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Analysis of Microbial Community Changes and Their Correlations with Volatile Flavouring Substances during Autonomous Fermentation of Western Sichuan Yi Suancai Based on High-Throughput Sequencing

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Abstract: Western Sichuan Yi suancai contains a group of highly functional microorganisms in an alpine and high-altitude ecological environment. Due to its unique salt-free fermentation process, it is nutritious and has a crispy texture. Six periods were established during fermentation: day 0, day 2, day 5, day 8, day 11, and day 14. The results showed that the antioxidant capacity and organic acid content increased during the fermentation process, while the pH gradually decreased, indicating that suancai from the later periods was better for supplementing the human body with acid substances and eliminating free radicals. Twenty-six flavour compounds were identified, including alcohols, esters, ketones, and acids. Ethyl alcohol, 3-methyl-1-butanol, ethyl methanoate, and acetic acid were the main contributors to the flavour, imparting floral and fruity notes to the suancai. Five dominant bacterial genera (*Lactobacillus*, *Leuconostoc*, *Weissella*, *Klebsiella*, and *unclassified_o__Lactobacillales*) were identified via high-throughput sequencing during the fermentation process, and there were nine dominant fungal genera (*Dipodascaceae_gen_Incertae_sedis*, *Mucor*, *Pichia*, *unclassified__f__Dipodascaceaet*, *Cyberlindnera*, *Diutina*, *Trichosporon*, *Saccharomycopsis*, and *Wickerhamomyces*). Correlation analysis showed that the antioxidant capacity was positively correlated with genera such as *Lactobacillus*, *Mucor*, and *Alternaria*, indicating that these microorganisms have important roles in enhancing the antioxidant properties of suancai. Meanwhile, some genera, such as *Microbacterium*, *Herbaspirillum*, *Mortierella*, and *Sampaiozyma*, promote the synthesis of alcohols, esters, acids, and ketones. This study revealed the interactions between microorganisms and metabolites during the fermentation of western Sichuan Yi suancai and provided a scientific basis for further understanding the fermentation mechanism of traditional suancai and improving the fermentation process.

Keywords: western Sichuan Yi suancai; high-throughput sequencing; microbial community structure; correlation analysis

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1. Introduction

Suancai plays an important role in the food culture of China. China's Sichuan suancai is one of the most famous types of suancai in Asia, with a history dating back more than 3000 years to the Shang Dynasty [1]. The main step in suancai production is to wash fresh vegetables and soak them in brine to make them undergo the fermentation process of *lactic acid bacteria* [2]. Western Sichuan Yi suancai is a traditional speciality in the Liangshan Yi region of Sichuan Province, China, and is one of the cuisines handed down from generation to generation by the local Yi people. It is mainly made of mustard and radish and is

fermented in a ceramic altar for 10 to 14 days in a cold region at an altitude of 1800 metres without adding salt. Then, chilli peppers and other seasonings are added to make it an appetising hot and sour dish that is often used as a cold dish or as a dish to be served under the table at family dinners or banquets. It is also a common treat for guests in local Yi customs. In addition, suancai is a great source of dietary fibre, vitamins, and amino acids [3]. With its unique fermentation process and strong local flavour, western Sichuan Yi suancai gradually won the favour of outsiders and spread to a wider area.

The microbial community composition and its dynamics are important determinants of suancai quality [4]. Lactobacilli play a central role in the microbiological system of suancai, converting substances such as carbohydrates, proteins, and fats into a wide range of volatile flavour substances [5]. Xiong et al. [6] observed microbial community changes during four rounds of fermentation of Laotan suancai and found that genera such as *Lactiplantibacillus* and *Companilactobacillus* dominated the process.

That study also indicated that organic acids contribute to microbial community succession by regulating the pH of the fermentation environment and inhibiting the growth of bacteria that are sensitive to acidic conditions. Song et al. [4] showed that the addition of *Lactobacillus plantarum* and *Pediococcus pentosaceus* not only significantly increased the organic acid content in suancai but also enhanced the level of bacterial diversity during the fermentation process. Liang et al. [7] found that the genera *Lactobacillus*, *Pediococcus*, and *Leuconostoc* dominated the fermentation process of northeastern suancai. They also found that co-inoculation of *L. plantarum* and *P. pentosaceus* increased the production of alcohols, esters, and aldehydes, thereby improving the flavour and texture of suancai. He et al. [8] revealed that the diversity of the bacterial community increased with increasing fermentation temperatures and indicated that *Lactobacillus* was the dominant genus during the later stages of suancai fermentation. However, there are still few research reports on Sichuan suancai, especially suancai from the Yi region of western Sichuan.

Until now, western Sichuan Yi suancai has mostly been made at home by Yi families and has lacked a set of standardised, regulated, and systematic production processes, which has limited the ability to produce high-quality and high-standard products. In addition, microorganisms are susceptible to changes in the spontaneous fermentation process due to a variety of factors such as the type of raw material, the fermentation process, and the geographic climate [9]. Therefore, studying the physicochemical properties, organic acid content, flavour composition, and microbial community structure of western Sichuan Yi suancai is necessary to clarify the main functional microorganisms, improve the fermentation process, and enhance the safety and quality of suancai. At present, the interrelationships between the microbial communities and the physicochemical properties and flavour components of suancai, as well as the functional properties of the dominant genera during fermentation, are yet to be explored.

In this study, we performed a detailed analysis of microbial composition and succession using high-throughput sequencing and identified volatile flavour components via headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS). In addition, we monitored the dynamics of basic physicochemical parameters and organic acids during fermentation with the aim of exploring (i) the evolution of the metabolite composition, (ii) changes in microbial community diversity and composition, and (iii) correlations between key microbial groups and metabolites during the fermentation of Yi suancai. This experiment provided a database for obtaining a deeper understanding of the functional microorganisms involved in the preparation and production of western Sichuan Yi suancai.

2. Materials and Methods

2.1. Sample Collection

The production process of western Sichuan Yi suancai is shown in Figure 1. Washed mustard greens are placed in a glass fermenter, and a bowl of suancai water left over from the last fermentation is added. Then, an appropriate amount of purified water is

added, the jar is sealed, and fermentation occurs in an anaerobic environment for about 15 days. Suancai and suancai juice that were independently fermented by ten Yi families in Liangshan Yi Autonomous Prefecture were randomly selected and mixed as samples. Samples were taken at six stages: day 0, day 2, day 5, day 8, day 11, and day 14. The samples were frozen with liquid nitrogen immediately after retrieval and stored at −80 °C for further analysis.

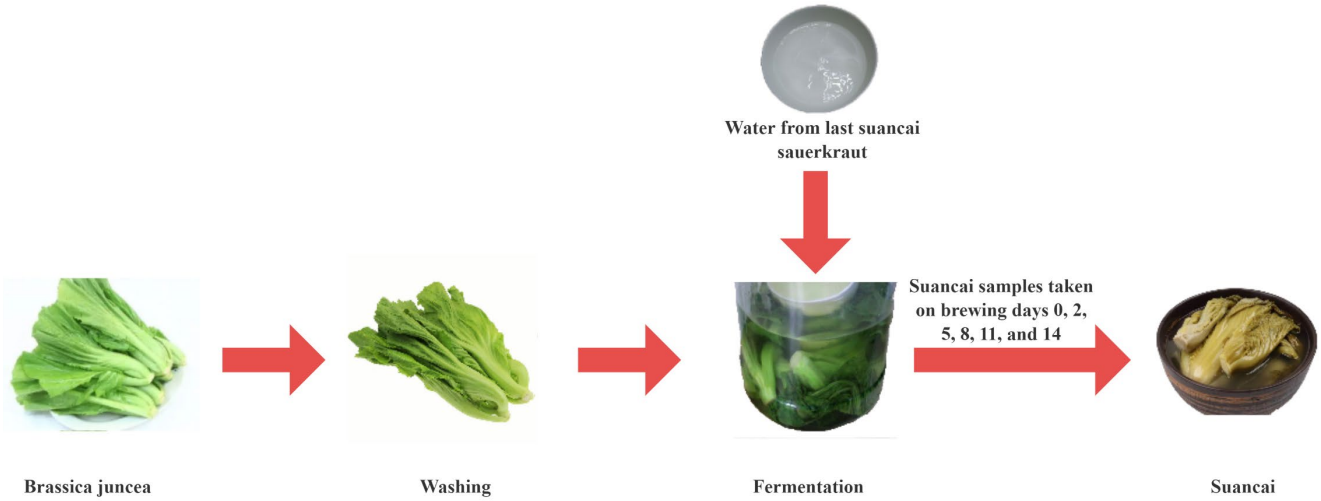

Figure 1. Flow chart of fermentation of western Sichuan Yi suancai.

2.2. Raw Materials and Reagents

The standards for the quantitative analysis of organic acids (HPLC, purity ≥ 98) and volatile compounds (GC, purity ≥ 98) used in the experiments were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Sodium hydroxide, phenolphthalein, potassium hydrogen phthalate, concentrated sulphuric acid, phenol, disodium hydrogen phosphate, sodium phosphate, sodium hypochlorite, sodium potassium tartrate, ammonium sulphate, potassium antimony tartrate, ammonium molybdate, and potassium dihydrogen phosphate (all AR) were purchased from China Pharmaceutical Group Chemical Reagent Co., Shanghai, China.

2.3. Measurement of Basic Physiological Indicators

2.3.1. Determination of pH Value

The pH values of the suancai were determined with a pH meter (pH-250L, Shanghai, China), and the average values of three measurements were recorded.

2.3.2. Determination of Reducing Capacity

The fermentation broth was diluted 5 times to generate the samples to be tested, and a total antioxidant capacity (T-AOC) assay kit (FRAP method) (BC1310 50T/48S, Beijing, China) was used for the assay, which was conducted according to the instruction manual. The absorbance values of the samples and a blank control were measured at 593 nm, and a 0.1 mg/mL vitamin C solution was used as the control.

2.3.3. Determination of Hydroxyl Radical and Superoxide Anion Radical Scavenging Rates

Referring to the method used by Tu et al. [10], with minor modifications, the fermentation broth was diluted 5 times to generate the sample to be tested. A Fenton solution was quickly mixed with the sample solution and subsequently left to stand at 37 °C for about 1 min, followed by the addition of 2 mL of Griess reagent. Then, the reaction was carried out at room temperature for 20 min, at the end of which the absorbance was measured at 550 nm. The formula for calculating the hydroxyl radical scavenging rate was as follows:

$$\text{Hydroxyl radical scavenging rate } (\%) = \frac{A_1 - A_2}{A_1 - A_3} \times 100\% \quad (1)$$

where A_1 is the absorbance of deionised water; A_2 is the absorbance after the sample is added; and A_3 is the absorbance of the blank control.

Next, 4.5 mL of 50 mmol/L pH 8.2 Tris-HCl buffer and 5.2 mL of ultrapure water were mixed in a test tube and held at 25 °C in a water bath for 10 min. Then, 0.4 mL of 6 mmol/L catechol and 1 mL of a sample solution were added to the above solution, shaken quickly, and left for 4 min. The reaction was subsequently terminated via the addition of 1 mL of 8 mol/L hydrochloric acid, and finally the absorbance of the mixture was measured at 320 nm. The formula for calculating the scavenging rate of the superoxide anion radicals was as follows:

$$\text{Superoxide anion radical scavenging rate } (\%) = \frac{A_1 - \frac{A_2}{A_0}}{A_1} \times 100\% \quad (2)$$

where A_1 is the absorbance of deionised water; A_2 is the absorbance after the sample is added; and A_0 is the absorbance of a Vc standard.

2.3.4. Determination of DPPH and ABTS Radical Scavenging Rates

Referring to the method used by Zhou et al. [11], with minor modifications, the fermentation broth was diluted 5 times to generate the sample to be tested. Then, 1.0 mL of a 1 mmol/L DPPH solution was added to 3.0 mL of broth, mixed well, and reacted for 30 min at room temperature while protected from light. The absorbance of the samples was measured at 517 nm, and the formula for calculating the DPPH radical scavenging activity was as follows:

$$\text{DDPH radical scavenging rate } (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (3)$$

where A_1 is the absorbance after adding the sample; A_2 is the absorbance without the sample; and A_0 is the absorbance of the blank control.

A 7 mmol/L ABTS solution was mixed with 2.45 mmol/L $K_2S_2O_8$ in equal volumes and placed in the dark to react for 12–16 h. The ABTS solution was diluted with anhydrous ethanol until the absorbance was 0.70 ± 0.02 (wavelength: 734 nm). Then, 0.8 mL of the sample solution and 3.2 mL of an ABTS solution were accurately measured, mixed well, and left to stand in the dark for 6 min. The absorbance was measured at 734 nm. In the blank group, an equal volume of ultrapure water was used instead of the sample solution. The formula for calculating the ABTS free radical scavenging activity was as follows:

$$\text{ABTS radical scavenging rate } (\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100\% \quad (4)$$

where A_1 is the absorbance after the addition of the sample and A_0 is the absorbance of the blank control.

2.4. Determination of Organic Acids

The determination of organic acids was based on the method used by Zong et al. [12], with minor modifications. An experiment was carried out to detect acetic, lactic, citric, tartaric, oxalic, malic, and succinic acids during fermentation of suancai using HPLC-MS (Agilent, Santa Clara, CA, USA). A ZORBAX SB-Aq column (4.6 × 250 mm, 5 μm, Thermo Fisher Scientific, Waltham, MA, USA) was used with a column temperature of 30 ± 0.5 °C, a detection wavelength of 215 nm, a total run time of 20 min, and a flow rate of 0.5 mL/min. Mobile phase A was acetonitrile, and mobile phase B was 0.05 mol/L KH_2PO_4 (pH 2.68).

2.5. Analysis of Volatile Compounds

Volatile compounds were determined according to the method used by Luo et al. [13], with minor modifications. The procedure was as follows: First, 5 g of suancai homogenate and a magnetic stirrer were placed into a 15 mL glass headspace vial, and 10 μL of an ethyl valerate solution (20 mg/L) was added as an internal standard. Next, the samples were equilibrated for 15 min at 40 °C and 40 rpm/min using a magnetic stirrer. The volatile com-

pounds in the headspace vials were then adsorbed through SPME fibres (Supelco, Bellefonte, PA, USA) coated with 50/30 μm divinylbenzene/carboxyphenyl/polydimethylsiloxane (DVB/CAR/PDMS) for 30 min at 45 °C. The extracted volatile fractions were held at 270 °C for 5 min in the injection port of a gas chromatograph–mass spectrometer (6890N/5975B, Agilent Technologies Co., Ltd.; Santa Clara, CA, USA).

GC-MS analysis: Helium (purity: 99.999%) was used with a flow rate of 1 mL/min. The temperature of the injection port was set at 270 °C, and a non-split injection was used. The initial temperature of the column oven was 40 °C, which was held for 3 min. The temperature was then ramped up to 100 °C at a rate of 5 °C/min, held for 1 min, ramped up to 175 °C at a rate of 3 °C/min, held for 1 min, ramped up to 215 °C at a rate of 10 °C/min, and held for 10 min. In addition, an EI ionisation source (70 eV) was used with a temperature of 230 °C, an interface temperature of 280 °C, and a full scan range of m/z 35–550.

The mass spectral data of the volatile flavouring substances obtained using the gas chromatography–mass spectrometry (GC-MS) technique were compared with the NIST 17.L standard database. They were analysed by searching for similarities and characteristic peaks, and a preliminary qualitative analysis was performed. Meanwhile, a quantitative analysis of the volatile substances was carried out using the internal standard method, in which the content of an internal standard substance (tertiary amyl alcohol) was combined with the content of each volatile substance to calculate their specific contents.

2.6. Metagenomic DNA Extraction, Amplification, and Sequencing

The juice of the western Sichuan Yi suancai was taken during the six fermentation periods, and the samples were centrifuged at 12,000 r/min for 10 min in order to remove the supernatant and collect the bacterial precipitate. DNA extraction was carried out according to the method described in the instructions of the DNA extraction kit (FastDNA® Spin Kit for Soil), and DNA was quantified via agarose gel electrophoresis to study the community structure of the bacteria and fungi in the suancai. Library construction was carried out with a NEXTFLEX Rapid DNA-Seq Kit. The 16S rRNA bacterial primers 338F (5′-ACTCCTACGGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify bacterial V3-V4 regions. The fungal universal primers ITS1FI2 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify fungal ITS sequences. The preparation and amplification procedures of the PCR mixtures were based on the method used by Wang et al. [14], and the PCR products were detected via 2% agarose gel electrophoresis. Based on the preliminary quantitative electrophoresis results, the PCR products were sent to Shanghai Majorbio Bio-pham Technology Co., Ltd. (Shanghai, China), to be sequenced for subsequent analysis.

2.7. Data Analysis

The data are presented as the means ± SDs of three independent experiments (n = 3). Significant differences among the groups were compared using a one-way analysis of variance (ANOVA) with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered significant. Graphs were generated using Origin 2022 software (Origin Lab, Northampton, MA, USA). PCA and OPLS-DA were conducted using SIMCA 14.1 (Umetrics AB, Umea, Vasterbotten, Sweden).

3. Results and Discussion

3.1. Dynamic Changes in Physicochemical Indicators during Fermentation of Western Sichuan Yi Suancai

A test was conducted to determine the pH and antioxidant capacity values of samples of juice from western Sichuan Yi suancai at different stages of fermentation, and the results are shown in Figure 2. As can be seen in Figure 2a, the pH showed a significant decreasing trend with increasing fermentation time from 3.96 ± 0.10 to 3.17 ± 0.09 ($p < 0.05$). This change was attributed to the large number of lactic acid bacteria, which converted soluble

carbohydrates into organic acids such as lactic acid and acetic acid. This resulted in a rapid decrease in pH [15], which was in line with the results of Zhao et al. [16]. Excess production of free radicals is closely linked to several organs in the body, such as the heart, lungs, and intestines [17], and removing excess free radicals from the body can help to reduce the incidences of a number of diseases, including cancer, cardiovascular disease, and diseases associated with aging [18]. As can be seen in Figure 2b,c,f, the reducing capacity, hydroxyl radical scavenging capacity, and DPPH radical scavenging capacity of the suancai juice significantly ($p < 0.05$) increased during the fermentation process. At the end of the fermentation period, the reducing capacity reached 152.70 ± 3.02 μmol/L, the hydroxyl radical scavenging capacity was 43.05 ± 2.21%, and the DPPH radical scavenging capacity was 74.78 ± 1.91%. As shown in Figure 2d,e, the superoxide anion radical scavenging capacity varied between 19.33 ± 0.22% and 78.65 ± 1.84%, with the highest value occurring on day 11, and the ABTS radical scavenging capacity ranged from 51.03 ± 1.00% to 91.88 ± 3.65%, with the peak value occurring on day 8. Both capacities showed trends of increases followed by decreases, and the reason for this change needs to be investigated further. In summary, the western Sichuan Yi suancai showed strong antioxidant properties, and the main carriers that exerted their antioxidant capacities might be related to the dominant microorganisms during the fermentation process.

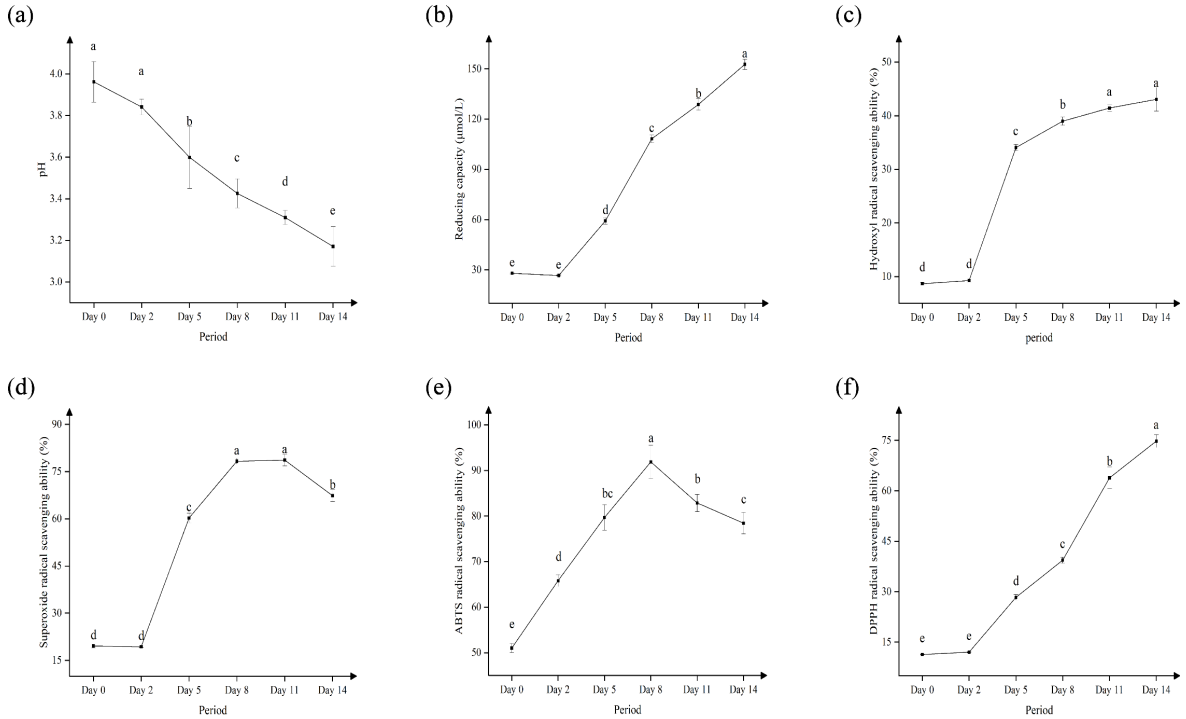

Figure 2. Changes in physicochemical indicators during fermentation of western Sichuan Yi suancai. Note: Different lowercase letters represent significant differences between factors at $p < 0.05$. (**a**) p H, (**b**) Reducing capacity, (**c**) Hydroxyl radical scavenging ability, (**d**) Superoxide radical scavenging ability, (**e**) ABTS radical scavenging ability and (**f**) DPPH radical scavenging ability.

3.2. Dynamic Changes in Organic Acids during Fermentation of Suancai of the Western Sichuan Yi Tribe

During the fermentation of suancai, the main metabolites produced by lactic acid bacteria are organic acids, which not only give suancai its unique flavour and texture [19] but also reflect the stability of the fermentation process. As can be seen in Table 1, the major organic acids in the suancai included lactic, citric, and oxalic acids, with lactic acid having the highest content, which was similar to the results of Zhao et al. [20]. Lactic acid, a key organic acid, is primarily produced by Lactobacillus, which provides a mild and distinctive sour flavour to fermented foods [21]. The lactic acid content rose significantly ($p < 0.05$)

throughout the process, reaching a maximum value of 51.28 ± 1.15 mg/g on day 11. Citric acid is present in a wide range of fermented products and is metabolically converted into flavour components such as ethyl acetate and acetaldehyde [22]. The concentration of citric acid increased during the fermentation process but did not vary much, with the maximum value of 15.89 ± 0.86 mg/g occurring on day 14. The concentration of oxalic acid ranged from 6.23 ± 0.11 mg/L to 7.82 ± 0.44 mg/L, which accounted for approximately 18 per cent of the total organic acids, and tended to decrease throughout the fermentation process. Acetic acid production usually involves acetic acid bacteria and some fermenting lactic acid bacteria, especially in aerobic environments [23], and the ratio of lactic acid to acetic acid in high-quality suancai is around 4:1. The acetic acid content increased during the first four periods and reached a maximum value of 5.10 ± 1.42 mg/g on day 11. Malic acid was first detected on day 2 at 5.76 ± 0.22 mg/g, and the concentration increased on days 11 and 14. Malolactic fermentation often occurs in a variety of fermentation processes, and this process may explain the fluctuations in the malic acid content [24]. Tartaric and succinic acids are important organic acids in the fermentation of suancai, but their contents were low compared to the other acids.

Table 1. Changes in organic acids (mg/g) during the fermentation process of western Sichuan Yi suancai.

	Day 0	Day 2	Day 5	Day 8	Day 11	Day 14
Lactic acid	32.12 ± 1.82 ^{c}	30.15 ± 2.13 ^{c}	35.82 ± 1.74 ^{b}	48.71 ± 2.18 ^{a}	51.28 ± 1.15 ^{a}	51.12 ± 2.58 ^{a}
Citric acid	9.18 ± 1.40 ^{e}	10.16 ± 0.22 ^{de}	11.68 ± 0.69 ^{cd}	13.25 ± 1.96 ^{bc}	14.95 ± 1.28 ^{ab}	15.89 ± 0.86 ^{a}
Oxalic acid	7.82 ± 0.44 ^{a}	7.22 ± 0.28 ^{c}	7.76 ± 0.35 ^{ab}	6.23 ± 0.11 ^{d}	7.42 ± 0.30 ^{abc}	7.35 ± 0.39 ^{bc}
Acetic acid	2.11 ± 0.62 ^{c}	3.12 ± 1.01 ^{bc}	3.32 ± 1.11 ^{abc}	3.96 ± 0.52 ^{abc}	5.10 ± 1.42 ^{a}	4.96 ± 0.85 ^{ab}
Malic acid	ND	5.76 ± 0.22 ^{b}	ND	ND	6.85 ± 1.12 ^{ab}	8.02 ± 0.56 ^{a}
Tartaric acid	1.18 ± 0.18 ^{ab}	ND	0.88 ± 0.15 ^{b}	ND	1.23 ± 0.10 ^{a}	0.95 ± 0.21 ^{b}
Succinic acid	0.18 ± 0.00 ^{c}	ND	0.58 ± 0.27 ^{b}	ND	ND	1.15 ± 0.16 ^{a}

Note: ND, not detected, i.e., concentrations below the detection limit. Different lowercase letters represent significant differences in factors at $p < 0.05$.

3.3. Dynamic Changes in Flavour Substances during the Fermentation Process of Western Sichuan Yi Suancai

Volatile flavour substances are key determinants of the taste, aroma, and overall quality of fermented foods [25]. A total of 26 major flavour substances, including 9 alcohols, 7 esters, 6 acids, 2 ketones, 1 sugar, and 1 ether, were detected during the fermentation process of western Sichuan Yi suancai via GC-MS analysis. As can be seen in Figure 3a, the variance contributions of the first principal component (PC1) and the second principal component (PC2) are 37.5% and 26.7%, respectively. The first two periods are located at the junction of the first and fourth quadrants, while the next two periods extend towards the third quadrant, followed by day 11 in the second quadrant and finally day 14 in the third quadrant. Suancai contains a complex combination of flavouring substances, which are mainly produced by the raw vegetable material, lactic acid bacteria, and the fermentation activity of yeast [26]. In Figure 3b, it can be seen that alcohols were the main contributors to the aroma of the western Sichuan Yi suancai, which was highly similar to the results of a study by Xing et al. [26]. In particular, [V2] ethyl alcohol, [V4] 3-methyl-1-butanol, [V5] 2-octanol, and [V7] 4-methyl-2-pentanol impart a pleasant floral and grassy aroma to suancai [27,28]. Most esters are produced through esterification during microbial fermentation, and they add a pleasant floral and fruity flavour to suancai [29]. [V16] ethyl methanoate, [V18] ethyl propionate, and [V19] propyl acetate were the main esters in this test, and they enhanced fruit flavours such as pickled pineapple and banana [30,31]. Acids such as [V10] acetic acid and [V15] myristic acid also contribute sourness and a fat-like aroma to suancai [32]. In addition, some ketones such as [V25] D-mannose and [V26] di-isopropyl ether were detected. The combined effect of these flavour substances gives suancai its layered aroma and moderately sweet and sour taste. In Figure 3b,c, it

can be seen that day 0 and day 2 are close to each other and are clustered together as one group, indicating that the compositions of the aroma substances were similar during these two periods and that the flavour textures were similar. Again, day 5 and day 8 show similarities. Day 11 and day 14 are far away from the other periods and day 11 is clustered into a separate category. It is speculated that this may be because the contents of some flavour substances changed significantly during the later stages of fermentation, such as [V2] ethyl alcohol, [V5] 2-octanol, [V11] 3-butenoic acid, [V12] lactic acid, [V16] ethyl methanoate, and [V6] 1,3-butylene glycol, or because new flavour substances such as [V14] 2-pelanoic acid and [V26] di-isopropyl ether were generated.

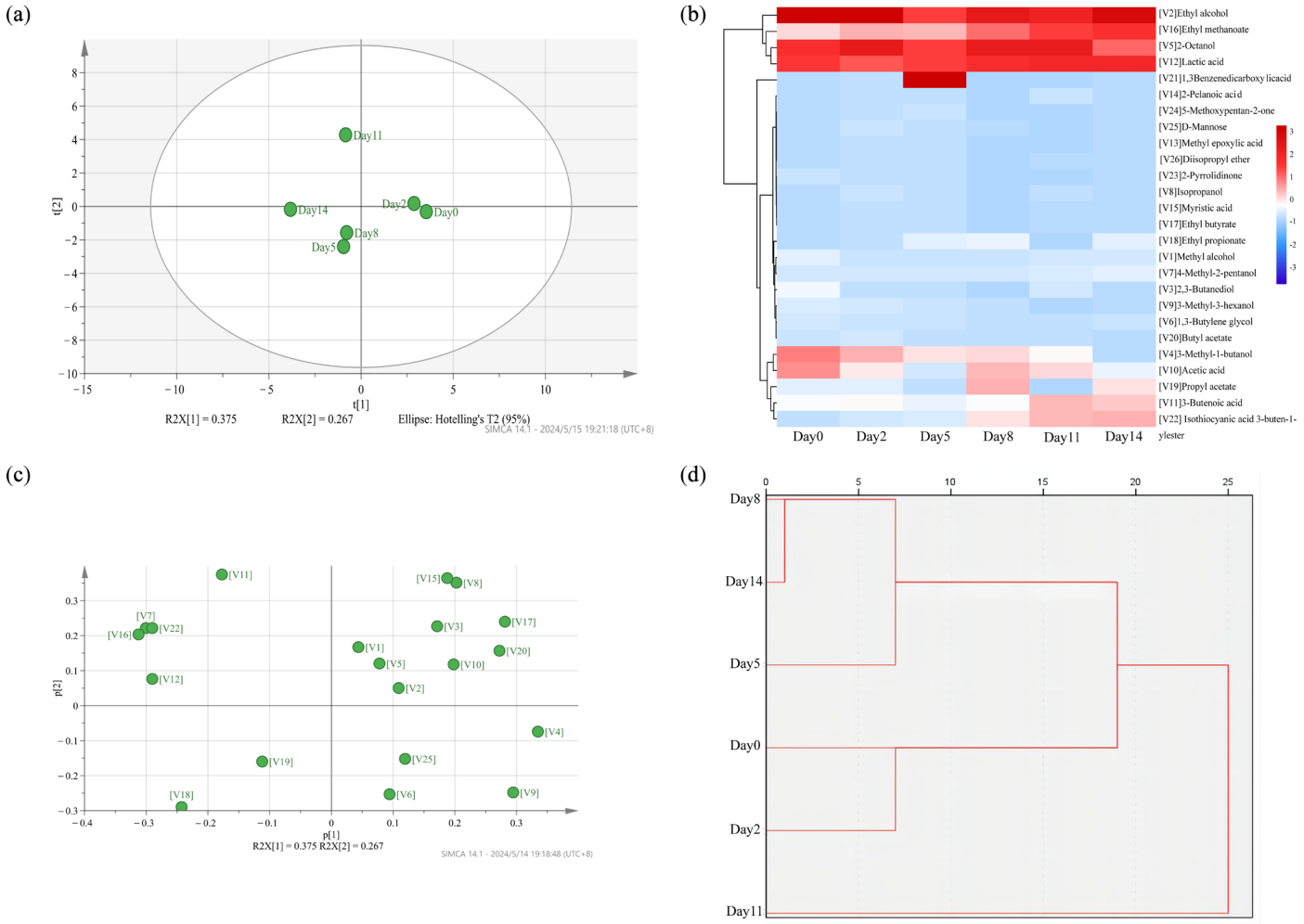

Figure 3. PCA (**a**), thermogram of volatile flavouring substances (**b**), loadings of volatile flavouring substances (**c**), and systematic clustering diagram (**d**) during fermentation of western Sichuan Yi suancai.

3.4. Dynamic Changes in Microbial Community Structure during Fermentation of Western Sichuan Yi Suancai

The taxonomy of each OTU-representative sequence was analysed against the 16S rRNA gene database (Silva v138) and the fungal UNITE database (v8.0) using RDP Classifier version 2.2 with a confidence threshold of 0.7. The base proportions of both Q20 and Q30 were above 96%, indicating that the sequencing data of the samples were of good quality (Table S2).

3.4.1. Dynamics of Bacterial Communities

High-throughput sequencing was performed on bacteria from western Sichuan Yi suancai juice during the six fermentation periods. Valid sequences were obtained by distinguishing the samples using the barcodes at the beginnings and ends of the sequences and

correcting the sequence orientations. A total of 267,709 valid sequences were obtained, with an average of about 44,618 contained in each sample, which were mainly concentrated in the interval of 421–440 bp. After clustering the OTUs, the number of taxonomic units was counted, which resulted in 1 domain, 1 kingdom, 5 phyla, 7 classes, 16 orders, 22 families, 30 genera, 46 species, and 56 OTUs. As can be seen in Figure 4a, there were five dominant bacterial genera (relative abundance ≥1%) in the six fermentation periods of the western Sichuan Yi suancai, which were *Lactobacillus*, *Leuconostoc*, *Weissella*, *Klebsiella*, and *unclassified__o_Lactobacillales*. They were similar to the dominant genera in Chaozhou kimchi [33]. *Lactobacillus* showed a decreasing and then increasing trend as the suancai fermentation proceeded, decreasing from 82.03% on day 0 to 75.00% on day 2 and finally rebounding to 96.68% on day 14. The maximum abundance of *Leuconostoc* (19.28%) occurred on day 2. The abundance of *Weissella* reached its maximum value of 5.91% at the beginning of the fermentation and did not exceed 1% in any of the five subsequent periods. The maximum abundances of both *Klebsiella* and *unclassified__o_Lactobacillales* occurred on day 2, at 3.22% and 1.36%, respectively.

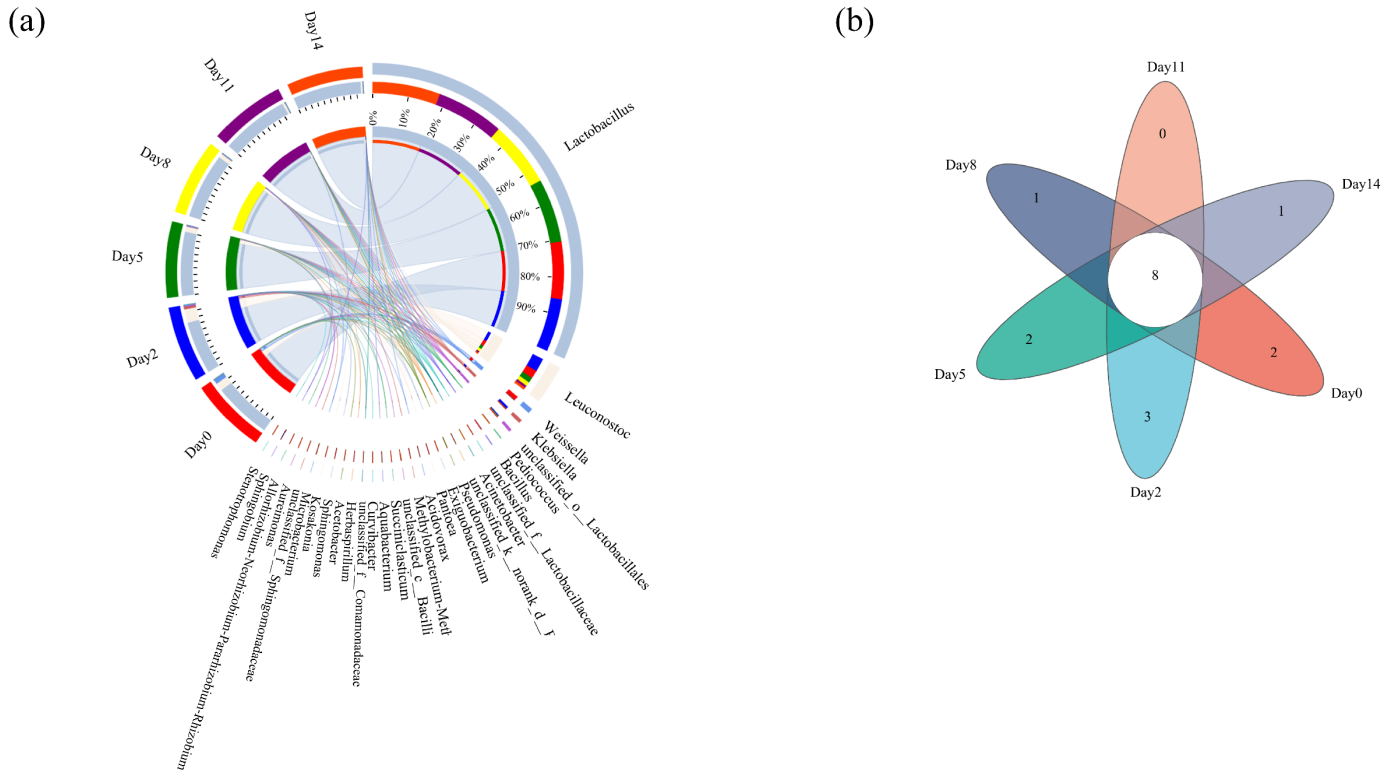

Figure 4. Circos (**a**) and Venn diagrams (**b**) of bacterial communities during fermentation of western Sichuan Yi suancai. Note: The different colours represent different periods and the numbers represent common genera at certain stages.

Lactobacillus plays a key role in fermented and pickled foods such as suancai [34,35]. It produces a variety of metabolites with anti-microbial activity, such as bacteriocins, hydrogen peroxide, and organic acids, which have been shown to have inhibitory effects on pathogens [36]. For example, bacteriocins are a class of small-molecule proteins that kill other bacteria or inhibit their growth. Lactic acid bacteria produce lactic acid as a by-product of their metabolism, which causes a decrease in the pH of the environment. This decrease in pH creates an acidic environment, which is detrimental to the growth of many pathogens, including *Klebsiella* [37]. *Leuconostoc* is a major producer of flavouring substances such as acetoin and diacetyl [38]. In fermentation experiments involving suancai with the addition of garlic, its growth was promoted, but the citric acid content was reduced [39]. *Weissella* is capable of adapting to environments with different oxygen

concentrations and produces lactic acid, ethanol, and acetic acid during the fermentation process, which improve the taste and flavour of food products [40] and were previously detected in fermented sausages [41]. As for *Klebsiella*, although it has a certain pathogenicity, its activity is only strong in conditions with sufficient oxygen and a high pH, so its concentration peaks at the beginning of fermentation and gradually decreases thereafter [42]. *Unclassified__o_Lactobacillales* was previously detected during milk fermentation, but its specific metabolic mechanisms have not been resolved in detail [43]. Many metabolic pathways during suancai fermentation are associated with low-abundance genera such as *Pseudomonas* and *unclassified__f__Lactobacillaceae*. These microorganisms are involved in amino acid metabolism, fatty acid synthesis, and sugar catabolism [44], and these pathways promote the formation of flavouring substances, thereby enhancing the taste and flavour of suancai.

As shown in Figure 4b, the six stages of the suancai fermentation process were shared by eight bacterial genera, namely Lactobacillus, Pediococcus, unclassified__f__Lactobacillaceae, Leuconostoc, Weissella, unclassified__o___Lactobacillales, Klebsiella, and unclassified__k__norank__d__Bacteria. On day 0, the exclusive bacterial genera were Acetobacter and Curvibacter. Acetobacter is capable of glycolysis, gluconeogenesis, and pyruvate metabolism, which helps to reduce the production of undesirable substances such as capric, caprylic, and butyric acids, thereby improving the taste and odour of fermented products [45]. By day 2, the new bacterial genera included unclassified__c__Bacilli, unclassified__f__Sphingomonadaceae, and Stenotrophomonas. On day 5, we could observe the occurrence of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium and Kosakonia. The salt concentrations in fermentation products can significantly affect the abundance of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium [46]. On day 8, the endemic bacterial genus was Methylobacterium-Methylorubrum. No new bacterial genera appeared on day 11, and on day 14, Aquabacterium became the endemic bacterial genus. The exact mechanism of the effect of Aquabacterium on suancai fermentation has not been clarified. However, it is able to synthesise glucan branching enzymes and can regulate the hydrolysis of starch [47]. Among the above bacterial genera, the presence of unclassified_c__Bacilli, Stenotrophomonas, and other genera thought to be able to contaminate suancai [48,49] may be indicative of immature conditions, such as the environment or the process by which the suancai is prepared, leading to the emergence of these bacteria. However, the growth and multiplication of these bacteria were effectively inhibited under low-oxygen and low-pH conditions during the later stages of fermentation.

3.4.2. Dynamics of Fungus Communities

High-throughput sequencing analyses of fungal taxa were carried out on six samples of western Sichuan Yi suancai juice from different periods, and a total of 714,765 valid sequences were obtained, with each sample containing about 119,127 sequences on average. The lengths of these sequences were mainly concentrated in the range of 141–160 bp. After clustering the OTUs and counting the bioclassification units, the results were as follows: 1 domain, 1 kingdom, 6 phyla, 20 classes, 37 orders, 60 families, 81 genera, 96 species, and 114 OTUs. It is noteworthy that the number of OTUs was significantly higher than that of the bacterial community, suggesting higher fungal diversity. As can be seen in Figure 5a, there were nine dominant fungal genera (relative abundance $\geq$1%) in the samples of western Sichuan Yi suancai juice from the six fermentation periods, namely *Dipodascaceae_gen_Incertae_sedis*, *Mucor*, *Pichia*, *unclassified_f__Dipodascaceaet*, *Cyberlindnera*, *Diutina*, *Trichosporon*, *Saccharomycopsis*, and *Wickerhamomyces*. This result had some similarities with previous studies [50,51]. By analysing the relative abundances of the fungi during the six fermentation periods of the western Sichuan Yi suancai, it was found that the relative abundance of *Dipodascaceae_gen_Incertae_sedis* was relatively low during the first two periods, at about 7%, but increased significantly from day 5 onwards and reached a maximum value of 76.96% on day 8. In contrast, *Mucor* was more abundant during the first two periods, especially on day 0, when it reached 78.99%. This was followed by a

significant decrease in abundance during the next five periods. The relative abundance of *Pichia* showed an increasing and then decreasing trend, with a peak abundance of 33.62% on the second day. *Unclassified_f__Dipodascaceae* first appeared on day 2 and showed a significant upward trend, peaking at 18.47% abundance on day 14. *Cyberlindnera* had an abundance of 8.72% on day 2 and abundances lower than 1% during the five remaining periods. As for the four fungi, *Diutina*, *Trichosporon*, *Saccharomycopsis*, and *Wickerhamomyces*, their relative abundances varied insignificantly, but all of them were relatively high during the pre-fermentation period.

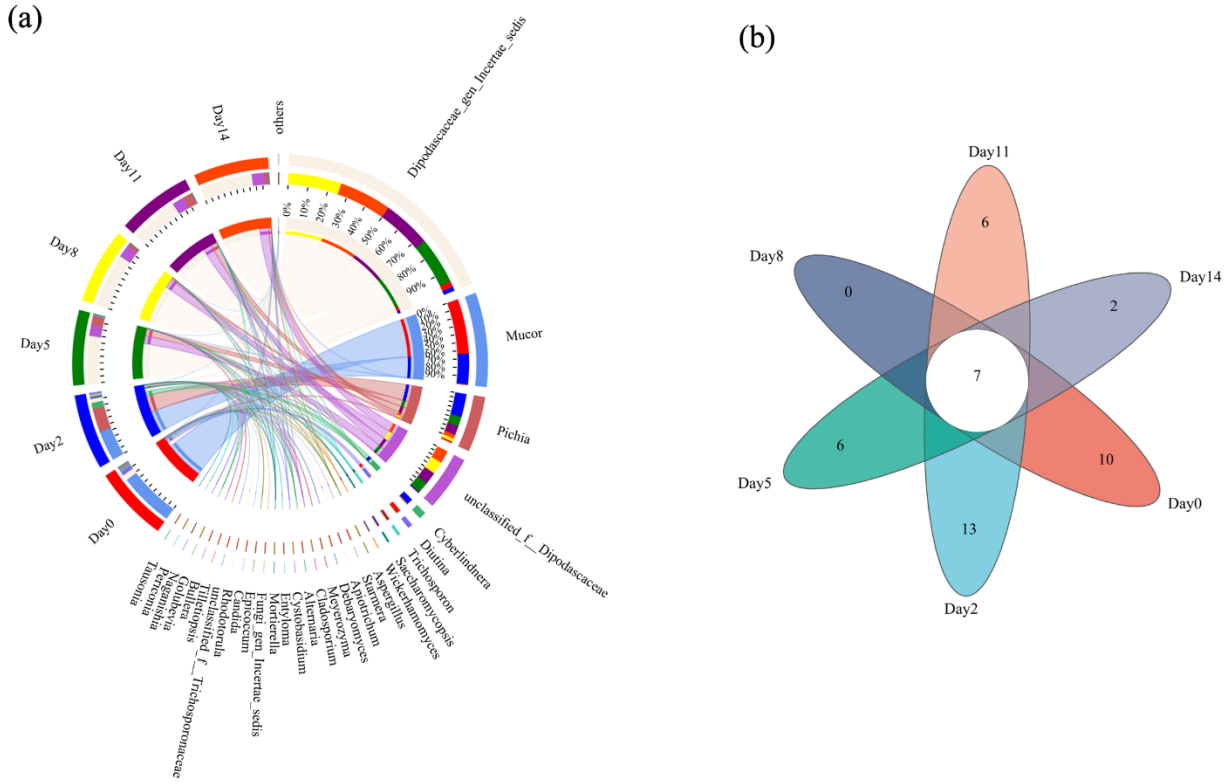

Figure 5. Circos (**a**) and Venn diagrams (**b**) of fungal communities during fermentation of western Sichuan Yi suancai. Note: The different colours represent different periods and the numbers represent common genera at certain stages.

Mucor dominates the pre-fermentation phase of suancai, secreting protease and lipase that break down proteins and fats, thereby increasing the nutritional value and flavour of the product [52]. *Mucor* is often found in fermented products such as tofu and soybeans [53]. *unclassified_f__Dipodascaceae* is rarely reported during suancai fermentation, but it is a dominant genus during sufu fermentation [54]. *unclassified_f__Dipodascaceae* is positively correlated with increases in substances such as ethyl 4-methylvalerate and nonanal, which contribute to the fruity flavour of fermented products [55]. *Cyberlindnera* has previously been found during wine production [56], but its specific metabolic mechanisms have not been thoroughly investigated. *Pichia*, *Cyberlindnera*, *Diutina*, *Saccharomycopsis*, and *Wickerhamomyces* all belong to a group of yeasts that are the main contributors of flavour substances in fermented products. These yeasts produce higher alcohols, esters, aldehydes, and acids during the fermentation process, further enhancing the taste and flavour of the product [57]. In addition, *Pichia* boosts the activity of Lactobacillus, which promotes terpene synthesis [58]. During the middle and late stages of suancai fermentation, *Dipodascaceae_gen_Incertae_sedis* becomes the dominant genus, but relatively little research has been conducted on this fungus. The *Dipodascaceae* family is the dominant fungal genus in kefir grains, and the production of 3-methyl-1-butanol may be associated with a high abundance of this family [59]. Some less abundant genera also play important roles in the

formation of suancai aroma. For example, *Aspergillus* is able to assist in the production of 1-octen-3-ol and 3-octanone, both of which impart mushroom and floral flavours [60]. In addition, *Starmera* shows a greater ester production ability than *Saccharomyces cerevisiae*, which can produce, for example, ethyl acetate and isoamyl acetate [61], and *Apiotrichum* has previously been found in fermentation tests of persimmon vinegar [62].

As can be seen in Figure 5b, seven fungal genera co-existed during the six fermentation stages of the suancai, including *Cladosporium*, *Alternaria*, *Dipodascaceae_gen_Incertae_sedis*, *Cyberlindnera*, *Wickerhamomyces*, *Pichia*, and *Trichosporon*. A variety of fungi were produced during suancai fermentation as follows: 10 species on day 0, 13 species on day 2, 6 species on day 5, no new fungi on day 8, 6 species on day 11, and 2 species on day 14. *Cystofilobasidium*, which occurs during the pre-fermentation phase of suancai, has also been detected in barley and has been noted to be effective in enhancing its flavour [63]. Among the fungi that appear during the middle and late stages of fermentation, *Papiliotrema* is believed to contribute to the formation of strawberry flavour substances, including compounds such as alcohols and esters [64]. In addition, *Cutaneotrichosporon* is able to adapt to a variety of harsh environments and possesses the ability to produce lipids [65].

3.5. Relevance Analysis

In order to further clarify the relationships between the microbial communities and the physicochemical properties and flavour substances of the western Sichuan Yi suancai, a microbial co-occurrence network analysis based on Spearman correlations ($|\rho| > 0.5$ and $p < 0.05$) was carried out on the suancai microorganisms at the genus level, and the results are shown in Figure 6.

As can be seen in Figure 6a, significant correlations were found between 12 microorganisms and six physicochemical properties, and three types of flavour substances. Among these microorganisms, the correlations between *Leuconostoc* and physicochemical properties were the most significant. Specifically, *Leuconostoc* showed a highly significant ($p < 0.01$) positive correlation with pH; a significant ($p < 0.05$) negative correlation with the superoxide radical scavenging ability; and highly significant ($p < 0.01$) negative correlations with the DPPH radical scavenging ability, the reducing capacity, and the hydroxyl radical scavenging ability. In terms of flavour substance species, acids had the highest correlation with bacteriophages. They showed a significant ($p < 0.05$) positive correlation with *Microbacterium* and significant ($p < 0.05$) negative correlations with *Aureimonas*, *Succiniclasticum*, and *unclassified_f__Lactobacillaceae*. In addition, *Lactobacillus* showed highly significant ($p < 0.01$) positive correlations with the DPPH radical scavenging ability, the reducing capacity, and the hydroxyl radical scavenging ability; a highly significant ($p < 0.05$) positive correlation with the superoxide radical scavenging ability; a highly significant ($p < 0.05$) positive correlation with the superoxide radical scavenging ability; and a highly significant ($p < 0.01$) negative correlation with pH. The trends of the effects for *Pediococcus* were the opposite of those seen for *Lactobacillus*. Finally, *Herbaspirillum* showed significant ($p < 0.05$) positive correlations with esters and ketones, suggesting that it may have a positive effect on the formation of specific flavour substances in suancai. It is worth noting that there were no significant relationships between the alcohols and these microorganisms at $|\rho| > 0.5$ and $p < 0.05$, so they are not shown on the graph.

As can be seen in Figure 6b, there were significant correlations between 25 microorganisms and six physicochemical properties and four flavour substance types. The fungus with the highest correlation with physicochemical properties was *Alternaria*, which showed a positive correlation with pH and negative correlations with the DPPH radical scavenging ability, the reducing capacity, the hydroxyl radical scavenging ability, and the superoxide radical scavenging ability. In terms of flavour substances, the species with the highest correlation with fungal genera was acids, which were negatively correlated with most fungal genera. In addition, highly significant ($p < 0.01$) positive correlations were found between *unclassified__f__Dipodascaceae* and the DPPH radical scavenging ability, *unclassified__f__Dipodascaceae* and the hydroxyl radical scavenging ability, and *Mortierella*

and alcohols. Highly significant ($p < 0.01$) negative correlations were observed between *Alternaria* and the reducing capacity, *Mucor* and the superoxide radical scavenging ability, *Mucor* and the ABTS radical scavenging ability, *unclassified_f__Dipodascaceae* and pH, *Cyberlindnera* and acids, *Aspergillus* and esters, and *Aspergillus* and ketones.

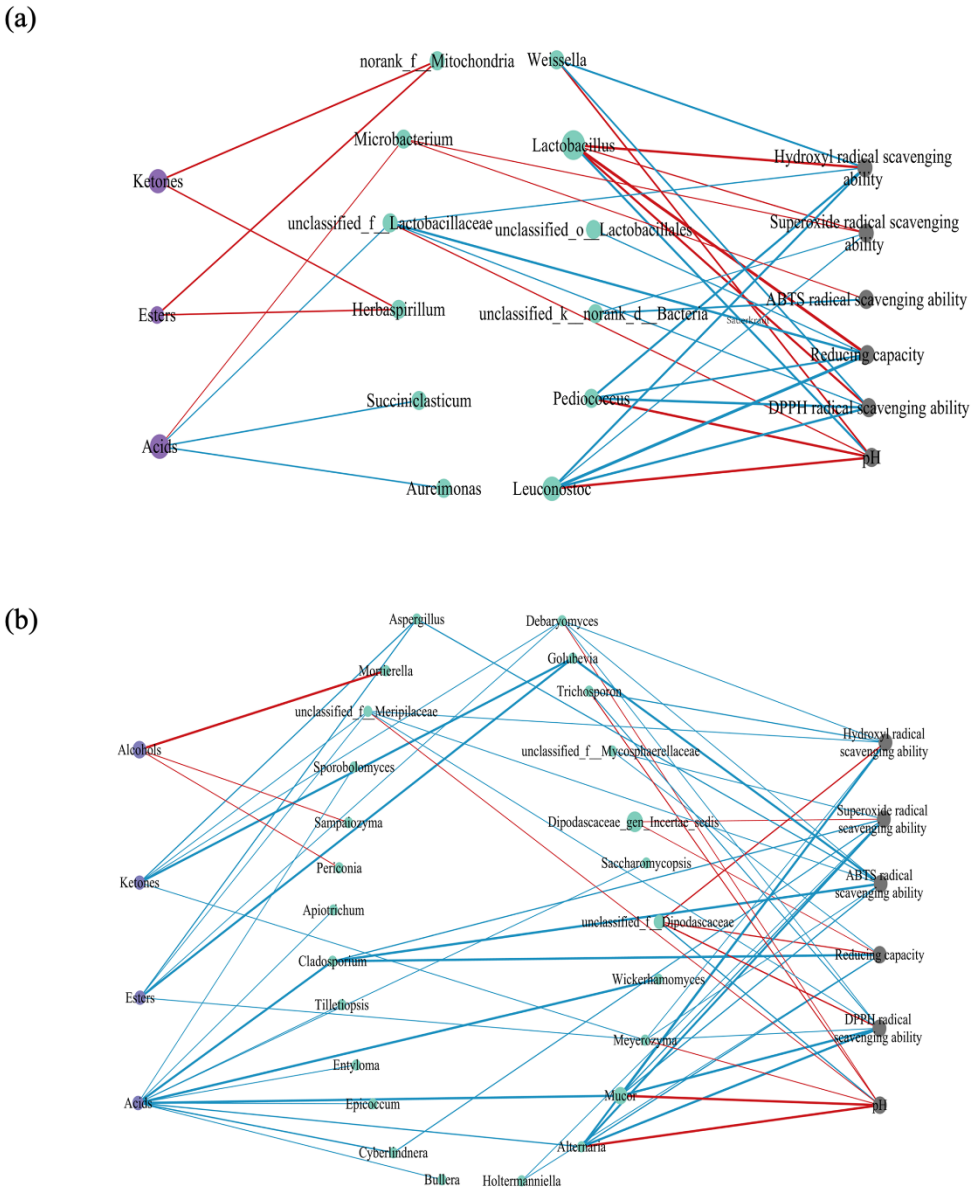

Figure 6. Correlation network analyses of bacterial genera (**a**) and fungal genera (**b**) with physicochemical properties and flavour substance species. Note: The size of a node indicates its relative abundance. Red represents a positive correlation, and blue represents a negative correlation. The thickness of a line represents the correlation.

In summary, two groups of microorganisms, *Lactobacillus* and *unclassified_f__Dipodascaceae*, showed significant negative correlations with pH, implying that a decrease in pH may be closely related to their growth and metabolic activities. *Lactobacillus*, *unclassified__f__Dipodascaceae*, and *Dipodasca-ceae_gen_Incertae_sedis* were positively correlated with the antioxidant activity of the suancai and may have been closely related to the strength of its antioxidant properties. In addition, *Microbacterium*, *Herbaspirillum*, *Mortierella*, *Sam-paiozyma*, and *Periconia* showed positive correlations with alcohols, esters, ketones, and acids, indicating that these genera play key roles in the production of flavour substances during suancai fermentation.

4. Conclusions

Due to the open fermentation environment and the dynamic fermentation process, the microbial community of the western Sichuan Yi suancai showed obvious diversity and dynamics. In this study, the basic physicochemical indicators and microbial community structure were detected in real time during the fermentation process of western Sichuan Yi suancai, and the correlations between the dominant microorganisms and the physicochemical properties, as well as volatile flavour substances, were comprehensively analysed, which strengthened the scientific research on the fermentation process of Yi suancai, improved the quality and safety of Yi suancai, and promoted the inheritance and development of a traditional dietary culture. However, this study only focused on the microbial community succession during the fermentation of western Sichuan Yi suancai, and the microbial metabolic pathways were not deeply investigated. Future studies could further explore the specific roles of these microorganisms during suancai fermentation and how to optimise the quality and flavour of suancai by controlling the microbial diversity during fermentation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10070353/s1, Table S1: Quantitative parameters of organic acid standards; Table S2: Quality analysis of sequencing data; Figure S1: The internal standards of the flavouring substances investigated in this work; Figure S2: Chart of flavouring substance samples.

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References

1. Ma, Q.; Zhu, Y.-T.; Li, Y.-D.; Zhang, Z.-L.; Huang, J.; Zuo, Y. Quantification of heavy metals and health risk assessment in Sichuan pickle. *J. Food Sci.* **2022**, *87*, 2229–2244. [CrossRef] [PubMed]
2. Rao, Y.; Qian, Y.; Tao, Y.; She, X.; Li, Y.; Che, Z.; Li, H.; Liu, L. Influence of oxygen exposure on fermentation process and sensory qualities of Sichuan pickle (paocai). *RSC Adv.* **2019**, *9*, 38520–38530. [CrossRef] [PubMed]
3. Zhao, M.; Cao, X.; Wu, Y.; Zou, S.; Li, Z.; Lin, X.; Ji, C.; Dong, L.; Zhang, S.; Yu, C.; et al. Effects of prebiotics on the fermentation of traditional suancai of Northeast China. *Food Sci. Hum. Wellness* **2024**, *13*, 1358–1367. [CrossRef]
4. Song, G.; He, Z.; Wang, X.; Zhao, M.; Cao, X.; Lin, X.; Ji, C.; Zhang, S.; Liang, H. Improving the quality of Suancai by inoculating with Lactobacillus plantarum and Pediococcus pentosaceus. *Food Res. Int.* **2021**, *148*, 110581. [CrossRef] [PubMed]
5. Cao, X.; Zhao, M.; Zou, S.; Li, Z.; Wu, Y.; Ji, C.; Chen, Y.; Dong, L.; Zhang, S.; Liang, H. Effect of Autochthonous Lactic Acid Bacteria-Enhanced Fermentation on the Quality of Suancai. *Foods* **2022**, *11*, 3310. [CrossRef] [PubMed]
6. Xiong, S.; Xu, X.; Du, T.; Liu, Q.; Huang, T.; Ren, H.; Xiong, T.; Xie, M. Organic acids drove the microbiota succession and consequently altered the flavor quality of Laotan Suancai across fermentation rounds: Insights from the microbiome and metabolome. *Food Chem.* **2024**, *450*, 139335. [CrossRef] [PubMed]
7. Liang, H.; He, Z.; Wang, X.; Song, G.; Chen, H.; Lin, X.; Ji, C.; Zhang, S. Bacterial profiles and volatile flavor compounds in commercial Suancai with varying salt concentration from Northeastern China. *Food Res. Int.* **2020**, *137*, 109384. [CrossRef] [PubMed]

8. He, Z.; Chen, H.; Wang, X.; Lin, X.; Ji, C.; Li, S.; Liang, H. Effects of different temperatures on bacterial diversity and volatile flavor compounds during the fermentation of suancai, a traditional fermented vegetable food from northeastern China. *LWT* **2020**, *118*, 108773. [CrossRef]
9. Xiao, M.; Huang, T.; Huang, C.; Hardie, J.; Peng, Z.; Xie, M.; Xiong, T. The microbial communities and flavour compounds of Jiangxi yancai, Sichuan paocai and Dongbei suancai: Three major types of traditional Chinese fermented vegetables. *LWT* **2020**, *121*, 108865. [CrossRef]
10. Tu, C.; Yu, T.; Feng, S.; Xu, N.; Massawe, A.; Shui, S.; Zhang, B. Dynamics of microbial communities, flavor, and physicochemical properties of kombucha-fermented Sargassum fusiforme beverage during fermentation. *LWT* **2024**, *192*, 115729. [CrossRef]
11. Zhou, H.; Wang, S.; Liu, W.; Chang, L.; Zhu, X.; Mu, G.; Qian, F. Probiotic properties of Lactobacillus paraplantarum LS-5 and its effect on antioxidant activity of fermented sauerkraut. *Food Biosci.* **2023**, *52*, 102489. [CrossRef]
12. Zong, K.; Jin, F.; Wang, D.; Hu, H.; Cui, H.; Yang, J. Effects of different pickling methods on physicochemical properties and flavor profiles of Tongling white ginger: Dry-salting, brine-pickling, and inoculation-pickling. *Food Sci. Nutr.* **2024**, *12*, 2597–2610. [CrossRef] [PubMed]
13. Luo, W.; Wu, W.; Du, X.; Yu, Y.; Wu, J.; Xu, Y.; Li, L. Regulation of the nitrite, biogenic amine and flavor quality of Cantonese pickle by selected lactic acid bacteria. *Food Biosci.* **2023**, *53*, 102554. [CrossRef]
14. Wang, X.; Dang, C.; Liu, Y.; Ge, X.; Suo, R.; Ma, Q.; Wang, J. Effect of indigenous Saccharomyces cerevisiae strains on microbial community successions and volatile compounds changes during Longyan wine fermentation. *Food Biosci.* **2024**, *57*, 1272127. [CrossRef]
15. Okoye, C.O.; Wang, Y.; Gao, L.; Wu, Y.; Li, X.; Sun, J.; Jiang, J. The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. *Microbiol. Res.* **2023**, *266*, 127212. [CrossRef]
16. Zhao, N.; Ge, L.; Lai, H.; Wang, Y.; Mei, Y.; Huang, Y.; Zeng, X.; Su, Y.; Shi, Q.; Li, H.; et al. Unraveling the contribution of pre-salting duration to microbial succession and changes of volatile and non-volatile organic compounds in Suancai (a Chinese traditional fermented vegetable) during fermentation. *Food Res. Int.* **2022**, *159*, 111673. [CrossRef] [PubMed]
17. Wang, Y.; Yang, G.; Shen, H.; Liang, Y.; Dong, H.; Guo, X.; Hao, Q.; Wang, J. Hybrids of selective COX-2 inhibitors and active derivatives of edaravone as COX-2 selective NSAIDs with free radical scavenging activity: Design, synthesis and biological activities. *Eur. J. Med. Chem.* **2024**, *266*, 116155. [CrossRef] [PubMed]
18. Xu, X.; Guo, Y.; Chen, M.; Li, N.; Sun, Y.; Ren, S.; Xiao, J.; Wang, D.; Liu, X.; Pan, Y. Hypoglycemic activities of flowers of Xanthoceras sorbifolia and identification of anti-oxidant components by off-line UPLC-QTOF-MS/MS-free radical scavenging detection. *Chin. Herb. Med.* **2024**, *16*, 151–161. [CrossRef] [PubMed]
19. Kong, Y.; Zhang, L.-L.; Sun, Y.; Zhang, Y.-Y.; Sun, B.-G.; Chen, H.-T. Determination of the Free Amino Acid, Organic Acid, and Nucleotide in Commercial Vinegars. *J. Food Sci.* **2017**, *82*, 1116–1123. [CrossRef]
20. Zhao, Y.; Zhao, Z.; Gao, Y.; Yang, G.; Liu, X.; Huang, R.; Liang, W.; Li, S. Assessment of autochthonous lactic acid bacteria as starter culture for improving traditional Chinese Dongbei Suancai fermentation. *LWT* **2023**, *178*, 114615. [CrossRef]
21. Zheng, Z.; Zhou, Q.; Li, D.; Wu, Y.; Zhong, K.; Gao, H. Effects of enhanced fermentation with Lactiplantibacillus plantarum WWPC on physicochemical characteristics and flavor profiles of radish paocai and dried-fermented radish. *Food Biosci.* **2024**, *59*, 103941. [CrossRef]
22. Liu, Y.; Chen, X.; Li, F.; Shi, H.; He, M.; Ge, J.; Ling, H.; Cheng, K. Analysis of Microbial Diversity and Metabolites in Sauerkraut Products with and without Microorganism Addition. *Foods* **2023**, *12*, 1164. [CrossRef] [PubMed]
23. Satora, P.; Strnad, S. The Influence of Fermentation Vessels on Yeast Microbiota and Main Parameters of Sauerkraut. *Appl. Sci.* **2023**, *14*, 236. [CrossRef]
24. Tkacz, K.; Chmielewska, J.; Turkiewicz, I.P.; Nowicka, P.; Wojdyło, A. Dynamics of changes in organic acids, sugars and phenolic compounds and antioxidant activity of sea buckthorn and sea buckthorn-apple juices during malolactic fermentation. *Food Chem.* **2020**, *332*, 127382. [CrossRef] [PubMed]
25. Han, Y.; Wang, C.; Zhang, X.; Li, X.; Gao, Y. Characteristic volatiles analysis of Dongbei Suancai across different fermentation stages based on HS-GC-IMS with PCA. *J. Food Sci.* **2022**, *87*, 612–622. [CrossRef] [PubMed]
26. Xing, Y.; Qiu, Y.; Yue, T.; Yi, R.; Xu, Q.; Rao, Y.; Huang, J.; Pan, H. Correlation between microbial communities and volatile flavor compounds in the fermentation of new pickle fermentation broth. *Food Biosci.* **2023**, *56*, 103167. [CrossRef]
27. Zhao, X.; Wang, Z.; Tang, F.; Cai, W.; Peng, B.; Shan, C. Exploring jujube wine flavor and fermentation mechanisms by HS-SPME-GC–MS and UHPLC-MS metabolomics. *Food Chem. X* **2024**, *21*, 101115. [CrossRef]
28. Yang, Y.; Wang, H.; Shuang, Q.; Xia, Y. Novel insights into flavor formation in whey fermented wine: A study of microbial metabolic networks. *LWT* **2024**, *197*, 115911. [CrossRef]
29. Qu, J.; Chen, X.; Wang, X.; He, S.; Tao, Y.; Jin, G. Esters and higher alcohols regulation to enhance wine fruity aroma based on oxidation-reduction potential. *LWT* **2024**, *200*, 116165. [CrossRef]
30. Wang, L.; Yin, H.; Shao, X.; Zhang, Z.; Zhong, X.; Wei, R.; Ding, Y.; Wang, H.; Li, H. Improving the aging aroma profiles of Italian Riesling and Petit Verdot Wines: Impact of spontaneous and inoculated fermentation processes. *Food Chem. X* **2023**, *20*, 100978. [CrossRef] [PubMed]
31. Li, P.; Jia, Y.; Cai, D.; Wang, X.; Liu, J.; Zhu, R.; Wang, Z.; He, Y.; Wen, L. Study on the relationship between flavor components and quality of ice wine during freezing and brewing of 'beibinghong' grapes. *Food Chem. X* **2023**, *20*, 101016. [CrossRef] [PubMed]

32. Liao, H.; Luo, Y.; Asif, H.; Luo, Y.; Xia, X. Novel insights into safety and quality enhancement of low-salt fermented chilies: High-order positively interacting lactic acid bacteria co-fermentation regulates microflora structure, metabolomics, and volatilomics profiles. *Food Biosci.* **2024**, *59*, 103861. [CrossRef]
33. Chen, Q.; Li, Y.; Yan, K.; Li, G.; Luo, D.; Bai, W.; Zeng, X.; Wu, Q.; Deng, J.; Dong, H. Variations of volatile flavors and microbial communities in Chinese Chaozhou pickle during natural fermentation revealed by GC-IMS and high-throughput sequencing. *LWT* **2024**, *191*, 115610. [CrossRef]
34. Liu, L.; She, X.; Chen, X.; Qian, Y.; Tao, Y.; Li, Y.; Guo, S.; Xiang, W.; Liu, G.; Rao, Y. Microbiota Succession and Chemical Composition Involved in the Radish Fermentation Process in Different Containers. *Front. Microbiol.* **2020**, *11*, 445. [CrossRef] [PubMed]
35. Li, J.; He, Z.; Yan, L.; He, Y.; Yang, J. Analysis of the microbial community structure and flavor components succession during salt-reducing pickling process of zhacai (preserved mustard tuber). *Food Sci. Nutr.* **2023**, *11*, 3154–3170. [CrossRef] [PubMed]
36. Petrova, P.; Arsov, A.; Tsvetanova, F.; Parvanova-Mancheva, T.; Vasileva, E.; Tsigoriyna, L.; Petrov, K. The Complex Role of Lactic Acid Bacteria in Food Detoxification. *Nutrients* **2022**, *14*, 2038. [CrossRef] [PubMed]
37. Zapaśnik, A.; Sokołowska, B.; Bryła, M. Role of Lactic Acid Bacteria in Food Preservation and Safety. *Foods* **2022**, *11*, 1283. [CrossRef] [PubMed]
38. Wätjen, A.P.; De Vero, L.; Carmona, E.N.; Sberveglieri, V.; Huang, W.; Turner, M.S.; Bang-Berthelsen, C.H. Leuconostoc performance in soy-based fermentations—Survival, acidification, sugar metabolism, and flavor comparisons. *Food Microbiol.* **2023**, *115*, 104337. [CrossRef] [PubMed]
39. Choi, H.-W.; Park, S.-E.; Kim, E.-J.; Seo, S.-H.; Cho, K.-M.; Kwon, S.J.; Lee, M.-H.; Son, H.-S. Effects of garlic on kimchi metabolites exerted through selective growth control of Lactobacillus and Leuconostoc. *LWT* **2024**, *198*, 116053. [CrossRef]
40. Nami, Y.; Panahi, B.; MohammadZadeh Jalaly, H.; Hejazi, M.A. Isolation and assessment of novel exopolysaccharide-producing Weissella confusa ABRIIFBI-96 isolated from an Iranian homemade dairy fermented food "Tof" as a main starter culture for probiotic fermented milk. *LWT* **2024**, *197*, 115910. [CrossRef]
41. Özpınar, F.B.; İspirli, H.; Kayacan, S.; Korkmaz, K.; Dere, S.; Sagdic, O.; Alkay, Z.; Tunçil, Y.E.; Ayyash, M.; Dertli, E. Physicochemical and structural characterisation of a branched dextran type exopolysaccharide (EPS) from Weissella confusa S6 isolated from fermented sausage (Sucuk). *Int. J. Biol. Macromol.* **2024**, *264*, 130507. [CrossRef] [PubMed]
42. Zong, C.; Tang, L.; Shao, T.; Xiao, Y.; Huang, Z.-Y.; Jiang, W.-Q.; Zhu, J.-G.; Dong, Z.-H.; Li, M.; Liu, Q.-H. Klebsiella as an α-tocopherol source facilitating Lactobacillus plantarum fermentation in rice straw silage1. *J. Integr. Agric.* in press. **2023**. [CrossRef]
43. Zheng, X.; Ge, Z.; Lin, K.; Zhang, D.; Chen, Y.; Xiao, J.; Wang, B.; Shi, X. Dynamic changes in bacterial microbiota succession and flavour development during milk fermentation of Kazak artisanal cheese. *Int. Dairy J.* **2021**, *113*, 104878. [CrossRef]
44. Wen, X.; Zhang, D.; Li, X.; Ding, T.; Liang, C.; Zheng, X.; Yang, W.; Hou, C. Dynamic changes of bacteria and screening of potential spoilage markers of lamb in aerobic and vacuum packaging. *Food Microbiol.* **2022**, *104*, 103996. [CrossRef] [PubMed]
45. Zhang, L.; Hong, Q.; Yu, C.; Wang, R.; Li, C.; Liu, S. Acetobacter sp. improves the undesirable odors of fermented noni (*Morinda citrifolia* L.) juice. *Food Chem.* **2023**, *401*, 134126. [CrossRef] [PubMed]
46. Mi, T.; Wang, D.; Yao, S.; Yang, H.; Che, Y.; Wu, C. Effects of salt concentration on the quality and microbial diversity of spontaneously fermented radish paocai. *Food Res. Int.* **2022**, *160*, 111622. [CrossRef] [PubMed]
47. Jiao, W.; Xie, F.; Gao, L.; Du, L.; Wei, Y.; Zhou, J.; He, G. Identification of core microbiota in the fermented grains of a Chinese strong-flavor liquor from Sichuan. *LWT* **2022**, *158*, 113140. [CrossRef]
48. Zhang, T.; Wu, H.; Ma, C.; Yang, Y.; Li, H.; Yang, Z.; Zhou, S.; Shi, D.; Chen, T.; Yang, D.; et al. Emergence of colistin-resistant Stenotrophomonas maltophilia with high virulence in natural aquatic environments. *Sci. Total Environ.* **2024**, *933*, 173221. [CrossRef] [PubMed]
49. Arias-Cartin, R.; Ferizhendi, K.K.; Séchet, E.; Pelosi, L.; Loeuillet, C.; Pierrel, F.; Barras, F.; Bouveret, E. Role of the Escherichia coli ubiquinone-synthesizing UbiUVT pathway in adaptation to changing respiratory conditions. *mBio* **2023**, *14*, e0329822. [CrossRef] [PubMed]
50. Yang, Y.; Fan, Y.; Li, T.; Yang, Y.; Zeng, F.; Wang, H.; Suo, H.; Song, J.; Zhang, Y. Microbial composition and correlation between microbiota and quality-related physiochemical characteristics in chongqing radish paocai. *Food Chem.* **2022**, *369*, 130897. [CrossRef] [PubMed]
51. Wang, D.; Chen, G.; Tang, Y.; Li, J.; Huang, R.; Ye, M.; Ming, J.; Wu, Y.; Xu, F.; Lai, X.; et al. Correlation between autochthonous microbial communities and flavor profiles during the fermentation of mustard green paocai (Brassica juncea Coss.), a typical industrial-scaled salted fermented vegetable. *LWT* **2022**, *172*, 114212. [CrossRef]
52. Chen, Z.; Song, J.; Ren, L.; Wang, H.; Zhang, Y.; Suo, H. Effect of the succession of the microbial community on physicochemical properties and flavor compounds of Mucor wutungkiao-fermented sufu. *Food Biosci.* **2023**, *51*, 102345. [CrossRef]
53. Heo, S.; Park, J.; Lee, K.-G.; Lee, J.-H.; Jeong, D.-W. Quality characteristics of soybean fermented by Mucor, Rhizopus, and Aspergillus from meju. *Heliyon* **2023**, *9*, e14092. [CrossRef] [PubMed]
54. Chen, Z.; Liu, L.; Du, H.; Lu, K.; Chen, C.; Xue, Q.; Hu, Y. Microbial community succession and their relationship with the flavor formation during the natural fermentation of Mouding sufu. *Food Chem. X* **2023**, *18*, 100686. [CrossRef] [PubMed]
55. Hu, X.; Liu, S.; Li, E. Microbial community succession and its correlation with the dynamics of flavor compound profiles in naturally fermented stinky sufu. *Food Chem.* **2023**, *427*, 136742. [CrossRef] [PubMed]

56. Vicente, J.; Ruiz, J.; Tomasi, S.; de Celis, M.; Ruiz-de-Villa, C.; Gombau, J.; Rozès, N.; Zamora, F.; Santos, A.; Marquina, D.; et al. Impact of rare yeasts in Saccharomyces cerevisiae wine fermentation performance: Population prevalence and growth phenotype of Cyberlindnera fabianii, Kazachstania unispora, and Naganishia globosa. *Food Microbiol.* **2023**, *110*, 104189. [CrossRef] [PubMed]
57. Zhao, W.; Liang, Z.; Qian, M.; Li, X.; Dong, H.; Bai, W.; Wei, Y.; He, S. Evolution of microbial communities during fermentation of Chi-flavor type Baijiu as determined by high-throughput sequencing. *LWT* **2022**, *170*, 114102. [CrossRef]
58. Xiao, Y.; Zhang, S.; Liu, Z.; Wang, T.; Cai, S.; Chu, C.; Hu, X.; Yi, J. Effect of inoculating Pichia spp. starters on flavor formation of fermented chili pepper: Metabolomics and genomics approaches. *Food Res. Int.* **2023**, *173*, 113397. [CrossRef] [PubMed]
59. Dertli, E.; Çon, A.H. Microbial diversity of traditional kefir grains and their role on kefir aroma. *LWT* **2017**, *85*, 151–157. [CrossRef]
60. Wang, Z.; Jin, Q.; Jiang, R.; Liu, Y.; Xie, H.; Ou, X.; Li, Q.; Liu, Z.; Huang, J. Characteristic volatiles of Fu brick tea formed primarily by extracellular enzymes during Aspergillus cristatus fermentation. *Food Res. Int.* **2024**, *177*, 113854. [CrossRef] [PubMed]
61. Gamero, A.; Quintilla, R.; Groenewald, M.; Alkema, W.; Boekhout, T.; Hazelwood, L. High-throughput screening of a large collection of non-conventional yeasts reveals their potential for aroma formation in food fermentation. *Food Microbiol.* **2016**, *60*, 147–159. [CrossRef] [PubMed]
62. Zhang, L.; Wang, M.; Song, H.; Liang, W.; Wang, X.; Sun, J.; Wang, D. Changes of microbial communities and metabolites in the fermentation of persimmon vinegar by bioaugmentation fermentation. *Food Microbiol.* **2024**, *122*, 104565. [CrossRef] [PubMed]
63. Hu, Y.; Jia, F.; Liu, Z.; Zhang, Y.; Li, L. Impact of storage time on microbial communities and flavor profiles in highland barley grains. *J. Stored Prod. Res.* **2024**, *107*, 102321. [CrossRef]
64. Xu, H.; Quan, Q.; Chang, X.; Ge, S.; Xu, S.; Wang, R.; Xu, Y.; Luo, Z.; Shan, Y.; Ding, S. Maintaining the balance of fungal community through active packaging film makes strawberry fruit pose pleasant flavor during storage. *Postharvest Biol. Technol.* **2024**, *211*, 112815. [CrossRef]
65. Xu, C.; Wang, Y.; Zhang, C.; Liu, J.; Fu, H.; Zhou, W.; Gong, Z. Highly-efficient lipid production from hydrolysate of Radix paeoniae alba residue by oleaginous yeast Cutaneotrichosporon oleaginosum. *Bioresour. Technol.* **2024**, *391*, 129990. [CrossRef] [PubMed]