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Correlation between Microorganisms and Volatile Compounds during Spontaneous Fermentation of Sour Bamboo Shoots

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Abstract: Chinese sour bamboo shoot is a traditionally, spontaneous fermented food that is particularly popular due to its complex and distinctive flavor. The volatile compounds of sour bamboo shoot originate mainly from the raw materials and the microbial fermentation. To reveal the correlation between microorganisms and flavor, third-generation sequencing and Gas Chromatography-Ion Mobility Spectrometry were applied to analyze the dynamics of microbial communities at the species level and volatile compounds during sour bamboo shoot fermentation. The abundance of *Lactobacillus acetotolerans* and *Lactobacillus fermentum* increased during the fermentation, while *Lactobacillus amyloliquefaciens* decreased at first but then began to rise. At the end of fermentation, *Lactobacillus amyloliquefaciens* and *Lactobacillus acetotolerans* became the predominant species. A total of sixty-seven volatile compounds, which included twenty-three esters, nineteen alcohols, eight ketones, six aldehydes, six aromatic hydrocarbons, four acids and one ether, were identified. These compounds constituted the primary flavor of sour bamboo, which created a complex flavor of sour bamboo shoot. Among them, the contents of acetic acid, propionic acid, and isoamyl alcohol gradually increased during the fermentation process, and they became the main volatile compounds. Furthermore, the correlation between microorganisms and volatile compounds was investigated through two-way Orthogonal Partial Least Squares (O2PLS), which revealed a positive correlation between *Lactobacillus amylolyticus* and ethyl propanoate. Additionally, the abundance of *Lactobacillus acetotolerans* and *Lactobacillus fermentum* was found to be positively correlated with 2-heptenal. These findings provide a theoretical basis for understanding the formation mechanism of sour bamboo shoot flavor and the standardized production of high-quality sour bamboo shoots.

Keywords: sour bamboo shoot; flavor; microbial diversity; third-generation sequencing; correlation analysis

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1. Introduction

Bamboo shoots, also known as "Cold Mountain Treasures" and "Forest Vegetables", have been a popular delicacy in China for centuries [1]. Sour bamboo shoot is a traditional, spontaneously fermented product with a complex flavor. During fermentation, a noxious odor is generated alongside a sour taste and fruity aroma which constitute a special complex aroma and taste. Sour bamboo shoots are characterized by their low fat and low sugar content, and they are high in dietary fiber, vitamins, and carbohydrates [1–3]. A large number of health benefits have been assigned to sour bamboo shoots, such as antioxidant, anticancer, blood pressure reduction, cardiovascular disease prevention, and weight loss [4,5]. In addition, bamboo shoots can be utilized in the food, pharmaceutical, and biofuel industries [4–6].

The volatile compounds of sour bamboo shoots originate mainly from the raw materials and during the fermentation process [7]. Fu et al. [8] identified a total of twenty-nine

aroma compounds in sour bamboo shoots with cresol, acetic acid, 2-heptanal, and linalool being the major odorous compounds, while Li et al. [9] identified a total of seventy aromatic compounds in sour bamboo shoots with cresol, acetic acid, and octanal being the primary volatile compounds and (E)-2-octanal being a secondary volatile compound. As such, cresol and acetic acid are usually recognized as the two main volatile components of sour bamboo shoots [8,9].

The development of the taste of sour bamboo shoots is closely linked to the activity of microorganisms. The microorganisms and volatile compounds present in spontaneously fermented sour bamboo shoots have been studied before [10–12]. Guan et al. [10] employed high-throughput sequencing technology to investigate the microbial species in sour bamboo shoot samples and reported that *Lactobacillus* and *Serratia* were the two most abundant genera in the microbial communities of fermented sour bamboo shoots. They reported that *Lactobacillus acetotolerans* and *Lactobacillus fermentum* were the most dominant species with a gradual increase during the fermentation, while *Serratia* first decreased before increasing in abundance over time. The fungal community in sour bamboo shoots is predominantly composed of the genera *Pichia* and *Candida*, with *Pichia norvegensis* and *Kazachstania humilis* (formerly known as *Candida humilis*) identified as the key species [10]. Despite slight variations influenced by the production area and fermentation conditions, microbial communities across different locations exhibit a notable degree of similarity. Guan et al. [11] determined the microorganisms at the phylum, genus, and species levels in sour bamboo shoot samples using high-throughput sequencing technology and found that *Lactobacillus*, *Lactococcus,* and *Serratia* were the main bacterial genera, *Lactobacillus acetotolerans* and *Lactobacillus brevis* were by far the predominant bacteria, and *Kazachstania*, *Debaryomyces*, and *Pichia* were the main fungal genera during the fermentation of sour bamboo shoots [11]. Traditional sequencing techniques are limited to ascertaining the genus level of microorganisms. In comparison to the classic sequencing technique, the third-generation sequencing technique has many advantages, such as greater throughput, accuracy, speed, and longer read lengths, allowing it to quantify the species level of microorganisms. In the literature we reviewed, the temperature of fermented vegetable typically follows a pattern of initial increase followed by a decrease. The early and intermediate stages of fermentation are characterized by a high presence of microbial species with high activity, and the temperature tends to rise. Conversely, in the later stages of fermentation, as microbial populations diminish, the temperature decreases [13].

Numerous studies have been conducted to investigate the microbial diversity and volatile compounds of sour bamboo shoots from different regions. However, the relationship between microbial flora and volatile compounds in sour bamboo shoots is still unclear. To further analyze the connection between microbial communities and volatile compounds, we aimed to investigate the microbial community with third-generation sequencing to identify dominant species dynamics during the sour shoot fermentation process. To observe the volatile components during fermentation, Gas Chromatography-Ion Mobility Spectrometry (GC-IMS) was employed, and multivariate statistical analysis was applied to understand both the major and minor volatile components of sour bamboo shoots and to identify the main volatile compounds of the sour bamboo shoot odor. O2PLS was employed to investigate the correlation between microbial composition and volatile components. Investigating the succession of microbial communities responsible for generating primary volatile compounds can provide valuable insight to aid in the selection of strains that produce desired flavor and functional substances.

2. Materials and Methods

2.1. Preparation of Sour Bamboo Shoots and Sampling

For the purpose of this study, we collaborated with a local manufacturer of sour bamboo shoots. Sour bamboo shoots were produced in the Liuluoxiang factory in Liuzhou, Guangxi. Fresh bamboo shoots, sourced from local suppliers, were used as the raw material. First, the bamboo shoots were washed with clean water. The cleaned bamboo shoots were

cut into 3–8 cm segments, and then were sliced into thin strips with thickness of 1–2 mm. In accordance with local production methods, the sliced bamboo shoots were packed in large earthenware jars (approximate dimensions: 80 cm internal diameter and 120 cm height), and filled with local well-water. The fermentations were carried out over three weeks at ambient temperature (21 ± 2 °C). Three large earthenware jars were randomly selected and dedicated for the purpose of this experiment, with solid shoot samples being withdrawn at weekly intervals. At each weekly sampling event three subsamples, removing approximately 5 g of bamboo shoots from three different depths from each individual jar, were taken and pooled and then processed into a slurry [14]. The same sampling regime was applied to all three, large earthenware jars in which the bamboo shoots were fermented. The pooled samples were stored at −20 °C for subsequent analysis.

2.2. Third-Generation Sequencing Analysis

Total DNA was extracted with the TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biotech Beijing Co., Ltd., Beijing, China) according to the manufacturer's instructions, which was then quantified with the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, OR, USA).

The V4–V5 hypervariable region of 16S rRNA gene was amplified by PCR using 27F:AGRGTTTGATYNTGGCTCAG and 1492R:TASGGHTACCTTGTTASGACTT from the genomic DNA extracted from each sample. Both the forward and reverse 16S primers were tailed with sample-specific PacBio barcode sequences to allow for multiplexed sequencing. The KOD One PCR Master Mix (TOYOBOLife Science, Osaka, Japan) was used to perform 25 cycles of PCR amplification, with initial denaturation at 95 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s, and a final step at 72 °C for 2 min. The V4 hypervariable region of 18S rRNA gene from the fungal genomic DNA extracted from each sample was amplified by PCR using two primers: Euk-A_(18S-F):AACCTGGTTGATCCTGCCAGT and Euk-B_(18S-R):GATCCTTCTGCAGGTTCACCTAC. The KOD One PCR Master Mix (TOYOBOLife Science, Osaka, Japan) was used to perform 25 cycles of PCR amplification, with initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, and a final step at 72 °C for 7 min. The total PCR amplicons were purified with Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, OR, USA). After the individual quantification step, amplicons were pooled in equal amounts. SMRTbell libraries were prepared from the amplified DNA by SMRTbell Express Template Prep Kit 2.0 according to the manufacturer's instructions (Pacific Biosciences, Menlo Park, CA, USA). Purified SMRTbell libraries from the pooled and barcoded samples were sequenced on a single PacBio Sequel II 8 M cell using the Sequel II Sequencing kit 2.0.

2.3. Bioinformatic Analysis

The bioinformatic analysis was performed with the aid of the BMK Cloud (Biomarker Technologies Co., Ltd., Beijing, China). The raw reads generated from sequencing were filtered and demultiplexed using SMRT Link software (version 8.0) with minimum passes ≥ 5 and minimum predicted accuracy ≥ 0.9 to obtain circular consensus sequencing (CCS) reads. Subsequently, lima (version 1.7.0) was employed to assign the CCS reads to the corresponding samples based on their barcodes. CCS reads containing no primers and those reads beyond the length range (1200–1650 bp) were discarded through the recognition of forward and reverse primers and quality filtering using the Cutadapt quality control process (version 2.7). The UCHIME algorithm (version 8.1) was used to detect and remove chimeric sequences to obtain clean reads. Sequences with similarity $\geq 97\%$ were clustered into the same operational taxonomic unit (OTU) by USEARCH (version 10.0), and the OTUs with abundance < 0.005% were filtered. Taxonomy annotation of the OTUs was performed based on the naive Bayes classifier in QIIME2 using the SILVA database (release 132) with a

confidence threshold of 70%. The alpha diversity was calculated and displayed by QIIME2 and R software (version 2018.11), respectively. Beta diversity was determined to evaluate the degree of similarity of microbial communities from different samples using QIIME. PCoA analysis of microbiota during fermentation was carried out at the OTU level based on the Bray-Curtis distance [15]. Furthermore, we employed Linear Discriminant Analysis (LDA) effect size (LEfSe) to test the significant taxonomic differences among groups. A logarithmic LDA score of 4.0 was set as the threshold for discriminative features.

2.4. Determination of Volatile Compounds

Gas Chromatography-Ion Mobility Spectrometry (GC-IMS) was applied to detect the volatile components in sour bamboo shoots. Five grams of each sample was placed in a 20 mL headspace vial. GC-IMS analysis was performed utilizing GC coupled with an ion mobility spectrometry instrument (Flavourspec®-G.A.S. Dortmund Company, Dortmund, Germany). Then, 2 µL of sour bamboo shoot fermentation broth was injected into the headspace vial (20 mL) and incubated at 40 °C for 10 min. Next, 100 µL of the sour bamboo shoot fermentation broth headspace was injected at a rate of 2 mL/min, while the temperature injector was held at 80 °C. Gas chromatography was carried out via a capillary column (FS-SE-54-CB-1, 30 m × 0.53 mm × 1 µm, CS-Chromatographie Service GmbH, Langerwehe, Germany) maintained at 45 °C with nitrogen as the carrier gas. With the initial flow rate (2.0 mL/min) over a 2 min period, the flow rate of the carrier gas was increased as follows: 2 mL/min for 10 min, 10 mL/min for 10 min, and finally, 100 mL/min, for a further 39 min [16]. The NIST gas phase retention index and IMS migration time database were used for qualitative analysis of volatile compounds, and the Gallery Plot function in the LAV program was used to make a map of volatile compounds.

The Odor Activity Value (OAV) is typically used to measure the potency of an aroma compound. This is calculated by dividing the content of each volatile component (Wi) in milligrams per kilogram by the odor threshold of the volatile component (Qi) in milligrams per kilogram [17]. When the OAV is equal or greater than 1, it is believed that the aroma substance has a significant impact on the aroma of the food. An OAV of more than 10 indicates a particularly important impact on the aroma.

2.5. Microorganisms and Flavor Correlation Analysis

O2PLS was carried out by the integration of microbiota (X) and flavors (Y) during sour bamboo shoot fermentation with SIMCA-14.1. The correlation matrix showed the pairwise correlation between all variables (X and Y). Terms with large variable importance in the projection (VIP), larger than 1 were recognized as the important factors. A high correlation coefficient ($|\rho| \geq 0.7$) with p value < 0.01 between microbiota and flavors was visualized through Cytoscape [18].

3. Results

3.1. Microbial Diversity of Sour Bamboo Shoots during Fermentation

Third-generation sequencing was carried out to reveal the dominant species during the fermentation of sour bamboo shoots. A total of 396 genera and 686 species of microbes were identified, comprising 42 bacterial genera, 354 fungal genera, encompassing 65 bacterial species and 621 fungal species.

Figure 1A illustrates the bacterial genus level of the three parallel samples over the same period. *Lactobacillus* was the most abundant genus in LLX1 (day 7) in all three jars, with an average of 94%. *Aeriscardovia* had an average of 2.5%. There was a significant decrease in the quantity of *Lactobacillus* in LLX2 (day 14) in all three jars, down to 84.1%, while the abundance of *Aeriscardovia* increased to 9.2%. In LLX3 (day 21), the average abundance of *Lactobacillus* increased to 97.9% across all three jars, and the abundance of *Aeriscardovia* decreased significantly to 0.012%. *Lactobacillus* is the most prevalent bacteria in the fermentation process, although its concentration varies over time. To acquire more comprehensive insight into the structure and fluctuating alterations of bacteria dur-

ing the process of fermentation, the bacterial species levels during the fermentation of sour bamboo shoots were analyzed, as illustrated in Figure 1B. On day 7 (LLX1), uncultured_bacterium_g_*Lactobacillus* was the most dominant species, followed by *Lactobacillus amylolyticus*, with an average abundance of 33.1%. On day 14 (LLX2), the abundance of *Lactobacillus amylolyticus* decreased to 11.3% across all three jars. The abundance of *Lactobacillus acetotolerans* increased to 15.4%. At the end of fermentation (day 21, LLX3), *Lactobacillus acetotolerans* became the most dominant species, with an average abundance of 39.6%. Additionally, the abundance of *Lactobacillus fermentum* was low in LLX1 and LLX2 but increased to 12% in LLX3. Furthermore, both the abundances of *Lactobacillus rapi* and *Bifidobacteriaceae genomosp* showed an upward trend in LLX1 and LLX2, but neither were detected in LLX3.

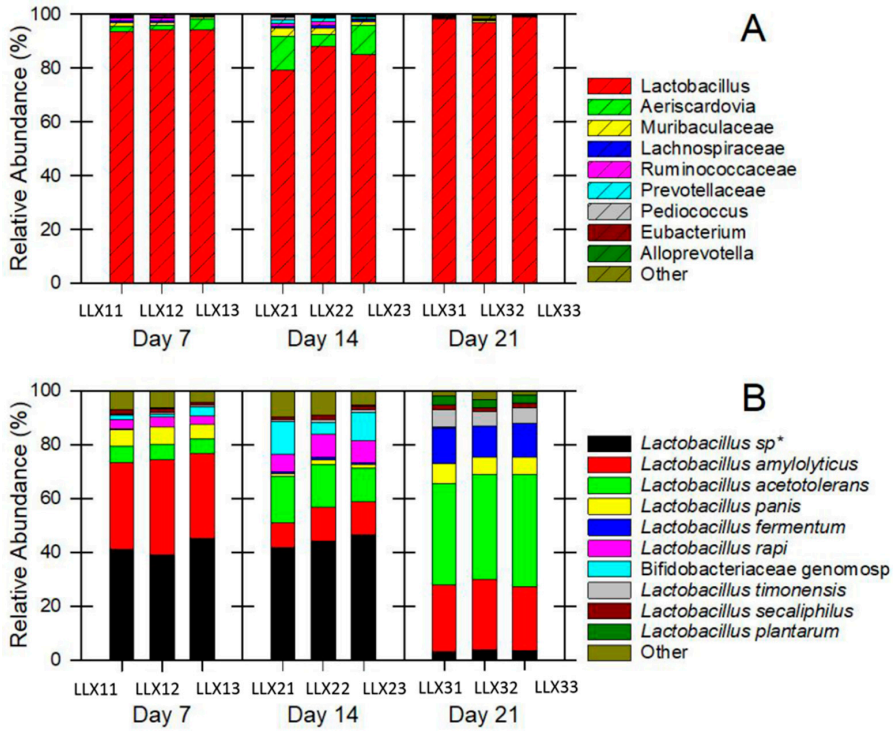


Figure 1. Community structure and dynamic changes in microorganisms at the genus level and species levels during fermentation of sour bamboo shoots. (**A**) Species distribution at the bacterial genus level; (**B**) species distribution at the bacterial species level. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation; *Lactobacillus* sp. *: uncultured_bacterium_g_*Lactobacillus*.

The microbial community at the fungal genus level is illustrated in Figure 2A. A diversity of fungi exists, although there was no clear dominant genus. In the LLX1 sample (day 7), *Aspergillus* (20%) and *Cladosporium* (9.1%) showed a greater abundance than other fungal genera. With the progression of fermentation, the presence of *Aspergillus* decreased to an average of 4.7%. In LLX3, the relative abundances of *Aspergillus*, *Cladosporium*, *Fusarium*, *Debaryomyces*, *Candida*, and *Alternaria* were 5.2%, 9.6%, 3.9%, 5.2%, 3.2%, and 3.4%, respectively. Figure 2B reveals the fungal species during fermentation. Initially, there were unclassified_g_*Aspergillus*, unclassified_g_*Cladosporium*, *Debaryomyces subglobosus*, *Candida parapsilosis*, unclassified_g_*Sampaiozyma*, *Alternaria alstroemeriae*, *Aspergillus subflavus*, and unclassified_c_*Sordariomycetes*. A notable decrease was observed in the abundance of both unclassified_g_*Aspergillus* and *Aspergillus subflavus* during fermentation. After 14 days of fermentation, unclassified_g_*Cladosporium* became the most abundant species. The abundance of *Candida parapsilosis* and *Alternaria alstroemeriae* increased with fermentation. Unclassified_*g*_*Apiotrichum* decreased initially before rising again, and unclassified_*c*_*Sordariomycetes* experienced a fluctuation, first increasing and

then decreasing. After 21 days of fermentation, the abundance of strains remained relatively constant, with *Debaryomyces subglobosus* and unclassified_*g*_*Cladosporium* still being the main strains. Unclassified_*g*_*Aspergillus* increased with the progression of fermentation, while *Candida parapsilosis* decreased initially before rising again. Additionally, unclassified_*c*_*Sordariomycetes* rose initially before decreasing.

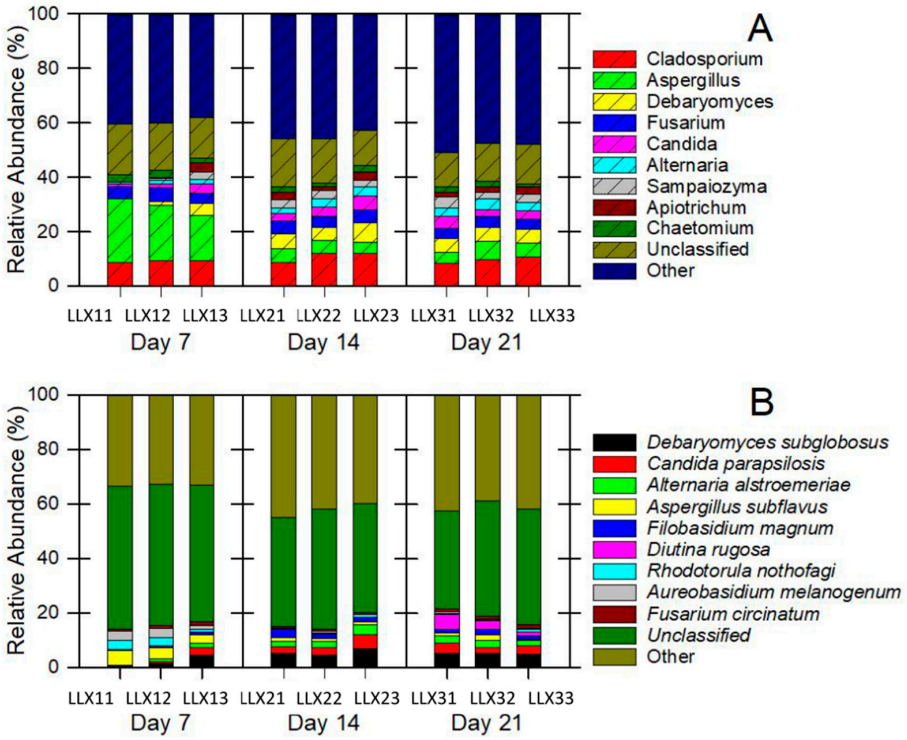


Figure 2. Community structure and dynamic changes in microorganisms at the genus level and species levels during fermentation of sour bamboo shoots. (**A**) Species distribution at the fungal genus level; (**B**) species distribution at the fungal species level. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation.

To gain a greater insight into the microbial community during the fermentation of sour bamboo shoots, PCA and PCoA were carried out to compare the microbial flora of sour bamboo shoots at different stages. In discriminative metabolomics research, multivariate statistical analyses are commonly employed to compare the chemical composition of individual samples. Among these tools, PCA stands out as the most widely utilized method. As shown in Figure 3A, the Bray-Curtis distance at the OTU level was applied to analyze the samples of different fermentation stages in different periods. The samples during fermentation were divided into three groups: early stage (7 d), middle stage (14 d), and end stage (21 d). Figure 3B shows the results of PCoA analysis, which was conducted to compare and analyze the differences of samples at different stages of fermentation, using Bray-Curtis distance at the OTU level. The PCA of fungal data revealed that LLX1 was distinct from the other two, more advanced fermentation stages, with LLX2 and LLX3 showing good repeatability and similar sample data. The collective data within the group suggest that the experiment was highly consistent and that the results were dependable. PCA did not show optimal aggregation of fungal species. Thus, PCoA was carried out to exhibit enhanced aggregation of fungal colonies. The simultaneous application of both PCA and PCoA has been successfully applied elsewhere [19].

Linear Discriminant Analysis effect size (LEfSe) was further utilized to analyze the specific microbial community differences in each fermentation stage, as demonstrated in Figure 4. Only the significant taxa with LDA scores > 4 are shown. LEfSe multiplex analysis demonstrated that the abundance of *Lactobacillus amylolyticus* in LLX1 was signif-

icantly different from that in the other two stages. LLX2 was distinguished by the class *Actinobacteria*, order *Bifidobacteria*, genera *Aeriscardovia*, and species *Lactobacillus rapi* and *Bifidobacteriaceae genomosp*. In contrast, LLX3 was dominated by *Lactobacillus*, including the species *Lactobacillus acetotolerans*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Lactobacillus timonensis*. For fungi, the taxa with an LDA score of 3.5 are illustrated in Figure 4C. The results indicated that the abundance of *Acaromyces* was higher in LLX1, and the species of *Acaromyces ingoldii* showed significant differences. For LLX2, the genera *Rhodotorula* exhibited a clear distinction from other samples. No significant difference in fungal taxa was found at day 21 (LLX3).

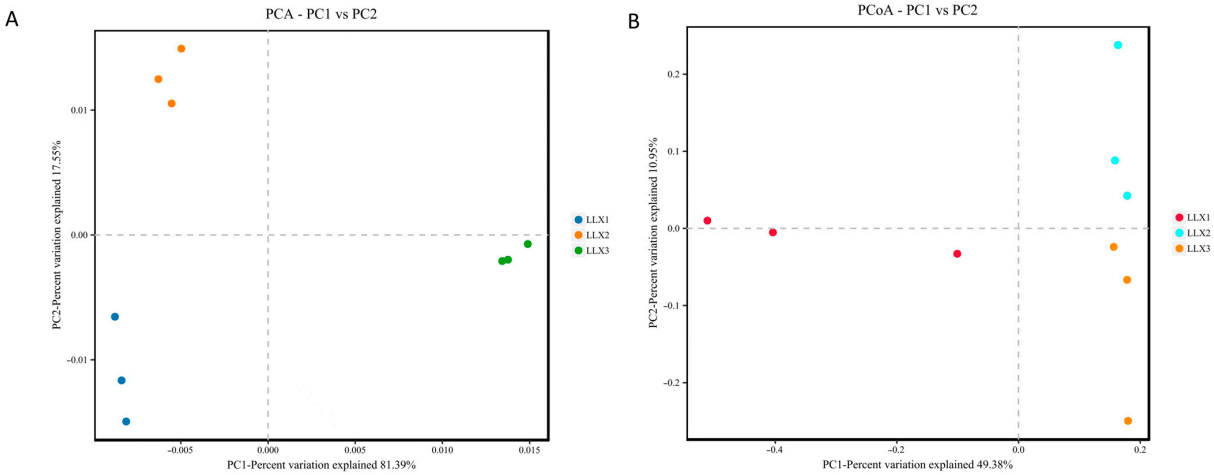


Figure 3. PCA and PCOA analysis of microbial community structure during fermentation of sour bamboo shoots. (**A**) PCA of bacterial communities at the OTU level; (**B**) PCoA of fungal communities at the OTU level. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation.

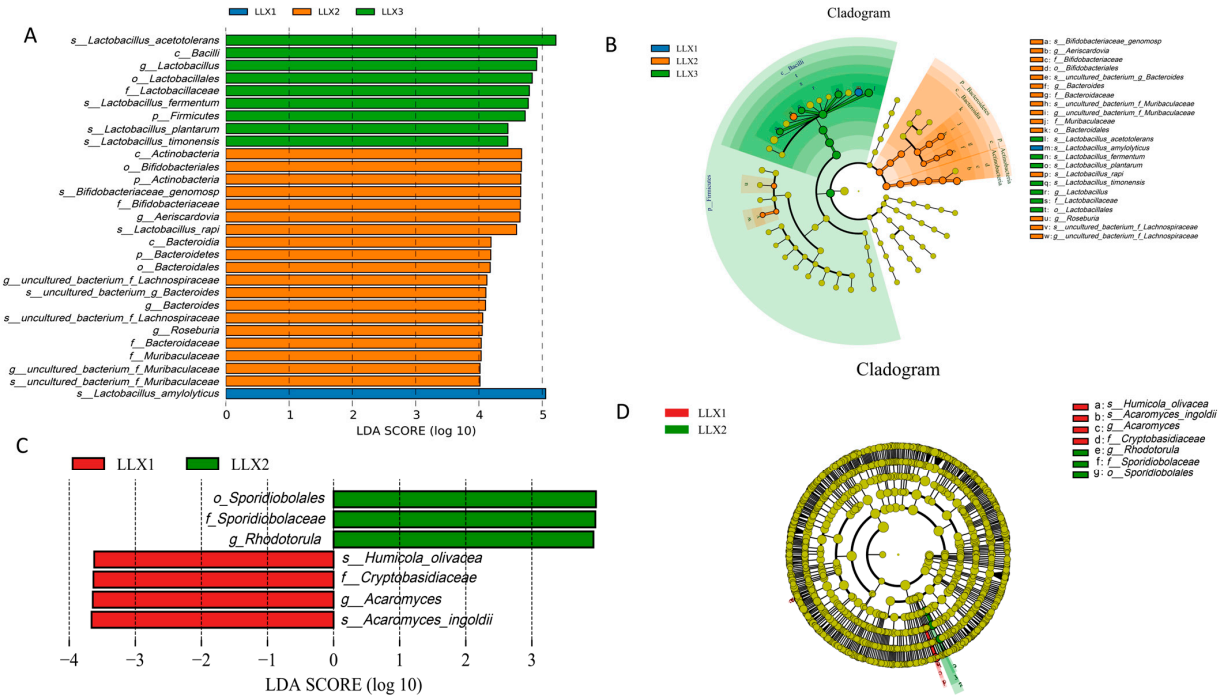


Figure 4. LEfSe of microbial communities during fermentation of sour bamboo shoots. (**A**) Bacterial LDA score; (**B**) bacterial Cladogram; (**C**) fungal LDA score; (**D**) fungal Cladogram. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation.

3.2. Changes in Volatile Compounds in Sour Bamboo Shoots during Fermentation

The volatile compounds of sour bamboo shoots during fermentation were detected by GC-IMS. A total of sixty-seven volatile components were detected, including twenty-three esters, nineteen alcohols, eight ketones, six aldehydes, six aromatic hydrocarbons, four acids, and one ether (Table 1). These five kinds of compounds constituted the primary flavor of sour bamboo shoots.

Table 1. Comparison of volatile compounds (mg/kg) (mean ± standard deviation) in different fermentation periods of sour bamboo shoots.

	Sample Code		
	LLX1 (Day 7)	**LLX2 (Day 14)**	**LLX3 (Day 21)**
acetone	0.1227 ± 0.0007	0.0656 ± 0.0006	nd
dimethyl sulfide	0.3827 ± 0.0017	0.2413 ± 0.0035	0.1695 ± 0.0005
propanal	0.4254 ± 0.0062	0.5958 ± 0.0024	0.3064 ± 0.0011
methyl acetate	1.0853 ± 0.0080	0.8701 ± 0.0013	0.3437 ± 0.0033
ethyl acetate	1.8533 ± 0.0138	1.7079 ± 0.0037	1.5443 ± 0.0057
ethanol M	3.9957 ± 0.0119	4.0285 ± 0.0236	6.8728 ± 0.0369
ethanol D	0.7732 ± 0.0106	0.7928 ± 0.0102	1.9826 ± 0.0733
ethyl propanoate	0.9631 ± 0.0015	0.5250 ± 0.0008	0.3418 ± 0.0036
isobutyl formate	0.0092 ± 0.0005	0.0144 ± 0.0002	0.0237 ± 0.0001
propyl acetate	2.2411 ± 0.0027	2.0356 ± 0.0207	0.9195 ± 0.0010
pentanal	nd	nd	0.0593 ± 0.0020
1-propanol M	4.1590 ± 0.0822	4.2639 ± 0.0313	2.1615 ± 0.0368
1-propanol D	0.9450 ± 0.0215	1.1847 ± 0.0079	0.3447 ± 0.0082
ethyl acrylate	0.1396 ± 0.0028	0.0605 ± 0.0002	nd
2,3-pentanedione	0.2116 ± 0.0119	0.1119 ± 0.0009	nd
methyl 3-methylbutanoate	0.0328 ± 0.0008	0.0108 ± 0.0005	nd
hexan-2-one	1.7564 ± 0.0078	1.8429 ± 0.0165	1.2088 ± 0.0245
butyl acetate M	0.1139 ± 0.0014	0.1172 ± 0.0012	0.0303 ± 0.0003
butyl acetate D	0.1014 ± 0.0009	0.0940 ± 0.0012	0.0474 ± 0.0011
3-methylpentan-2-one	0.0074 ± 0.0002	0.0068 ± 0.0003	0.0070 ± 0.0004
isobutanol M	0.0209 ± 0.0004	0.0188 ± 0.0002	0.0312 ± 0.0010
isobutanol D	nd	nd	0.0138 ± 0.0008
isobutyl propionate	nd	nd	0.0111 ± 0.0006
ethyl pentanoate	0.0084 ± 0.0003	0.0111 ± 0.0003	0.0077 ± 0.0001
1-butanol M	0.0938 ± 0.0005	0.1112 ± 0.0005	0.0927 ± 0.0009
1-butanol D	0.1764 ± 0.0012	0.1788 ± 0.0031	0.0445 ± 0.0007
1-butanol T	0.1535 ± 0.0007	0.1329 ± 0.0012	0.0520 ± 0.0005
ethyl 3-methylbutanoate	nd	nd	0.0646 ± 0.0003
1-penten-3-ol	0.0121 ± 0.0007	0.0152 ± 0.0006	0.0643 ± 0.0004
ethyl 2-methylpentanoate	nd	nd	0.0105 ± 0.0003
2-heptanone	0.0046 ± 0.0003	0.0060 ± 0.0002	0.0069 ± 0.0004
(E)-2-pentenal	0.0053 ± 0.0002	0.0071 ± 0.0007	0.0162 ± 0.0005
3-methyl-1-butanol M	0.1004 ± 0.0009	0.1062 ± 0.0012	0.1688 ± 0.0022
3-methyl-1-butanol D	0.1547 ± 0.0009	0.1526 ± 0.0012	0.1674 ± 0.0013
pentyl acetate	0.0392 ± 0.0004	0.0308 ± 0.00001	nd
butyl butanoate	0.0099 ± 0.0008	0.0081 ± 0.0002	0.0123 ± 0.0006
heptanal	0.0053 ± 0.0002	0.0057 ± 0.0004	0.0102 ± 0.0001
1-Octen-3-one	0.0235 ± 0.0005	0.0172 ± 0.0002	0.0043 ± 0.0001
hexyl acetate	0.0289 ± 0.0004	0.0258 ± 0.0010	0.0171 ± 0.0004
2,6-dimethylpyrazine M	0.0193 ± 0.0002	0.0219 ± 0.0005	0.0574 ± 0.0011
2,6-dimethylpyrazine D	nd	nd	0.0082 ± 0.0006
3-hydroxy-2-butanone	0.0611 ± 0.0002	0.1019 ± 0.0003	0.0918 ± 0.0003
1-hexanol	0.0089 ± 0.0005	0.0080 ± 0.0006	0.0179 ± 0.0006
octanal	0.0050 ± 0.0005	0.0052 ± 0.0002	0.0099 ± 0.0002
heptyl acetate	0.0299 ± 0.0018	0.0326 ± 0.0013	0.0255 ± 0.0005
2,5-dimethylpyrazine	0.1402 ± 0.0029	0.1645 ± 0.0017	0.0657 ± 0.0005
acetic acid M	2.6432 ± 0.1092	2.6694 ± 0.0140	2.7786 ± 0.0096
acetic acid D	2.4885 ± 0.1222	2.4360 ± 0.0188	2.3823 ± 0.0088
propionic acid M	0.7832 ± 0.0019	0.6103 ± 0.0286	0.3803 ± 0.0285
propanoic acid D	0.7920 ± 0.0547	0.3346 ± 0.0465	0.1038 ± 0.0165
2-acetylfuran	0.1371 ± 0.0040	0.1034 ± 0.0020	0.0473 ± 0.0022
2-furanmethanol	nd	7.6312 ± 0.5442	7.7465 ± 0.0191

LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation; nd: not detected.

To further analyze the differences in the volatile composition of the three bamboo shoot fermentation stages, LLX1, LLX2, and LLX3, a fingerprint map (Figure 5) was created by selecting the volatile signal peaks in the spectrum of each sample to provide a comprehensive and intuitive overview.

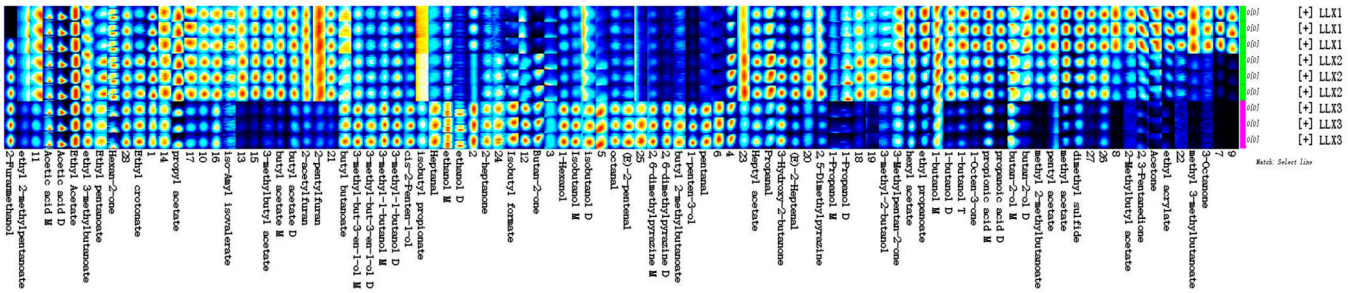


Figure 5. Fingerprint analysis of volatile compounds in sour shoot fermentation broth. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation.

As shown in Table 1, the samples of the LLX1 stage contained relatively high concentrations of 3-octanone, methyl 3-methylbutanoate, ethyl acrylate, acetone, 2,3-pentanedione, 2-methylbutyl acetate, dimethyl sulfide, methyl acetate, pentyl acetate, methyl 2-methylbutyrate, 2-butanol, propionic acid, 1-octen-3-one, 1-butanol, ethyl propionate, hexyl acetate, and 3-methyl-2-pentanone. The samples from the LLX2 stage contained relatively high concentrations of 3-methyl-2-butanol, 1-propanol, 2,5-dimethylpyrazine, (E)-2-heptenal, 3-hydroxy-2-butanone, propanal, and heptyl acetate. Among these substances, 1-propanol and 3-hydroxy-2-butanone were the main flavor compounds present in higher concentrations. The samples from the LLX3 stage contained relatively high concentrations of pentanal, 1-penten-3-ol, butyl 2-methylbutyrate, 2,6-dimethylpyrazine, trans-2-pentenalpentenal, octanal, isobutanol, 1-hexanol, 2-butanone, isobutyl formate, 2-heptanone, ethanol, heptanal, isobutyl propionate, 3-methylbutanol, 3-methyl-3-buten-1-ol, and butyl butyrate.

The concentrations of acetic acid remained steady over the entire fermentation period at about 5.1 mg/kg, while propionic acid was present at 1.5 mg/kg at day 7 and progressively decreased to 0.5 mg/kg by day 21 (Table 1). Overall, the concentrations of both acetic and propionic acids (known for their distinguished and pungent odors) surpassed those of other volatile organic acids during all stages of the fermentations, potentially attributing to the odor in the sour bamboo shoot fermentation broth.

The analysis of sour bamboo shoot samples revealed moderate levels of ethanol (4.8 mg/kg at LLX1 and LLX2 and increasing to 8.8 mg/kg by LLX3) and 1-propanol (5.2, 5.5, and 2.5 mg/kg at LLX1, LLX2, and LLX3, respectively) during the fermentation period. Notably, ethanol content exhibited a gradual increase over time, with both alcohol compounds emitting an ethanol-like aroma devoid of any discernible pungency.

The Odor Activity Value (OAV) of isoamyl alcohol increased steadily throughout the bamboo shoot fermentation process (Table 2) with the OAV of isoamyl alcohol in excess of 1 during the middle and late stages of fermentation, which could have a significant impact on the aroma of the food. Hence, it is likely that the isoamyl alcohol present in the fermentation broth of sour bamboo shoots is responsible for the odor.

The concentration of aldehydes such as propanal, pentanal, heptanal, and octanal in the bamboo shoots may be minimal; however, due to the low aroma threshold value of aldehydes, their contribution to the taste of sour bamboo shoots is significant. If the levels of aldehydes in foodstuff are too high, it can cause unpleasant flavors [12].

The esters methyl acetate, ethyl acetate, ethyl propanoate, and propyl acetate were found to be present at all stages of sour bamboo shoot fermentations; however, the relative concentrations were constantly decreasing over time (Table 1). However, the ketone, 2-hexanone was found to be persistently present at all stages of the fermentations at an OAV in excess of 50 (Tables 1 and 2). This suggests that 2-hexanone may be one of the main contributors to the distinctive odor in the fermentation broth of sour bamboo shoots.

Table 2. Dynamic changes in OAV values of volatile compounds during fermentation of sour bamboo shoots.

Compound	Aroma	Aroma Threshold (mg/kg)	OAV *		
			LLX1	LLX2	LLX3
dimethyl sulfide	sulphide odor	0.003	127	80	56
propanal	pungent odors in wood	0.001	425	596	306
ethyl propanoate	slightly fatty odor, pineapple fruit aroma, slightly astringent taste, sesame-like aroma	0.007	138	75	49
ethyl acrylate	pungent and pungent odor	0.00026	537	233	nd
2-hexanone	an irritating odor similar to acetone and is a strong irritant to the eyes and nose	0.024	73	77	50
ethyl valerate	natural fruit aroma	0.00011	77	101	70
ethyl isovalerate	strong fruit and wine aroma	0.000013	nd	nd	4969
isoamyl-alcohol M	pungent and often disagreeable odor	0.0017	59	62	99
isoamyl-alcohol D	pungent and often disagreeable odor	0.0017	91	90	98
octanal	fruity aroma, sweet orange aroma	0.00001	498	524	992
acetic acid M	a strong pungent odor	0.006	440	445	463
acetic acid D	a strong pungent odor	0.006	415	406	397
propionic acid M	an unpleasant sour and pungent odor	0.0057	137	107	67
propanoic acid D	an unpleasant sour and pungent odor	0.0057	139	59	18

* OAV scores are the concentrations of the compounds presented in Table 1 divided by the aroma thresholds. All OAVs are rounded to the nearest whole number. nd: not detected.

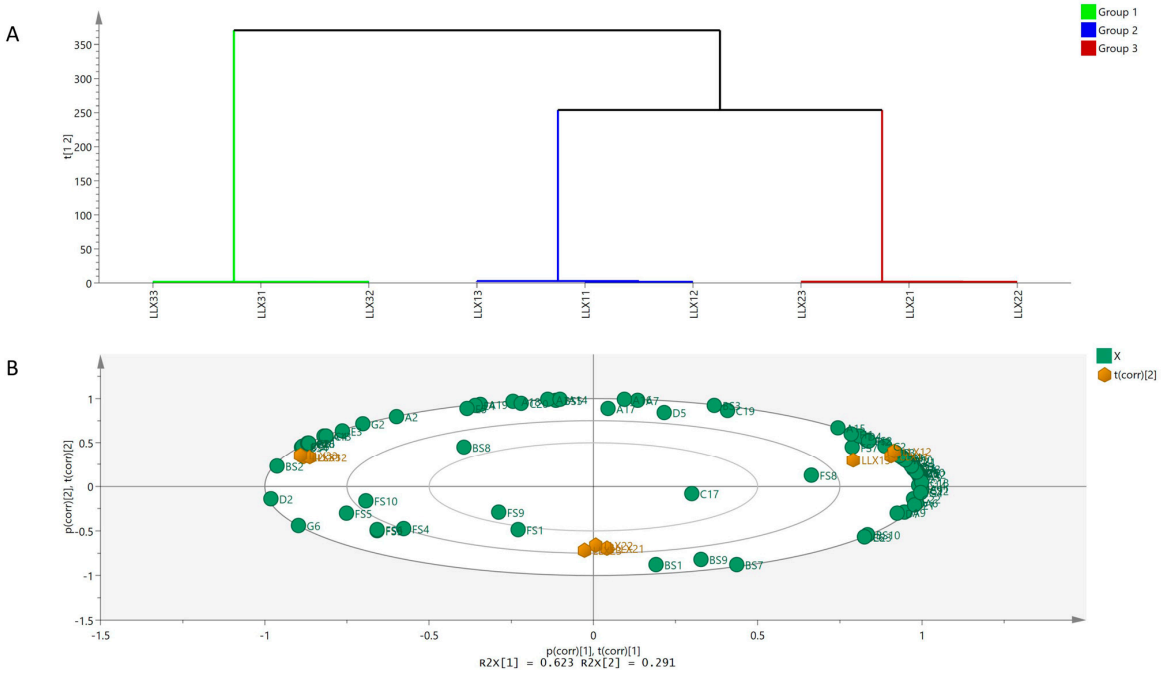
The content of dimethyl sulfide decreased during the fermentation of sour bamboo shoots, yet its OAV exceeded a score of 50 as the fermentation time progressed, which could have a considerable influence on the food aroma. Thus, dimethyl sulfide in the fermentation broth of sour bamboo shoots may also be one of the main contributors to the distinctive odor.

The results of the PCA, presented in Figure 6A, were used to analyze the differences between samples from different fermentation stages. The results of the PCA of volatile compounds showed that the volatile compounds at day 21 of fermentation were divided into three distinct groups. The scatter points corresponding to the three groups of samples throughout the fermentation period demonstrated mutual clustering within the groups, indicating a high degree of coherence within the groups and a strong similarity between the sample data.

The Biplot in Figure 6B indicates that the concentrations of acids and esters in the first group of sour bamboo shoot samples (LLX1) were relatively high, such as ethyl acetate (C2), ethyl propionate (C3), acetic acid D (H2), propionic acid M (H3), and propionic acid D (H4), while 1-butanol T (A12), 2,3-pentanedione (D3), 1-octen-3-one (D6), and dimethyl sulfide (I1) were also present in significant amounts. The sour bamboo shoot samples that had been fermented for 14 days (LLX2) had a substantial amount of ethyl crotonate (C17). On the other hand, the samples that had been fermented for 21 days (LLX3), had relatively high levels of ethanol D (A2), 1-penten-3-ol (A13), isobutyl formate (C4), butan-2-one (D2), (E)-2-pentenal (E3), 2,6-dimethylpyrazine M (G2), 2,6-dimethylpyrazine D (G3), and 2-furanmethanol (G6).

Dimethyl sulfide, propanal, ethyl propionate, ethyl acrylate, 2-hexanone, ethyl valerate, ethyl isovalerate, octanal, isoamyl alcohol, acetic acid, and propionic acid in LLX1 have OAVs exceeding a score of 10 (Table 2). Propionaldehyde, ethyl acrylate, and acetic acid have an OAV of more than 100, making them extremely important aroma substances and contributing significantly to the overall aroma. LLX2 has the same 10 substances with the propanal and octanal OAVs exceeding 500, which are essential aroma substances and play an important role in the overall aroma of sour bamboo shoots. Additionally, dimethyl sulfide, propanal, ethyl propionate, valeraldehyde, 2-hexanone, ethyl valerate, ethyl isovalerate, isoamyl alcohol, heptanal, and octanal all have high OAVs in LLX3. The OAVs of twelve aroma substances, including acetic acid and propionic acid, are no less than 1. However, ethyl isovalerate and octanal have an OAV of more than 10, making them crucial to the overall aroma. Table 2 displays the aroma characteristics of the main volatile compounds. The presence of propionaldehyde, with an OAV greater than 1, was

observed after 14 days of fermentation and was found to have a significant impact on the aroma of the food. Ethyl acrylate was detected after 7 days of fermentation; however, its OAV decreased and was not detected after 21 days. Heptanal showed an increasing OAV throughout the three stages of fermentation, with an OAV greater than 10, making it an important aroma substance. Acetic acid and propionic acid were also found to be important aroma substances, with their OAVs remaining stable during the three stages of fermentation.



BS3	*Lactobacillus_amylolyticus*	A3	butan-2-ol M	C3	ethyl propanoate	D3	2,3-Pentanedione
BS4	*Lactobacillus_fermentum*	A4	butan-2-ol D	C4	Isobutyl formate	D4	3-Methylpentan-2-one
BS5	*Lactobacillus_panis*	A5	1-Propanol M	C5	propyl acetate	D5	2-heptanone
BS6	*Lactobacillus_plantarum*	A6	1-Propanol D	C6	methyl 2-methylbutanoate	D6	1-Octen-3-one
BS7	*Lactobacillus_rapi*	A7	Isobutanol M	C7	ethyl acrylate	D7	3-Hydroxy-2-butanone
BS8	*Lactobacillus_secaliphilus*	A8	isobutanol D	C8	methyl 3-methylbutanoate	D8	Hexan-2-one
BS9	*uncultured_bacterium_f_Muribaculaceae*	A9	3-methyl-2-butanol	C9	butyl acetate M	E1	Propanal
BS10	*uncultured_bacterium_g_Lactobacillus*	A10	1-butanol M	C10	butyl acetate D	E2	pentanal
		A11	1-butanol D	C11	isobutyl propionate	E3	(E)-2-pentenal
FS1	*Unclassified_g_Cladosporium*	A12	1-butanol T	C12	3-methylbutyl acetate	E4	Heptanal
FS2	*Unclassified_g_Aspergillus*	A13	1-penten-3-ol	C13	2-Methylbutyl acetate	E5	octanal
FS3	*Debaryomyces_subglobosus*	A14	3-methyl-1-butanol M	C14	Ethyl pentanoate	E6	(E)-2-Heptenal
FS4	*Candida_parapsilosis*	A15	3-methyl-1-butanol D	C15	ethyl 3-methylbutanoate	G1	2-pentylfuran
FS5	*Unclassified_g_Sampaiozyma*	A16	3-methyl-but-3-en-1-ol M	C16	ethyl 2-methylpentanoate	G2	2,6-dimethylpyrazine M
FS6	*Alternaria_alstroemeriae*	A17	3-methyl-but-3-en-1-ol D	C17	Ethyl crotonate	G3	2,6-dimethylpyrazine D
FS7	*Aspergillus_subflavus*	A18	cis-2-Penten-1-ol	C18	pentyl acetate	G4	2,5-Dimethylpyrazine
FS8	*Unclassified_g_Chaetomium*	A19	1-Hexanol	C19	butyl butanoate	G5	2-acetylfuran
FS9	*Unclassified_g_Apiotrichum*	H1	Acetic acid M	C20	butyl 2-methylbutanoate	G6	2-Furanmethanol
FS10	*Unclassified_c_Sordariomycetes*	H2	Acetic acid D	C21	hexyl acetate	I1	dimethyl sulfide
		H3	propionic acid M	C22	iso-Amyl isovalerate		
		H4	propanoic acid D	C23	(E)-2-Heptenal		

Figure 6. Dynamics of volatile flavors during the fermentation of sour bamboo shoots. (**A**) HCA plot; (**B**) Biplot of PCA. LLX1: 7th day of fermentation; LLX2: 14th day of fermentation; LLX3: 21st day of fermentation.

3.3. Correlation Analysis of Microorganisms and Volatile Compounds in Sour Bamboo Shoots under Different Fermentation Times

The O2PLS model was applied to investigate the relationship between microorganisms and volatile compounds in the fermentation process, with the aim of elucidating the role of microorganisms in the fermentation process. The independent variable X was composed of 10 bacterial genera and 14 fungal genera, with an abundance higher than 1%, while the dependent variable Y was represented by 66 volatile compounds detected during the fermentation process. The R_2 (cum) and Q_2 (cum) of the model both exceed 0.5, indicating that it is suitable for the correlation between microorganisms and volatile compounds in the fermentation process of sour bamboo shoots and thus possess good explanatory and predictive power.

The data presented in Figure 7B reveal that the range of VIP, the independent variable importance index of 24 genera in microorganisms, ranges from 0.10 to 1.187. It is worth noting that fourteen variables, comprising seven bacterial and four fungal variables, had VIP values higher than 1.0, thus implying that these variables are of great significance in the production of volatile compounds during fermentation. The seven bacterial variables were *Lactobacillus acidfast* (BS2), *Lactobacillus fermentum* (BS4), *Lactobacillus plantarum* (BS6), *uncultured_bacterium_g_Lactobacillus* (BS10), *Lactobacillus rapi* (BS7), *Lactobacillus amylolyticus* (BS3), and *Lactobacillus panis* (BS5), and their VIP values were 1.187, 1.182, 1.180, 1.156, 1.039, 1.027, and 1.021, respectively. The VIP values of other bacterial genera were lower than 1.0. The highest VIP value among the independent variables was observed for *Lactobacillus fermentum*, suggesting that it had the most significant impact on volatile compounds during fermentation. The bacterial genera with VIP values higher than 1.0 were unclassified_*g*_*Aspergillus* (FS2), *Aspergillus subflavus* (FS7), unclassified_*g*_*Sampaiozyma* (FS5), and *Debaryomyces subglobosus* (FS3), with values of 1.135, 1.089, 1.028, and 1.003, respectively.

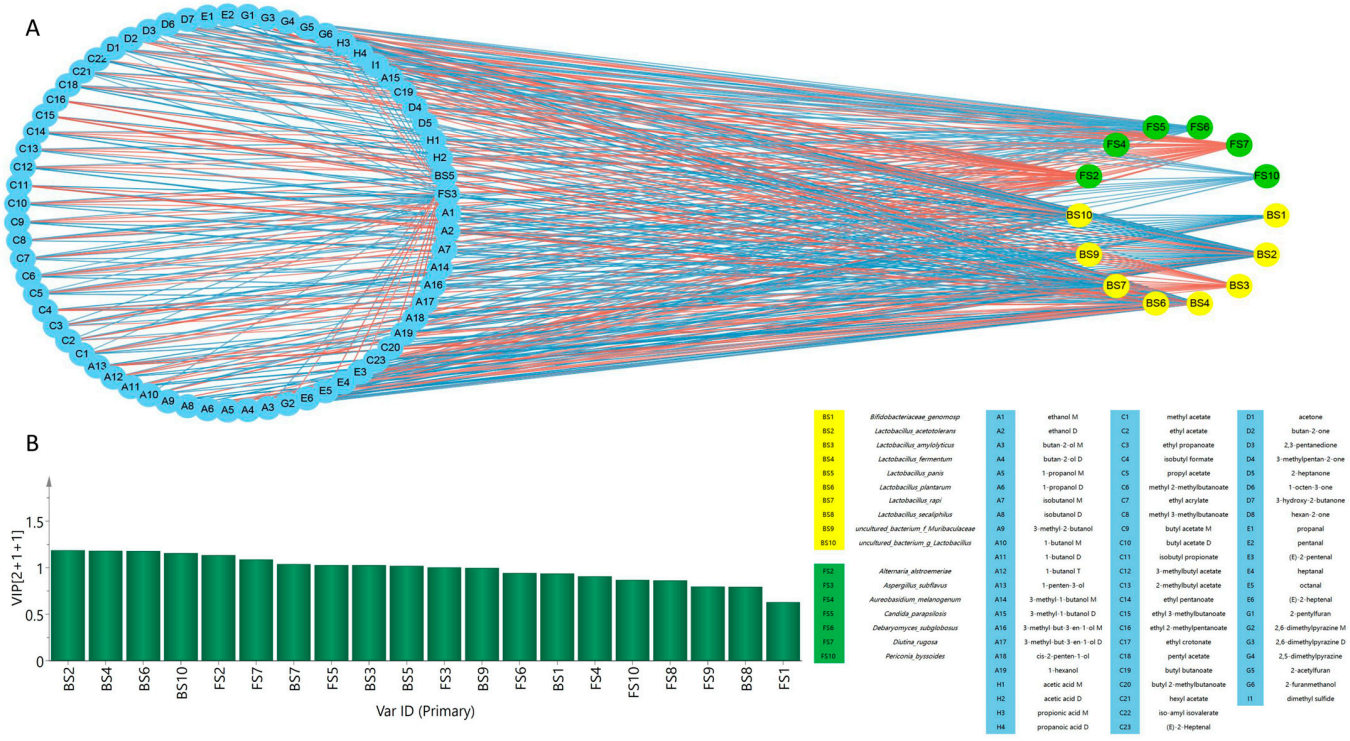


Figure 7. Correlation between microorganisms and flavors in sour bamboo shoot fermentation broth. (**A**) Correlation network between microorganisms and volatile compounds through the O2PLS model. The circles on the left represent microorganisms related to volatile compounds ($|\rho| \geq 0.7$), the circles on the right represent volatile compounds related to microorganisms, the red line represents a positive correlation, and the blue line represents a negative correlation; (**B**) Microbial Independent Variable Importance Index (VIP).

The correlation coefficient matrix of the O2PLS model in Figure 7A allows us to measure the degree of correlation between the microorganisms of any genus in the independent variable X and any volatile substance in the variable Y, with Cytoscape (version 3.7.2) providing a visual representation of these values. There was a strong correlation between nine bacterial species and seven fungal species with volatile compounds, and the correlation coefficient was $|\rho| \leq 0.7$. *Aspergillus* (FS2) and *Aspergillus subflavus* (FS7) had strong positive correlations ($\rho \geq 0.7$) with 32 and 27 volatile compounds, respectively. These compounds included butan-2-ol M (A3), butan-2-ol D (A4), 1-propanol M (A5), 1-butanol M (A10), 1-butanol T (A12), 3-methyl-1-butanol D (A15), methyl acetate (C1), ethyl acetate (C2), ethyl propanoate (C3), propyl acetate (C5), methyl 2-methylbutanoate (C6), ethyl acrylate (C7), methyl 3-methylbutanoate (C8), butyl acetate D (C10), 2-methylbutyl acetate (C13), hexyl acetate (C21), acetone (D1), 2,3-pentanedione (D3), 3-methylpentan-2-one (D4), 1-octen-3-one (D6), 2-pentylfuran (G1), 2-acetylfuran (G5), acetic acid M (H1), acetic acid D (H2), propionic acid M (H3), propionic acid MD (H4), and dimethyl sulfide (I1). *Debaryomyces subglobosus* (FS3), *Candida parapsilosis* (FS4), unclassified_*g*_*Sampaiozyma* (FS5), *Alternaria alstroemeriae* (FS6), and unclassified_*c*_*Sordariomycetes* (FS10) were significantly negatively correlated with most substances, such as alcohols, acids, and esters.

The bacterial variables *Lactobacillus acetotolerans* (BS2), *Lactobacillus amylolyticus* (BS3), *Lactobacillus fermentum* (BS4), *Lactobacillus plantarum* (BS6), and *Lactobacillus rapi* (BS7) were found to have strong correlations with 27 volatile compounds. Twelve substances showed a positive correlation with *Lactobacillus acetotolerans* (BS2) with a correlation coefficient greater than 0.9, including isobutanol D (A8), 1-penten-3-ol (A13), isobutyl formate (C4), isobutyl propionate (C11), ethyl 3-methylbutanoate (C15), ethyl 2-methylpentanoate (C16), butan-2-one (D2), pentanal (E2), (E)-2-pentenal (E3), 2,6-dimethylpyrazine M (G2), and 2,6-dimethylpyrazine D (G3). A moderate positive correlation was also observed with ethanol D (A2). *Lactobacillus amylolyticus* (BS3) had a strong positive correlation with seven alcohols, including ethanol M (A1), isobutanol M (A7), 3-methyl-1-butanol M (A14), 3-methyl-1-butanol D (A15), ethyl 3-methyl-but-3-en-1-ol M (A16), 3-methyl-but-3-en-1-ol D (A17), and 2 cis-2-penten-1-ol (A18). A positive correlation was also observed with butyl butanoate (C19), butyl 2-methylbutanoate (C20), 3-methylpentan-2-one (D4), and 2-heptanone (D5). Furthermore, *Lactobacillus fermentum* (BS4) and *Lactobacillus plantarum* (BS6) had strong positive correlations with 1-penten-3-ol (A13), isobutyl formate (C4), ethyl 3-methylbutanoate (C15), and ethyl 2-methylpentanoate (C16). On the other hand, *Bifidobacteriaceae genomosp* (BS1), *Lactobacillus acetotolerans* (BS2), and *Lactobacillus rapi* (BS7) had significant negative correlations with most substances, such as alcohols, acids, and esters.

Acaromyces ingoldii (FS6) and *Lactobacillus amylolyticus* (BS3) had a strong correlation with most of the alcohols, esters, and acids in sour bamboo shoots, particularly ethyl acetate (C2).

4. Discussion

Sour bamboo shoots have become increasingly sought-after for their distinctive flavor and nutritional value. The microorganisms in the fermentation environment and the transformations they undergo are integral for the final flavor of sour shoots. To gain a better understanding of this, third-generation sequencing technology was applied to define the fermentation broth of sour bamboo shoots that were fermented for 21 days in September from Guangxi. The results showed that *Lactobacillus* was the dominant bacteria in the entire fermentation process. The predominant species were *Lactobacillus amyloliquefaciens* and *Lactobacillus acetotolerans*. At the fungal genus level, *Aspergillus* and *Cladosporium* were the dominant bacteria during the early stage of fermentation (7 days), while *Debaryomyces* and *Cladosporium* predominated during the middle stage (14 days). In the late fermentation stage (21 days), the genera *Aspergillus*, *Cladosporium*, and *Debaryomyces* were the most abundant. This study provides insight into the trends of dominant species during each stage. Chen et al. [20] showed that at the genus level of bacteria, the top 10 species at abundance levels in all samples were *Lactobacillus*, *Lactococcus*, *Enterococcus*,

Chloroplast, *Mitochondria*, *Acinetobacter*, *Weissella*, *Pseudomonas*, and unclassified genus. The *Lactobacillus* genus (relative abundance > 30.35%) in sour bamboo shoots rapidly increased and stabilized in the later stage of fermentation and became the unique dominant bacteria. Guan et al. [11] used third-generation sequencing technology to analyze the microbial community in sour bamboo shoots after 15–30 days of fermentation on sour bamboo shoots from 13 different regions in summer. The results showed that in sour bamboo shoots, the number of bacteria was higher than that of fungi. It is evident that the two dominant genera at the bacterial genus level were *Lactobacillus* and *Serratia*, while the two predominant species were *Lactobacillus fermentum* and *Lactobacillus acetotolerans*. At the fungal genus level, the most prevalent genera were *Aspergillus*, *Debaryomyces*, *Pichia,* and *Candida*. At the species level, the most common bacteria were *Candida tropicalis*, *Pichia norvegensis*, and *Debaryomyces hansenii*. Our findings indicated a lower presence of *Saccharomyces* than the study, which may be a result of the different geographical and climatic conditions of fermentation, as well as the various microorganisms present in the raw materials. It is possible that the ideal fermentation time for sour bamboo shoots is in May and June, when the temperature is approximately 22–28 °C, which is suitable for the proliferation of fungi. Therefore, the high concentration of fungi, especially the predominant genus *Saccharomyces*, and the diverse microbial species may result in the production of dissimilar volatile compounds. The effect of *Saccharomyces cerevisiae* on the flavor of sour bamboo shoots can be further explored. Chen et al. [21] used metagenomic sequencing technology to sequence the fermentation broth of sour bamboo shoots in six different regions, including Guilin and Liuzhou, Guangxi, in summer in August. The study revealed that the majority of microorganisms in the sour bamboo shoot fermentation broth were bacteria, making up 98.89% of the total. *Firmicutes*, *Bacteroides*, *Actinomycetes*, *Proteobacteria,* and *Ascomycota* were the dominant phyla, with the genus *Lactobacillus* having a substantial presence. *Pediococcus*, *Leuconostoc,* and *Lactococcus* were also discovered. *Lactobacillus fermentum* was the most abundant species, followed by *Lactobacillus plantarum* and *Lactobacillus amylolytica*. Therefore, no classification or detailed analysis of fungi was carried out. The reason may be that the sampling time of this study is the hot season from July to August, the temperature can reach 30–37 °C, and the high temperature is suitable for the growth of bacteria. Therefore, more bacteria were detected. In our study, both fungi and bacteria were specifically analyzed, and a total of 403 microorganisms were detected, of which bacteria accounted for 10.92% of the total microorganisms and fungi accounted for 89.08% of the total microorganisms. The end of September, with its lower temperature, is a favorable environment for fungi to thrive, thus explaining the high proportion of fungi observed.

By utilizing GC-IMS technology, the volatile compounds in the fermentation broth of sour bamboo shoots were determined and studied, resulting in the detection of 67 volatile substances. These substances were mainly composed of aldehydes, acids, esters, and alcohols. Eight volatile compounds, acetic acid, propionic acid, 2-furunmethanol, ethyl propionate, isoamyl alcohol, 2-hexanone, propanal, and octanal, were identified as the main flavor contributors to the flavor of sour bamboo shoots. Moreover, ethanol was also identified as a significant flavoring substance. Wang et al. [22] showed that a total of thirty five main volatile compounds were measured by GC-MS, including fifteen kinds of alcohols, four kinds of aldehydes, four kinds of ketones, five kinds of esters, five kinds of acids, and two kinds of other chemicals. The substances with the highest relative contents were 1-hexanol, hexanoic acid, 2-heptanol, 2-pentylfuran, and acetic acid. SHIH-GUEI FU et al. [8] used gas chromatography-olfactory (GCO) technology to determine the volatile compounds in the fermentation broth of sour bamboo shoots prepared by fermenting bamboo shoots from Taiwan for 12 days. It was determined that sour bamboo shoots contained seventy volatile flavors, the ten most significant of which were cresol, methional, 2-heptanol, acetic acid, (E,Z)-2,6-nonadienal, linalool, phenyl acetaldehyde, and three unidentified substances. The volatile compounds in the raw materials of bamboo shoots before fermentation were also measured, mainly consisting of phenethyl alcohol, 4-ethylguaiacol, 2-methoxy-4-cresol,

3-ethylphenol, ethyl acetate, ethanol, acetic acid, benzaldehyde, and phenyl acetaldehyde; thus, it is more evident which volatiles are produced after fermentation. Cai et al. [23] used headspace solid-phase microextraction GC-MS to determine the characteristic flavor components of three sour bamboo shoots in Guangxi. Twenty-nine volatile components were identified in our study, of which phenols, acids, alcohols, and esters were the predominant ones. Cresol, acetic acid, ethanol, propanol, and propyl acetate were the main volatile compounds. Comparatively, similar volatile compounds were observed in the previous two studies, such as the prevalence of esters and acids such as acetic acid and ethyl acetate. Phenolics, such as cresols, were identified in both studies and are thought to be the main volatiles responsible for the odor of sour bamboo shoots. No phenolics were detected in this study, and it was deduced that the flavor difference may have resulted from the differences in the production process of sour bamboo shoots or the volatile compounds produced by different raw materials, the nutrients in the raw material, or the microbial species involved in the spontaneous fermentation process, all of which can influence the volatile compounds produced. Wang et al. detected the volatile compounds in naturally fermented hydrochloric acid-free bamboo shoots, and the results showed that the content of phenolic compounds was the highest, followed by alcohols and aldehydes. The content of phenolic compounds decreased first and then increased with fermentation time, while the content of alcoholic compounds increased first and then decreased. Therefore, 1-heptanal was the main volatile substance, and the contents of 2,4-dimethylbenzaldehyde and 2,4-di-tert-butylphenol were second only to 1-heptanal, and they were considered to have a great contribution to the flavor of sour bamboo shoots [24].

According to recent research, there is spontaneous fermentation of the majority of sources of microorganisms and volatile compounds in sour bamboo shoots [10]. In this study, the general trends were similar to those of the forward studies. However, the correlation between microorganisms and volatile compounds was not studied, nor was the effect of a variety of volatile compounds created during microbial succession on the flavor of sour shoots. To examine the connection between acetic acid, propionic acid, ethyl propionate, and dimethyl sulfide and *Exobasidiomycetes*, *Aspergillus flavus*, and *Staphylococcus aureus*, a series of analyses and explorations were performed. A significant correlation was observed between acetic acid and propionic acid and *Lactobacillus amyloliquefaciens*, while 2-hexanone was found to have a strong correlation with both *Lactobacillus amyloliquefaciens* and *Fusarium sphaeroides*. Furthermore, isovaleric acid has a strong association with *Lactobacillus acidfast*, *Lactobacillus amyloliquefaciens*, *Lactobacillus fermentum*, *Lactobacillus bread*, and *Lactobacillus plantarum*.

Guan et al. [25] investigated the correlation between microorganisms and volatile compounds by taking eight samples and analyzing them during the 30-d fermentation process of fresh bamboo shoots from Liuzhou, Guangxi, China, and showed that *Lactobacillus* was positively correlated with 15 volatile compounds, including hexanal, acetaldehyde, heptanal, acetic acid, ethyl acetate, hexanoic acid, and ethyl ester. *Enterobacter* had a significant relationship with six aldehydes, including hexanal, acetaldehyde, heptanal, and n-valeraldehyde, and *Clostridium* was strongly correlated with four volatile compounds, including acetic acid, pentyl acetate, ethyl hexanoate, and dimethyl sulfide. In contrast, *Bacillus immobilis* and *Acetobacter* had a negative effect on the formation of sour shoot flavor, as they were negatively correlated with 14 and 10 volatile compounds, respectively. Similarly, our study found that *Lactobacillus* was strongly correlated with acetic acid, ethyl acetate, and hexanal. *Enterobacter* and *Akebia* in our study were not significant enough to be considered microorganisms with a major influence on flavor.

5. Conclusions

To investigate the microbial community and function during sour bamboo shoot fermentation, third-generation sequencing and GC-IMS were applied to identify the composition of microbial communities and volatile compounds. The dominant bacterial genera throughout the fermentation process were *Lactobacillus* and *Aeroscardovia*. *Lactobacillus*

amylolyticus and *Lactobacillus acetotolerans* were the dominant species. The major fungi identified were *Aspergillus* and *Cladosporium*, with *Debaryomyces subglobosus* and *Candida parapsilosis* as the dominant species. A total of 67 volatile compounds were identified, with acids, alcohols, aldehydes, esters, and ketones being the five most prevalent compounds. Acetic acid, propionic acid, dimethyl sulfide, ethyl propionate, isoamyl alcohol, and 2-hexanone were identified as the main volatile compounds in the sour shoot fermentation broth, all of which had complex odors. Additionally, O2PLS was used to analyze the correlation between microbial communities and volatile compounds. It was concluded that *Lactobacillus amylolyticus* and *Fusarium circinatum* had a strong association with 2-hexanone. *Lactobacillus acetotolerans*, *Lactobacillus amylolyticus*, *Lactobacillus fermentum*, *Lactobacillus panis*, and *Lactobacillus plantarum* had a stronger correlation with 3-methyl-1-butanol. This research revealed the microbial diversity and flavor profile during fermentation, which provides a basis for the selection of strains that improve the taste and quality of sour bamboo shoots.

Author Contributions: X.P. was responsible for the experimental design and article writing. F.V. assisted with data interpretation and article writing. X.Z. performed the experiment and data analysis with the help of Q.W. and Y.X. (Yong Xiong). Y.X. (Yuanhong Xie) and H.Z. assisted in the experimental design and guidance. J.J. assisted with the data analysis. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wang, Y.; Chen, J.; Wang, D.; Ye, F.; He, Y.; Hu, Z.; Zhao, G. A systematic review on the composition, storage, processing of bamboo shoots: Focusing the nutritional and functional benefits. *J. Funct. Foods* **2020**, *71*, 104015. [CrossRef]
2. Singhal, P.; Bal, L.M.; Satya, S.; Sudhakar, P.; Naik, S.N. Bamboo shoots: A novel source of nutrition and medicine. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 517–534. [CrossRef]
3. Chongtham, N.; Bisht, M.S.; Premlata, T.; Bajwa, H.K.; Sharma, V.; Santosh, O. Quality improvement of bamboo shoots by removal of antinutrients using different processing techniques: A review. *J. Food Sci. Technol.* **2022**, *59*, 1–11. [CrossRef]
4. Singhal, P.; Satya, S.; Naik, S.N. Fermented bamboo shoots: A complete nutritional, anti-nutritional and antioxidant profile of the sustainable and functional food to food security. *Food Chem.* **2021**, *3*, 100041. [CrossRef]
5. Sanlier, N.; Gokcen, B.B.; Sezgin, A.C. Health benefits of fermented foods. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 506–527. [CrossRef]
6. Tang, J.; Zhang, Z.; Zheng, S.; Gao, N.; Li, Z.; Li, K. Changes of main nutrient components and volatile flavor substances in processing of canned bamboo shoots. *Fermentation* **2021**, *7*, 293. [CrossRef]
7. Behera, P.; Balaji, S. Health Benefits of Fermented Bamboo Shoots: The Twenty-First Century Green Gold of Northeast India. *Appl. Biochem. Biotechnol.* **2021**, *193*, 1800–1812. [CrossRef]
8. Fu, S.G.; Yoon, Y.; Bazemore, R. Aroma-active components in fermented bamboo shoots. *J. Agric. Food Chem.* **2002**, *50*, 549–554. [CrossRef]
9. Li, S.; Tian, Y.; Sun, M.; Liu, J.; Bai, Y.; Liu, X.; Guo, Y. Characterization of key aroma compounds in fermented bamboo shoots using Gas Chromatography-Olfactometry-Mass Spectrometry, odor activity values, and Aroma Recombination Experiments. *Foods* **2022**, *11*, 2106. [CrossRef]
10. Guan, Q.; Zheng, W.; Mo, J.; Huang, T.; Xiao, Y.; Liu, Z.; Peng, Z.; Xie, M.; Xiong, T. Evaluation and comparison of the microbial communities and volatile profiles in homemade suansun from Guangdong and Yunnan provinces in China. *J. Sci. Food Agric.* **2020**, *100*, 5197–5206. [CrossRef]
11. Guan, Q.; Zheng, W.; Huang, T.; Xiao, Y.; Liu, Z.; Peng, Z.; Gong, D.; Xie, M.; Xiong, T. Comparison of microbial communities and physiochemical characteristics of two traditionally fermented vegetables. *Food Res. Int.* **2020**, *128*, 108755. [CrossRef] [PubMed]
12. Kim, K.H.; Kim, Y.H. Composition of key offensive odorants released from fresh food materials. *Atmos. Environ.* **2014**, *89*, 443–452. [CrossRef]
13. Lee, D.; Kim, S.; Cho, J.; Kim, J. Microbial population dynamics and temperature changes during fermentation of kimjang kimchi. *J. Microbiol.* **2008**, *46*, 590–593. [CrossRef] [PubMed]

14. Chavhan, D.M.; Hazarika, M.; Brahma, M.L.; Hazarika, R.A.; Rahman, Z. Effect of incorporation of fermented bamboo shoot on physicochemical and microbial quality of pork pickle. *J. Food Sci. Technol.* **2015**, *52*, 1223–1227. [CrossRef]
15. Martínez-Sanz, J.; Talavera-Rodríguez, A.; Díaz-Álvarez, J.; Rosas Cancio-Suárez, M.; Rodríguez, J.M.; Alba, C.; Sánchez-Conde, M. A gut microbiome signature for HIV and metabolic dysfunction-associated steatotic liver disease. *Front. Immunol.* **2023**, *14*, 1297378. [CrossRef]
16. Li, W.; Li, J.; Qin, Z.; Wang, Y.; Zhao, P.; Gao, H. Insights into the Composition and Antibacterial Activity of Amomum tsao-ko Essential Oils from Different Regions Based on GC-MS and GC-IMS. *Foods* **2022**, *11*, 1402. [CrossRef] [PubMed]
17. Zhu, J.; Wang, L.; Xiao, Z.; Niu, Y. Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography–olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC–MS) and flame photometric detection (FPD). *Food Chem.* **2018**, *245*, 775–785. [CrossRef] [PubMed]
18. Pang, X.N.; Han, B.Z.; Huang, X.N.; Zhang, X.; Hou, L.F.; Cao, M.; Gao, L.J.; Hu, G.H.; Chen, J.Y. Effect of the environment microbiota on the flavour of light-flavour Baijiu during spontaneous fermentation. *Sci. Rep.* **2018**, *8*, 3396. [CrossRef]
19. Zhang, D.; Cheng, H.; Zhang, Y.; Zhou, Y.; Wu, J.; Liu, J.; Feng, W.; Peng, C. Ameliorative effect of Aconite aqueous extract on diarrhea is associated with modulation of the gut microbiota and bile acid metabolism. *Front. Pharmacol.* **2023**, *14*, 1189971. [CrossRef]
20. Chen, C.; Cheng, G.; Liu, Y.; Yi, Y.; Chen, D.; Zhang, L.; Wang, X.; Cao, J. Correlation between microorganisms and flavor of Chinese fermented sour bamboo shoot: Roles of Lactococcus and Lactobacillus in flavor formation. *Food Biosci.* **2022**, *50 Pt A*, 101994.
21. Chen, X.; Zhu, Z.; Zhang, Y.; Wu, C.; Guo, Q. Microbial Community Diversity Analysis of Traditional Fermented Bamboo Shoots Using Metagenomic Technology. *Food Sci. Biotechnol.* **2020**, *39*, 89–98. (In Chinese)
22. Wang, L.; Luo, Y.-Y.; Zhang, Y.; Zou, C.-X.; Wang, P.-J.; Qin, L.-K.; Zhu, Q.-J.; Jia, Y.-L. Quality analysis of ultra-fine whole pulp of bamboo shoots (Chimonobambusa quadrangularis) fermented by *Lactobacillus plantarum* and *Limosilactobacillus reuteri*. *Food Biosci.* **2023**, *52*, 102458. [CrossRef]
23. Cai, Y.; Jia, L.; Du, M.; Guo, H. Analysis of volatile flavor components of guangxi sour bamboo shoots. *China Condiment* **2021**, *46*, 142–150. (In Chinese) [CrossRef]
24. Xu, X.; Long, Z.; Du, W.; Chen, Q.; Zhang, Y.; Hu, S. Dynamics of physicochemical properties, flavor, and microbial communities of Salt-Free bamboo shoots during natural fermentation: Correlation between microorganisms and metabolites. *Fermentation* **2023**, *9*, 733. [CrossRef]
25. Guan, Q.; Huang, T.; Peng, F.; Huang, J.; Liu, Z.; Peng, Z.; Xie, M.; Xiong, T. The microbial succession and their correlation with the dynamics of flavor compounds involved in the natural fermentation of suansun, a traditional Chinese fermented bamboo shoots. *Food Res. Int.* **2022**, *157*, 111216. [CrossRef]