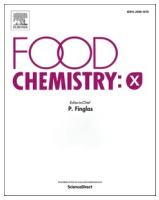
Food Chemistry: X 24 (2024) 101827

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of temperature on the quality and microbial community during Daocai fermentation

Xueli Wang [a,b,1], Xueting Liu [a,b,1], Chunmei Sun [a,b], Yanwei Cheng [c], Zhen Li [d], Shuyi Qiu [a,b], Yongguang Huang [a,b,*]

[a] Guizhou Province Key Laboratory of Fermentation Engineering and Biopharmacy, Guizhou University, Guiyang 550025, China
[b] College of Liquor and Food Engineering, Guizhou University, Guiyang 550000, China
[c] Department of Food and Drug, Guizhou Vocational College of Agriculture, Guiyang 551400, China
[d] Zhenyuan County Li's Food Co., Ltd. Qiandongnan Miao and Dong Autonomous Prefecture, 557700, China

ARTICLE INFO

Keywords:
Biogenic amine
Correlation analysis
High-throughput sequencing
HS-GC-IMS
Volatile flavor compounds

ABSTRACT

Daocai is a traditional salted pickle in the southeastern region of Guizhou with a unique aroma, color, and taste. The quality of Daocai is greatly influenced by the fermentation temperature. In this study, high-throughput sequencing and headspace-gas chromatography-ion mobility spectrometry were used to investigate the changes in microbial community succession and volatile flavor compounds during Daocai fermentation under temperature-controlled (D group) and non-temperature-controlled (C group).We found that the predominant genera in the C group samples were *Latilactobacillus*(40.57 %), *Leuconostoc*(21.25 %), *Cystofilobasidium*(22.12 %), *Vishniacozyma*(23.89 %), and *Leucosporidium*(24.95 %), whereas *Weissella*(29.39 %), *Lactiplantibacillus*(45.61 %), *Mucor*(68.26 %), and *Saccharomyces*(23.94 %) were the predominant genera in the D group. A total of 92 VFCs were detected in Daocai samples, including 5 isothiocyanates, 16 esters, 14 alcohols, 24 aldehydes, 17 ketones, 3 acids, 2 pyrazines, 1 pyridines, 1 thiazoles, 3 furans, 4 alkenes, and 2 nitriles. Further analysis revealed *Latilactobacillus*, *Leuconostoc*, *Lactococcus*, *Cystofilobasidium*, *Leucosporidium*, *Holtermanniella*, and *Dioszegia* as key bacteria involved in flavor formation. They are closely related to the formation of flavors such as aldehydes, furans, pyridines, and alkenes. This study contributes to our understanding of the relationship between bacterial communities and the flavor formation during Daocai fermentation.

1. Introduction

Fermentation effectively solves the problem of the short shelf life of fresh vegetables. Fermented vegetables have a richer and more pleasant taste, and contain dietary fiber, minerals, amino acids, vitamins, carotenoids, glucosinolates, phenolic compounds, and their decomposition products, which have positive effects such as anti-obesity, anti-cancer, and anti-aging properties(Liang, He, Wang, Song, Chen, Lin, Ji, & Zhang, 2020). Bangcai(common name), scientifically named *Brassica juncea* L. *var. crassicaulis Chen & Yang*, is a nutritious and flavorful stem-leaf vegetable from the Brassica genus (Chen et al., 2020). Zhenyuan Daocai differs from Sichuan Paocai, Northeast sauerkraut, and Jiangxi Yancai, which are primarily made from bangcai as the main ingredient, combined with salt, white wine, and other seasonings. The production process involves 14 complex steps, including salting, fermentation, sun drying, kneading, and steaming. Zhenyuan Daocai has been popular in Guizhou cuisine for more than 500 years, it was created by the Taoists of Qinglong Cave during the Ming Dynasty(Liu et al., 2023).

Microorganisms, crucial to pickle fermentation, have recently attracted significant attention from domestic and international experts (Chen et al., 2024). The succession of microbial communities during fermentation is complex and is influenced by various factors, such as temperature, humidity, fermentation process, storage equipment, pH, geographical distribution, and types of fermented vegetables(Liu, Li,

Abbreviations: HTS, high-throughput sequencing; GC, gas chromatography; HS, headspace; IMS, ion mobility spectroscopy; BAs, biogenic amines; HPLC, high-performance liquid chromatography; TA, total acidity; AAN, amino acid nitrogen; VFCs, volatile flavor compounds; OTUs, operational taxonomic units; PCA, principal component analysis; LAB, lactic acid bacteria; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

* Corresponding author at: College of Liquor and Food Engineering, Guizhou University, 550025, China.
E-mail address: yghuang1@gzu.edu.cn (Y. Huang).

[1] These authors contributed equally to this work and were considered co-first authors.

https://doi.org/10.1016/j.fochx.2024.101827
Received 17 July 2024; Received in revised form 6 September 2024; Accepted 9 September 2024
Available online 24 September 2024

et al., 2019). Small changes in the diversity and quantity of microbial communities can impact the contents of sugars, organic acids, amino acids, biogenic amines (BAs), and nitrites in fermented foods even impact the sensory characteristics of the food, including its smell, taste, texture, and color(An et al., 2021). Therefore, a comprehensive understanding of microbial community changes during pickle fermentation is crucial for regulating the fermentation process and stabilizing pickle quality.

Fermentation temperature has a profound impact on microbial community structure and microbial metabolites, significantly contributing to product quality. De Filippis et al. ((De Filippis et al., 2018) promoted further growth of acetic acid bacteria by controlling the fermentation temperature, which increased the organic acid content in kombucha tea. Wang et al.(Wang et al., 2020)proposed that Northeast sauerkraut and Paocai are primarily fermented at low to medium temperatures (5–15 °C), whereas Sichuan Paocai is mainly fermented at medium to high temperatures (15–30 °C). Temperature not only affects the fermentation rate and maturation time of Paocai, but also changes the diversity and biomass of microorganisms, which are important indicators of kimchi processing. Sun et al.(Sun et al., 2022).found that temperature has a significant impact on the formation of main flavor compounds during yogurt fermentation. Du et al.(Du et al., 2022) demonstrated that changes in temperature impact yeast metabolites. The contents of ethanol, ethyl acetate, and ethyl butyrate in wine increase, while the contens of acetic acid, phenylethyl alcohol, phenylethyl acetate, capric acid, ethyl ester, and ethyl palmitate become lower with a decrease in temperature.

Although existing research has explored the impact of temperature on microbial communities and metabolites in fermented foods, these studies often focus on specific foods and time points, lacking comprehensive observations of microbial and metabolite dynamics throughout the entire fermentation process. Furthermore, the effects of temperature on microbial succession and metabolites during Daocai fermentation remain poorly understood. Up to now, there has been limited research on the mechanisms of flavor formation and development during the processing of Daocai. To address these research gaps, this study will use HTS, HPLC, and HS-GC-IMS technologies to systematically investigate the impact of temperature on microbial communities and metabolites during Daocai fermentation.

High-throughput sequencing (HTS) has become a mainstream method for elucidating the structure and diversity of microbial communities in fermented foods due to its speed, accuracy, efficiency, and comprehensiveness(Ryu et al., 2021). In recent years, HTS technology has been used to study the correlation between microbial communities and volatile metabolites during vegetable fermentation. For example, it has been shown that the dominant microorganisms in sauerkraut, including LAB and *Pediococcus*, have a significant impact on the levels of various flavor metabolites. Characteristic LAB in fermented vegetables are closely associated with the presence of more than 20 flavor compounds(Xiao et al., 2020). The abundance of LAB, which is the dominant genus in sauerkraut, is significantly positively correlated with the contents of alcohols, esters, phenols, nitriles, sulfides, and isothiocyanates (Liang, He, Wang, Song, Chen, Lin, Ji, & Li, 2020; Liang, He, Wang, Song, Chen, Lin, Ji, & Zhang, 2020).

Recently, Headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) has attracted much attention because it combines the high separation ability of GC and the fast response ability of IMS. There have been studies that have combined HTS and HS-GC-IMS to analyze the correlation between microbial communities and VFCs in fermented foods. Xiao et al. (Xiao, Chen, et al., 2022) utilized HTS and HS-GC-IMS to investigate the impact of bacteria and fungi in the distinctive Xiaoqu starter culture on the flavor characteristics of traditional Xiaoqu rice wine. Sun et al.(Sun et al., 2023) used HS-GC-IMS and HTS to investigate the characteristics of changes in VFCs and microbial communities during the storage of pickles.

The research objectives of this study were: (1) to use HTS to describe the changes in microbial communities during Daocai fermentation with and without temperature control; (2) to determine VFCs using HS-GC-IMS and BAs using high-performance liquid chromatography (HPLC), and to explore the contents of physicochemical indicators such as pH, total acidity (TA), chloride content, amino acid nitrogen (AAN), and nitrite content, as well as the changes in VFCs and BAs during Daocai fermentation; (3) to establish the relationships between the dominant flora and VFCs, BAs, and physicochemical indicators during Daocai fermentation. The results of this study can help establish a framework for assessing the quality and authenticity of Daocai products based on laboratory pilot production and food company production. This information can support the development of regulatory frameworks, standards, and certification systems to ensure the integrity and transparency of the fermented vegetable industry. Furthermore, this study provides a theoretical basis for the improvement of Daocai processing technology.

2. Materials and methods

2.1. Experimental design and sample collection

Fresh bangcai were obtained from Li's Food Co., Ltd. (Zhenyuan County, Qiandongnan Prefecture, Guizhou Province, China) and were used for natural fermentation under laboratory temperature-controlled conditions. Fifteen kilograms of bangcai were cleaned, air-dried, and cut into pieces approximately 1 cm wide. NaCl was then added to a concentration of 2.5 % (2.5 g/100 g), and bangcai pieces were packed in sterile sealed bags, which were placed under different temperature conditions, namely 18 °C, 23 °C, 28 °C, 33 °C, and 37 °C, for fermentation. Ten sensory panelists (five males and five females, aged 25–29 years) with experience in sensory evaluation were selected from the Food Science and Engineering Laboratory to evaluate the aforementioned fermented Daocai samples. The color, aroma, taste, and texture of the Daocai were evaluated on a nine-point scale. The scoring criteria are shown in Table S1. According to the score, the temperature of 28 °C was determined as the optimal temperature for natural fermentation under controlled conditions (Table S2). The sensory analysis in this study complied with sensory ethical standards, all participants participated voluntarily and their consent and knowledge were obtained. The information and privacy of participants in the study were anonymized and appropriate measures were taken to protect it. Consent was obtained from all participants before the open publication of the experimental data.

The bangcai were processed according to the above method, they were fermented at 28 °C for 5 days. Daocai and brine samples were collected randomly from temperature-controlled fermentation samples (designated as D-1d to D-5d) and non-temperature-controlled natural fermentation samples (produced using the same batch of fresh bangcai plants and the same processing, at Zhenyuan County Li's Food Co., Ltd., designated as C-1d to C-5d) on days 1 to 5 of the fermentation process. The samples were transported to the laboratory on dry ice and stored at −80 °C. The collected samples were divided into two parts. The Daocai samples were used to analyze the contents of TA, chloride, nitrite, AAN, soluble solids, BAs, and VFCs. The brine samples were used for pH measurements and microbial diversity analysis. The brine samples for microbial diversity analysis were centrifuged at 12,000 ×*g* for 2 min, the supernatant was then removed, and microbial cells were flash-frozen in liquid nitrogen and stored at −80 °C for DNA extraction.

2.2. Reagents

Acetonitrile was chromatographically pure and purchased from Tiandi Reagent Company(USA). Potassium ferrocyanide, zinc acetate, sodium borate, NaOH, NaCl, and potassium chromate were all purchased from Jinshan Chemical Reagent Co., Ltd.(Chengdu, China); silver nitrate standard solution, formaldehyde, p-aminobenzenesulfonic acid, naphthylethylenediamine hydrochloride, sodium nitrite, and dansyl

chloride were purchased from Macklin Biochemical Technology Co., Ltd.(Shanghai, China). Nitric acid and acetone were purchased from Wansheng Chuandong Chemical Co., Ltd.(Chongqing, China); cadaverine, tyramine, histamine, spermidine, putrescine, tryptamine, etc. were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). All chemical reagents were at least analytically grade.

2.3. Analysis of physicochemical parameters

The physicochemical indexes (pH, TA, soluble solids content, AAN, nitrite content, chloride content) of the Daocai were determined following the method described by Liu et al. (Liu et al., 2021).

2.4. Analysis of BAs

BAs were extracted according to the method previously described by Sun et al. (Sun et al., 2019) with slight modifications. Briefly, 5 g of a uniformly crushed sample was mixed with 50 mL of 0.4 mol/L perchloric acid. The mixture was then subjected to ultrasonic extraction for 30 min. After that, it was centrifuged at 4 °C and 5000 r/min for 10 min, and the supernatant was collected.

The pre-column derivatization method was slightly modified as previously described by Zhao et al. (Zhao, Lai, et al., 2021). Briefly, 1 mL of the extract in a 5 mL volumetric flask was mixed with 200 μL of a 2 mol/L NaOH solution, 300 μL of the saturated $NaHCO_3$ buffer solution, and 2 mL of a dansyl chloride derivative at a concentration of 10 mg/mL (prepared in acetone). The above mixture was incubated at 40 °C in the dark for 45 min, then 100 μL of ammonia water was added to terminate the reaction, the volume was adjusted to 5 mL with acetonitrile, and then passed through a 0.22 μm membrane filter.

Analysis of BAs was performed by HPLC (Agilent, 1260 Infinity, Agilent Technologies Co., Ltd., USA), equipped with a C18-AQ column (250 mm × 4.6 mm, 5 μm) (Shimadzu, Japan), and an ultraviolet light detector. The injection volumes of the sample and standard solution were both 20 μL, The column temperature was 35 °C, and the ultraviolet light wavelength was 254 nm for detection. Mobile phase A was water (100 %), mobile phase B was acetonitrile (100 %), and the flow rate was 1.0 mL/min. The gradient elution proceeded as follows: 0 min, 30 % A; 15 min, 25 % A; 22 min, 15 % A; 25–28 min, 10 % A; 35 min, 40 % A. All BAs were quantified using external standards, and the results were then expressed in mg/kg.

2.5. Analysis of VFCs

VFCs were determined by HS-GC-IMS (FlavourSpec®, Germany) (Chen et al., 2023). Briefly, 2 g of the broken and homogenized fermented Daocai sample was placed into a 20 mL headspace bottle, incubated at 60 °C for 15 min, and then the sample was injected into the analyzer. The incubation speed was 500 r/min, and the injection volume was 500 μL.

The conditions of IMS were as follows: carrier gas N_2 (purity ≥99.999 %); injection needle temperature 85 °C; MXT-5 chromatographic column (15 m × 0.53 mm, 1 μm); carrier gas flow rate: 0–2 min, 2 mL/ min; 2–20 min, 100 mL/min. IMS conditions: the drift tube length is 98 mm, with a drift tube temperature of 45 °C; the drift gas used in N_2 (purity ≥99.999 %); drift gas flow rate is 150 mL/min; β-rays, 3H as the radioactive source; positive ionization mode. The volatile component content was calculated as the relative peak area (%) after normalization.

2.6. DNA extraction and identification of microorganisms

DNA was extracted following the instructions of the TGuide S96 Soil Genomic DNA Extraction Kit (Tiangen Biochemical Technology Co., Ltd. Beijing, China). The V3–V4 hypervariable region of the bacterial 16S rRNA gene and the ITS1 region of the fungal rRNA gene were amplified using the universal primer pairs 338F (5′-ACTCCTACGGGAGGCAGCA-3′)/806R (5′-GGACTACHVGGGTWTCTAAT-3′) and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)/ITS2(5′-GCTGCGTTCTTCATC-GATGC-3′), respectively. The PCR amplification system contained 10 ng of template DNA, 4 μL of the KOD FX Neo buffer, 2 μL of dNTPs at 2.5 mM each, 0.2 μL of KOD FX Neo polymerase, 0.3 μL of each 10 μM primer, and ddH_2O to make a total volume of 10 μL. The PCR amplification program consisted of an initial denaturation step at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 7 min. The integrity of the PCR products was evaluated by electrophoresis in a 1.8 % agarose gel, and quantitative analysis was performed using ImageJ software. PCR products were purified using the E.Z.N.A. TM Cycle-Pure Kit (Omega, Inc., USA) and gel-purified using the Monarch® DNA Gel Extraction Kit (New England Biolabs, Inc., USA). The amplified DNA fragments were used to construct a sequencing library, which was quality-checked using the Qsep-400 method and sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) at BMK-Bio Technology Co., Ltd. (Beijing, China). At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated. After paired-end reads assembly and quality control, the high-quality sequences were obtained, then assembled and applied to operational taxonomic unit (OTU) and taxonomic analysis (Bolyen et al., 2019). The alpha diversity indices, Beta diversity were calculated by QIIME2 software (https://qiime2.org/) to determine the species complexity between different groups(Xiao, Chen, et al., 2022; Xiao, Lapu, et al., 2022).

2.7. Statistical analysis

All tests were performed in triplicate, and the results were presented as means ± standard deviation. Significant differences among means were tested by ANOVA using SPSS Statistics Software (SPSS Inc., Chicago, IL, USA) at $p<0.05$ and Tukey's test was applied for comparison of means. Figures were generated using Origin 2018 software (OriginLab Corporation,USA). The GC-IMS data were analyzed using Laboratory Analytical Viewer, and Library Search Software (with built-in NIST2014 and IMS databases) to match and analyze the detected volatile components.

3. Result and discussion

3.1. Changes in physicochemical parameters during Daocai fermentation

Several physicochemical parameters measured during fermentation showed significant differences between the traditionally fermented non-temperature-controlled (group C) and temperature-controlled (group D) Daocai samples (Fig. 1). Firstly, the pH and chloride content in groups C and D significantly decreased during fermentation (Fig. 1A, F). The initial pH value was 5.6, and by the 5th day of fermentation, the pH of both groups had dropped to 3.93 and 3.33, respectively. The chloride content in samples from groups C and D decreased rapidly during the first 2 days of fermentation, with reductions of 53 % and 25 %, respectively. After the 3rd day of fermentation, the chloride content remained relatively stable. We also found that the chloride content in group D samples was significantly higher than that in group C samples. This could be attributed to the larger volume of fermentation conducted by the manufacturer, resulting in the extraction of a larger brine volume from bangcai and, consequently, lower chloride content in the latter group. Secondly, the TA and AAN content in samples from both groups C and D overall increased (Fig. 1B, D), with group D samples consistently exhibiting higher TA and AAN content than group C samples. By the 5th day of fermentation, the TA contnet in group D reached its maximum, which was twice higher than TA of group C samples. Meanwhile, the AAN content in group D was 0.287 g/100 g, which was 22 % higher than that in group C. The nitrite content (Fig. 1E) in both groups C and D

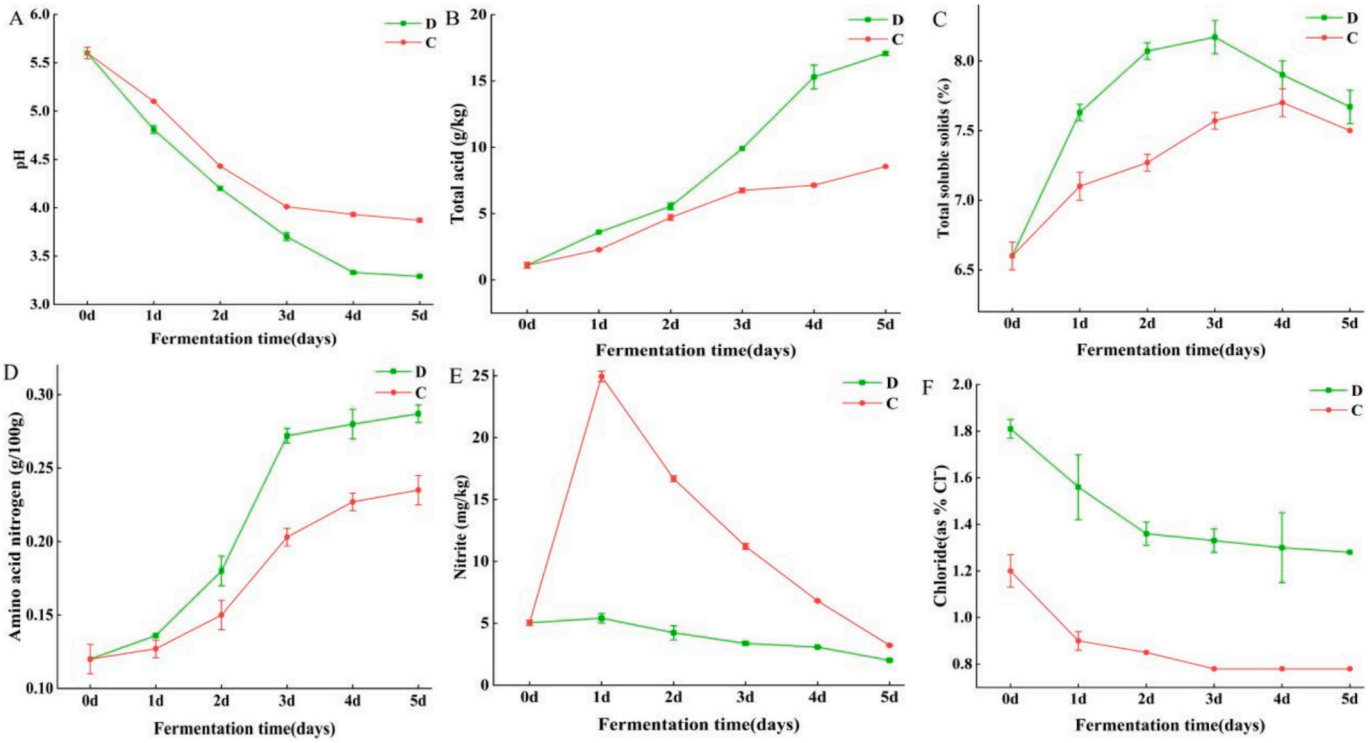


Fig. 1. Fermentation process of Daocai - Monitoring changes in pH (A), total acidity (B), soluble solids (C), amino acid nitrogen (D), nitrite (E), and chloride (F).

showed an initial increase followed by a decreasing trend. Throughout fermentation, the nitrite content in the group D samples was lower than that in the group C samples. The peak nitrite content in group C (24.95 mg/kg) was 4.6 times higher than that in group D. Furthermore, the study also found that the content of soluble solids in group C and group D samples increased and then decreased (Fig. 1C). This may be related to the decomposition of protein and polysaccharide by microorganisms in the early stage of Daocai fermentation, resulting in the soluble sugar and amino acid contents of the Daocai samples gradually increased. However, during the later stages of fermentation, microbes utilize and consume these substances for growth, leading to a decrease in the total soluble solid content (Ooi et al., 2020).

In studies of fermented vegetable products, when the brine pH is lower than 4.0 and TA is higher than 3 g/kg, the astringent taste of fresh vegetables disappears from fermented vegetables, and the palatability improves(Zhang et al., 2016). In this study, after 5 days of fermentation of bangcai, the pH of the product ranged from 3.29 to 3.87, and the TA content reached 8.55–17.07 g/kg. This is similar to the findings of Cao et al. (Cao et al., 2017),who reported a pH range of 3.2 to 4.2 for Sichuan pickles, with an average pH of 3.48. They also noted a total acidity range of 6.5 to 25 g/kg, with an average of 12.71 g/kg.

3.2. Changes in BAs during Daocai fermentation

BAs are low-molecular-weight nitrogenous organic compounds, mainly produced by microorganisms (amine-producing bacteria) under appropriate conditions and are common in various fermented foods (Dabadé et al., 2021). They are important active ingredients of cells, but excessive intake can cause adverse reactions. Currently, some reports indicate that paocai samples contain high levels of BAs, which may react with nitrite to generate carcinogenic compounds-nitrosamine (Jastrzębska et al., 2023). Therefore, it is imperative to control the contents of nitrite and BAs in paocai. Table 1 shows the changes in the BAs content of the samples during the fermentation of raw bangcai and Daocai. Putrescine, cadaverine, histamine, and tyramine were detected in fresh bangcai, and the total biogenic amine content was 9.837 mg/kg. After the fermentation, the total BA content increased significantly ($p < 0.05$), and the total BA content of samples in groups C and D were 60.439 mg/kg and 27.53 mg/kg, respectively.

The main BAs detected in samples from group C were putrescine

Table 1
Changes in biogenic amine concentrations (mg/kg) during dynamic fermentation of Daocai.

Sample	Biogenic amine concentrations(mg/kg)					
	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Total BA
0 d	4.277 ± 0.259^{g}	0.824 ± 0.028^{f}	1.615 ± 0.228^{c}	3.122 ± 0.048^{bc}	0.000^{d}	9.837 ± 0.081^{f}
C-1d	6.257 ± 1.021^{e}	12.54 ± 0.386^{d}	0.268 ± 0.01^{c}	2.262 ± 0.187^{cd}	0.000^{d}	21.328 ± 0.944^{e}
C-2d	13.597 ± 0.226^{d}	20.333 ± 0.389^{b}	0.441 ± 0.01^{c}	2.258 ± 0.245^{cd}	1.391 ± 0.088^{a}	38.020 ± 0.567^{c}
C-3d	56.293 ± 0.765^{a}	24.382 ± 0.440^{a}	0.523 ± 0.036^{c}	2.816 ± 0.134^{cd}	1.057 ± 0.075^{b}	85.070 ± 1.185^{a}
C-4d	42.113 ± 0.493^{b}	18.161 ± 0.522^{bc}	0.540 ± 0.011^{c}	2.881 ± 0.484^{cd}	0.868 ± 0.163^{c}	64.562 ± 0.966^{b}
C-5d	40.599 ± 0.355^{c}	15.808 ± 0.478^{cd}	0.409 ± 0.012^{c}	2.296 ± 0.424^{cd}	1.326 ± 0.141^{a}	60.439 ± 0.595^{b}
D-1d	4.233 ± 0.672^{g}	0.000^{f}	5.708 ± 1.390^{b}	1.161 ± 0.553^{e}	0.000^{d}	11.102 ± 2.437^{f}
D-2d	4.944 ± 0.317^{f}	3.897 ± 0.847^{ef}	9.154 ± 1.826^{a}	3.922 ± 0.735^{b}	0.000^{d}	21.917 ± 1.948^{e}
D-3d	4.656 ± 0.915^{g}	5.231 ± 0.883^{e}	8.351 ± 1.836^{a}	2.413 ± 0.632^{cd}	0.000^{d}	20.651 ± 6.089^{e}
D-4d	4.282 ± 0.833^{g}	4.822 ± 0.840^{e}	7.961 ± 1.680^{a}	1.987 ± 0.188^{de}	0.000^{d}	19.052 ± 3.356^{e}
D-5d	5.902 ± 1.088^{ef}	6.633 ± 0.219^{e}	9.878 ± 1.151^{a}	5.118 ± 0.666^{a}	0.000^{d}	27.530 ± 5.254^{d}

Note: Values are shown as mean ± standard deviation; different letters (a–d) within the same column indicate significant differences ($p < 0.05$).

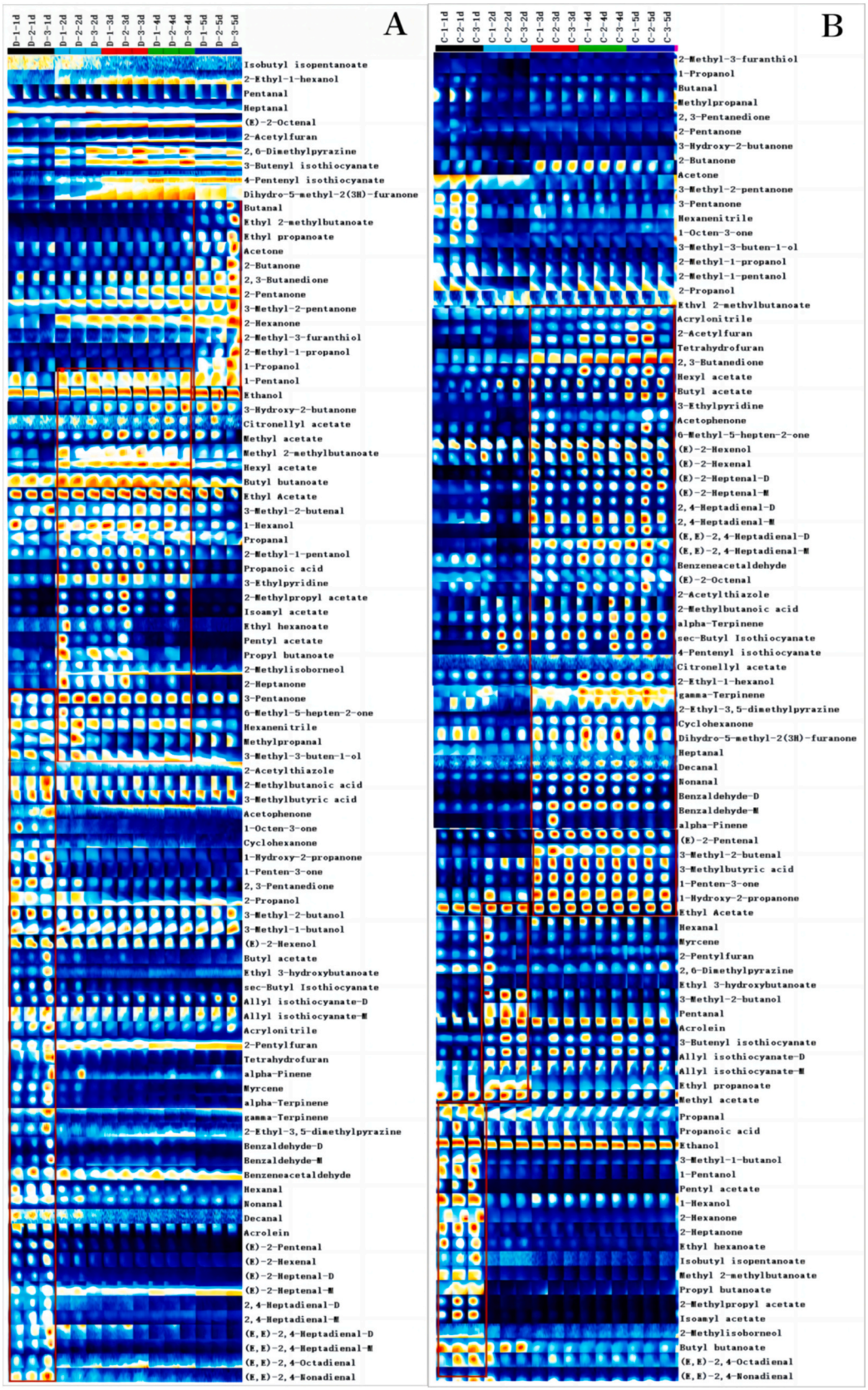


Fig. 2. Changes in VFCs concentrations during Daocai fermentation. A) GC-IMS-based fingerprints of VFCs changes in Group D. B) GC-IMS-based fingerprints of VFCs changes in Group C. C) Heat map and hierarchical clustering of GC-IMS analysis showing changes in VFCs during Daocai fermentation for Group D. D) Heat map and hierarchical clustering of GC-IMS analysis showing changes in VFCs during Daocai fermentation for Group C. E) PCA plots of VFCs changes in Group D. F) PCA plots of VFCs changes in Group C.

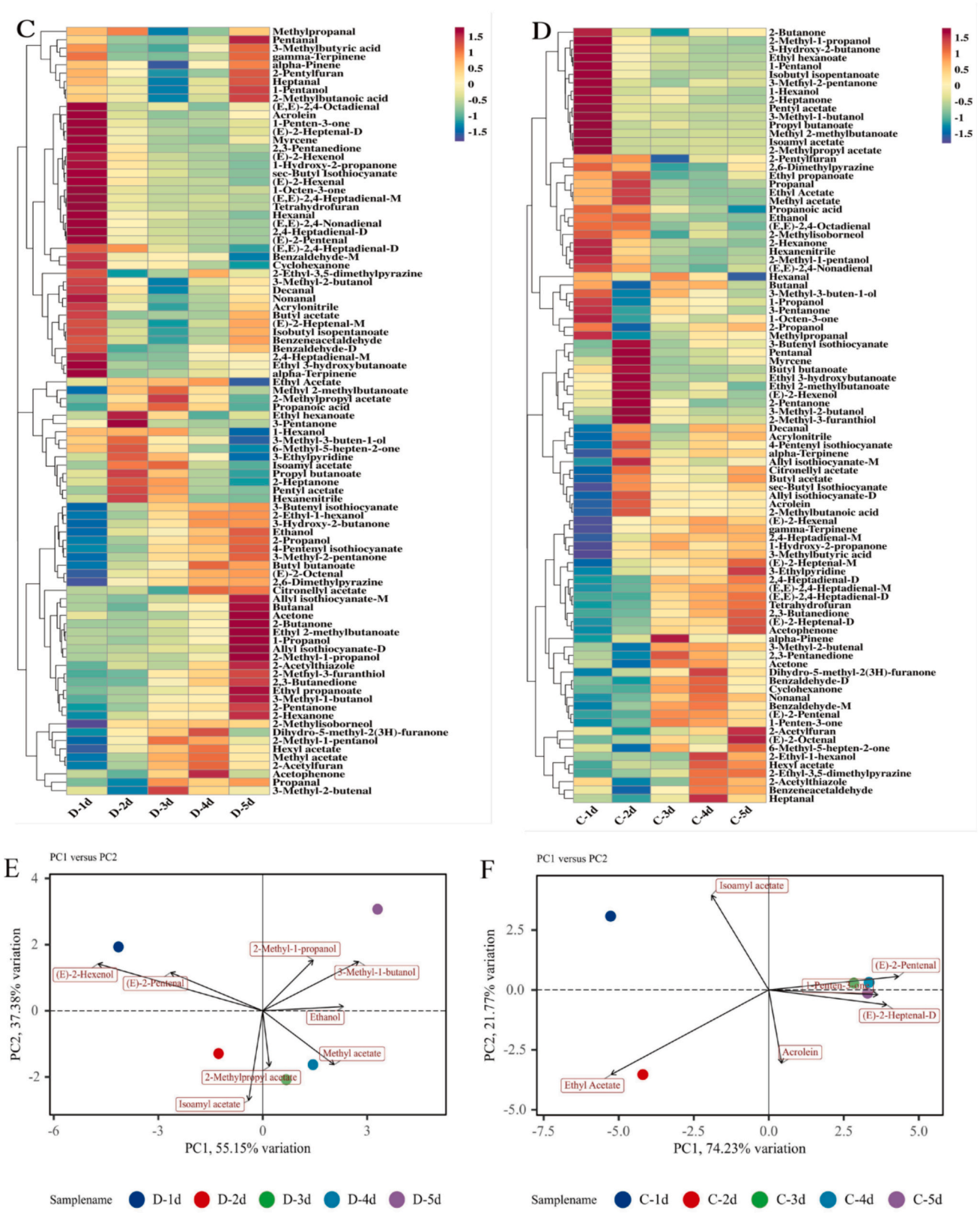


Fig. 2. (*continued*).

(40.599 mg/kg) and cadaverine (15.808 mg/kg); while the BAs detected in samples from group D were putrescine (5.902 mg/kg), cadaverine (6.633 mg/kg), histamine (9.878 mg/kg), and tyramine (5.118 mg/kg). Additionally, our study revealed that the histamine and tyramine contents of the Daocai samples from group D, after fermentation, were significantly higher than those in group C. This suggests that the higher fermentation temperature may have promoted the synthesis of histamine and tyramine. These findings are consistent with previous studies (Tian et al., 2021), which reported that higher processing temperatures promoted proteolytic and decarboxylase activity, resulting in increased

histamine and tyramine content.

Putrescine, cadaverine, tyramine, and histamine were the main BAs identified in Daocai samples, which is consistent with previous findings (Kim et al., 2022). In our experiments, the contents of putrescine and cadaverine increased, which was in contrast to some previously published data(Zhang et al., 2021). These discrepancies may be explained by differences in fermentation temperature, processing technology, raw materials, and microbial communities (Gao et al., 2023). Significant differences were observed in the contents of putrescine, histamine, cadaverine, tyramine, spermidine, and total BA content between the two groups after fermentation. This indicates that fermentation temperature has a significant impact on the BA content in the product. Studies have shown that the content of BAs in the product fermented at 30–35 °C was significantly lower than that in the product fermented at 20 °C (Zhao et al., 2022). Similarly, in our study, the total BA content in the temperature-controlled (28 °C) fermentation group D was significantly lower than that in the non-temperature-controlled fermentation group C (15–18 °C).

3.3. Changes in VFCs during Daocai fermentation

A total of 92 VFCs were detected in Daocai samples from non-temperature-controlled natural fermentation (group C) and temperature-controlled natural fermentation (group D), including 5 isothiocyanates, 16 esters, 14 alcohols, 24 aldehydes, 17 ketones, 3 acids, 2 pyrazines, 1 pyridines, 1 thiazoles, 3 furans, 4 alkenes, and 2 nitriles (Table S3). Among these, four kinds of compounds contained monomers and dimers; allyl isothiocyanate, (E,E)-2,4-heptadiene aldehydes, 2,4-heptadienal, and (E)-2-heptenal. The composition of VFCs in samples from groups C and D changed during Daocai fermentation (Fig. 2A–D). For example, isothiocyanate, phyllyl acetate, and aldehydes (such as acetaldehyde, butyraldehyde, nonanal, phenylacetaldehyde, and furfural) were prominent VFCs in the raw bangcai, accounting for 18.69 %, 16.07 %, and 21.39 % of the total volatile flavor compound content, respectively. However, the contents of these compounds decreased during the Daocai fermentation process. Furthermore, the relative contents of alcohols, including ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, esters such as ethyl acetate, hexyl acetate, methyl acetate, ketones like 3-hydroxy-2-butanone, 2-acetylfuran, and heterocyclic compounds such as 2,6-dimethylpyrazine, increased as fermentation progressed and reached peak values on the final day of fermentation. PCA showed that the first principal component (PC1) contributed to 55.15 % and 74.23 %, whereas PC2 contributed to 37.38 % and 21.77 % of the total variation in groups D and C, respectively (Fig. 2E, F). The cumulative contributions of these two PCs were 92.53 % and 96.00 % respectively, indicating that these PCs represented most of the effective information from the original data for both sample groups. However, the distribution of VFCs in group C and group D samples was different, and the distribution of VFCs changed with the fermentation time. E-2-hexenol, E-2-hexenal, isopentyl acetate, methyl propionate, methyl acetate, ethanol, 3-methyl-1-butanol, and 2-methyl-1-propanol were the characteristic flavors of sample groups D (Fig. 2E). In turn, sample group C was characterized by the presence of isopentyl acetate, ethyl acetate, acrolein, (E)-2-hexenal, 1-penten-3-one, and (E)-2-octenal-D (Fig. 2F). These observations indicate distinct distribution patterns of volatile compounds between groups C and D, with significant differences in their characteristic flavors. This indicates that fermentation temperature affects the content of volatile flavor compounds. The content of heterocyclic substances increased with increasing temperature, while the content of terpenes decreased with increasing temperature. This is consistent with the results of Liu et al.(Liu et al., 2024), who found that terpenes showed lower concentrations with increasing temperature, while heterocyclic substances displayed the opposite trend.

Isothiocyanates, especially allyl isothiocyanate and phenethyl isothiocyanate, are the major VFCs in fresh bangcai greens that impart a pungent, garlic-like, and horseradish-like aroma to the product. This is also the unique aroma of pickled mustard greens(Mi et al., 2022). During fermentation, the isothiocyanate content decreased, while the relative contents of esters, alcohols, aldehydes, ketones, acids, pyrazine, terpenes, and other compounds increased in our experiments. The formation mechanism of VFCs is shown in Fig. 6. For example, esters are typically formed through the esterification of alcohols or the oxidation of lipids and amino acids. They possess floral, fruity, and sweet aromas, contributing to the pleasant flavor of Daocai (Chen et al., 2023). Alcohols, as byproducts of yeast metabolism, are synthesized through sugar metabolism or amino acid dehydrogenation and decarboxylation, and play a vital role in enhancing flavor and aroma, providing typical floral, fruity and mellow aromas (Tian et al., 2024). The content of ethanol, (E)-2-hexenol, and 3-methyl-1-butanol is relatively high. Among them, 3-methyl-1-butanol is the vital precursor of isoamyl acetate, which is mainly generated by Ehrlich pathways of leucine (Xiong et al., 2024). Aldehydes are produced through biosynthesis and Strecker degradation pathways. Due to their relatively low olfactory threshold, they play an important role in the formation of Daocai flavors, contributing fruity, honey, floral and other aromas (Wang et al., 2023). Most ketones are oxidation products of oleic and linoleic fatty acids, giving off aromas such as floral, fruity, and creamy (Zheng et al., 2024). Acids are usually generated through the metabolism of lipids and carbohydrates (Song et al., 2024). The presence of acids provides the foundation for the subsequent formation of flavor compounds such as esters and aldehydes, which significantly contribute to the flavor of Daocai. Pyrazine is a characteristic product of the Maillard reaction, and pyrazines usually show odors such as roasted, sauce, and nuts. Terpenes are associated with pleasant fruity, floral, and spicy aromas of the product (Zhao, Lai, et al., 2021; Zhao, Wei, et al., 2021). Thus, changes in these compounds during fermentation indicate the transformation of the original bitterness and spiciness of bangcai into fresh, sweet, and aromatic flavors. Fermentation enhances the unique sensory characteristics of the final product, thereby increasing consumer acceptance and preference.

3.4. Microbial profile of Daocai fermented with different temperatures

3.4.1. Sequence quality control

Bacterial and fungal diversity in 39 samples (nine raw material samples and 30 Daocai samples) was assessed using an Illumina Novaseq 6000 system. A total of 3,119,260 bacterial sequences (average 79,981 reads) and 3,118,823 fungal sequences (average 79,969 reads) were obtained from the 39 samples. After quality filtering, 3,109,585 clean tags were obtained from the 16S rRNA sequence (an average of 79,733 tags per sample). These sequences were clustered into 5577 bacterial operational taxonomic units (OTUs) according to the standard of 97 % similarity threshold, with numbers ranging from 44 to 330. Furthermore, 3,105,014 clean tags (79,616 tags per sample on average) were obtained from the ITS sequences and clustered into 3047 fungal OTUs, with numbers ranging from 30 to 129. The sequencing depth was evaluated using a sparse curve, which flattened as the sequencing depth increased (Supplementary Fig. S1), indicating that the sequencing data sufficiently reflected most of the microbial information in the sample. The coverage index of each sample was greater than 99.9 %, indicating that the sequencing results were sufficiently reliable.

3.4.2. Alpha diversity analysis of microbial community

The alpha diversity indexes include Chao1, OTUs, Shannon, and Simpson. Dynamic changes in alpha diversity indices within the temperature-controlled natural fermentation group (group D) and the non-temperature-controlled natural fermentation group (group C) were visualized using box-whisker plots (Fig. 3). The trend of the Chao1, Shannon, and Simpson indexes of bacteria in group C was similar to that in group D, showing a trend of first decreasing and then increasing with the extension of fermentation time. The reason may be that the bacteria need time to adapt to the fermentation environment, and then the abundance increases. The OTUs, Chao1, Shannon, and Simpson at the

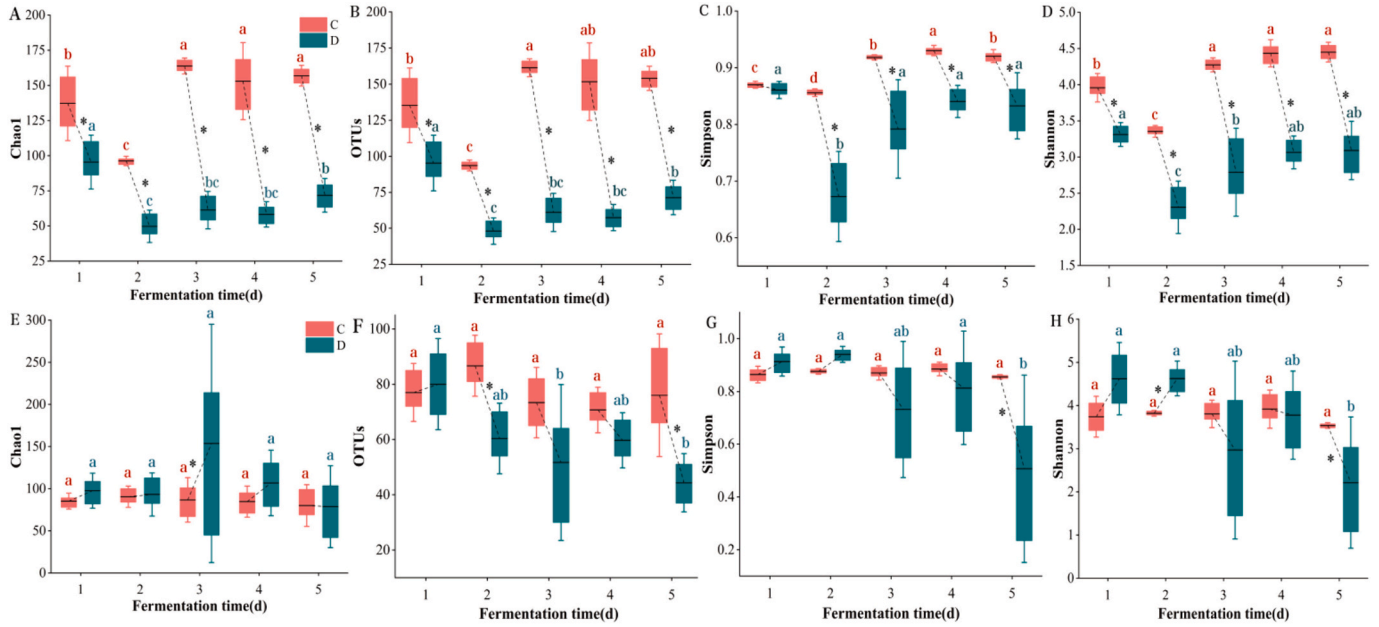


Fig. 3. Boxplots of bacterial community Chao1 (A), OTUs (B), Shannon (C), Simpson (D), and fungal community Chao1 (E), OTUs (F), Shannon (G), Simpson (H) in Daocai samples. Different lowercase letters (a-d) indicate significant differences among bacterial community($p < 0.05$). * indicates significant difference among groups ($p < 0.05$). Alpha-diversity feature data are shown in Table S4.

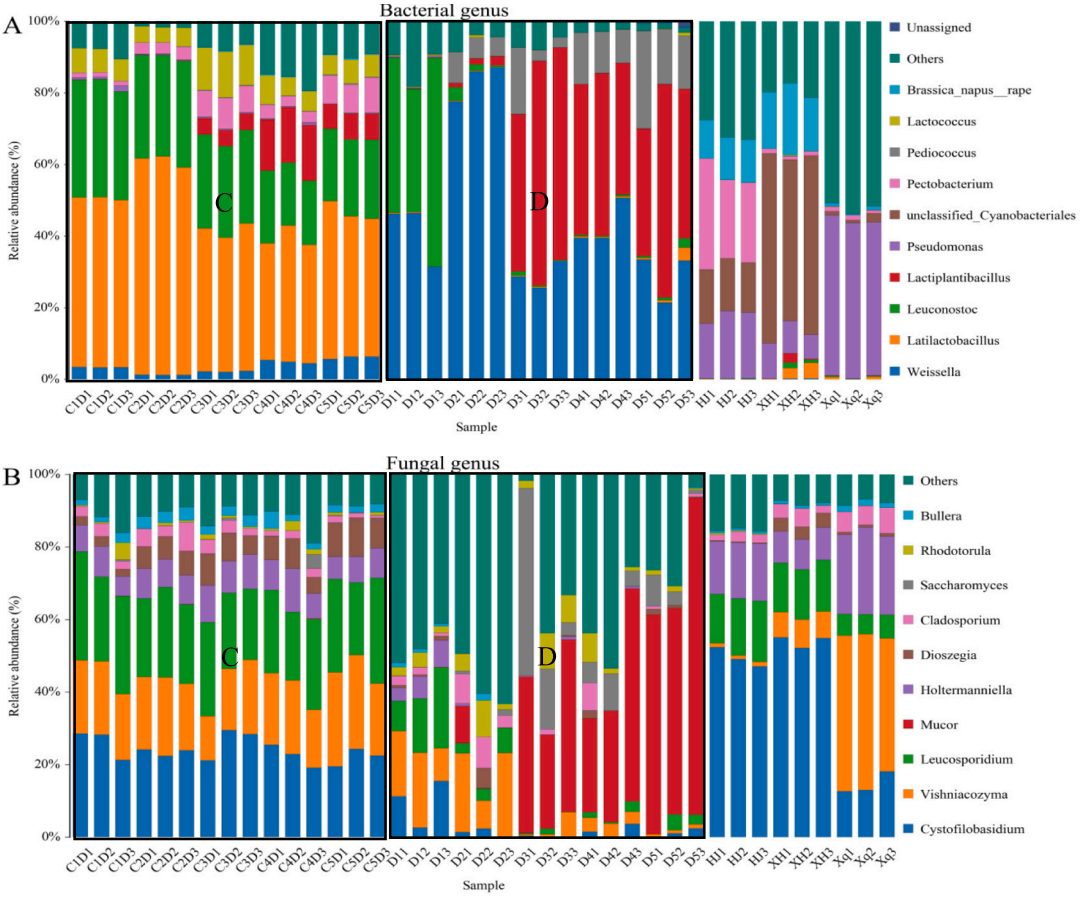


Fig. 4. Succession of bacterial (A) and fungal (B) community structures at the genus level. Only the top ten species, based on abundance, are displayed, while other species are combined as 'Others.' HJ represents the environmental sample from the bangcai storage place; XH corresponds to the sample of the bangcai after cleaning, and Xq represents the sample of the bangcai before cleaning.

end of fermentation in group C were significantly higher than those on the first day, while the Chao1 and OTUs of group D samples were significantly lower than those on the first day of fermentation. In addition, the diversity of bacteria in group D was significantly lower than that in group C ($p < 0.05$). In terms of fungal diversity, there were no significant differences ($p \geq 0.05$) among the samples in the C group as the fermentation time extended, indicating that the manufacturer's non-temperature-controlled natural fermentation has a stable fermented fungal system. However, in group D, the Chao1 index did not vary significantly with the progress of fermentation, while all other indices showed no significant differences in the first 4 days of fermentation, but decreased significantly($p < 0.05$) on the last day of fermentation and were significantly lower than those of group C. This indicated that laboratory temperature-controlled fermentation had an impact on the fungal diversity of Daocai. Overall, the microbial diversity of Daocai fermented in non-temperature-controlled(15–18 °C) fermentation in the manufacturer is higher than that of Daocai fermented in temperature-controlled(28 °C) fermentation in the laboratory. This is similar to the results of Zhang et al.(Zhang et al., 2024), who found that increased temperature during fermentation led to a loss of biodiversity, and low fermentation temperature was beneficial for preserving a wide range of microbial diversity.

3.4.3. Comparison of microbial profiles

Previous studies have defined certain functional microbes with a relative abundance greater than 10 % as characteristic microbes (Zhu et al., 2022). Phyla *Firmicutes* and *Proteobacteria* were predominant throughout the fermentation process in groups C and D (Supplementary Fig. S2A). The abundance of *Firmicutes* increased from an initial value of 4.90 % to 97.45 %, while the abundance of *Proteobacteria* decreased from 40.34 % to 1.56 %. The predominant bacterial taxa and their succession at the phylum level were similar between groups C and D, but differences were observed at the genus level (Fig. 4A). *Latilactobacillus* (2.49 % - 40.57 %, indicating the change in abundance from fresh bangcai (XH) to the end of fermentation) and *Leuconostoc* (0.81 % - 21.25 %) were the dominant during fermentation in group C. Both genera transiently increased in abundance, *Leuconostoc* reached its highest relative abundance of 32.03 % on the first day, whereas *Latilactobacillus* peaked at 59.76 % on the second day. As fermentation progressed, the abundance of lactobacilli-related genera, such as *Pectobacterium*, *Lactococcus*, and *Lactiplantibacillus* increased. In summary, lactobacilli (including lactobacilli-related genera and *Weissella*) were found to be abundant in group C, accounting for 82.74–98.09 % of the whole bacterial community, the relative abundances initially increased and then decreased. In group D, *Weissella* (0.16 %–29.39 %) and *Lactiplantibacillus* (0.97 %–45.61 %) were the dominant genera at the genus level. *Weissella* reached its highest abundance at 83.63 % on the second day, whereas *Lactiplantibacillus* peaked at 55.29 % on the third day. Additionally, the relative abundance of *Leuconostoc* decreased, whereas that of *Pediococcus* increased. In group D, *lactobacilli* (including lactobacilli-related genera and *Weissella*) were also abundant, accounting for 87.46–97.37 % of the bacterial community, with an increasing trend in relative abundance. In conclusion, lactobacilli were the characteristic bacteria during the Daocai fermentation process in both groups C and D.

The fungi were classified into eight phyla (Supplementary Fig. S2B). The dominant fungal communities and their succession differed between the samples from groups C and D. *Basidiomycota* was the major phylum in group C, accounting for 92.27–97.99 % of the total fungal community. In group D, the major phyla were *Basidiomycota*, *Ascomycota*, and *Mucoromycota,* and their dynamics during fermentation varied. The abundance of *Basidiomycota* decreased from 94.97 % to 13.00 %, while *Ascomycota* initially increased and then decreased (from 4.88 % to 35.95 % on the third day, then decreased to 15.14 %), and *Mucoromycota* increased from 0.01 % to 68.26 %. Fungal community analysis showed that *Cystofilobasidium* (54.05 %–22.12 %), *Vishniacozyma* (7.37 %–23.89 %), and *Leucosporidium* (13.91 %–24.95 %) were the dominant genera in group C (Fig. 4B). In group D, *Mucor* (0.01 %–68.26 %) gradually became the dominant genus in the fungal community. In addition, *Holtermanniella* (C:8.59 %–7.12 %; D:8.59 %–0.01 %), *Dioszegia* (C:3.71 %–9.53 %; D:3.71 %–0.87 %), *Cladosporium* (C:3.66 %–5.23 %; D:3.66 %- 6.66 %), *Saccharomyces* (C:0.01 %–1.88 %; D:0.01 %–23.94 %), and *Rhodotorula* (C:0.05 %–1.8 %; D:0.05 %–6.51 %) were detected in both groups. Overall, the abundance of yeast-related genera was higher in group C, accounting for 81.09–88.32 % of the fungal community, while yeast-related genera accounted for 10.84–50.11 % of the fungal community in group D.

In this study, we found that *Firmicutes* and *Proteobacteria* were the dominant bacterial phyla in the bacterial community, with the relative abundance of *Firmicutes* being 90 %. Other characteristic microorganisms included LAB, such as *Lactobacillus*, *Latilactobacillus*, *Leuconostoc*, *Weissella*, and *Lactobacillus plantarum*. The succession of bacterial communities in Daocai was consistent with that reported in other fermented vegetables (Liu, Li, et al., 2019; Liu, Peng, et al., 2019).

Usually, the quality of fermented vegetables often depends on the initially small fraction of LAB species, which quickly become the dominant bacteria during the fermentation process(Song et al., 2020). In this study, the relative abundance of LAB species was 2.08 % of the total bacterial community before fermentation, which increased to 82.74–98.09 % as Daocai fermentation progressed. *Pseudomonas* was the predominant bacterial genus identified in the fresh bangcai samples, accounting for 43.58 % of the total abundance (Fig. 4A). However, *Pseudomonas* rapidly disappeared after fermentation due to the ecological succession of microorganisms. Many studies have found that bacteria are the predominant microorganisms during vegetable fermentation, and the abundance of bacterial communities in fermented vegetables is much higher than that of fungi (Pardali et al., 2017). However, yeast fungi not only participate in vegetable fermentation but also have a significant impact on the quality of the final product (Guan et al., 2020). In support of this notion, we identified a core group of fungi associated with the Daocai fermentation process, including *Cystofilobasidium*, *Vishniacozyma*, *Leucosporidium*, *Holtermanniella*, *Dioszegia*, and *Cladosporium*. Recent studies have shown that certain yeasts interact with LAB to produce significant aromatic compounds (Sieuwerts et al., 2018).

3.5. Correlation between microbial abundance and Daocai quality

Coefficients of Spearman rank correlation (r) between the 18 most abundant microbial genera and flavor, physicochemical indicators, and biogenic amines of fermented Daocai were calculated. According to the criteria of $P < 0.05$ and $|r| > 0.5$, construct a relevant network diagram. The VFCs are mainly derived from the raw materials and microbial metabolites during the fermentation process. Therefore, microbial communities are closely related to the flavor characteristics of Daocai. As shown in Fig. 5A, a correlation network diagram was constructed to demonstrate the significant influence of microorganisms on the types of volatile flavor compounds during Daocai fermentation. We found that the relative abundances of *Latilactobacillus*, *Leuconostoc*, *Lactococcus, Cystofilobasidium*, *Leucosporidium*, *Holtermanniella*, *Dioszegia*, and *Bullera* were positively correlated with the relative contents of aldehydes, pyridines, furans, and alkenes. These results are consistent with a previous study (Tang et al., 2024), which found that various LAB genera (*Lactobacillus*, *Leuconostoc*, and *Lactococcus*) were strongly associated with most flavor compounds during fermentation. Additionally, these microbiota exhibited a negative correlation with nitriles.

Generally, the formation of BA in food is related to factors such as temperature, pH, chloride content, and free amino acids (Ahmad et al., 2020). As shown in Fig. B, the cadaverine content exhibited a positive correlation with the nitrite content ($r = 0.518$) and a negative correlation with the contents of chloride ($r = -0.876$) and soluble solids ($r = -0.623$). The histamine content showed a positive correlation with the

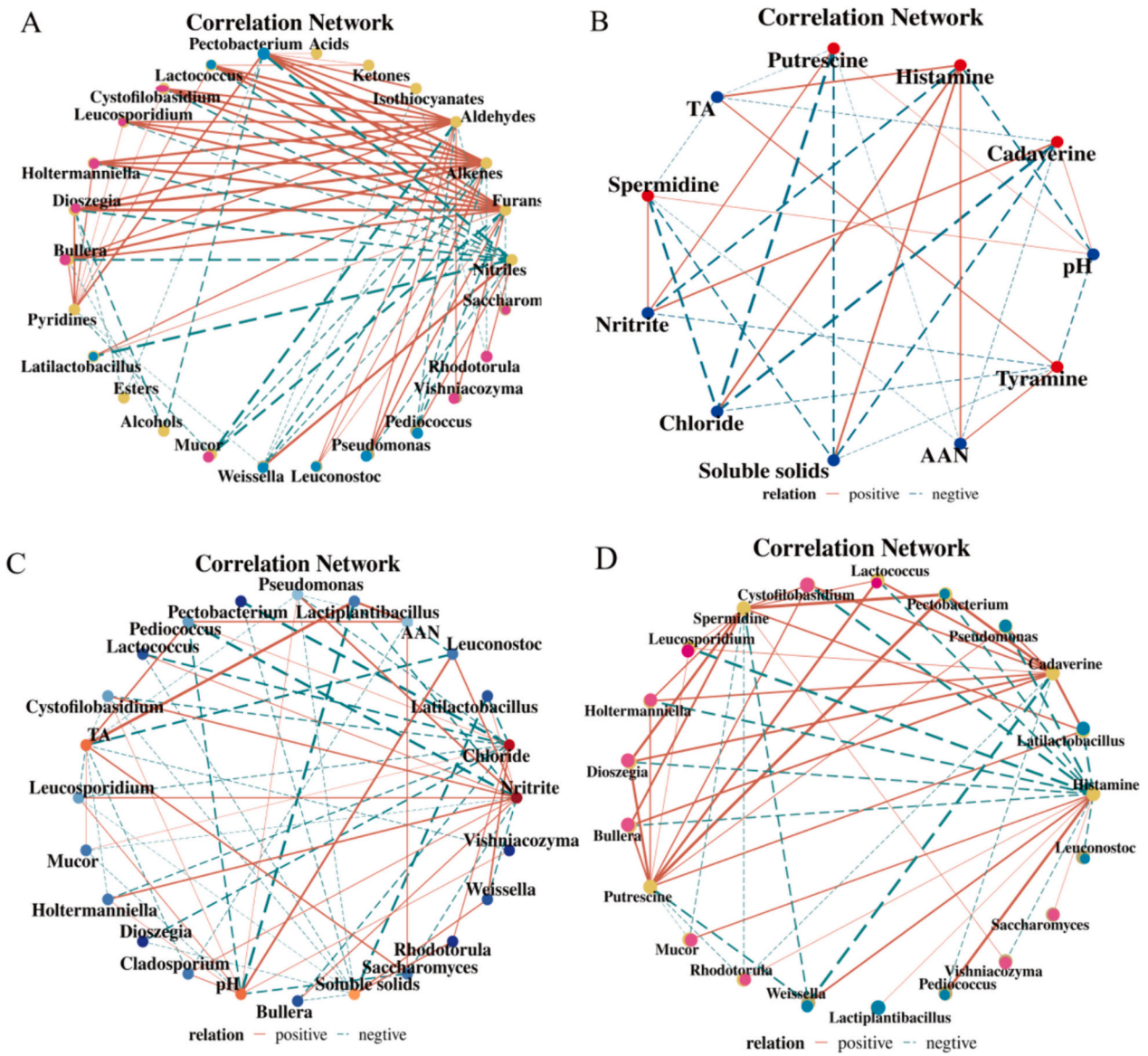


Fig. 5. Spearman correlation network between microbial types (8 types of bacteria and 10 types of fungi) and flavor types(A) physical and chemical indicators (C), and biogenic amines (D) during the fermentation process of Daocai. Additionally, Spearman correlation network diagram between physical and chemical indicators and biogenic amines (B) is shown. In the diagrams, the red solid line and cyan dotted line represent positive and negative correlations, respectively, and the width of the line corresponds to the strength of the Spearman correlation value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contents of TA (r = 0.541), chloride (r = 0.608), soluble solids (r = 0.584), but a negative correlation with the nitrite content (r = −0.661) and pH (r = −0.532). The putrescine content exhibited a negative correlation with the contents of chloride (r = −0.923) and soluble solids (r = −0.572). The spermidine contnet showed a negative correlation with chloride content (r = −0.784).

As shown in Fig. 5C, the pH is highly negatively related to the abundance of *LactiPlantibacillus* (r = −0.914). The TA content exhibited a strong positive correlation ($|r| > 0.8$) with *Lactiplantibacillus* (r = 0.922), and a highly negative correlation with *Leuconostoc* (r = −0.815). In our experiments, the pH and TA values in group D differed from those in group C. Correlation analysis revealed a significant positive correlation between *Lactiplantibacillus* and TA content. In fact, the acids in kimchi are mostly lactic acid and acetic acid, which not only contribute to the sour taste of the product but also interact with aldehydes, alcohols, and ketones to create additional flavor compounds in fermented vegetable products. Furthermore, the production of organic acids establishes a low pH acidic environment, effectively enhancing the inhibition of various spoilage microorganisms. The AAN content and the abundance of *Lactiplantibacillus* showed a positive correlation (r = 0.837). The chloride content was highly negatively correlated with *Pectobacterium* (r = −0.909). The nitrite content showed a negatively correlation with the abundance of *Pediococcus* (r = −0.898), *Weissella*(r = −0.678), *Lactiplantibacillus*(r = −0.710). In this study, the nitrite content of all Daocai samples decreased significantly during fermentation and was far below the National Health Standard for the nitrite safety limit of 20 mg/kg (Luo et al., 2023). This was consistent with the observation that LAB gradually become the dominant bacteria in the fermentation process. Acetic acid-producing bacteria, such as *Lactobacillus plantarum,* produce nitrite reductase to degrade nitrites (Wu et al., 2015). Studies have shown that adding LAB during the fermentation process can decrease the content of BAs and nitrites in fermented foods (Qin et al., 2023). This is similar to the findings of our study, which found that the contents of BAs and nitrite decreased as the abundance of

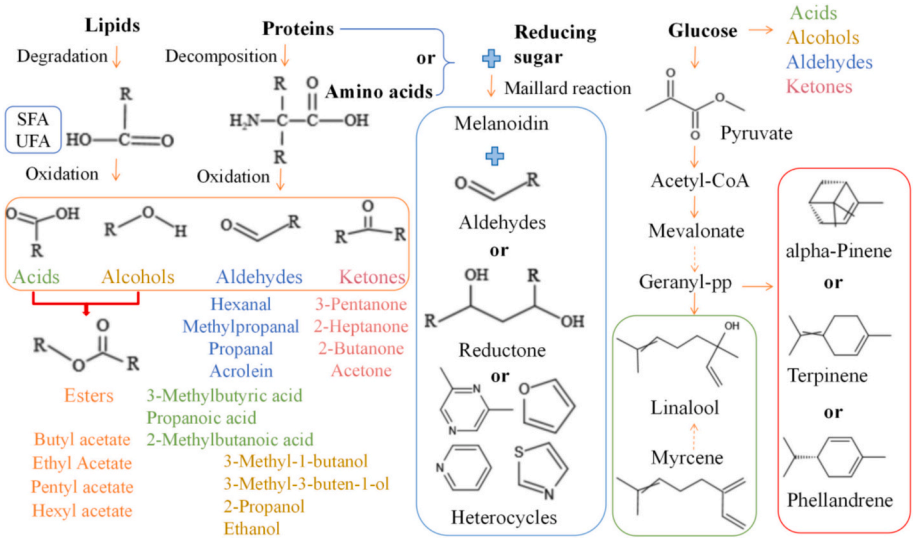


Fig. 6. Flavor formation mechanism of Daocai. SFA, saturated fatty acids; UFA, unsaturated fatty acids.

LAB increased. The soluble solids content highly negatively correlated with the abundance of *Latilactobacillus* ($r = -0.857$). This is consistent with the research results of Ooi et al.(Ooi et al., 2020), who found that *Latilactobacillus* can significantly reduce the content of soluble solids during the fermentation process.

As shown in Fig. 5D, the cadaverine content exhibited a strong negative correlation with the abundance of *Weissella* ($r = -0.854$), and a strong positive correlation with the abundances of *Latilactobacillus* ($r = 0.80$) and *Pectobacterium* ($r = 0.850$). The histamine content showed a strong negative correlation with the abundances of *Cystofilobasidium* ($r = -0.811$) and *Leucosporidium* ($r = -0.835$). The putrescine content was highly positively correlated with the abundance of *Pectobacterium* ($r = 0.866$). The spermidine content showed a strong positive correlation with the abundances of *Pectobacterium* ($r = 0.865$) and *Dioszegia* ($r = 0.820$). Hu et al.(Hu et al., 2021) utilized metagenomic sequencing to demonstrate that the gene responsible for encoding the key enzyme in the BA production process is the amino acid decarboxylase gene. Genetic analysis of the metagenome-assembled genomes revealed that the genomes of *Lactobacillus* and *Lactococcus* contain amino acid decarboxylase genes, which contributed to histamine and putrescine in Sufu. This confirms our findings that in our experiments, histamine showed a positive correlation with *Pediococcus* ($r = 0.829$), *Weissella* ($r = 0.715$), *Lactiplantibacillus* ($r = 0.512$), and putrescine showed a positive correlation with *Lactococcus* ($r = 0.798$), *Latilactobacillus* ($r = 0.675$) (Fig. 5D). Some reports indicate that *Pseudomonas* promotes the accumulation of histamine, putrescine, and cadaverine (Li et al., 2023). However, our associated network graph shows a negative correlation between *Pseudomonas* ($r = -0.793$) and histamine, which was consistent with some special fermented foods, such as shrimp paste (Sang et al., 2020). The observed discrepancies in these results suggest that the microbial community's ability to produce biogenic amines may be influenced by the types of fermented foods and their ingredients. However, the ability of *Pectobacterium*, *Cystofilobasidium*, *Leucosporidium*, and *Dioszegia* to produce BA has not been reported and needs further study.

4. Conclusion

We investigated the changes in microbial community structure and physicochemical parameters during the natural fermentation of Daocai under temperature-controlled (group D) and non-temperature-controlled (group C) conditions. HTS revealed that the predominant genera in the samples from group C were *Latilactobacillus*, *Leuconostoc*, *Cystofilobasidium*, *Vishniacozyma*, and *Leucosporidium*, whereas *Weissella*, *Lactiplantibacillus*, *Mucor*, and *Saccharomyces* were the predominant genera in group D. The relative abundances of *Latilactobacillus*, *Leuconostoc*, *Weissella*, *Lactiplantibacillus*, *Leucosporidium*, and *Saccharomyces* were strongly correlated with pH, TA, nitrites, BAs, and VFCs in the samples. In general, higher contents of TA, chloride, AAN, and soluble solids were observed in samples from group D, whereas pH, nitrite, and BAs contents were higher in samples from group C. Furthermore, our results suggest that the accumulation of TA and VFCs, such as alcohols, esters, aldehydes, and ketones, contributed to the unique sensory characteristics of Daocai. These findings confirm the significant impact of temperature on the microbial community structure and quality of fermented Daocai, providing fundamental data for the standardization and improvement of gustatory characteristics of fermented vegetables. However, this study has the following limitations. First, the effects of factors such as humidity and fermentation equipment on microbial community structure and VFCs were not considered. Second, sequencing targeted amplicons based on the V3-V4 hypervariable region and the ITS1 region may not identify all microbial species in the sample and cannot determine the viability of microorganisms. In the future research, the activity of bacteria can be determined using the live bacteria staining method. The complexity of VFCs involves the combination of GC-IMS technology with GC-O-MS and GC–MS technology.

Ethical statement

The sensory analysis in this study complied with sensory ethical standards. Additionally, all sensory evaluators were professionally trained and their rights and privacy were adequately respected and protected. The purpose, process, risks and benefits of the study were adequately explained to all participants and their informed consent was obtained. Participation was voluntary and participants could withdraw from the study at any time. This study compared two methods of temperature-controlled natural fermentation and non-temperature-controlled natural fermentation of bangcai, and did not involve human life science and medical research. In addition, the sensory analysis participants did not incur additional risks or discomfort. Therefore, this study did not require ethical approval.

CRediT authorship contribution statement

Xueli Wang: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Xueting Liu:** Writing – original draft, Methodology, Investigation, Data curation,

Conceptualization. **Chunmei Sun:** Project administration, Conceptualization. **Yanwei Cheng:** Supervision, Project administration, Funding acquisition. **Zhen Li:** Resources, Project administration. **Shuyi Qiu:** Supervision, Project administration. **Yongguang Huang:** 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.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Science and Technology Support Project of Guizhou Province, China ([2020] 1Y154), ([2020] 1Y116), ([2021] General 273), ([2021] General 121), and ([2022] General 148), Guizhou University Natural Science Program (Gui Da Ren Ji He Zi [2023] 49), Basic research project of Guizhou University(Gui Da Ji Chu [2024]27), and Guizhou University Laboratory Open Project (SYSKF2024-60). The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101827.

References

Ahmad, W., Mohammed, G. I., Al-Eryani, D. A., Saigl, Z. M., Alyoubi, A. O., Alwael, H., … El-Shahawi, M. S. (2020). Biogenic amines formation mechanism and determination strategies: Future challenges and limitations. *Critical Reviews in Analytical Chemistry, 50*(6), 485–500. https://doi.org/10.1080/10408347.2019.1657793

An, F., Sun, H., Wu, J., Zhao, C., Li, T., Huang, H., Fang, Q., Mu, E., & Wu, R. (2021). Investigating the core microbiota and its influencing factors in traditional Chinese pickles. *Food Research International, 147*, Article 110543. https://doi.org/10.1016/j.foodres.2021.110543

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Arumugam, M. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Cao, J., Yang, J., Hou, Q., Xu, H., Zheng, Y., Zhang, H., & Zhang, L. (2017). Assessment of bacterial profiles in aged, home-made Sichuan paocai brine with varying titratable acidity by PacBio SMRT sequencing technology. *Food Control, 78*, 14–23. https://doi.org/10.1016/j.foodcont.2017.02.006

Chen, F., Zhu, Z., Tong, L., Guo, X., Xu, S., Chen, J., Wu, J., & Zhu, Z. (2020). Production of allohexaploid Brassica hybrid between tuber mustard (*Brassica juncea* L. var. crassicaulis Chen &Yang) and Chinese kale (*Brassica oleracea var. alboglabra* bailey). *Scientia Horticulturae, 270*, Article 109412. https://doi.org/10.1016/j.scienta.2020.109412

Chen, Q., Li, Y., Yan, K., Li, G., Luo, D., Bai, W., Zeng, X., Wu, Q., Deng, J., & Dong, H. (2024). Variations of volatile flavors and microbial communities in Chinese Chaozhou pickle during natural fermentation revealed by GC-IMS and high-throughput sequencing. *LWT, 191*, Article 115610. https://doi.org/10.1016/j.lwt.2023.115610

Chen, Z., Liu, L., Du, H., Lu, K., Chen, C., Xue, Q., & Hu, Y. (2023). Microbial community succession and their relationship with the flavor formation during the natural fermentation of Mouding sufu. *Food Chemistry: X, 18*, Article 100686. https://doi.org/10.1016/j.fochx.2023.100686

Dabadé, D. S., Jacxsens, L., Miclotte, L., Abatih, E., Devlieghere, F., & De Meulenaer, B. (2021). Survey of multiple biogenic amines and correlation to microbiological quality and free amino acids in foods. *Food Control, 120*, Article 107497. https://doi.org/10.1016/j.foodcont.2020.107497

De Filippis, F., Troise, A. D., Vitaglione, P., & Ercolini, D. (2018). Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. *Food Microbiology, 73*, 11–16. https://doi.org/10.1016/j.fm.2018.01.008

Du, Q., Ye, D., Zang, X., Nan, H., & Liu, Y. (2022). Effect of low temperature on the shaping of yeast-derived metabolite compositions during wine fermentation. *Food Research International, 162*, Article 112016. https://doi.org/10.1016/j.foodres.2022.112016

Gao, X., Li, C., He, R., Zhang, Y., Wang, B., Zhang, Z., & Ho, C. (2023). Research advances on biogenic amines in traditional fermented foods: Emphasis on formation mechanism, detection and control methods. *Food Chemistry, 405*, Article 134911. https://doi.org/10.1016/j.foodchem.2022.134911

Guan, Q., Zheng, W., Huang, T., Xiao, Y., Liu, Z., Peng, Z., Gong, D., Xie, M., & Xiong, T. (2020). Comparison of microbial communities and physiochemical characteristics of two traditionally fermented vegetables. *Food Research International, 128*, Article 108755. https://doi.org/10.1016/j.foodres.2019.108755

Hu, M., Dong, J., Tan, G., Li, X., Zheng, Z., & Li, M. (2021). Metagenomic insights into the bacteria responsible for producing biogenic amines in sufu. *Food Microbiology, 98*, Article 103762. https://doi.org/10.1016/j.fm.2021.103762

Jastrzębska, A., Kmieciak, A., Brzuzy, K., Gralak, Z., Krzemiński, M. P., & Szłyk, E. (2023). Determination of selected biogenic amines in fermented vegetables juices. *Food Control, 154*, Article 109980. https://doi.org/10.1016/j.foodcont.2023.109980

Kim, H. M., Han, D. M., Baek, J. H., Chun, B. H., & Jeon, C. O. (2022). Dynamics and correlation of microbial communities and metabolic compounds in doenjang-meju, a Korean traditional soybean brick. *Food Research International, 155*, Article 111085. https://doi.org/10.1016/j.foodres.2022.111085

Li, Y., Zhao, N., Li, Y., Zhang, D., Sun, T., & Li, J. (2023). Dynamics and diversity of microbial community in salmon slices during refrigerated storage and identification of biogenic amine-producing bacteria. *Food Bioscience, 52*, Article 102441. https://doi.org/10.1016/j.fbio.2023.102441

Liang, H., He, Z., Wang, X., Song, G., Chen, H., Lin, X., Ji, C., & Li, S. (2020). Effects of salt concentration on microbial diversity and volatile compounds during suancai fermentation. *Food Microbiology, 91*, Article 103537. https://doi.org/10.1016/j.fm.2020.103537

Liang, H., He, Z., Wang, X., Song, G., Chen, H., Lin, X., Ji, C., & Zhang, S. (2020). Bacterial profiles and volatile flavor compounds in commercial Suancai with varying salt concentration from northeastern China. *Food Research International, 137*, Article 109384. https://doi.org/10.1016/j.foodres.2020.109384

Liu, D., Shen, Q., Lin, K., Wang, F., Bu, Z., Peng, J., Brennan, C., Benjakul, S., Xiao, G., & Ma, L. (2024). The aroma profiles of dried gonggans: Characterization of volatile compounds in oven-dried and freeze-dried gonggan. *Food Research International, 191*, Article 114716. https://doi.org/10.1016/j.foodres.2024.114716

Liu, X., Qian, M., Shen, Y., Qin, X., Huang, H., Yang, H., He, Y., & Bai, W. (2021). An high-throughput sequencing approach to the preliminary analysis of bacterial communities associated with changes in amino acid nitrogen, organic acid and reducing sugar contents during soy sauce fermentation. *Food Chemistry, 349*, Article 129131. https://doi.org/10.1016/j.foodchem.2021.129131

Liu, X., Wang, X., Cheng, Y., Wu, Y., Yan, Y., & Li, Z. (2023). Variations in volatile organic compounds in Zhenyuan Daocai samples at different storage durations evaluated using E-nose, E-tongue, gas chromatography, and spectrometry. *LWT, 173*, Article 114186. https://doi.org/10.1016/j.lwt.2022.114186

Liu, Z., Li, J., Huang, T., Xiao, Y., Peng, Z., Xie, M., & Xiong, T. (2019). Comparison of the bacterial communities in home-made Nanfeng yancai with and without salt. *Food Research International, 125*, Article 108509. https://doi.org/10.1016/j.foodres.2019.108509

Liu, Z., Peng, Z., Huang, T., Xiao, Y., Li, J., Xie, M., & Xiong, T. (2019). Comparison of bacterial diversity in traditionally homemade paocai and Chinese spicy cabbage. *Food Microbiology, 83*, 141–149. https://doi.org/10.1016/j.fm.2019.02.012

Luo, W., Wu, W., Du, X., Yu, Y., Wu, J., Xu, Y., & Li, L. (2023). Regulation of the nitrite, biogenic amine and flavor quality of Cantonese pickle by selected lactic acid bacteria. *Food Bioscience, 53*, Article 102554. https://doi.org/10.1016/j.fbio.2023.102554

Mi, T., Wang, D., Yao, S., Yang, H., Che, Y., & Wu, C. (2022). Effects of salt concentration on the quality and microbial diversity of spontaneously fermented radish paocai. *Food Research International, 160*, Article 111622. https://doi.org/10.1016/j.foodres.2022.111622

Ooi, T. S., Ting, A. S. Y., & Siow, L. F. (2020). Influence of selected native yeast starter cultures on the antioxidant activities, fermentation index and total soluble solids of Malaysia cocoa beans: A simulation study. *LWT, 122*, Article 108977. https://doi.org/10.1016/j.lwt.2019.108977

Pardali, E., Paramithiotis, S., Papadelli, M., Mataragas, M., & Drosinos, E. H. (2017). Lactic acid bacteria population dynamics during spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine. *World Journal of Microbiology and Biotechnology, 33*(6). https://doi.org/10.1007/s11274-017-2276-8

Qin, S., Zeng, X. M., Jiang, M., Rui, X., Li, W., Dong, M. S., … Zhang, Q. Q. (2023). Genomic and biogenic amine-reducing characterization of Lactiplantibacillus planatraum JB1 isolated from fermented dry sausage. *Food Control, 154*, Article 109971. https://doi.org/10.1016/j.foodcont.2023.109971

Ryu, J., Kim, E., Yang, S., Lee, S., Yoon, S., Jang, K., & Kim, H. (2021). High-throughput sequencing of the microbial community associated with the physicochemical properties of meju (dried fermented soybean) and doenjang (traditional Korean fermented soybean paste). *LWT, 146*, Article 111473. https://doi.org/10.1016/j.lwt.2021.111473

Sang, X., Li, K., Zhu, Y., Ma, X., Hao, H., Bi, J., Zhang, G., & Hou, H. (2020). The impact of microbial diversity on biogenic amines formation in grasshopper sub shrimp paste during the fermentation. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.00782

Sieuwerts, S., Bron, P. A., & Smid, E. J. (2018). Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT, 90*, 201–206. https://doi.org/10.1016/j.lwt.2017.12.022

Song, H. S., Whon, T. W., Kim, J., Lee, S. H., Kim, J. Y., Kim, Y. B., … Roh, S. W. (2020). Microbial niches in raw ingredients determine microbial community assembly during kimchi fermentation. *Food Chemistry, 318*, Article 126481. https://doi.org/10.1016/j.foodchem.2020.126481

Song, X., Liao, D., Zhou, Y., Huang, Q., Lei, S., & Li, X. (2024). Correlation between physicochemical properties, flavor characteristics and microbial community structure in Dushan shrimp sour paste. *Food Chemistry: X, 23*, Article 101543. https://doi.org/10.1016/j.fochx.2024.101543

Sun, Q., Sun, F., Zheng, D., Kong, B., & Liu, Q. (2019). Complex starter culture combined with vacuum packaging reduces biogenic amine formation and delays the quality deterioration of dry sausage during storage. *Food Control, 100*, 58–66. https://doi.org/10.1016/j.foodcont.2019.01.008

Sun, X., Qi, X., Han, Y., Guo, Z., Cui, C., & Lin, C. (2023). Characteristics of changes in volatile organic compounds and microbial communities during the storage of pickles. *Food Chemistry, 409*, Article 135285. https://doi.org/10.1016/j.foodchem.2022.135285

Sun, Y., Peng, C., Wang, J., Guo, S., Sun, Z., & Zhang, H. (2022). Mesopic fermentation contributes more to the formation of important flavor compounds and increased growth of *Lactobacillus casei* Zhang than does high temperature during milk fermentation and storage. *Journal of Dairy Science, 105*(6), 4857–4867. https://doi.org/10.3168/jds.2021-20949

Tang, J., Wu, X., Lv, D., Huang, S., Zhang, Y., & Kong, F. (2024). Effect of salt concentration on the quality and microbial community during pickled peppers fermentation. *Food Chemistry: X, 23*, Article 101594. https://doi.org/10.1016/j.fochx.2024.101594

Tian, H., Xiong, J., Sun, J., Du, F., Xu, G., Yu, H., Chen, C., & Lou, X. (2024). Dynamic transformation in flavor during hawthorn wine fermentation: Sensory properties and profiles of nonvolatile and volatile aroma compounds coupled with multivariate analysis. *Food Chemistry, 456*, Article 139982. https://doi.org/10.1016/j.foodchem.2024.139982

Tian, X., Gao, P., Xu, Y., Xia, W., & Jiang, Q. (2021). Reduction of biogenic amines accumulation with improved flavor of low-salt fermented bream (*Parabramis pekinensis*) by two-stage fermentation with different temperature. *Food Bioscience, 44*, Article 101438. https://doi.org/10.1016/j.fbio.2021.101438

Wang, D., Chen, G., Tang, Y., Li, H., Shen, W., Wang, M., Liu, S., Qin, W., & Zhang, Q. (2020). Effects of temperature on paocai bacterial succession revealed by culture-dependent and culture-independent methods. *International Journal of Food Microbiology, 317*, Article 108463. https://doi.org/10.1016/j.ijfoodmicro.2019.108463

Wang, P., Kan, Q., Yang, L., Huang, W., Wen, L., Fu, J., Liu, Z., Lan, Y., Huang, Q., Ho, C., & Cao, Y. (2023). Characterization of the key aroma compounds in soy sauce by gas chromatography-mass spectrometry-olfactometry, headspace-gas chromatography-ion mobility spectrometry, odor activity value, and aroma recombination and omission analysis. *Food Chemistry, 419*, Article 135995. https://doi.org/10.1016/j.foodchem.2023.135995

Wu, R., Yu, M., Liu, X., Meng, L., Wang, Q., Xue, Y., Wu, J., & Yue, X. (2015). Changes in flavour and microbial diversity during natural fermentation of suan-cai, a traditional food made in Northeast China. *International Journal of Food Microbiology, 211*, 23–31. https://doi.org/10.1016/j.ijfoodmicro.2015.06.028

Xiao, L., Lapu, M., Kang, S., Jiang, P., Li, J., Liu, Y., Liu, D., & Liu, M. (2022). Effects of Tartary buckwheat on physicochemical properties and microbial community of low salt natural fermented soybean paste. *Food Control, 138*, Article 108953. https://doi.org/10.1016/j.foodcont.2022.108953

Xiao, R., Chen, S., Wang, X., Chen, K., Hu, J., Wei, K., Ning, Y., Xiong, T., & Lu, F. (2022). Microbial community starters affect the profiles of volatile compounds in traditional Chinese Xiaoqu rice wine: Assement via high-throughput sequencing and gas chromatography-ion mobility spectrometry. *LWT, 170*, Article 114000. https://doi.org/10.1016/j.lwt.2022.114000

Xiao, Y., Huang, T., Huang, C., Hardie, J., Peng, Z., Xie, M., & Xiong, T. (2020). The microbial communities and flavour compounds of Jiangxi yancai, Sichuan paocai and Dongbei suancai: Three major types of traditional Chinese fermented vegetables. *LWT, 121*, Article 108865. https://doi.org/10.1016/j.lwt.2019.108865

Xiong, S., Xu, X., Du, T., Liu, Q., Huang, T., Ren, H., Xiong, T., & Xie, M. (2024). Organic acids drove the microbiota succession and consequently altered the flavor quality of Laotan Suancai across fermentation rounds: Insights from the microbiome and metabolome. *Food Chemistry, 450*, Article 139335. https://doi.org/10.1016/j.foodchem.2024.139335

Zhang, C., Gu, C., Wang, M., Chen, J., Chang, H., Chang, Z., Zhou, J., Yue, M., Zhang, W., Zhang, Q., & Feng, Z. (2024). Effect of temperature regulation on microbial community, volatile flavours, amino acid profiles, and iridoid glycosides during noni (*Morinda citrifolia* L.) fruit fermentation. *Food Chemistry, 24*, Article 140966. https://doi.org/10.1016/j.foodchem.2024.140966

Zhang, C., Zhang, J., & Liu, D. (2021). Biochemical changes and microbial community dynamics during spontaneous fermentation of Zhacai, a traditional pickled mustard tuber from China. *International Journal of Food Microbiology, 347*, Article 109199. https://doi.org/10.1016/j.ijfoodmicro.2021.109199

Zhang, Q., Chen, G., Shen, W., Wang, Y., Zhang, W., & Chi, Y. (2016). Microbial safety and sensory quality of instant low-salt Chinese paocai. *Food Control, 59*, 575–580. https://doi.org/10.1016/j.foodcont.2015.06.041

Zhao, N., Lai, H., Wang, Y., Huang, Y., Shi, Q., He, W., Zhu, S., Li, Y., Zhu, Y., Li, H., & Ge, L. (2021). Assessment of biogenic amine and nitrite production in low-salt Paocai during fermentation as affected by reused brine and fresh brine. *Food Bioscience, 41*, Article 100958. https://doi.org/10.1016/j.fbio.2021.100958

Zhao, Y., Liu, S., Han, X., Zhou, Z., & Mao, J. (2022). Combined effects of fermentation temperature and *Saccharomyces cerevisiae* strains on free amino acids, flavor substances, and undesirable secondary metabolites in huangjiu fermentation. *Food Microbiology, 108*, Article 104091. https://doi.org/10.1016/j.fm.2022.104091

Zhao, Y., Wei, W., Tang, L., Wang, D., Wang, Y., Wu, Z., & Zhang, W. (2021). Characterization of aroma and bacteria profiles of Sichuan industrial paocai by HSSPME-GC-O-MS and 16S rRNA amplicon sequencing. *Foodservice Research International, 149*, Article 110667. https://doi.org/10.1016/j.foodres.2021.110667

Zheng, L., Wang, S., Yang, Y., Zheng, X., Xiao, D., Ai, B., & Sheng, Z. (2024). Volatile aroma compounds of passion fruit seed oils: HS-GC-IMS analysis and interpretation. *Food Chemistry: X, 21*, Article 101212. https://doi.org/10.1016/j.fochx.2024.101212

Zhu, C., Cheng, Y., Zuo, Q., Huang, Y., & Wang, L. (2022). Exploring the impacts of traditional crafts on microbial community succession in Jiang-flavored Daqu. *Food Research International, 158*, Article 111568. https://doi.org/10.1016/j.foodres.2022.111568