

Article

Dynamics of Physicochemical Properties, Flavor, and Microbial Communities of Salt-Free Bamboo Shoots during Natural Fermentation: Correlation between Microorganisms and Metabolites

Xiaofeng Xu ^{1,†}, Zhijian Long ^{1,2,†}, Wanning Du ¹, Qiyang Chen ¹ , Yu Zhang ¹ and Shanglian Hu ^{1,2,*}

¹ School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China; 18788981570m@sina.cn (X.X.); long20053182@swust.edu.cn (Z.L.); dwncie@sina.com (W.D.); chenqiyang@swust.edu.cn (Q.C.); vividz2010@126.com (Y.Z.)

² Engineering Research Center for Biomass Resource Utilization and Modification of Sichuan Province, Mianyang 621010, China

* Correspondence: hushanglian@swust.edu.cn

† These authors contributed equally to this work.

Abstract: Sour bamboo shoot is a Chinese fermented vegetable with unique flavors and is favored by local consumers. In this study, at different fermentation times, the texture of bamboo shoots and the changing rules of pH, titratable acid (TA), reduced sugar, and nitrite in bamboo shoot fermentation broth were explored. Headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to investigate the dominant aroma compounds. 16S rRNA high-throughput sequencing technology (HTS) was employed to investigate the core microbial communities. The results show that the chewiness, fracturability, hardness, and pH decreased, while TA increased during the 60-day fermentation. The contents of reducing sugar and nitrite peaked on the 14th day of fermentation and then decreased. A total of 80 volatile compounds were detected during sour bamboo shoot fermentation, with 2,4-Di-tert-butylphenol having the highest concentration. Among them, 12 volatile compounds ($VIP \geq 1$) were identified as characteristic aroma substances of sour bamboo shoots. The dominant bacterial phyla in sour bamboo shoots were Firmicutes and Proteobacteria, while *Bacillus* and *Acinetobacter* were the dominant genus. Correlation analysis showed that Firmicutes exhibited a positive correlation with 3,6-Nonadien-1-ol, (E,Z)-, Oxalic acid, isobutyl hexyl ester, and (-)-O-Acetylmalic anhydride, whereas *Bacillus* exhibited a negative correlation with Silanediol, dimethyl-, and Oxime-, methoxy-phenyl-. A detailed picture of the microbial community of fermented bamboo shoots has been provided by this study, and it may provide insight into the Chinese traditional fermented vegetable microbial structure.

Keywords: fermentation; sour bamboo shoots; volatile compounds; core microbiota



Citation: 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. <https://doi.org/10.3390/fermentation9080733>

Academic Editors: Ana Leahu, Maria Soledad Prats Moya and Cristina Ghinea

Received: 24 July 2023

Revised: 2 August 2023

Accepted: 4 August 2023

Published: 6 August 2023



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1. Introduction

Fermented vegetables originated during the Shang and Zhou periods, around 3100 years ago, and have become one of the important traditional fermented foods both domestically and internationally [1]. Fermented bamboo shoots are now commonly used in Southern Chinese households and are a traditional fermented food in Guangxi and surrounding regions. They are soaked in spring water or low-salt water and naturally fermented to acquire a sour taste. The distinctive aroma and flavor of sour bamboo shoots are beloved by locals [2].

The flavor of fermented vegetables is usually closely related to microorganisms. Different regions' fermented vegetables exhibit differences in dominant microbial communities

and flavor-associated microbial communities. For example, *Lactobacillus* in Sichuan paocai (pickled vegetables), Jiangxi-jiancai (fermented vegetables), and Northeast-jiancai are considered as the core functional microbial groups influencing flavor [3]. The main microbial communities in vegetable fermentation were lactic acid bacteria (LABs), including *Leuconostoc*, *Weissella*, *Lactococcus*, *Lactobacillus*, etc., and these microbial communities contribute to the maturation of fermented vegetables [4–7]. Sour bamboo shoots contain a rich resource of lactic acid bacteria, including *Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Weissella*. Microbial metabolism is crucial in the flavor of sour bamboo shoots. For example, in a study by Xu et al. (2020), the authors investigated the progression of bacterial and fungal communities and changes in key odorants during the fermentation of red pepper. The results revealed that species such as *Aspergillus*, *Bacillus*, *Brachybacterium*, *Microbacterium*, and *Staphylococcus* were responsible for producing the majority of the key odorants throughout the fermentation process [8]. *Lactobacillus* and *Streptococcus* showed a significant positive correlation with certain organic acids, low-threshold aldehydes, phenols, and high-threshold alcohols and esters. The complex microbial community provides the foundation for flavor development, and the two are closely intertwined [9]. Therefore, it is important to investigate the relationship between the flavor of sour bamboo shoots and microorganisms.

2. Materials and Methods

2.1. Preparation of Bamboo Shoots

Fresh Jipo bamboo shoots were collected from Leshan, Sichuan Province, China, and quickly transported to the laboratory within 24 h. Approximately 10 kg of bamboo shoots were peeled off, leaving the tender edible part; then, the shoots were washed and cut into 13 cm long, 0.5 cm wide, and 0.5 cm thick strips. These strips were placed in plastic bottles with a total volume of 0.5 L (50 g) and filled with sterile water [10,11]. The bottles were sealed and fermented at room temperature for 60 days. During the fermentation process, samples were collected on the 1st, 7th, 14th, 21st, 28th, and 60th day, resulting in a total of six samples labeled as JPW1, JPW2, JPW3, JPW4, JPW5, and JPW6; for sampling, three unopened bottles were selected at random each time. The collected bamboo shoot samples were immediately subjected to texture analysis, while the collected fermentation liquid samples were stored in a $-80\text{ }^{\circ}\text{C}$ freezer for subsequent analysis.

2.2. Texture Analysis

The texture of sour bamboo shoots during fermentation was evaluated using a TA-XT Plus texture analyzer. Uniform sour bamboo shoot strips (30 mm \times 5 mm \times 5 mm) were positioned beneath the P36R probe for texture measurement. The analysis was conducted with the following measurement parameters: pre-test speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 1 mm/s, and a compression of 70% during testing, pause time of 5 s, data acquisition rate of 400 pps, and trigger force of 5 g.

2.3. Determination of pH and Total Acid (TA)

The pH of the samples was determined using a precise pH meter (PHS-25, Greifensee, Switzerland). The total acidity (TA) was measured according to the standard GB/T 12456-2021 [12].

2.4. Determination of Reducing Sugar Content

The reducing sugar content of the fermentation liquids was measured following GB 5009.7-2021 [13], and the direct titration method was used for determination.

2.5. Determination of Nitrite Content

The nitrite content in bamboo shoot fermentation liquid was measured using the spectrophotometric method according to GB 5009.33-2016 [14]. Place 18 g of bamboo shoot fermentation liquid in a 250 mL volumetric flask, add 2.5 mL of saturated borax solution,

and then add 12 mL of 70 °C water. Mix well, heat in a boiling water bath for 15 min, remove and cool in cold water, and then let it cool to room temperature. Quantitatively transfer the above extraction solution to a beaker, add 1 mL of 106 g/L potassium ferrocyanide solution, shake well, and then add 5 mL of 220 g/L zinc acetate solution to precipitate proteins. Add water to make up the volume to 40 mL, shake well, let it stand for 30 min, remove the upper fat layer, filter the supernatant with filter paper, and reserve the filtrate. Add 8 mL of the filtered solution into a 10 mL stoppered colorimetric tube, then add 0.4 mL of 4 g/L sulfanilic acid solution, mix well, and after standing for 3–5 min, add 0.2 mL of 2 g/L naphthyl ethylenediamine hydrochloride solution. Add water to the mark, mix well, let it stand for 15 min, and measure the absorbance at 538 nm using a 1 cm cuvette. Each sample was measured three times. Based on the measured absorbance, find the corresponding mass of nitrite (micrograms) in the bamboo shoot fermentation liquid from the nitrite standard curve, and use the formula to calculate the nitrite content (mg/kg).

2.6. Determination of Volatile Compounds

To determine the volatile compounds in bamboo shoot fermentation liquid during different fermentation processes, this study appropriately modified the method reported by He et al. [15]. Analysis was performed using the headspace solid-phase microextraction (HS-SPME) [16] technique and gas chromatography-mass spectrometry (GC-MS). First, after adding 100 µL of an internal standard solution containing 38.12 µg of cyclohexanone to the samples for quantitative analysis, 8 mL of bamboo shoot fermentation liquid and 3 g of sodium chloride were placed in a 10 mL headspace vial for pretreatment. The vial was then preheated for 10 min in a 60 °C water bath. Next, a divinylbenzene/carboxylic acid/polydimethylsiloxane (CAR/PDMS/DVB, 65 µm) fiber was used for headspace adsorption of volatile compounds for 30 min, followed by desorption of the volatile compounds from the fiber onto a 250 °C heated GC injection port for 5 min. For gas chromatography analysis, a temperature program was set as follows: initial temperature 40 °C, increased at a rate of 12 °C/min to 100 °C and held for 2 min, increased at a rate of 5 °C/min to 120 °C, further increased at a rate of 8 °C/min to 180 °C and held for 5 min, then increased at a rate of 12 °C/min to 210 °C, and finally increased at a rate of 8 °C/min to 250 °C for the final temperature. A flexible capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 µm) was used, with the mass spectrometer set at a source temperature of 230 °C, an ionization potential of 70 eV, and a mass scan range of 40–400 m/z. Lastly, preliminary identification of the compounds was performed using the NIST 17 mass spectral library, and volatile compounds with a match quality greater than 85% were selected for qualitative analysis.

2.7. Microbial Analysis

The total genomic DNA from the bamboo shoot fermentation liquid was extracted according to the cetyltrimethylammonium bromide (CTAB) method. And triplicate samples of bamboo shoot fermentation liquid at different fermentation stages were collected, with 5 mL of DNA extracted from each sample. Next, PCR amplification was performed on the extracted DNA using the universal primers 338F and 806R, and only samples with brightness readings between 400 and 550 bp were selected for sequencing. To obtain more accurate and reliable results, the Illumina platform for PE250 bp sequencing was used to provide paired-end reads of 250 bp. Finally, using Trimatic v0.33 software for filtering, cutadapt 1.9.1 software to eliminate primer sequences, and the dada2 method in QIIME2 2020.6 for denoising the analysis, the raw reads were obtained. An operational taxonomic unit (OTU) is a grouping of sequences with at least 97% similarity.

2.8. Statistical Analysis

The IBM SPSS Statistics 25.0 software was utilized for statistical analysis. Statistically significant differences between two groups of data were determined using one-way analysis of variance (ANOVA) and post hoc Tukey's honestly significant difference (HSD) tests.

Additionally, Pearson correlation networks and cell landscape (3.9.0) interaction networks were employed to explore the interactions between physicochemical properties, flavor, and microbiota. Throughout this process, mean values \pm standard deviation (SD) were used to represent all measured values. A significance level of 0.05 was used to determine statistical significance, with a p -value below this threshold considered as statistically significant.

3. Results and Discussion

3.1. Physicochemical Property Analysis

3.1.1. Texture Analysis of Bamboo Shoots

Evaluating the quality of bamboo shoots involves considering various factors, including texture. Hardness, fracturability, and chewiness are particularly important aspects of texture assessment [10]. As shown in Figure 1A, the hardness of bamboo shoots significantly ($p < 0.05$) decreases within the first 1–14 days and slowly decreases from day 14 to day 60. Similarly, the fracturability and chewiness of bamboo shoots gradually decrease in a relatively stable and slow manner (Figure 1B,C). The softening of bamboo shoots may be caused by a decrease in cell membrane permeability due to a pH decrease during fermentation or by the hydrolysis of native pectin in bamboo shoots [17].

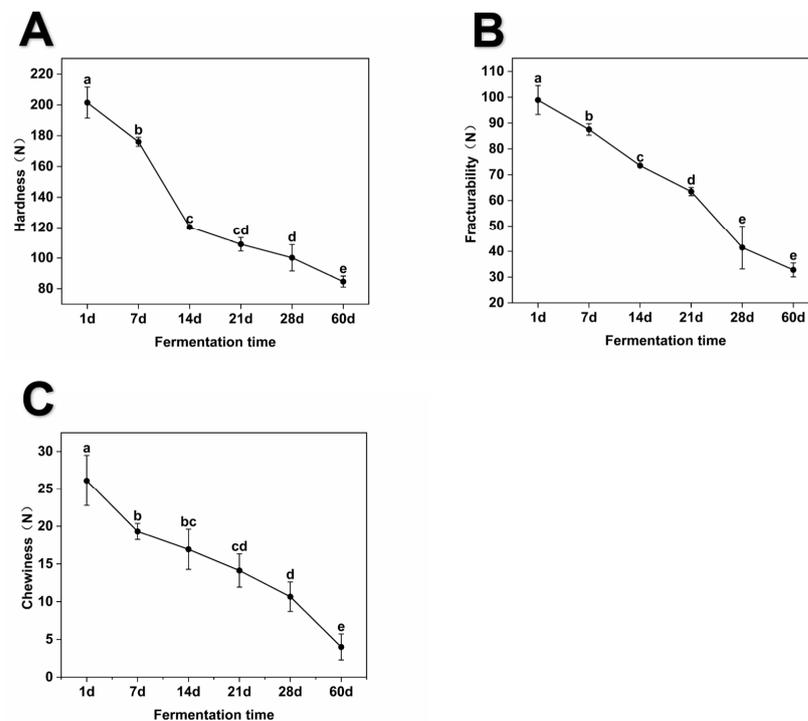


Figure 1. Texture properties of the sour bamboo shoot during the fermentation process. (A) Changes in hardness of sour bamboo shoots during fermentation. (B) Changes in fracturability of sour bamboo shoots during fermentation. (C) Changes in chewiness of sour bamboo shoots during fermentation. Statistically significant differences are indicated by different letters at $p < 0.05$.

3.1.2. Physicochemical Properties of Fermentation Liquids

The flavor quality of bamboo shoots is closely related to their acidity, and an appropriate increase in acidity helps enhance the flavor of the product [18]. During the fermentation process, lactic acid bacteria decompose and utilize carbohydrates in bamboo shoots to produce organic acids, thus forming the flavor of bamboo shoots. With the accumulation of organic acids, the pH and titratable acidity (TA) change [19]. As shown in Figure 2A, the pH of the fermentation broth remains stable throughout the fermentation process and then decreases significantly ($p < 0.05$), while the total acidity increases (Figure 2B). The decline in pH levels could potentially be attributed to the presence of *Bacillus* spores and lactic acid bacteria [20]. During the fermentation of bamboo shoots, the entry of lactic acid bacteria

or airborne microorganisms into the fermentation system causes changes in acidity. As fermentation progresses, the ethanol content gradually decreases, indicating the generation of acidic components, leading to an increase in the acidity of the sample with prolonged fermentation time [21]. However, when the acidity reaches a certain level, the growth of lactic acid bacteria may be inhibited due to the reduction of nutrients in the fermentation environment, resulting in a slow increase in acidity in the later stages [22].

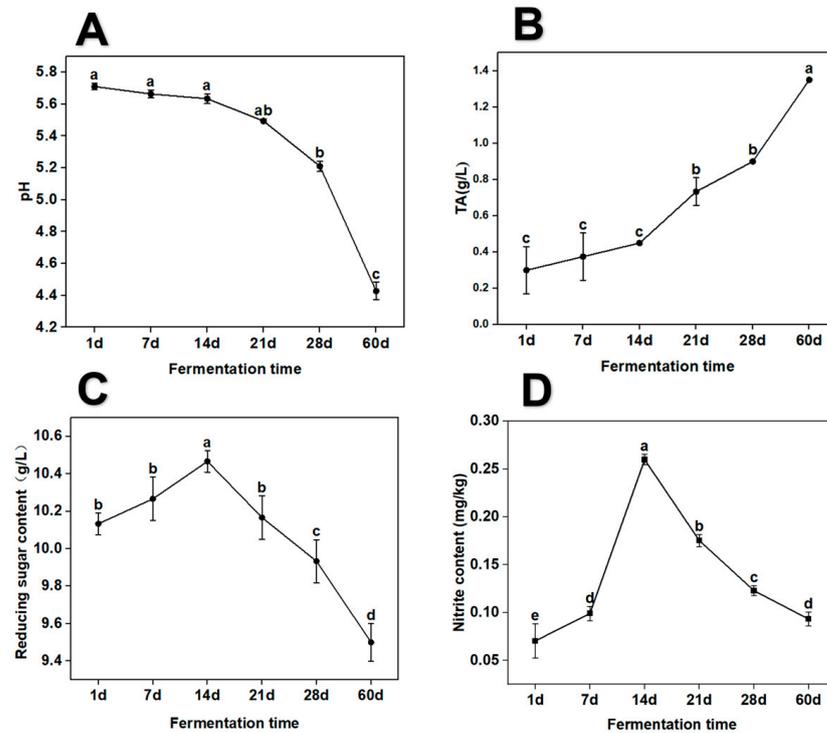


Figure 2. Changes in the pH (A), TA (B), reducing sugar content (C), and nitrite content (D) during different fermentation periods. Statistically significant differences are indicated by different letters at $p < 0.05$.

As shown in Figure 2C, the content of reducing sugars shows an upward trend from day 1 to day 14 during fermentation, possibly due to the flow of some bamboo shoot juice into the fermentation broth after cutting. Additionally, during the fermentation process, polysaccharides and disaccharides in bamboo shoots undergo hydrolysis, leading to the generation of reducing sugars and an increase in their content [23]. From day 14 to day 60, there is a significant downward trend in reducing sugar content, possibly because the growth metabolism of microorganisms during fermentation consumes the carbon source. With the rapid reproduction of microorganisms, the carbon source is continuously utilized and depleted, resulting in a significant decrease in reducing sugar content.

Nitrite is a recognized chemical carcinogen mainly produced by nitrite-reducing bacteria during vegetable fermentation [24]. The content of nitrites may be related to the growth of spoilage microorganisms [25]. As shown in Figure 2D, the nitrite content of bamboo shoots increases during the first 1–14 days of fermentation, possibly due to the rapid proliferation of harmful microorganisms such as *Enterobacteriaceae* in the initial stage of fermentation, leading to a significant increase in nitrite content [26]. A “nitrite peak” appears on day 14, followed by a significant decrease. On the one hand, this may be due to the presence of microorganisms with efficient nitrite degradation capabilities during fermentation, effectively reducing the production of nitrites in bamboo shoots [17]. On the other hand, as fermentation progresses, lactic acid bacteria gradually form dominant microbial populations, inhibiting the growth of harmful microorganisms and thereby suppressing nitrite production [26].

highest content, followed by alcohols and aldehydes. The content of phenolic volatile compounds generally decreased at first and then increased. The variety of phenolic compounds increased during fermentation, with the detection of 2,4-Di-tert-butylphenol, 2-Methoxy-4-vinylphenol, and 1,3,5-Benzotriol, 3TMS derivative. Among them, 2,4-Di-tert-butylphenol had the highest content, and it has been found to be a candidate compound related to anti-diabetic enzymes [29]. The content of alcohol and aldehyde volatile compounds was next after phenols. Alcohols contribute pleasing aromas but have a higher odor threshold, and their impact on characteristic aromas in fermented vegetables is not significant [30]. As the fermentation progressed, the number of alcohol volatile compounds increased, including 1-Heptanol, 2-Octen-1-ol, (E)-, 1-Decanol, 1-Nonanol, Cedrol, among others. The content of aldehyde volatile compounds generally increased and then decreased, but it increased compared to day 1 of fermentation. The aldehyde compound with the highest content was 2,5-Dihydroxybenzaldehyde, 2TMS derivative, followed by Benzaldehyde, 2,4-dimethyl-. Most aldehyde compounds can provide a fresh, fruity, and nutty aroma to fermented vegetables [31]. Aldehyde compounds are mainly formed through the carbon chain oxidation or decarboxylation of unsaturated fatty acids, which are exothermic reactions during fermentation and easily reduced to alcoholic compounds. Although their content in bamboo shoots is not high, these compounds have a low threshold [32]. Ester substances are one of the characteristic flavor components in fermented foods [30]. Esters are primarily formed by microbial metabolism during fermentation through the combined action of acyl-coenzyme A and alcohol acyltransferases, as well as by the reaction of acids with alcohols catalyzed by lipases [9]. Ester compounds in bamboo shoots primarily serve to decrease strong odors, enhance aroma, and provide a balancing effect. They also contribute to increasing the content of health-promoting compounds [33]. Ketones contribute to aroma, but their content in bamboo shoots is relatively low, so their impact on the flavor of bamboo shoots may be limited [34]. Acid compounds are mainly formed by microbial biochemical reactions utilizing starch, fat, protein, and other organic substances [35]. The quantity of other volatile compounds remained relatively stable with a slight decrease. Due to variations in raw materials, processing methods, and environmental changes during fermentation, the content and variety of volatile flavor components in fermented bamboo shoots may differ.

As shown in Figure 3C, the analysis shows that PC1 and PC2 explain 42.2% and 21.2% of the total variance, respectively, with a cumulative value of 63.4%. The distance between day 60 of fermentation and other fermentation days is relatively large, indicating significant changes in the physicochemical properties and flavor compared to day 1 of fermentation. The distance between day 7 and day 14 is relatively close, suggesting similar flavors, which is consistent with the results of the column chart of volatile compounds (Figure 3B). At day 1 of fermentation, the content of volatile compounds was closely related to pH, reducing sugar content, and nitrite content. Representative volatile compounds at day 7 and day 14 of fermentation were aldehydes. At day 21 and day 28 of fermentation, the representative volatile compounds were ketones, esters, alcohols, acids, and other compounds. At day 60, the representative volatile compounds were phenols, strongly associated with total acid content.

3.2.2. Characteristic Volatile Compounds of Fermented Bamboo Shoots

By conducting an OPLS-DA analysis and obtaining VIP (variable importance in the projection) values, compounds with a VIP value of ≥ 1 were considered as contributing to the formation of the aroma in fermented bamboo shoots. According to our knowledge, 2,4-dimethylbenzaldehyde and 2,4-di-tert-butylphenol were detected as the main volatile compounds in fermented bamboo shoots for the first time, and these differences could be attributed to factors such as the type of raw materials, the growth environment of the raw materials, and fermentation conditions [36].

The table below presents the volatile compounds with VIP values ≥ 1 , arranged according to their VIP values. As shown in Table 1, the compound with the highest VIP

value is 1-Heptanol, which has a floral aroma [34] that contributes to the floral aroma of fermented bamboo shoots and is considered to have the greatest contribution to the flavor formation of fermented bamboo shoots. Phenolic volatile compounds have the highest content in bamboo shoots, and phenolic volatile substances typically produce off-flavors and irritating odors [34]. 2,4-Di-tert-butylphenol is the second compound after 1-Heptanol, considered to have a significant contribution to the flavor of fermented bamboo shoots. Benzaldehyde, 2,4-dimethyl- also has a VIP value greater than 1 and is considered to contribute to the flavor of fermented bamboo shoots with an almond/spicy flavor [37]. Alcohols have pleasant aromas. Silanediol, dimethyl-, and 3,6-Nonadien-1-ol, (E,Z)- have a relatively smaller contribution, but they provide a unique aroma to fermented bamboo shoots. Ester compounds with complex flavors have relatively less content throughout the fermentation process but have a variety of types. Although Oxalic acid, isobutyl hexyl ester, and 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester have VIP values greater than 1, their impact on the flavor is relatively minor, contributing slightly to the aroma of fermented bamboo shoots. O-Xylene has a faint aromatic odor, and Isophorone has a tobacco/woody aroma. D-Limonene contributes relatively less, has a lemon-like taste, and is a natural ingredient found in many citrus fruits, vegetables, and spices. It has a small content in bamboo shoots and may have a limited impact on the flavor of fermented bamboo shoots [34].

Table 1. Characteristic compounds in fermented bamboo shoots.

Compounds	VIP
1-Heptanol	4.64
2,4-Di-tert-butylphenol	3.50
Silanediol, dimethyl-	3.18
Oxime-, methoxy-phenyl-	2.96
Benzaldehyde, 2,4-dimethyl-	2.84
Oxalic acid, isobutyl hexyl ester	2.63
(-)-O-Acetylmalic anhydride	2.57
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	2.46
3,6-Nonadien-1-ol, (E,Z)-	1.71
o-Xylene	1.12
Isophorone	1.11
D-Limonene	1.05

3.3. Microorganisms

The microorganisms in bamboo shoots are mainly bacteria, including the genera *Lactobacillus*, *Weissella*, *Leuconostoc*, *Lactococcus*, *Enterobacter*, and *Serratia*, which are commonly enriched in fermented vegetables [9]. Throughout the bamboo shoot fermentation process, lactic acid bacteria mainly produce metabolites such as lactic acid, acetic acid, and ethanol, which help maintain the acidic environment necessary for fermentation [38,39]. These metabolites play an important role in giving bamboo shoots a certain flavor, as well as serving as the basis for the formation of acids and esters during the fermentation process.

Alpha diversity is a comprehensive index of microbial richness and evenness in the habitat [40]. Chao1 index and ACE index are used to compare community abundance [41], while Shannon index and Simpson index are used to compare community diversity [42]. As shown in Table S2, the coverage of all experimental samples is 1, indicating that the sequencing results can basically represent the actual situation of the samples [43]. Chao1, ACE, Shannon, and Simpson all showed a trend with an initial increase, then a decrease, and then an increase again. By comparing the Chao1, Simpson, and Shannon indices of the six fermentation stages, it can also be observed that the species diversity, species richness, and evenness of bamboo shoots at 21 days of fermentation were significantly higher than other fermentation stages. This phenomenon is due to the proliferation of microorganisms and increased species richness during the 1-day to 21-day fermentation stage. During the 21-day to 28-day fermentation stage, the bacterial community structure gradually became more

singular, with some bacteria becoming dominant as the fermentation environment changed. During the 28–60 day fermentation stage, there may be the growth of miscellaneous bacteria in the fermentation bottles, leading to an increase in species richness [44]. PCoA and UPGMA analysis (Figures S2 and S3) revealed significant differences in microbial structure during the later stages of fermentation (21–60 days), with minimal fluctuation during the earlier stages.

3.3.1. Analysis of Beta Diversity during the Fermentation Process of Sour Bamboo Shoots

The following Figure 4A shows the top ten abundant phyla detected at the phylum level. At the phylum level, representatives include Cyanobacteria, Proteobacteria, Firmicutes, and Bacteroidetes [9]. Except for day 1 of fermentation, Firmicutes has the highest relative abundance in all other stages, indicating that Firmicutes is the dominant phylum during the 7–60 day stage of fermentation. At day 1 of fermentation, Proteobacteria has the highest relative abundance at 66.45%, followed by Firmicutes at 28.54%. Proteobacteria plays a dominant role during this stage. Except for day 1 of fermentation, Firmicutes has the highest relative abundance in all other stages, indicating that Firmicutes is the dominant phylum during the 7–60 day stage of fermentation. Therefore, Firmicutes plays a major role in the entire fermentation process, followed by Proteobacteria.

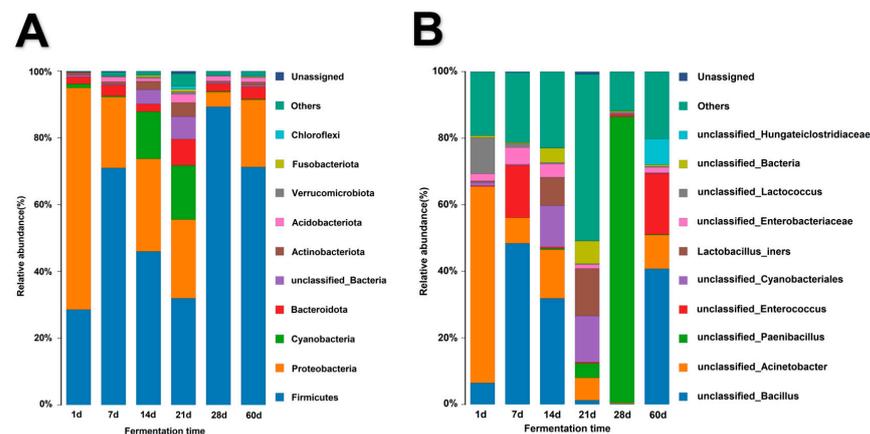


Figure 4. The phylum level (A) and genus level (B) of microbial community.

The following Figure 4B shows the top ten abundant genera detected at the genus level. It can be seen from the figure that the dominant bacterial genera vary under different fermentation conditions. At day 1 of fermentation, the dominant bacterial genus is *unclassified_Acinetobacter*. At day 7, 14, 60 of fermentation, the dominant bacterial genus is *unclassified_Bacillus*. At day 21 of fermentation, the dominant bacterial genus is *Lactobacillus_iners*. At day 28 of fermentation, the dominant bacterial genus is *unclassified_Paenibacillus*. Studies have shown that the microbial community in traditional fermented bamboo shoots has a high diversity, mainly including genera like *Lactobacillus*, *Lactococcus*, *Weissella*, *Shuttleworthia*, *Enterobacter*, and *Actinobacillus* [45]. The dominant genera identified in this sequencing are consistent with previous reports. The relative abundance of *unclassified_Enterobacteriaceae* gradually decreases with fermentation time, as lactic acid produced by lactic acid bacteria inhibits the growth of *unclassified_Enterobacteriaceae*. *Lactobacillus* is mostly facultative anaerobes with strong acid tolerance, making them more suitable for growth and metabolism in the low oxygen environment of the mid- to late-fermentation stages [46]. The relative abundance of *Enterococcus* shows a decreasing trend initially and then increases with fermentation. *Enterococcus* can produce short-chain fatty acids and organic acids in the human body and degrades sugars through glycolysis and pentose phosphate pathways [47].

3.3.2. Representative Bacteria in Sour Bamboo Shoots

LDA effect size (LEfSe) was employed as a statistical tool to assess dissimilarities among bacterial groups, discover distinctive features with diverse abundances and associated categories, and explore potential biomarkers [48]. Species with LDA values greater than four were selected for mapping [11]. As shown in Figure 5, on the first day of fermentation, sour bamboo shoots included two species biomarkers, two genus biomarkers, one family biomarker, one order biomarker, one class biomarker, and one phylum biomarker. On the 21st day of fermentation, sour bamboo shoots included five family biomarkers, four species biomarkers, four genus biomarkers, two order biomarkers, three class biomarkers, and three phylum biomarkers. On the 28th day of fermentation, sour bamboo shoots included one species biomarker, one genus biomarker, one family biomarker, one order biomarker, one class biomarker, and one phylum biomarker. On the 60th day of fermentation, sour bamboo shoots included one species biomarker, one genus biomarker, one family biomarker, and one domain biomarker. Combining the analysis of Alpha diversity and Beta diversity, as well as the succession between dominant bacterial phyla and genera, it can be inferred that the microbial succession process during the fermentation of bamboo shoots consists of Proteobacteria being gradually replaced by Firmicutes.

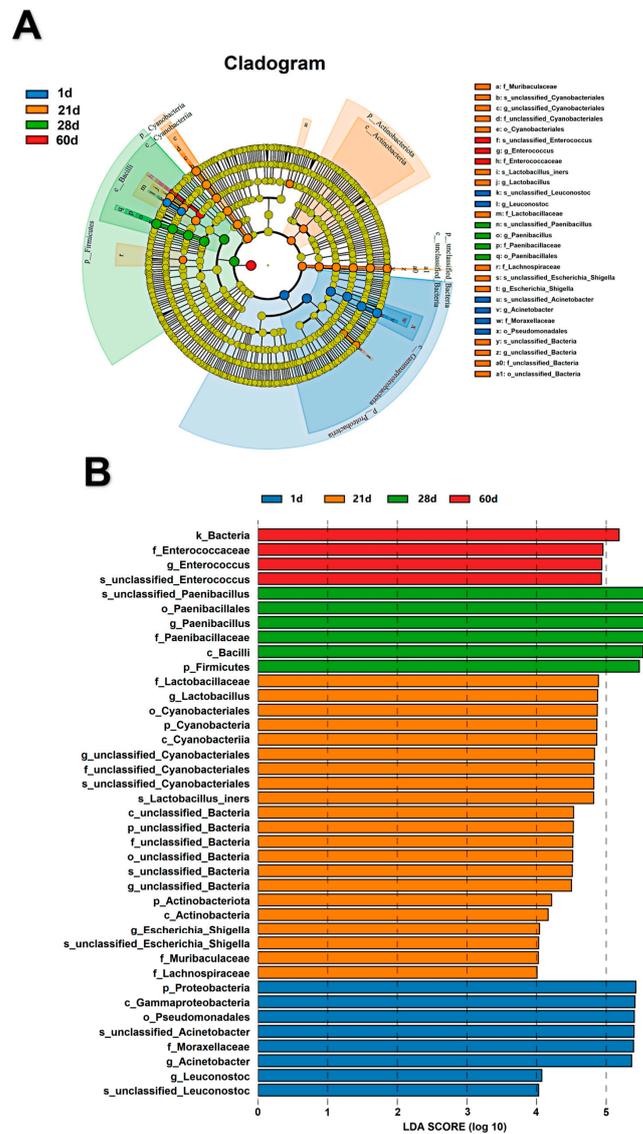


Figure 5. The composition of microbial community in LEfSe at the phylum (A) and genus (B) levels.

3.3.3. The Relationship between the Physicochemical Properties, Flavor, and Microbial Composition of Bamboo Shoots

The physicochemical properties and major volatile compounds during fermentation are closely related to the quantity and types of microbial communities. By using Pearson correlation analysis, the relationship between the physicochemical properties of bamboo shoots, characteristic volatile compounds (VIP ≥ 1), and dominant bacterial phyla (top ten) and genera (top ten) of the microbial community were studied.

The microbial population may be a key factor in the formation of volatile flavor substances in sour shoots [3,49–51]. As shown in Figure 6A, the vertical axis represents volatile flavor compounds with VIP values greater than one, as well as basic indicators such as pH, total acid, nitrite, and reducing sugar. The horizontal axis represents the top ten dominant bacterial phyla. $p \leq 0.05$ represents a significant correlation. When the correlation coefficient is 1, it indicates a positive correlation, while when the correlation coefficient is -1, it indicates a negative correlation. At the phylum level, Firmicutes and Proteobacteria are the dominant bacterial phyla. As shown in the figure, Firmicutes showed a significant positive correlation with 3,6-Nonadien-1-ol, (E,Z)-, and a significant negative correlation with D-Limonene. Proteobacteria showed a significant positive correlation with 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and a significant negative correlation with 3,6-Nonadien-1-ol, (E,Z)-. Alcohol compounds are related to the aroma of bamboo shoots, while ester compounds mainly reduce the irritating odor [30,33]. This indicates that Firmicutes is beneficial to the aroma production of bamboo shoots. Proteobacteria is beneficial for reducing the irritating odor of pickled bamboo shoots. Cyanobacteria showed a significant positive correlation with nitrite content, indicating that Cyanobacteria is beneficial for the nitrite content in bamboo shoots. Firmicutes showed a negative correlation with fracturability, hardness, and chewiness, indicating that it is not conducive to the texture of bamboo shoots, which will cause the bamboo shoots to become soft and decrease their taste. Proteobacteria showed the opposite trend, and it showed a positive correlation with the three texture indicators of bamboo shoots, indicating that it is conducive to the texture of bamboo shoots.

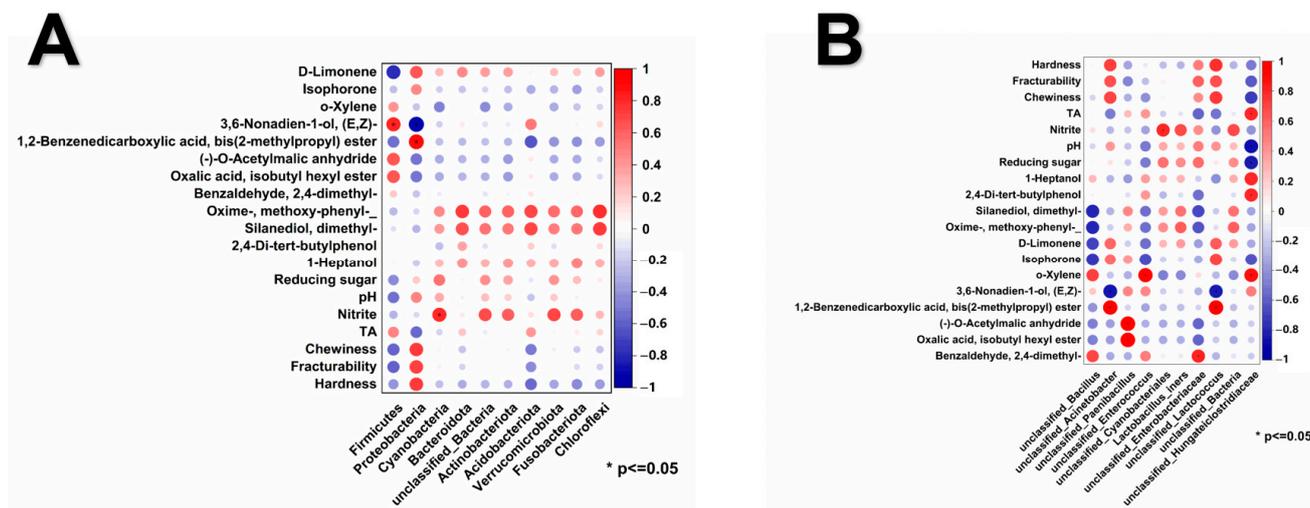


Figure 6. The relative abundance of the top 10 phyla (A) and genera (B) and the Pearson correlation analysis of volatile organic compounds (VIP ≥ 1).

As shown in Figure 6B, at the genus level, the vertical axis represents volatile flavor compounds with VIP values greater than one, as well as basic indicators such as pH, total acid, nitrite, and reducing sugar. The horizontal axis represents the top ten dominant bacterial genera. *Unclassified_Acinetobacter* and *unclassified_Lactococcus* showed a significant positive correlation with 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and a significant negative correlation with 3,6-Nonadien-1-ol, (E,Z)-, indicating that these two

genera are not beneficial for the aroma of bamboo shoots [28]. *Unclassified_Paenibacillus* showed a significant positive correlation with (-)-O-Acetylmalic anhydride and Oxalic acid, isobutyl hexyl ester. *Unclassified_Enterococcus* showed a significant positive correlation with o-Xylene. *Unclassified_Cyanobacteriales* showed a significant positive correlation with nitrite. This indicates that *unclassified_Cyanobacteriales* will influence the safety of bamboo shoots. When its content increases, it will lead to an increase in nitrite content in bamboo shoots, which will have an impact on consumer's health when the nitrite content reaches a certain level [24]. *Unclassified_Enterobacteriaceae* showed a significant positive correlation with Benzaldehyde, 2,4-dimethyl-. *Unclassified_Hungateiclostridiaceae* showed a significant positive correlation with o-Xylene, 2,4-Di-tert-butylphenol, and total acid, and a significant negative correlation with pH and reducing sugar. This indicates that *unclassified_Hungateiclostridiaceae* is conducive to the formation of the flavor of bamboo shoots but not to the texture. *Unclassified_Acinetobacter* and *unclassified_Lactococcus* showed a positive correlation with the chewiness, hardness, and fracturability of bamboo shoots, indicating that both genera are beneficial to the texture of bamboo shoots.

4. Conclusions

Through the above experimental investigation, the relationship between the physicochemical properties of bamboo shoots, characteristic volatile compounds, and core microbial communities was studied. It was found in the study that there were significant changes in the physicochemical properties of sour bamboo shoots during the 60-day fermentation. 1-Heptanol was considered the most significant volatile compound contributing to the formation of bamboo shoots flavor. Firmicutes showed a significant positive correlation with 3,6-nonadien-1-ol (E, Z). Proteobacteria showed a significant positive correlation with 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester and a significant negative correlation with 3,6-nonadien-1-ol (E, Z). Proteobacteria showed a positive correlation with the texture of sour bamboo shoots. *Unclassified_Bacillus* showed a negative correlation with Silanediol, dimethyl-, and oxime-, methoxy-phenyl-. *Classified_Acinetobacter* showed a significant positive correlation with 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester and a significant negative correlation with 3,6-nonadien-1-ol (E, Z). The findings of this study provide valuable references for the advancement of industry-scale production of sour bamboo shoots.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation9080733/s1>. Figure S1: Correlation among reducing sugar, hardness, fracturability, chewiness, TA, pH, and nitrite of sour bamboo shoots conducted using Pearson's correlation analysis; Figure S2: PCoA analysis of the microbial community in sour bamboo shoots during fermentation process; Figure S3: UPGMA analysis of the microbial community in sour bamboo shoots during fermentation process; Table S1: Volatile compounds of sour bamboo shoots during fermentation measured using GC-MS; Table S2: Alpha diversity of the microbial community in sour bamboo shoots during the fermentation process.

Author Contributions: Conceptualization, Q.C. and S.H.; investigation, Z.L., X.X. and W.D.; writing—original draft preparation, X.X. and Z.L.; writing—review and editing, Z.L. and Q.C.; funding acquisition, Y.Z. and S.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key R&D Program of China (2021YFD2200504_1 and 2021YFD2200505_2), Science and Technology Project of Sichuan Province, China (2023JDRC0130, 2021YFYZ0006, and 2022NSFSC0093), and Ph.D. Foundation (no. 22zx7).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is shown in the article.

Acknowledgments: The authors would like to acknowledge the National Key R&D Program of China, Sichuan Provincial Department of Science and Technology for the research project funds

received for the project. The authors would like to thank the Southwest University of Science and Technology for the internal project fund to support the research.

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

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