMF and LIMF and association analysis of flavor substances. A. Volcano plot of differentially expressed genes in the transcriptomic analysis of the LD muscle of HIMF and LIMF beef; B. KEGG enrichment analysis of differentially expressed genes; C. Pearson correlation coefficient analysis of the top 50 differentially expressed genes and flavor compounds.

5. Conclusion

In this study, we used multi-omics dimensions to analyze the effects of different IMF on beef flavor components and meat quality based on different IMF contents in the XBC population. For the first time, we used GC×GC-TOF MS to identify beef's volatile flavor compounds and found no significant difference in flavor between beef from different parts. However, there were significant differences in beef with different IMFs, especially in castrated bulls. Beef with high IMF showed more robust flavor characteristics such as sweet, green, fruity, and waxy, which may cause the richer flavor type of high IMF beef. The metabolomics results also showed that beef with different IMF contents greatly differed in metabolites. intramuscular fat (IMF) affects meat quality through several biochemical and physiological processes. IMF contributes to flavor development by providing a reservoir of flavor precursors, such as free fatty acids and lipid oxidation products, which are released during cooking. These compounds interact with heat to form complex flavor molecules. Additionally, IMF affects tenderness and juiciness through its influence on meat's texture. The fat infiltrates muscle fibers, creating a marbled effect that disrupts the muscle protein matrix, reducing

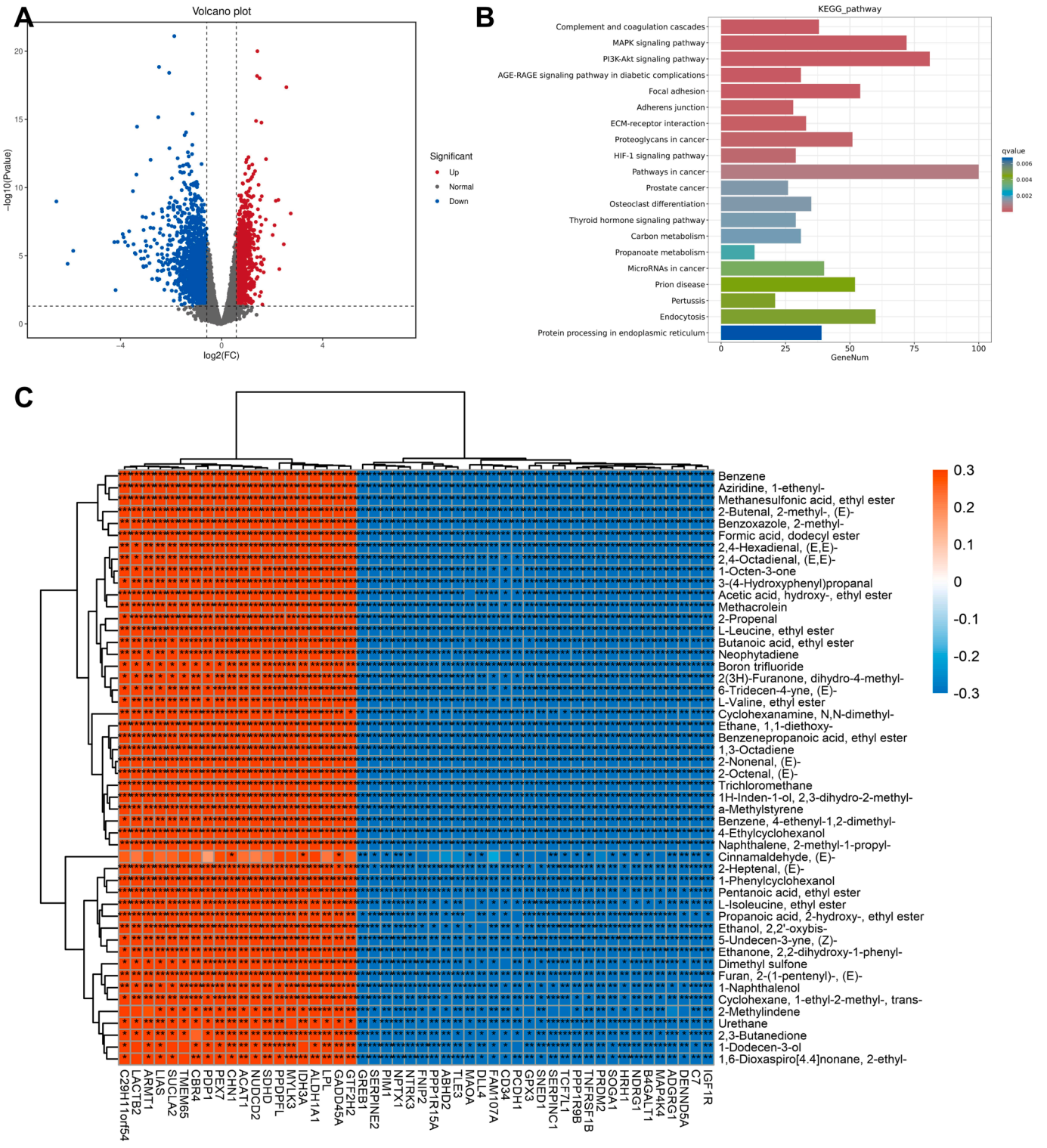

Fig. 6. RNA-Seq analysis of HIMF and Steer and association analysis of flavor A. Volcano plot of differentially expressed genes in the transcriptomic analysis of the LD muscle of HIMF and Steer beef; B. KEGG enrichment analysis of differentially expressed genes; C. Pearson correlation coefficient analysis of the top 50 differentially expressed genes and flavor compounds.

toughness and enhancing moisture retention. This marbling also helps in the even distribution of heat during cooking, further improving the texture and juiciness of the meat. After RNA-Seq analysis at the gene expression level and association with flavor substances, we found that genes such as AQP4, FZD2, FADS1, BPG1, CEBPD, and FABP4 were highly correlated with the flavor formation of XBC beef. Our results mapped the flavor characteristics of XBC beef, proved the contribution of IMF content to beef flavor, and provided a basis for the subsequent improvement of XBC meat quality.

CRediT authorship contribution statement

Zhen Ma: Writing – original draft, Visualization, Validation, Software, Data curation, Conceptualization. **Xiao Wang:** Formal analysis.

Lei Chen: Data curation. **Lixing Yuan:** Methodology. **Fanrong Cui:** Investigation. **Zongsheng Zhao:** Validation. **Xiangmin Yan:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, 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.

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.fochms.2024.100220.

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