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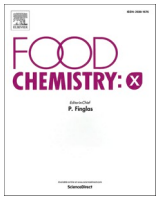

Non-targeted metabolomics reveals the effects of fermented methods on the flavor, quality, and metabolites of whipping cream

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ABSTRACT

Flavored fermented whipping cream has received particular attention. However, the effects of different fermentation methods on the quality and flavor of whipping cream remain elusive. This study characterized the flavor, quality, and metabolites of whipping cream produced by different fermentation methods based on non-targeted metabolomics. The results showed that the quality, color, and flavor of fermented whipping cream were significantly improved. Notably, the GMF fermentation group had the highest variety and content of flavoring substances. A total of 729 shared metabolites were identified, mainly including glycerophospholipids (151, 20.71 %), fatty acyls (114, 15.64 %), organoacyls (89, 12.21 %). Whipping cream by the mixed bacteria fermentation contained lower lipids and higher flavor amino acids, while the contents of bitter peptides and some organic acids and their derivatives were significantly lower. The results of this study provide a valuable reference for enhancing the quality, flavor, flavor of whipping cream using mixed bacteria fermentation methods.

1. Introduction

Fermented whipping cream has gained significant attention due to its unique texture and flavor, making it a popular ingredient in various culinary applications. In recent years, a large number of flavored cream cheeses have been produced by co-fermentation with lactic acid bacteria, with emphasis on fat modification through lactobacilli fermentation to enhance the flavor and quality of whipping cream (Wang, Fan, et al., 2023). Despite the growing interest in flavored fermented whipping cream, there is a paucity of research on the mechanisms behind flavor formation when using various blended lactic acid bacteria fermentation techniques.

Flavor formation and metabolite changes in fermented whipping cream are closely related to the different probiotics added and the fermentation method. Homofermentative *Lactobacillus rhamnosus* and heterofermentative *Leuconostoc mesenteroides* are two commonly used fermentation strains. Studies have shown that L. *rhamnosus* is a non-toxic probiotic with no side effects, which has a variety of functional properties such as regulating intestinal flora, preventing and treating diarrhea, eliminating toxins and boosting immunity (Peng et al., 2022). *L. mesenteroides* has the capacity to ferment sugars, thereby producing a variety of acids and alcohols. It has also been shown to have high antioxidant capacity and resistance to pathogenic bacteria. Consequently, it is widely used in flavoring agents, plasma substitutes, and other areas (Akpinar & Yerlikaya, 2021). Both strains are well established in sour cream, yoghurt and cheese applications (Xilin et al., 2025). For instance, the utilization of *L. mesenteroides* has been shown to enhance the storage stability of yoghurt, while concomitantly imparting a more pronounced flavor profile (Sarhir et al., 2023). A study using *S. boulardii* and *L. rhamnosus* fermentation for probiotic cream production resulted in the formation of different aromatic compounds by different fermentation methods (Goktas et al., 2022). Wang, Fan, et al. (2023) prepared fermented whipping cream using *Lactococcus lactis,* and the product has pleasant aroma and taste, with a smooth texture and overall high acceptability. Although these fermentation techniques are mature, the fermentation of whipping cream still suffers from excessive fat oxidation and easy rancidity, and the potential of flavoring bacteria to enhance the flavor in cream cheese products still needs to be explored, and there are still fewer studies on the flavor-forming mechanism of whipping cream fermented by mixed bacteria. It is therefore vital to

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explore the flavor formation mechanism of whipping cream fermented with mixed bacteria in different ways, in order to enhance the flavor and quality of whipping cream.

Non-targeted metabolomics is a scientific field that focuses on the detection and analysis of metabolites, and it can be very useful for studying correlations between metabolites. A study using a broadly applicable metabolomics approach elucidates fluctuations in milk emulsions over time (Yang et al., 2024). Functional metabolites have been reported to be metabolomically firm by mixed fermentation of *L. rhamnosus* and *L. plantarum* (Wang et al., 2022). In contrast, Wang et al. (2021) used metabolomics techniques to identify methional, hexanal, acetoin, 1-octen-3-one, furaneol, hexanoic acid in the study of cream cheese. However, studies that utilize metabolomics techniques to investigate changes in whipping cream metabolites by fermentation mode are still scarce. Non-targeted metabolomics has the capacity to comprehensively analyze the mechanism of metabolite changes in whipping cream, thereby providing a scientific basis for optimizing its flavor.

The present study investigated the effects of different fermentation methods on the quality, flavor and metabolites of fermented whipping cream. For this, the basic physicochemical properties, volatile flavor substances and metabolites of the product were examined. Through these experiments, we aim to gain a more profound comprehension of the mechanisms underlying changes in volatile flavor substances and metabolites of whipping cream prepared by different fermentation methods. This study will be crucial for improving the flavor and quality as well as enhancing the competitiveness of fermented whipping cream in the market.

2. Materials and methods

2.1. Whipping cream and strains

The whipping cream (MILKGROUND: 250 g/box, Ground Dairy Industry, Jilin, China) brand was selected for this experiment. *L. rhamnosus* (SHMCC D17392) and L. *mesenteroides* (SHMCC D16375) (Shanghai Preservation Biotechnology Center, Shanghai, China) were used as fermentation strains.

2.2. Preparation of fermented whipping cream

A total of two strains were used for the fermentation of whipping cream, *L. rhamnosus* (SHMCC D17392) and *L. mesenteroides* (SHMCC D16375) were activated and passaged twice in MRS liquid medium with good viability according to the manufacturer's requirements, and then the bacterial suspension was prepared and adjusted to a concentration of 10^7 CFU/mL.

Fermented whipping cream was prepared with little modification in reference to previous studies (Kang et al., 2023; Wang, Fan, et al., 2023; Wang, Zhang, et al., 2023), 80 mL of whipping cream was taken into a 100 mL covered glass jar, 10 % (*v*/v) sugar was added, the initial pH of the whipping cream was adjusted to 0.65 with citric acid, homogenized at 15–18 MPa for 1 min (High speed disperser XHF-DY, NingBo Scientz Biotechnology, Zhejiang, China), then sterilized at 95 °C for 10 min and cooled to about 30 °C. The following inoculations were then performed: a) 8 % (*v*/v) of *L. rhamnosus* only (LGF); b) 8 % (v/v) of *L. mesenteroides* only (LMF); c) 4 % (v/v) of *L. rhamnosus* initially and 4 % (v/v) of *L. mesenteroides* 4 h later (GMF); d) 4 % (v/v) of *L. mesenteroides* initially and 4 % (v/v) of *L. rhamnosus* 4 h later (MGF); e) simultaneous inoculation 4 % (v/v) of both bacteria (COF). The pH of the samples at the end point of fermentation was set at 4.40 ± 0.05, and the count of viable lactic acid bacteria was higher than the recommended 10^6 CFU/g, then transferred to a refrigerator at 4 °C for 12 h of post-ripening (Wang, Fan, et al., 2023). To minimize errors and interfering factors, the control group was unfermented whipping cream and the fermented and unfermented groups were set up as three parallel groups each. The fermented samples were then processed according to the test requirements.

2.3. Determination of basic physicochemical indicators

The pH of whipping cream was measured with a pH meter (PHS-25; Yichang; Shanghai, China). The Fourier infrared spectra were determined with reference to previous studies and with minor modifications (Sen et al., 2021). The determination of total and reducing sugars was conducted through the utilization of a colorimetric method, employing 3,5-dinitrosalicylic acid as the analytical reagent (Kasegn et al., 2024); lactose was determined using liquid chromatography-quadrupole electrostatic field orbit trap mass spectrometry (Vanquish Q Exactive Plus, Thermo Fisher, Bremen, Germany) (Nomi et al., 2024). Sample color was measured using a hand-held colorimeter (ZE7700, Nippon Denshoku Industries Co., Japan); the concentrations of organic acids were measured in accordance with the Chinese national standard GB 5009.157–2016; the sample photos were taken using a mobile phone model IQOO 12 (Vivo Mobile Communications, Guangdong, China).

2.4. Detection of volatile flavor substances

Referring to previous preparation method with minor modifications (Yang et al., 2023), the determination of flavoring substances was carried out by GC–MS/MS (Agilent 8890-7000D). A 20 mL vial was filled with 6.00 g of whipping cream, 2 g of sodium chloride and 5 μL of 1000 mg/mL 1,2-dichlorobenzene (internal standard). The vial was preheated at 65 °C for 5 min, and then equilibrated by inserting the extraction head for 35 min, and then detected on the GC–MS/MS.

The volatiles were then characterized based on matching the mass spectrometry data with the NIST14 database, and only compounds with >80 % similarity were characterized. This was carried out using an internal standard semi-quantitative method, with content expressed as μg/g. three parallel determinations were performed for each period sample.

2.5. Preparation of whipping cream metabolites

Metabolomics samples of whipping cream were prepared according to previous method (Zhou et al., 2025). The whipping cream samples were mixed 1: 4 with methanol solution and shaken, stabilized at −20 °C for 30 min, centrifuged at 20,000 ×*g* for 15 min, repeated twice, and the supernatant transferred to a feed bottle. An aliquot of the supernatant from each sample was mixed to prepare the QC samples, all procedures were performed on ice.

2.6. Metabolite extraction and non-targeted metabolomics analysis

Non-targeted metabolomics analyses were carried out as described in previous study (Zhou et al., 2025). Data were collected using a liquid phase (ACQUITY UPLC System I Class) and a high-resolution mass spectrometer, Q-Exactive Plus (Thermo Fisher Scientific, Bremen, Germany), on an ACQUITY UPLC T3 (column 100 mm × 2.1 mm, 1.8 μm, Waters, UK). The column temperature was set at 40 °C and the flow rate was 0.3 mL/min. The mobile phases used included phase A (5 mmol/L ammonium acetate, 5 mmol/L acetic acid and water) and phase B (acetonitrile). Positive and negative ion modes were performed separately for sample collection. During the acquisition process, QC samples were scanned every 10 samples to correct for systematic errors in quality differences between QCs in the whole batch of experiments.

2.7. Data processing

Metabolite identification was carried out using Compound Discoverer 3.1.0 software (Thermo Fisher Scientific, USA). The acquired MS data pretreatments including peak picking, peak grouping, retention time correction, second peak grouping, and annotation of isotopes and adducts was performed using XCMS software. The raw data with relative

standard deviations of higher than 30 % were removed. Only the peak area data with less than or equal to 50 % null values in each group or across all groups were saved to facilitate subsequent statistical analysis (Zhou et al., 2025).

2.8. Statistical analysis

Analysis of variance was performed using IBM SPSS Statistics 20. In the non-targeted metabolomics section, several types of statistical tests were performed, such as principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA). Differential metabolites with VIP > 1, $p < 0.05$ (t-test), FC ≤ 0.5 or ≥ 2 were screened, PCA, PLS-DA, volcano diagrams and Wayne diagrams were used to show the separation between groups. Metabolite annotation and metabolic pathway searches were conducted using the KEGG online database (https://www.kegg.jp/kegg/pathway.html) and Metaboanalyst 3.0 (http://www.metaboanalyst.ca/). The clustering heat maps of volatiles and metabolites were plotted by the LianChuan BioCloud platform tool (https://www.omicstudio.cn/index).

3. Results and discussion

3.1. The physical and chemical indicators and sample charts

To observe the quality changes of whipping cream after fermentation, samples of whipping cream fermented under optimal process conditions were selected for basic indexes and compared with unfermented samples. The results of FTIR spectroscopic detection were shown in Fig. 1A, the peaks appearing at 3200–3600 cm^{-1} are mainly -OH expansion modes generated by water and hydroxyl groups, which contain -OH groups of glucose and fructose (Al Lafi et al., 2024). The reduced peak intensity in this range for the fermented samples may be related to the metabolism of the saccharide material substances in the system. The chromatographic bands detected in the range of 1500–1700 cm^{-1} were mainly ketones, aldehydes, carboxylic acids and other carbonyl-containing compounds, the presence of distinct characteristic peaks with reduced intensity in this range and a shift in vibrational frequency towards lower wave numbers indicate the production and change of the substances of interest as described above. The peaks 1000–1300 cm^{-1} may indicate changes in glycosidic bonding, with 1065 cm^{-1} and 1115 cm^{-1} demonstrating -CC groups that are unique to glucose and fructose, respectively (Al Lafi et al., 2024). The sugar content of dairy products is one of the most important indicators that consumers are most concerned about. Lactose, as a reducing sugar widely found in dairy products, imparts specific flavor substances to fermented products and directly determines product characteristics (Mahato et al., 2020). Sugar content was shown in Fig. 1B, total sugar content was the lowest in the GMF group and the reducing sugar content was significantly higher in all fermentation groups except LGF fermentation group. Lactose content was significantly reduced after fermentation with mixed bacteria, but higher than that of the LMF group. This is because *L. rhamnosus* cannot metabolize lactose well (Hussain et al., 2021), whereas metabolism of lactose by *L. mesenteroides* can produce D-lactic acid, acetic acid, etc. (Alexandri et al., 2022). The fermented whipping cream samples and color changes were shown in Fig. 1C and Fig. 1D. Compared with the control group, the fermented samples showed a significant decrease in L^* and a^* values and a significant increase in b^* value, which may be attributed to the metabolic conversion of the shiny lipids in the whipped cream by lactobacilli to yellow ketones, resulting in a decrease in the glossiness and an increase in the yellowness of the cream (Wang, Fan, et al., 2023). In addition, the acidic state caused by the decrease in pH of fermented whipping cream destabilized the natural carotenoids in the cream leading to decrease a^* value (Zhang, Zhang, Mujumdar, & Liu, 2024). As demonstrated in Table S1, the content of organic acids was presented. A decrease in malic acid content and an increase in lactic and succinic acid content were observed in the fermented samples, suggesting that the physiological activities of the strains brought about a unique change in the fermentation system, and that this change may also have a strong link with the flavor and quality of fermented whipping cream. The above results indicate that whipping creams produced by different fermentation methods have significant differences in physicochemical indices and phenological aspects, which are often closely linked to changes in flavor and composition.

3.2. Volatile aroma substances

During the fermentation of whipping cream by lactic acid bacteria, *L. rhamnosus* (homozygous fermentation) dominates the production of acidity and inhibits spoilage bacteria through the EMP pathway, but the contribution of volatile flavor substances is limited; whereas *L. mesenteroides* (heterozygous fermentation) metabolizes sugars to produce a variety of acids and alcohols through the ketoacyl phosphate enzyme pathway and generates a variety of volatile compounds such as ethyl diphthongs, esters, and so on (e.g., floral, fruity), which synergistically balances the acidity with the aroma (Peng et al., 2022; Akpinar & Yerlikaya, 2021). Based on the above data, the volatile aroma components of different fermented whipping creams were qualitatively and quantitatively analyzed by GC–MS/MS in this study. A total of 46 volatile aroma compounds were detected, including 16 ketones, 11 acids, 10 alcohols, 6 esters, and 3 other aroma compounds (Table S2). There were 7 substances common to the fermentation group. The LMF and GMF fermentation groups contained 2 unique flavor fermentables and the unfermented group contained only one unique volatile fermentable (Fig. 2A), which were clustered as shown in Fig. 2B.

The main sources of alcohol during whipping cream fermentation can be divided into the following categories: lactose metabolism and amino acid metabolism (Wang, Fan, et al., 2023). Ten alcohols were detected, (*S*)-1,2-propanediol was the most abundant alcohol in the COF of the mixed bacterial fermentation group with a range of 1815.44 μg/g, its aroma is characterized by sweetness (Xu et al., 2021). Glycerol is the second most abundant alcohol after (*S*)-1,2-propanediol in the GMF fermentation group with a range of 1530.56 μg/g, glycerol contributes to a rounder mouthfeel in cream and imparts a slightly sweet taste (Lei et al., 2024). 2,3-Butanediol be produced either by the glycolytic pathway of lactic acid bacteria or by citric acid metabolism (Mar et al., 2020). It has an aroma typical of butter, and its content rises significantly after both monoclonal and mixed-bacterial fermentation. The GMF fermentation group produced significantly higher types of alcohols than the control and other fermentation groups.

Esters contribute mainly to fruity odours in the formation of dairy product aromas (Tian et al., 2023). A total of six esters including allyl acetate, δ-octan lactone, and butyl decan lactone were detected in this experiment with few types and low contents. The findings of the study demonstrated that propyl lactate was the most abundant ester, which reached 1941.04 μg/g in the COF fermentation group, and was able to provide a sweet, slightly acidic flavor profile to the fermented cream. Propyl pyruvate, the second most abundant ester after propyl lactate, was the most abundant in the MGF fermentation group, reaching 902.51 μg/g, which imparts a caramel floral odour to the fermented whipping cream. Among them, lactones are mainly produced by the β-oxidation process of unsaturated fatty acids and have a pronounced fruity and creamy flavor (Verma et al., 2022).

Ketones were found in the greatest variety and abundance of volatiles, which may be due to the fact that lactic acid bacteria metabolize fats to produce ketones (mainly 2-ketones). A total of 16 ketones were detected in the fermented whipping cream, most of these substances have pleasant odours such as floral, fruity and creamy aromas (Wang et al., 2021). 2-Heptanone provides pear-like fruity aroma and sweetness, and is the most typical characteristic aroma substance in cream and cheese products, 2-nonanone imparts fruit, flower, oil and herb-like aroma to fermented cream, and 2-undecanone has peach aroma, with the highest levels of all three in the GMF fermentation group (Hussain

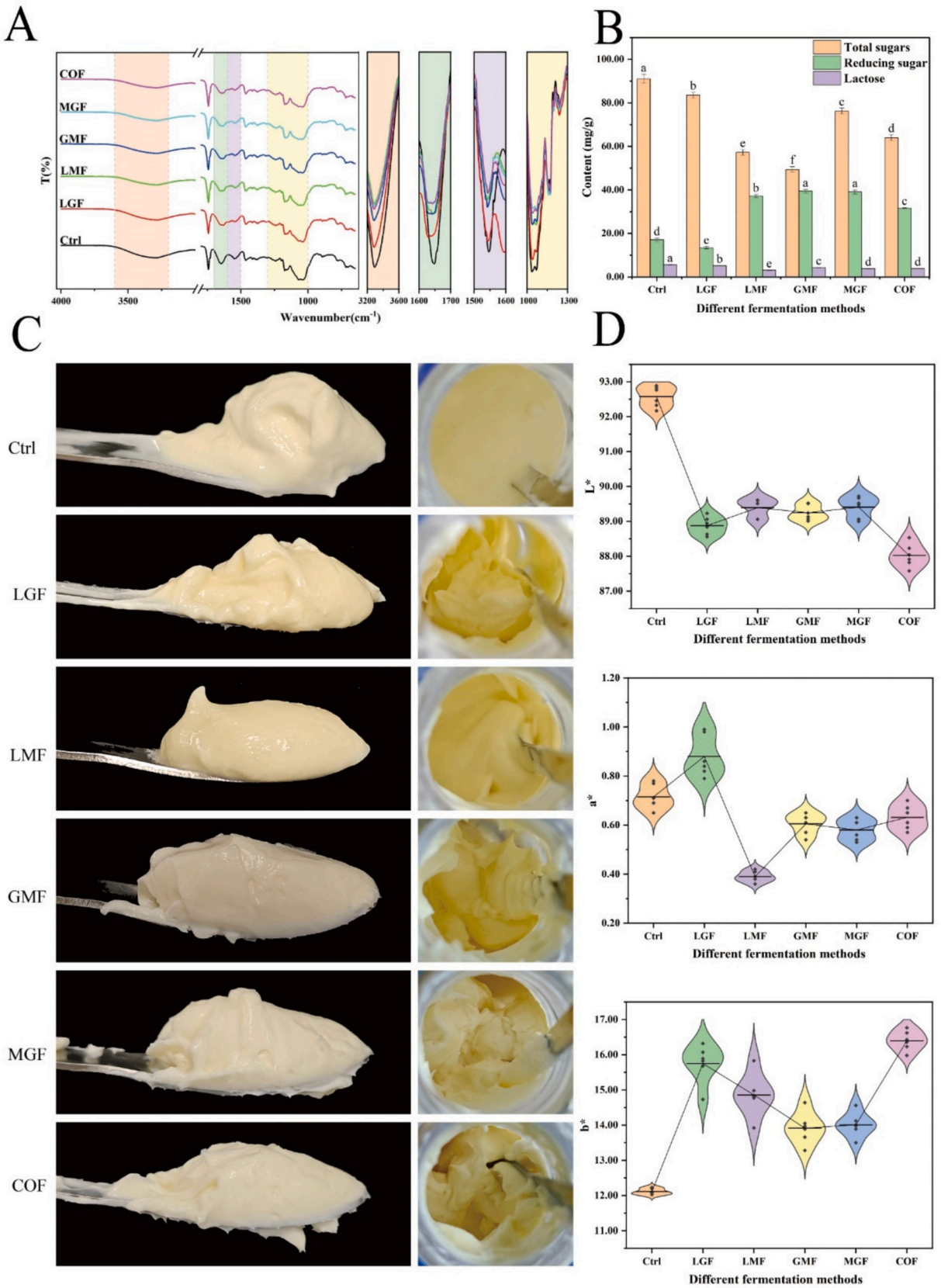

Fig. 1. Changes in basic physicochemical indicators. FTIR spectroscopy (A), total sugar, reducing sugar, lactose content (B), photographs of samples (C), changes in color (D). Ctrl denotes the non-fermented group; LGF denotes the fermented group inoculated with *L. rhamnosus* only; LMF denotes the fermented group inoculated with *L. mesenteroides* only; GMF denotes the fermented group inoculated with *L. rhamnosus* for 4 h and then continued to be inoculated with *L. mesenteroides*; MGF denotes the fermented group inoculated with *L. rhamnosus* for 4 h and then continued to be inoculated with *L. mesenteroides*; COF denotes the fermented group inoculated with both organisms.

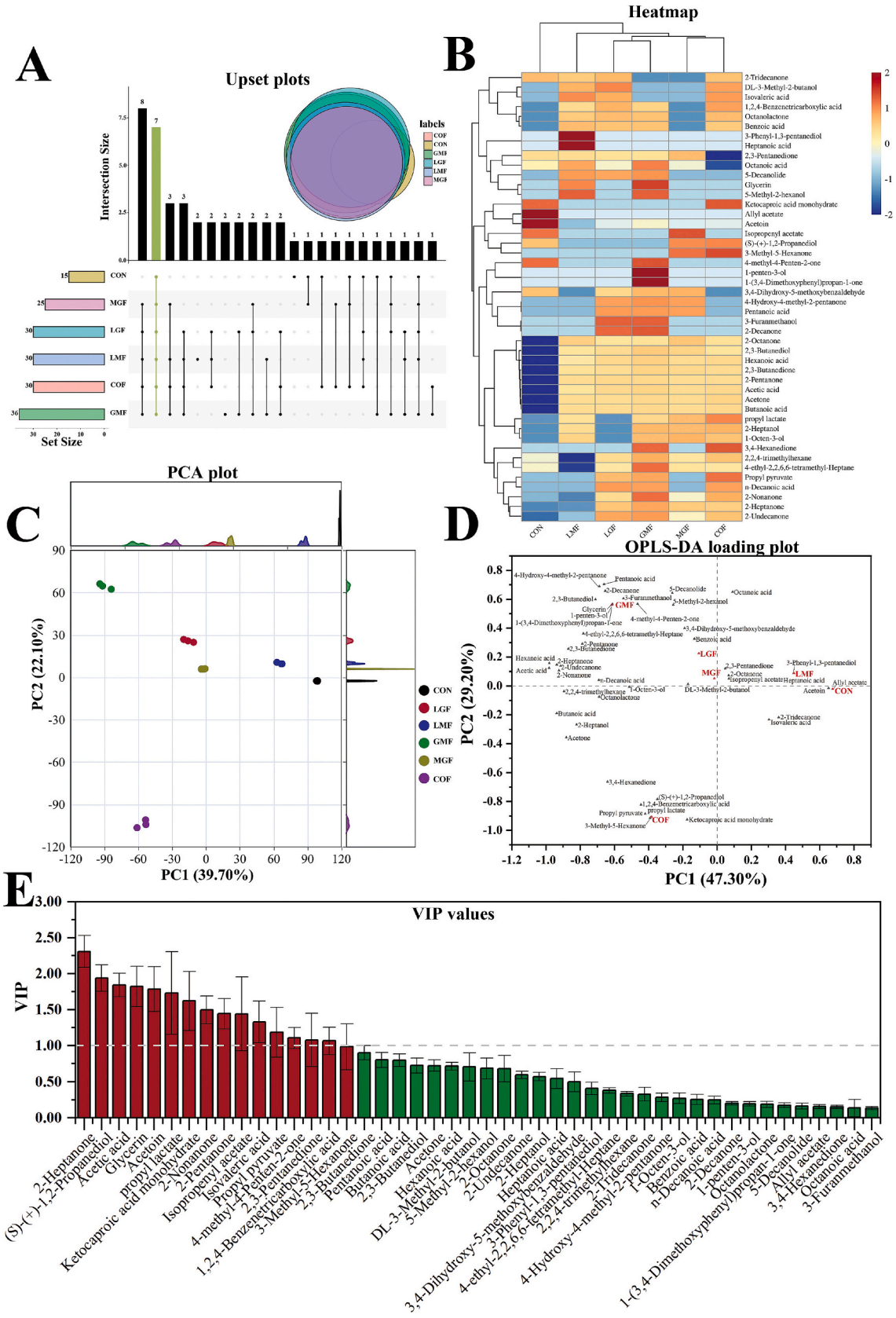

Fig. 2. The aromatic substances in different fermentation groups. (A) Upset plots of aromatic substances in different fermentation groups; (B) Heat map of content (color scale changes from blue to red, indicating sequential increase in content); (C) PCA plot; (D) OPLS-DA loading plot; (E) Distribution of VIP values (red represents the characteristic flavors with VIP > 1). (For an explanation of the colors in this legend, the reader is referred to the online version of this article). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 2021). Interestingly, the content of acetoin decreased after fermentation and the content of 2,3-butanedione increased after fermentation. Acetoin is an intermediate product of the metabolism of *Lactobacillus heterofermentans*, which can be converted to 2,3-butanedione by the action of acetyl coenzyme dehydrogenase, this is a compound with a strong buttery aroma and is one of the main reasons for the flavor produced by lactic acid bacteria (Rutkowska et al., 2022).

Acids are extremely important for their flavor and octanoic acid, acetic acid, valeric acid, hexanoic acid and n-decanoic acid were detected after fermentation of whipping cream. During the ripening of the whipping cream, valeric, capric and capric acids usually originate from lipolysis, protein hydrolysis or lactose fermentation pathways and have a fruity and greasy flavor, while acetic acid is usually produced by lactose by microbial action and generally has a sour and pungent taste. Acetic acid, valeric acid, and hexanoic acid were significantly higher in GMF than in the other fermentation groups ($p < 0.05$). Heptanoic acid was only detected in LMF, which had a rancid fatty flavor. Interestingly, the presence of octanoic acid was detected in the unfermented mixture, which could be due to natural presence or high temperature treatment (e.g. pasteurization), the observed result is in line with previous findings indicating that the synthesis of volatile acids occurs from strain-dependent metabolic pathways inherent in specific fermenting microorganisms during biochemical transformations (Zheng et al., 2024).

Three other substances, 5-hydroxyvanillin, 2,2,4-trimethylhexane and 4-ethyl-2,2,6,6-tetramethyl-heptane were also detected, and were not major flavoring substances in fermented whipping cream due to their generally low levels.

In addition, the volatile flavor substances of different whipping creams were analyzed by marginal PCA in this study and the results are shown in Fig. 2C. As demonstrated by the figure, the cumulative variance contribution of PC1 (47.30 %) and PC2 (29.20 %) is 76.50 %, and the parallel sample distributions were clustered with each other, indicating the effectiveness of this PCA separation model. The unfermented and fermented samples were well separated from each other on both PC1 and PC2, and their mountain range plots were far away from each other, suggesting that the volatile compounds of the whipping cream samples from the unfermented group and from the different fermentation groups were more different. Fig. 2D and Fig. 2E showed the OPLS-DA loading plots and VIP ranking plots of volatile compounds at different fermentation stages, and usually substances with VIP ≥ 1 are considered as key substances (Chen et al., 2024). The cumulative statistic of the model, $R^2X = 0.998$, the parameter of model explanatory rate, $R^2Y = 0.998$, and the parameter of predictive ability, $Q^2 = 0.997$, are all higher than 0.5, which indicates that the model demonstrates excellent predictive ability and validity. The OPLS-DA results showed that t volatile compounds were well differentiated between samples, with more flavor compounds similar to those of the GMF fermentation group, indicating that this fermentation group was highly correlated with many flavor compounds, and that this type of fermentation was able to increase the complexity of flavor compounds in the fermentation system. A total of 16 key substances with VIP ≥ 1 were screened, which could be used to discriminate different fermentation modes, namely 2-heptanone, (*S*)-(+)-1,2-propanediol, acetic acid, malonyl alcohol, acetoin, propyl lactate, ketomalonic acid, 2-nonanone, 2-pentanone, isopropenyl acetate, isovaleric acid, propyl pyruvate, 4-methyl-4-penten-2-one, 2,3 -pentanedione, 1,2,4-benzenetricarboxylic acid, 3-methyl-5-hexanone. These substances, which were found in high levels in the GMF fermentation group, essentially had floral-fruity, sour, sweet and fatty flavors, with 2-heptanone being considered to have a pear-like fruity aroma. Thus, these findings enabled us to determine that the main sources of flavor in fermented whipping cream are fat, floral-fruity, sour-sweet and fatty flavors. The results showed that the two Lactobacillus strains could effectively enhance the flavor profile of fermented whipping cream and improve the organoleptic acceptability of its products.

3.3. Non-targeted metabolic analysis

3.3.1. HCA clustering, PCA and OPLS-DA analysis

Based on previous studies, the present study used non-target metabolomics to reveal the production and transformation processes of potential metabolites in whipping cream. The results showed that a total of 15,861 metabolites were identified in both negative and positive ion modes, and a total of 729 secondary metabolites were annotated by MS/MS mode identification. As shown in Fig. 3A, these metabolites were mainly distributed as: 6 indoles and derivatives; 6 azoles; 5 pyrrolidines; 151 glycerophospholipids; 8 keto acids and derivatives; 7 organic sulfuric acids and derivatives; 6 phenols; 21 organonitrogen compounds; 19 benzene and substituted derivatives; 12 glycerolipids; 114 fatty acyls; 5 coumarins and derivatives; 4 pyrans; 4 organic phosphoric acids and derivatives; 89 organooxygen compounds; 67 carboxylic acids and derivatives; 28 sphingolipids; 9 steroids and steroid derivatives; 9 diazines; 8 prenol lipids; 11 pyridines and derivatives; 10 hydroxy acids and derivatives, and 130 others (Fig. S1A). Most of these substances are lipids and their derivatives and organic oxygen compounds, in agreement with Zhou et al. (2025).

Subsequently, this study comprehensively analyzed the differences in metabolites of different fermented whipping cream samples by combining cluster analysis, PCA analysis and OPLS-DA analysis. As shown in Fig. S1B, the unfermented group, LMF, LGF, and mixed bacterial fermentation group (GMF, MGF, and COF) formed clusters, respectively, the above phenomena indicate that the metabolic profiles differed significantly before and after fermentation. In order to depict the distribution of principal components between samples from fermented and different fermentation groups, PCA (Fig. S1C) and OPLS-DA (Fig. S1D) analyses were performed, and a clear distinction was found between the unfermented and different fermentation groups. In PCA, both fermented and unfermented whipping cream samples were significantly different, similar to the results of previous studies (Wu et al., 2024; Zhou et al., 2025), and there was a partial overlap between the GMF and the COF, suggesting the similarity of their metabolites. All biological replicates were within 95 % confidence intervals, with good biological reproducibility within groups. In conclusion, whipping creams prepared by different fermentation methods showed significant differences in metabolomics, which provided a prerequisite for subsequent differential metabolite screening.

3.3.2. PLS-DA analysis

To obtain more reliable differences between metabolite groups, metabolite profiles in whipping cream samples were modelled and predicted using the PLS-DA model with supervised analysis (Kang et al., 2023). The quality indices R^2 of the PLS-DA models in Fig. S2(A1-E1) were all greater than 0.99, Q^2 was greater than 0.6, indicating that the model had good fitting and predictive ability. In addition, 200 reciprocal substitution tests were conducted in this study and the results were shown in Fig. S2(A2-E2), intercept Q^2 was less than −0.6 and Intercept R^2 was greater than 0.7 between the different comparison groups, indicating that there was no overfitting of the model. The results reflected the comparative reliability of the metabolomics data and modelling, confirming significant differences in the metabolite profiles of whipping cream samples processed by different fermentation methods, and providing a basis for subsequent screening of differential metabolites.

3.3.3. Screening of differential metabolites in whipping cream

Based on PLS-DA analysis, metabolites with FC (ratio) ≥ 2 or FC (ratio) ≤ 0.5, VIP ≥ 1, and $p < 0.05$ (*t*-test) were identified as differential metabolites in this study. Then, this study visualized and analyzed the differential metabolites of each comparison group in the form of volcano plots (Fig. 3B and C), and identified 156 (GMF VS Ctrl), 70 (GMF VS LGF), 80 (GMF VS LMF), 44 (GMF VS MGF), and 21 (GMF VS COF) significant differential metabolites. The results showed that the

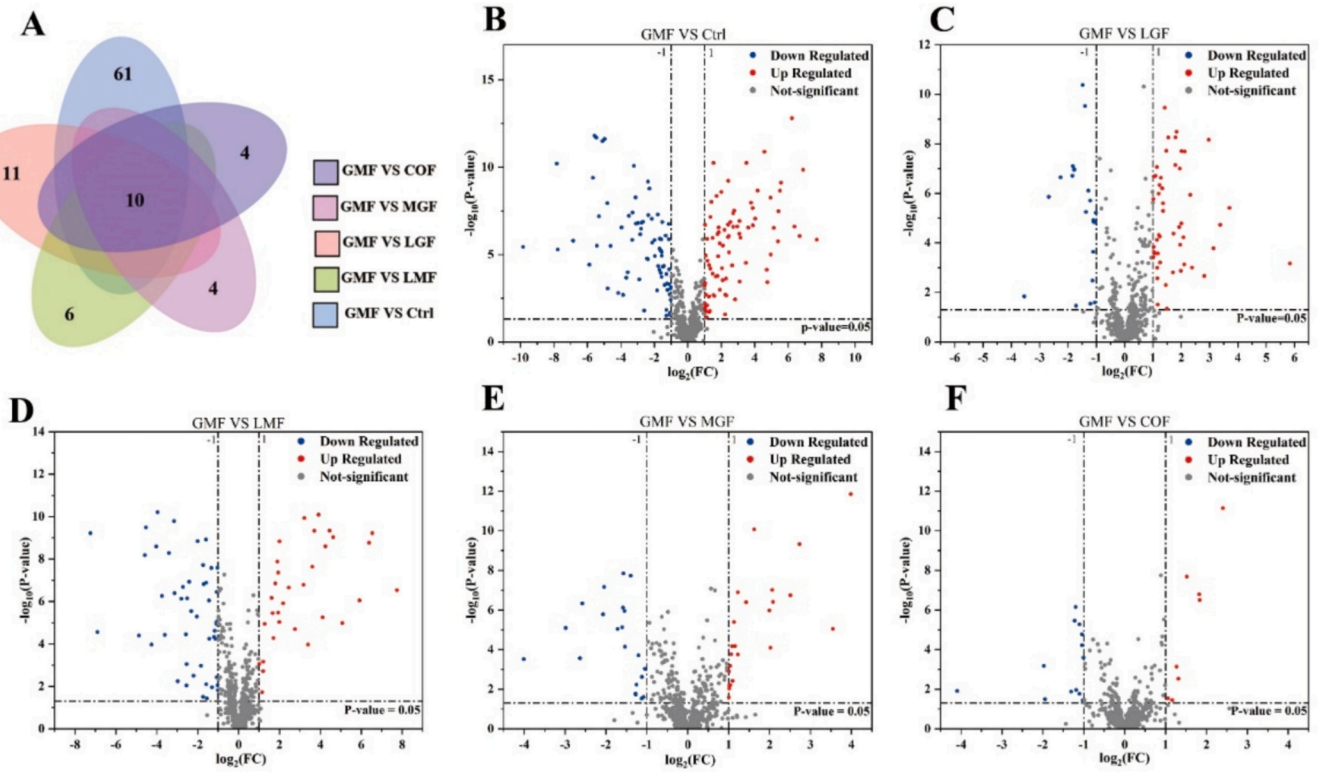

Fig. 3. The different metabolites in different fermentation groups. Venn diagram (A) and Volcano plot (B, C, D). Differential comparison of different metabolites in six groups of FWC samples: GMF VS Ctrl, GMF VS LGF, GMF VS LMF, GMF VS MGF, and GMF VS COF. In the pyramid plot, red dots represent significantly up-regulated differential metabolites, blue dots represent significantly down-regulated differential metabolites, and grey dots represent insignificant differential metabolites. The Venn diagram shows the total number of partitions that categorized the differential metabolites in the different comparison groups, and the numbers indicate the number of cross-biotic metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

metabolites in the samples from the GMF fermentation group were able to show significant regulatory differences compared to both the control and other fermentation groups. These results indicate that the two lactic acid bacteria differed significantly in metabolic levels during fermentation of whipping cream which may be related to the intercellular contact interaction, co-operation and synergistic symbiosis between the two strains in the fermentation system, these dynamic changes fundamentally differentiated the metabolites of the GMF from those of the CON, LGF and LMF group (Wang, Zhang, et al., 2023). In addition, the significant differences between the mixed-bacteria fermentation groups can be explained by the fact that L. *rhamnosus* in the GMF rapidly acidified the fermentation environment, which triggered the acid-tolerant reaction mechanism of L. *mesenteroides* to promote the synthesis of acetoin and dextran (Sarhir et al., 2023; Yang et al., 2022), while the early colonization of L. *mesenteroides* in the MGF converted sugars to acetic acid or ethanol, which activated propionic acid production or esterase expression pathways in L. *rhamnosus*, forcing subsequent inoculation of L. *rhamnosus* to decompose other substrates to produce esters, ketones, branched-chain fatty acids, ketones, etc. (Goktas et al., 2022; Hussain et al., 2021). For COF, our simultaneous inoculation of both bacteria in the initial pH environment exacerbated microbial competition, thereby inhibiting the full expression of certain pathways through resource constraints to produce metabolites different from GMF. This finding suggests that strain inoculation timing can be a key parameter in regulating metabolite diversity in mixed fermentations. In addition, after advanced Wayne diagram statistics, 10 differential metabolites were present in the different comparison groups: 4 dipeptides, 3 glycerophospholipids, 2 lys-phospholipids, and 1 fatty amide (Fig. 3A).

3.4. Abundance and correlation analysis of differential metabolites

High-frequency clustering heatmaps are important tools used to demonstrate the similarity between metabolites and samples. As shown in Fig. 4, the grouping and classification of the samples were demonstrated by hierarchical clustering of the metabolite levels in the samples from different treatment groups, the color scale, ranging from red to blue, indicates increasing and decreasing relative expression levels, respectively (Zhang, Zhang, Xiao, et al., 2024). The results showed that the intra-group convergence of whipping cream from different treatment groups was well reproducible and the inter-groups could be well distinguished. Among them, MGF and COF converged firstly indicating that these had more similarity, and Ctrl clustered lastly indicating that it was more different from other samples, which was consistent with the previous findings. In addition, the whipped cream metabolites fermented by the two Lactobacillus strains in the GMF fermentation group were significantly different in many aspects compared to the unfermented group and the other fermentation groups, which laid the groundwork for the subsequent analyses.

Furthermore, the present study analyzed the interactions between significantly different metabolites in whipping cream under different fermentation methods, with molecular network formation as shown in Fig. 5. The larger the dots, the more the number of related objects, the thicker the line, the stronger the correlation between the metabolites, and the positive and negative correlation between the metabolites characterizes the synthesis and conversion relationship between the metabolites. When a synthetic pathway is active, the metabolites involved get accumulated simultaneously (i.e., a positive correlation); whereas in catabolic reactions, when the substrate is consumed to a lesser extent, the corresponding product is increased (i.e., a negative

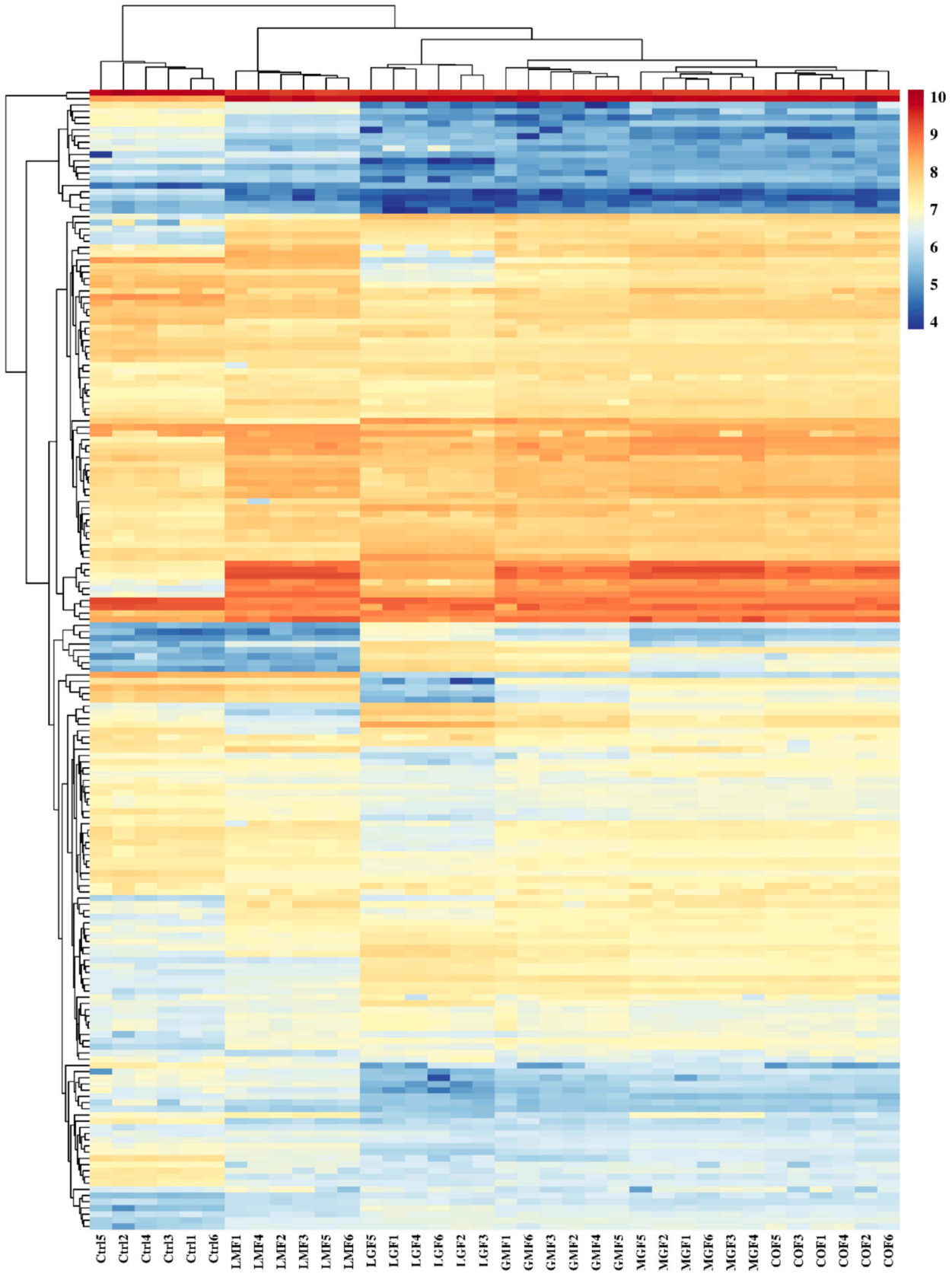

Fig. 4. Thermograms of metabolites Thermograms of FWC samples under different fermentation methods.

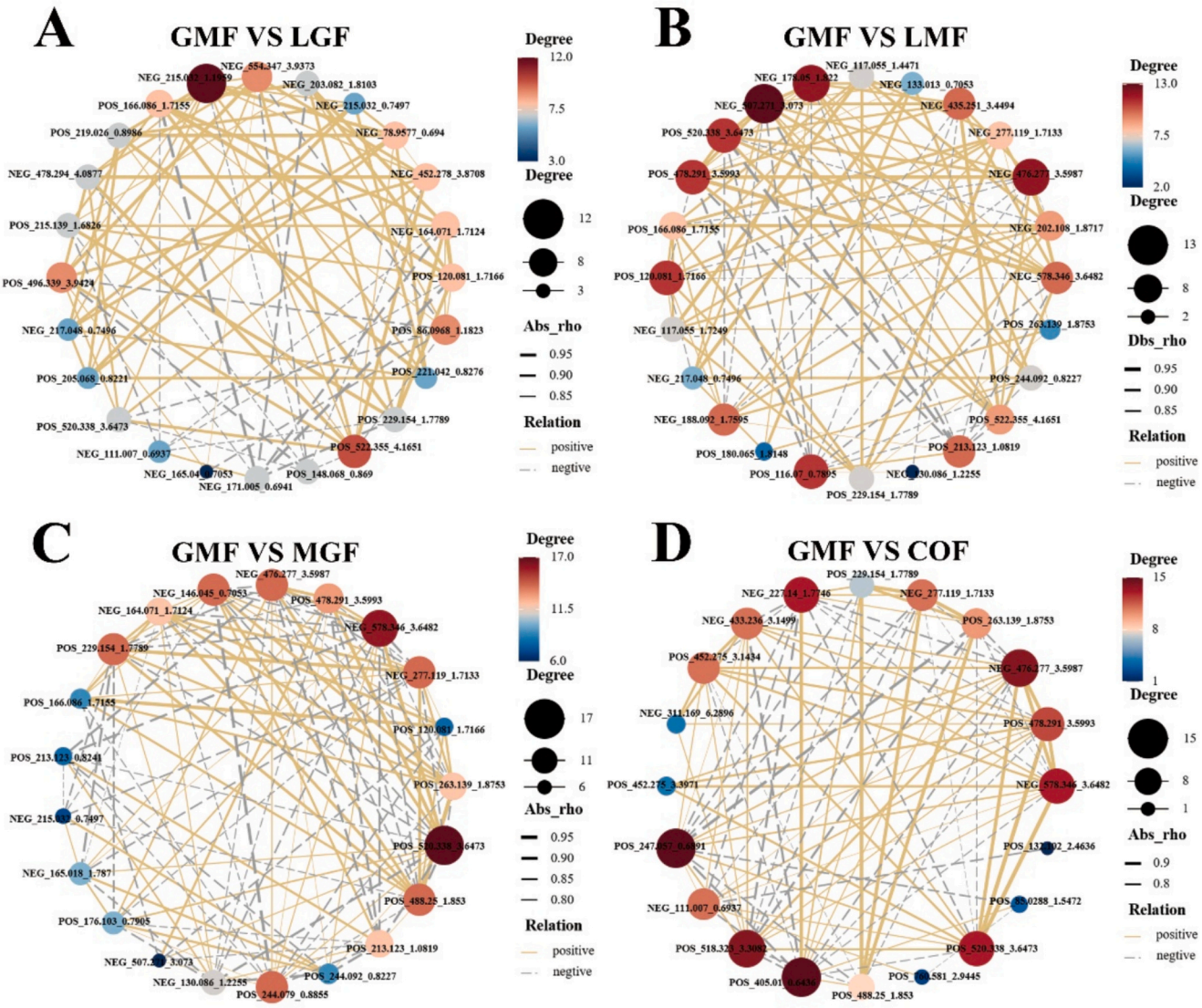

Fig. 5. Spearman's correlation network plots of significantly different metabolites. A, GMF VS LGF; B, GMF VS LMF; C, GMF VS MGF; D, GMF VS COF. The larger dots and redder colors represent more correlated metabolites. Thicker lines indicate stronger correlations between metabolites, and the color of the lines characterizes positive and negative correlations.

correlation), and the positive-negative correlation between metabolites characterizes the synthetic and transformational relationships between metabolites (Wang, Zhang, et al., 2023). As shown in Fig. 5A, 2-hydroxyphenethylamine was positively correlated and most strongly correlated with phenylalanine in the correlation analysis of significantly different metabolites in the GMF VS LGF comparison group. Increased levels of phenylalanine, a precursor for the synthesis of catecholamines such as tyrosine and dopamine, may promote the accumulation of antioxidants in fermented samples, which are important for cognitive health and stress response (Mamy et al., 2024). The highest amount of phenylalanine was found in GMF compared to Ctrl and mixed bacterial fermentation groups. D-mannitol is positively correlated with bergapten, D-mannitol has beneficial physiological effects such as bacteriostatic and antioxidant properties and has been recognized as GRAS (Generally Recognized as Safe) by the FDA, which facilitates its use in food products (Zhang et al., 2023). In addition, phenylalanine was significantly negatively correlated with disodium glycerol-3-phosphate and 2-furoic acid. Fig. 5B showed that the GMF VS LMF comparison group had the highest number of objects related to the differential metabolite, LPG (18: 2). Among them, LPG was strongly positively correlated with 1-oleoyl-sn-glycero-3-phosphocholine, p-acetaminobenzoic acid and negatively correlated with Pro-Pro. LPG (18: 2) is a lys-phospholipid, and this differential metabolite showed a down-regulation in the comparator group, whereas PG (18: 1), PG (35: 2) were up-regulated. It has been shown that heating operation depletes lysozyme-GPL during fermentation of lactic acid bacteria, but a dramatic increase in the amount of phospholipid PG, a unique phospholipid in the cell membrane of the bacterium, reverses phospholipid degradation, which is consistent with the results of this study (Liu et al., 2024). As shown in Fig. 5C, in the correlation analysis of significantly different metabolites between the GMF VS MGF comparison groups, the most relevant subjects were associated with the lysophospholipid LPC (18: 2), LPC (18: 2) was positively correlated with PE (18: 2/0: 0), Pro-Pro, etc., and its metabolic network relationship was similar to that of the comparator group GMF VS LGF, Pro-Pro was strongly negatively correlated with *N*-acetylgalactosamine. Between the GMF VS COF comparison groups (Fig. 5D), the highest number of relevant objects was glycerophosphoglycerol, a metabolite that showed metabolic down-regulation, which is consistent with previous research (Ding et al., 2022). In this metabolome, the metabolite that showed the strongest positive correlation was LPC (18: 2/0: 0), and the metabolites that showed the strongest negative correlation were glycerophosphoglycerol

and Phe-Pro.

3.5. Metabolic pathway analysis of whipping cream from different fermentation methods

3.5.1. KEGG pathway enrichment of differentially expressed metabolites

Enrichment analysis facilitates the subsequent screening of pathways to identify those which demonstrate the highest association with diverse metabolites (Xu et al., 2023). On this basis, different metabolites were linked to specific pathways in the KEGG database and the major pathways of the different metabolites were obtained by enrichment analysis. The larger the enrichment factor value, the higher the enrichment of the pathway; the larger the dot, the more differential metabolites in the pathway; the color of the dot represents the enrichment significance q-value, and the smaller the q-value, the greater the enrichment significance (Wang, Zhang, et al., 2023).

As shown in Fig. 6, the metabolic pathway of fermented whipping cream involves 3 main functions: metabolism, environmental information processing and genetic information processing. In the context of GMF VS Ctrl species, the primary enrichment pathways were as follows: glycerophospholipid metabolism, galactose metabolism, glycerolipid metabolism, metabolic pathways, D-amino acid metabolism, citrate acid cycle (TCA cycle) and ornithine, lysine and nicotinic acid alkaloid biosynthesis (Fig. 6A). In the GMF VS LGF (Fig. 6B) and GMF VS LMF (Fig. 6C) comparator groups, the enrichment of differential metabolites was significantly reduced in metabolic pathways, and glycerophospholipid metabolism, galactose metabolism, glycerolipid metabolism, metabolic pathways, biosynthesis of ornithine, lysine, and nicotinic acid alkaloids, the TCA cycle and D-amino acid metabolism were significantly lower, metabolites were promoted. GMF mainly enhanced glycerophospholipid metabolism, D-amino acid metabolism, arginine and proline metabolism, and arginine biosynthesis, as compared to MGF in the mixed bacterial fermentation group (Fig. 6D). Compared with the mixed-organism fermentation group sample (COF), the GMF mainly promoted metabolic processes such as glycerophospholipid metabolism (Fig. 6E). Glycerophospholipid metabolism, amino acid metabolism, sugar metabolism and organic acid metabolism levels are dominant in these metabolite enrichment pathways, which is similar to previous research (Kang et al., 2024). The results presented herein clearly demonstrate that the process of converting lipids, glycolysis/gluconeogenesis of sugars and metabolism of derived and amino acids during whipping cream fermentation represent the necessary hubs of biosynthesis and metabolism of the bacterial cells, the composition and content of lipids and organic acids are key factors in determining the unique flavor of fermented dairy products (Zhang, Zhang, Xiao, et al., 2024).

3.5.2. KEGG metabolic network analysis

Through functional analyses of metabolites and their enrichment in the pathway, we identified common differential metabolites such as glycerol-3P, glycerophospholipids, glutamic acid, mannose, raffinose, citrate, furoate, bergapten, etc., which can be mainly categorized as lipids and their related metabolites, amino acids, sugars, organic acids. Based on the association between differential metabolites and KEGG pathways, this study constructed metabolic networks of significantly different metabolites in different whipping cream samples by combining the metabolic pathways reported in the KEGG database, analyzed the main effects of different fermentation modes on the fermented samples, and explored in depth the evolutionary mechanisms among the metabolites (Fig. 7). Where, red, and black color indicated key functional metabolites and related functional metabolites, respectively. The levels of the different metabolites were represented as heat maps in the figure, with blue to red representing increasing levels.

Lactic acid bacteria metabolize lipids during the fermentation of whipping cream to produce important by-products, and lipids are also affected by temperature and homogenization factors during processing. Ketones have been shown to be produced by lipid oxidation and esterification reactions carried out by microorganisms in the product, with 2-ketones (e.g. 2-pentanone, 2-heptanone and 2-nonanone) being the most abundant, produced by fatty acid oxidation or bioenzymatic oxidation and having a buttery, pungent or blue cheese flavor (Fu et al., 2022). In the present study, the lipid differential metabolites showing significant down-regulation in the post-fermentation samples were PA (18: 2/16: 0), PE (18: 2/0: 0), PE (18: 1/0: 0), PS (18: 1/0: 0), LPA 16: 0, LPA 18: 2, LPG 18: 2, Relative to single-bacteria fermentation, these substances were found in lower levels after mixed-bacteria fermentation, while their corresponding ketone species and levels were higher. Therefore, we hypothesized that lipids were oxidized and microbially esterified by both lactic acid bacteria to form some ketones such as 2-heptanone and 2-nonanone (Table S2), which in turn enhanced the variety and content of the fermented whipping cream flavoring substances, which was more pronounced in the GMF fermentation group. *L. rhamnosus* can inhibit the activity of key enzymes of phospholipid biosynthesis through rapid acid production, while acidic conditions lead to the conversion of phospholipids into more fatty acids, which in turn lead to the formation of ketones with the participation of lipoxygenase, which is the main reason why the GMF fermentation group is different from the other sample groups (Fu et al., 2022; Wang, Fan, et al., 2023).

Meanwhile, peptides and amino acids are derived from the degradation of proteins during whipping cream fermentation, most of which will serve as flavorings or precursors of flavorings, and changes in these metabolites play a crucial role in the flavor assessment of fermented products. Among them, glutamic acid is an important metabolite in the amino acid metabolic pathway that can be converted into bioactive and flavor compounds by enzymes produced by lactic acid bacteria (Lee et al., 2022), its content decreases after fermentation. In the fermentation group containing *L. rhamnosus*, the levels of L-arginine, D-proline, and L-tryptophan were significantly higher relative to the control group, which may be due to the fact that *L. rhamnosus* has specific proteases and peptidases such as serine protease, proline-specific peptidases PepR and PepX, which are well capable of converting proteins and peptides into amino acids. Kim et al. (2023) demonstrated that co-administration of *L. rhamnosus* and dietary proteins promotes amino acid uptake by the intestinal enterocytes, resulting in health benefits. In addition, the aromatic amino acid L-tryptophan is essential for protein synthesis in humans and animals and is important in the treatment of insomnia and other sleep-related disorders, depression, behavioral disorders and premenstrual syndrome (PMS) (Yousef et al., 2024). Among the peptides characterized in this study, Phe-Pro, Leu-Pro, Pro-Pro, Tyr-Pro were described as bitter peptides (Wei et al., 2023), Val-Pro, Phe-Ala were described as fresh flavor peptides, bitter flavor peptides. The detection of these peptides in the fermented whipping cream was higher in the single-organism fermentation group LGF and lower in the mixed-organism fermentation group, which suggest that in terms of amino acid metabolism, the *L. rhamnosus* fermentation may have a detrimental effect on the mouthfeel of the whipping cream, and this undesirable flavor could be ameliorated by the mixed-organism fermentation.

Secondly, it is imperative to acknowledge the significance of sugar metabolism as a pivotal pathway in the fermentation process of lactic acid bacteria. The content of lactose in fermented whipping cream was significantly lower, which is consistent with the previous findings. D-sorbitol, D-mannose, fructose, raffiuose, and D-mannitol can be categorized as galactose and sugar polyols, which are considered safe to consume and endowed with health benefits (Urminská et al., 2022). Compared to the control and *L. mesenteroides* fermentation groups, the levels of these substances were higher in samples of fermented whipping cream containing *L. mesenteroides*, especially in the MGF fermentation group. This may be due to the ability of *L. mesenteroides* to catalyze sugars into dietary polyol structures, a potentially probiotic property of heterogeneous fermentation strains (Martínez-Miranda et al., 2022).

In addition, the acids in the TCA cycle play a crucial role in the flavor and quality of whipping cream. Compared with the control group, the

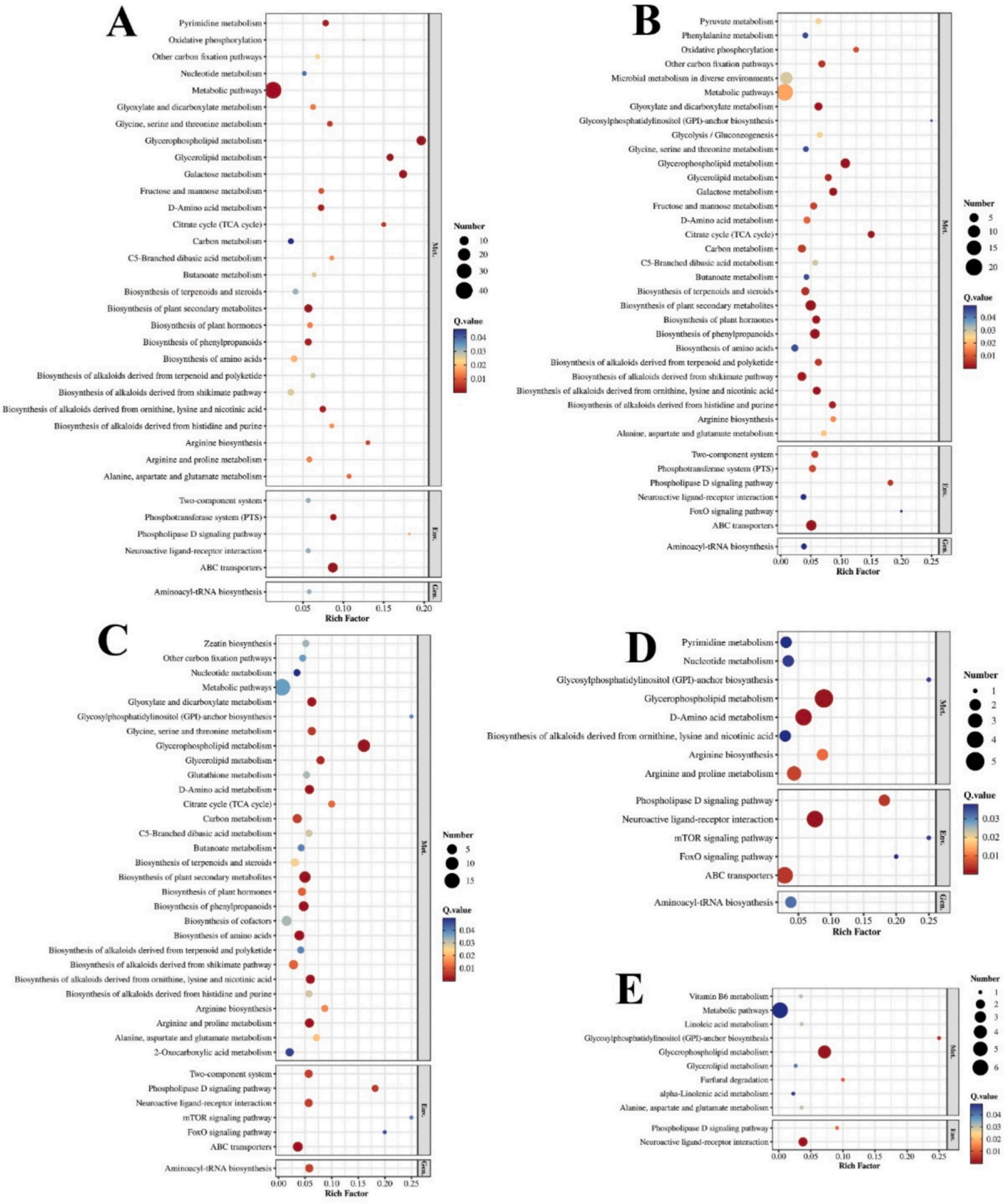

Fig. 6. KEGG pathways for differential metabolite enrichment in different comparison groups of FWC samples. A, B, C, D, and E indicate the metabolic pathways with significant differential metabolite enrichment in GMF VS Ctrl, GMF VS LGF, GMF VS LMF, GMF VS MGF, GMF VS COF comparison groups, respectively. The horizontal coordinate indicates the enrichment factor and the vertical coordinate represents the pathway's name. Each point represents a pathway. The color of the dot reflects the size of the *P*-value, and the bluer the color, the more significant the enrichment. The size of the dot reflects the number of differential metabolites enriched to the metabolic pathway, and the larger the dot, the more metabolites are enriched to the metabolic pathway.

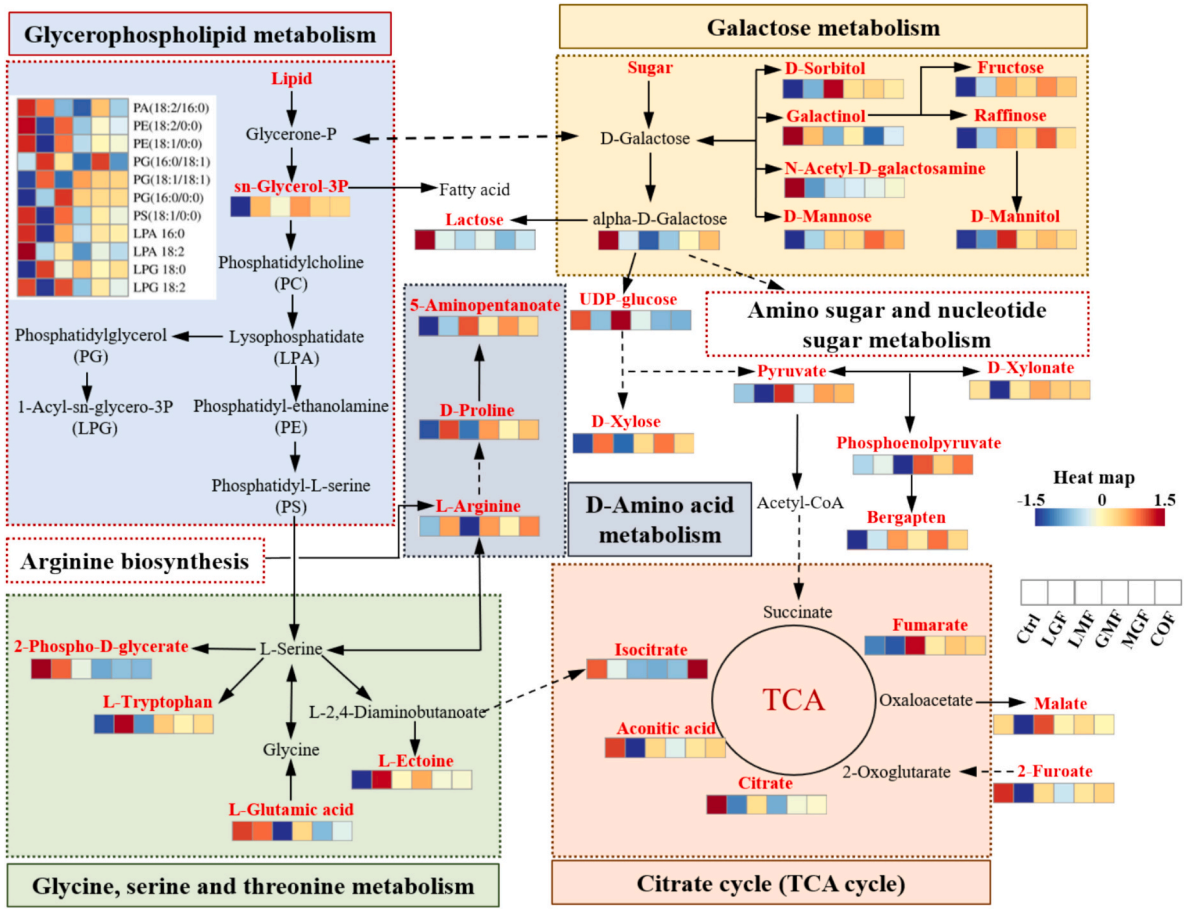

Fig. 7. Analysis of the relationship network between key functional metabolites and correlated professional metabolites using KEGG-based analysis of the metabolic pipeline. The essential metabolites versus the functionally related metabolites are depicted in red and black, respectively. The levels of various metabolites are presented as a heat map, with increasing levels from blue to red. From left to right are Ctrl, LGF, LMF, GMF, MGF, and COF.

LGF fermentation group contained significantly lower levels of aconitic, citric, nicotinic, 2-furoic, and malic acids, which is consistent with the results of the previous study (Table S1). This may be due to the fact that *L. rhamnosus*, as a homofermentative lactic acid bacterium, produces large amounts of lactic acid mainly through the EMP pathway without TCA cycle metabolism, and acids such as malate may also be used as an energy source in the presence of lactic acid for secondary fermentations such as malic-lactic acid fermentation (MLF). In contrast, *L. mesenteroides* can promote the production of acids such as fumarate and malate via the ketoglutarose phosphate pathway (ED pathway) and the TCA cycle (Yang et al., 2022).

In summary, *L. rhamnosus* mainly promoted the conversion of lipids and the formation of amino acids during the fermentation of whipping cream, and *L. mesenteroides* mainly promoted the metabolism and production of sugars and acids. In addition, the mixed *Lactobacillus* fermentation group had lower levels of fatty acids, glycerophospholipids and lysophosphatidylglycerol, higher levels of flavor amino acids and their metabolites, and significantly lower levels of bitter peptides and some organic acids and their derivatives, where too high a level of acids would produce a harsh taste, and the reverse would make for a homogeneous flavor profile. Therefore, the content level of metabolites in the GMF fermentation group can show a better advantage in ensuring the richness of volatile flavor substances type and content.

4. Conclusion

This study provides valuable insights into the mechanisms of flavor and quality improvement in fermented whipping cream through mixed-bacteria fermentation, highlighting the importance of specific metabolic pathways and the potential of *L. mesenteroides* in enhancing the sensory attributes and functional properties of the cream. In this study, the fundamental physicochemical indexes and volatile flavor substances were determined, and the metabolites of fermented whipping cream were combined with stoichiometric and pathway analyses. It was thus determined that the differences in the synthesis of flavor substances and metabolite content of different strains were related to the metabolic pathways. The whipping cream fermented by different ways exhibited changes in -OH groups, amide I, amide II and -CC groups in Fourier infrared spectrograms, and the fermented whipping cream had lower total sugar and lactose content, higher reducing sugar content, moderate acidity, lower L^* and a^* values, and higher b^* values, and the texture of the whipping cream was improved significantly by the mixed-bacteria fermentation. The volatile substances were positively affected in the mixed-bacteria fermentation group, and the types and contents of volatile flavor substances, such as ketones, were significantly higher, and by comparing the different fermentation methods, it could be found that the GMF fermentation group had the richest types and higher contents of volatile flavor substances. Metabolomics analyses showed the identification of different metabolites as glycerophospholipids, fatty acids, amino acids, sugars, and glycopolyols among the different controls. Differential metabolites among different samples were mainly enriched in four metabolic pathways: glycerophospholipid metabolism, amino acid metabolism, sugar metabolism and organic acid metabolism, which are important metabolic pathways affecting the flavor and quality of different FWC samples. These results can advance our understanding of chemical transformations in the mixed fermentation of FWC by

L. rhamnosus and *L. mesenteroides*. Conversely, metabolic pathway and key metabolite analyses indicated that the co-fermentation of *L. rhamnosus* and *L. mesenteroides* resulted in a softer, more rounded final product, with a reduced impact of undesirable flavors such as sharp acidity and bitter peptides. Sugars can be converted into glycopolyols by means of fermentation involving *L. mesenteroides*, which also promotes to a certain extent the conversion of lipids into ketones to give the whipping cream samples a sweet and savory odour. The study demonstrated the potential for *L. mesenteroides* to be utilized in the production of polyols, providing novel research ideas and possibilities for microbial fermentation in the production of beneficial metabolites.

CRediT authorship contribution statement

Xin Zhou: Writing – original draft, Software, Resources, Methodology, Investigation. **Jian-Guo Zhang:** Validation, Software, Methodology, Investigation. **Fei Hu:** Validation, Software, Resources. **Zhi-Jing Ni:** Validation, Software, Resources. **Kiran Thakur:** Writing – review & editing, Validation. **Zhao-Jun Wei:** Writing – review & editing, Supervision, Project administration, Funding acquisition, 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.

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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.2025.102376.

Data availability

Data will be made available on request.

References

Akpinar, A., & Yerlikaya, O. (2021). Some potential beneficial properties of *Lacticaseibacillus paracasei* subsp. paracasei and *Leuconostoc mesenteroides* strains originating from raw milk and kefir grains. *Journal of Food Processing and Preservation, 45*(12), Article e15986. https://doi.org/10.1111/jfpp.15986

Al Lafi, A. G., Naser, I., & Abboud, H. (2024). Application of two dimensional correlation Fourier transform infrared spectroscopy (2D-COS-FTIR) to the quantification of carbohydrates in milk powder. *Journal of Food Composition and Analysis, 134*, Article 106541. https://doi.org/10.1016/j.jfca.2024.106541

Alexandri, M., Hubner, D., Schneider, R., Frohling, A., & Venus, J. (2022). Towards efficient production of highly optically pure d-lactic acid from lignocellulosic hydrolysates using newly isolated lactic acid bacteria. *New Biotechnology, 72*, 1–10. https://doi.org/10.1016/j.nbt.2022.08.003

Chen, X., Yang, D., Huang, L., Li, M., Gao, J., Liu, C., Bao, X., Huang, Z., Yang, J., & Huang, H. (2024). Comparison and identification of aroma components in 21 kinds of frankincense with variety and region based on the odor intensity characteristic spectrum constructed by HS-SPME-GC-MS combined with E-nose. *Food Research International, 195*, Article 114942. https://doi.org/10.1016/j.foodres.2024.114942

Ding, R., Li, M., Zou, Y., Wang, Y., Yan, C., Zhang, H., Wu, R., & Wu, J. (2022). Effect of normal and strict anaerobic fermentation on physicochemical quality and metabolomics of yogurt. *Food Bioscience, 46*, Article 101368. https://doi.org/10.1016/j.fbio.2021.101368

Fu, Y., Cao, S., Yang, L., & Li, Z. (2022). Flavor formation based on lipid in meat and meat products: A review. *Journal of Food Biochemistry, 46*(12), Article e14439. https://doi.org/10.1111/jfbc.14439

Goktas, H., Dikmen, H., Bekiroglu, H., Cebi, N., Dertli, E., & Sagdic, O. (2022). Characteristics of functional ice cream produced with probiotic *Saccharomyces boulardii* in combination with *Lactobacillus rhamnosus* GG. *LWT-Food Science and Technology, 153*, Article 112489. https://doi.org/10.1016/j.lwt.2021.112489

Hussain, N., Li, R., Takala, T. M., Tariq, M., Zaidi, A. H., & Saris, P. E. (2021). Generation of lactose-and protease-positive probiotic *Lacticaseibacillus rhamnosus* GG by conjugation with *Lactococcus lactis* NCDO 712. *Applied and Environmental Microbiology, 87*(6). https://doi.org/10.1128/AEM.02957-20. e02957–02920.

Kang, H. Y., Ao, X. L., Tang, Q., Li, H., Fan, Y., Liu, A. P., … Zhao, N. (2024). Effects of yeast screened from traditional fermented milk on commercial fermented milk as adjunct flavor culture. *Food Bioscience, 57*, Article 103551. https://doi.org/10.1016/j.fbio.2023.103551

Kang, M., Wang, H., Chen, C., Suo, R., Sun, J., Yue, Q., & Liu, Y. (2023). Analytical strategies based on untargeted and targeted metabolomics for the accurate authentication of organic milk from Jersey and yak. *Food Chemistry: X, 19*, Article 100786. https://doi.org/10.1016/j.fochx.2023.100786

Kasegn, M. M., Simachew, A., Redda, Y. T., Gebremedhn, H. M., Berhanu, A. D., & Mossa, M. M. (2024). Evaluation of sugar content and bioethanol production of Ethiopian local varieties "Nech Tinkish" and "Hawaye" sweet sorghum (*Sorghum bicolor* (L.)). *Biotechnology for the Environment, 1*(1), 2. https://doi.org/10.1186/s44314-024-00001-6

Kim, H., Kim, J., Lee, M., Jeon, H. J., Moon, J. S., Jung, Y. H., & Yang, J. (2023). Increased amino acid absorption mediated by *Lacticaseibacillus rhamnosus* IDCC 3201 in high-protein diet-fed mice. *Journal of Microbiology and Biotechnology, 33*(4), 511. https://doi.org/10.4014/jmb.2212.12020

Lee, S. P., Yoon, W.-K., Lee, J., & Garcia, C. V. (2022). Fermented milk product enriched with γ-PGA, peptides and GABA by novel co-fermentation with *Bacillus subtilis* and *Lactiplantibacillus plantarum*. *Fermentation, 8*(8), 404. https://doi.org/10.3390/fermentation8080404

Lei, Y., Li, T., Chen, L., Deng, M. S., Jin, L., Li, Y. H., & Li, D. (2024). Isolation and identification of non-*Saccharomyces* yeasts and their flavour characteristics while brewing Yinhong plum wine. *Flavour and Fragrance Journal, 39*(4), 244–260. https://doi.org/10.1002/ffj.3786

Liu, Y. H., Liu, T. T., Niu, J. Q., Zhang, X. S., Xu, W. S., Song, S., & Wang, Z. (2024). Characterization of phospholipidome in milk, yogurt and cream, and phospholipid differences related to various dairy processing methods. *Food Chemistry, 454*, Article 139733. https://doi.org/10.1016/j.foodchem.2024.139733

Mahato, D. K., Keast, R., Liem, D. G., Russell, C. G., Cicerale, S., & Gamlath, S. (2020). Sugar reduction in dairy food: An overview with flavoured milk as an example. *Foods, 9*(10), 1400. https://doi.org/10.3390/foods9101400

Mamy, D., Boateng, I. D., & Chen, X. (2024). Metabolomic changes in Citrus reticulata peel after conventional and ultrasound-assisted solid-state fermentation with aspergillus Niger: A focus on flavonoid metabolism. *Food Chemistry, 467*, Article 142224. https://doi.org/10.1016/j.foodchem.2024.142224

Mar, M. J., Andersen, J. M., Kandasamy, V., Liu, J., Solem, C., & Jensen, P. R. (2020). Synergy at work: Linking the metabolism of two lactic acid bacteria to achieve superior production of 2-butanol. *Biotechnology for Biofuels, 13*, 1–10. https://doi.org/10.1186/s13068-020-01689-w

Martínez-Miranda, J. G., Chairez, I., & Durán-Páramo, E. (2022). Mannitol production by heterofermentative lactic acid bacteria: A review. *Applied Biochemistry and Biotechnology, 194*(6), 2762–2795. https://doi.org/10.1007/s12010-022-03836-5

Nomi, Y., Anazawa, T., Shinzawa, K., Tamura, M., & Matsumoto, H. (2024). Identification of lactose-derived α-dicarbonyl compounds in dairy products and elucidation of their formation mechanism. *Journal of Agricultural and Food Chemistry, 73*(1), 781–789. https://doi.org/10.1021/acs.jafc.4c08966

Peng, X., Ed-Dra, A., Song, Y., Elbediwi, M., Nambiar, R. B., Zhou, X., & Yue, M. (2022). *Lacticaseibacillus rhamnosus* alleviates intestinal inflammation and promotes microbiota-mediated protection against *Salmonella* fatal infections. *Frontiers in Immunology, 13*, 973224. https://doi.org/10.3389/fimmu.2022.973224

Rutkowska, J., Antoniewska-Krzeska, A., Żbikowska, A., Cazón, P., & Vázquez, M. (2022). Volatile composition and sensory profile of lactose-free kefir, and its acceptability by elderly consumers. *Molecules, 27*(17), 5386. https://doi.org/10.3390/molecules27175386

Sarhir, S. T., Belkhou, R., Bouseta, A., & Hayaloglu, A. A. (2023). Evaluation of techno-functional and biochemical characteristics of selected lactic acid bacteria (*Lactococcus lactis* subsp. lactis and *Leuconostoc mesenteroides* subsp. mesenteroides) used for the production of Moroccan fermented milk: Lben. *International Dairy Journal, 140*, Article 105592. https://doi.org/10.1016/j.idairyj.2023.105592

Sen, S., Dundar, Z., Uncu, O., & Ozen, B. (2021). Potential of Fourier-transform infrared spectroscopy in adulteration detection and quality assessment in buffalo and goat milks. *Microchemical Journal, 166*, Article 106207. https://doi.org/10.1016/j.microc.2021.106207

Tian, H., Xiong, J., Yu, H., Chen, C., & Lou, X. (2023). Flavor optimization in dairy fermentation: From strain screening and metabolic diversity to aroma regulation. *Trends in Food Science & Technology, 141*, Article 104194. https://doi.org/10.1016/j.tifs.2023.104194

Urminská, D., Haring, N., VladimĀr, F., & Urminská, J. (2022). Fermentable oligosaccharides, disaccharides, monosaccharides and polyols and their role in food digestion. *Journal of Microbiology, Biotechnology and Food Sciences, 11*(4), e5521. https://doi.org/10.55251/jmbfs.5521

Verma, D. K., Al-Sahlany, S. T. G., Niamah, A. K., Thakur, M., Shah, N., Singh, S., … Aguilar, C. N. (2022). Recent trends in microbial flavour compounds: A review on chemistry, synthesis mechanism and their application in food. *Saudi Journal of Biological Sciences, 29*(3), 1565–1576. https://doi.org/10.1016/j.sjbs.2021.11.010

Wang, J., Yang, Z., Xu, L., Wang, B., Zhang, J., Li, B., Cao, Y., & Tan, L. (2021). Key aroma compounds identified in Cheddar cheese with different ripening times by aroma extract dilution analysis, odor activity value, aroma recombination, and

omission. *Journal of Dairy Science, 104*(2), 1576–1590. https://doi.org/10.3168/jds.2020-18757

Wang, J. J., Zhang, W. W., Guan, Z. J., Thakur, K., Hu, F., Khan, M. R., … Wei, Z. J. (2023). Exploring the effects of the fermentation method on the quality of Lycium barbarum and Polygonatum cyrtonema compound wine based on LC-MS metabolomics. *Food Chemistry, 428*, Article 136770. https://doi.org/10.1016/j.foodchem.2023.136770

Wang, M. S., Fan, M., Zheng, A. R., Wei, C. K., Liu, D. H., Thaku, K., & Wei, Z. J. (2023). Characterization of a fermented dairy, sour cream: Lipolysis and the release profile of flavor compounds. *Food Chemistry, 423*, Article 136299. https://doi.org/10.1016/j.foodchem.2023.136299

Wang, R., Sun, J., Lassabliere, B., Yu, B., & Liu, S. Q. (2022). UPLC-Q-TOF-MS based metabolomics and chemometric analyses for green tea fermented with *Saccharomyces boulardii* CNCM I-745 and *Lactiplantibacillus plantarum* 299V. *Current Research in Food Science, 5*, 471–478. https://doi.org/10.1016/j.crfs.2022.02.012

Wei, G., Chitrakar, B., Regenstein, J. M., Sang, Y., & Zhou, P. (2023). Microbiology, flavor formation, and bioactivity of fermented soybean curd (furu): A review. *Food Research International, 163*, Article 112183. https://doi.org/10.1016/j.foodres.2022.112183

Wu, T., Guo, S., Kwok, L. Y., Zhang, H., & Wang, J. (2024). Temperature-dependent metabolic interactions between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. bulgaricus in milk fermentation: Insights from gas chromatography–ion mobility spectrometry metabolomics. *Journal of Dairy Science, 108*(1), 242–256. https://doi.org/10.3168/jds.2024-25153

Xilin, T., He, X. L., Bi, Y. Q., Gao, Y., Chen, A., Urtnasan, M., … Li, M. H. (2025). Research progress on chemical composition, microbial diversity and effects on human health of koumiss. *Food & Medicine Homology, 2*(2). https://doi.org/10.26599/FMH.2025.9420027

Xu, H., Dai, Y., Qiu, S., Sun, B., & Zeng, X. (2021). Distribution and quantification of 1,2-propylene glycol enantiomers in baijiu. *Foods, 10*(12), 3039. https://doi.org/10.3390/foods10123039

Xu, W., Dong, Q., Zhao, G., & Han, B. (2023). Analysis of metabolites of bactrain camel milk in Alxa of China before and after fermentation with fermenting agent TR1 based on untargeted LC-MS/MS based metabolomics. *Heliyon, 9*(8). https://doi.org/10.1016/j.heliyon.2023.e18522

Yang, G., Zhang, J., Dai, R., Ma, X., Huang, C., Ren, W., Ma, X., Lu, J., Zhao, X., & Renqing, J. (2023). Comparative study on nutritional characteristics and volatile flavor substances of yak milk in different regions of Gannan. *Foods, 12*(11), 2172. https://doi.org/10.3390/foods12112172

Yang, X., Hu, W., Xiu, Z., Ji, Y., & Guan, Y. (2022). Interactions between *Leu. Mesenteroides* and *L. plantarum* in Chinese northeast sauerkraut. *LWT-Food Science and Technology, 168*, Article 113901. https://doi.org/10.1016/j.lwt.2022.113901

Yang, Y., Lv, K., Zhang, Y., Wang, X., & Deng, L. (2024). Widely targeted metabolomics reveals dynamic alterations in colostrum, transitional, and mature milk of jennies. *LWT-Food Science and Technology, 200*, Article 116179. https://doi.org/10.1016/j.lwt.2024.116179

Yousef, P., Rosen, J., & Shapiro, C. (2024). Tryptophan and its role in sleep and mood. *Studies in Natural Products Chemistry, 80*, 1–14. https://doi.org/10.1016/B978-0-443-15589-5.00001-3

Zhang, L., Zhang, M., Mujumdar, A. S., & Liu, Y. (2024). Ozone combined with ultrasound processing of high-sugar concentrated orange juice: Effects on the antimicrobial capacity, antioxidant activity, and *in vitro* bioaccessibility of carotenoids. *Food and Bioprocess Technology, 17*(10), 3046–3059. https://doi.org/10.1007/s11947-023-03307-7

Zhang, X., Zhang, C., Xiao, L., Wang, X., Ma, K., Ji, F., Azarpazhooh, E., Ajami, M., Rui, X., & Li, W. (2024). Gas chromatography-mass spectrometry and non-targeted metabolomics analysis reveals the flavor and nutritional metabolic differences of cow's milk fermented by *Lactiplantibacillus plantarum* with different phenotypic. *Food Bioscience, 60*, Article 104433. https://doi.org/10.1016/j.fbio.2024.104433

Zhang, Y. Y., Zhang, P., Le, M. M., Qi, Y., Yang, Z., Hu, F. L., … Bao, G. H. (2023). Improving flavor of summer Keemun black tea by solid-state fermentation using *Cordyceps militaris* revealed by LC/MS-based metabolomics and GC/MS analysis. *Food Chemistry, 407*, Article 135172. https://doi.org/10.1016/j.foodchem.2022.135172

Zheng, A. R., Wei, C. K., Wang, M. S., Ju, N., & Fan, M. (2024). Characterization of the key flavor compounds in cream cheese by GC-MS, GC-IMS, sensory analysis and multivariable statistics. *Current Research in Food Science, 8*, Article 100772. https://doi.org/10.1016/j.crfs.2024.100772

Zhou, Z., Sarwar, A., Hu, G., Zhang, J., Hu, H., Aziz, T., Wu, J., Yang, Z., & Yang, Z. (2025). Identification of potential key metabolites in synbiotic yoghurt made with probiotic *Saccharomyces cerevisiae* var. boulardii CNCM I-745 and prebiotic inulin by non-targeted metabolomics analysis. *Food Chemistry, 464*, Article 141923. https://doi.org/10.1016/j.foodchem.2024.141923