

## Article

# Correlation Analysis of Microbiota and Volatile Flavor Compounds of Caishiji Soybean Paste

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**Abstract:** Microbial diversity plays a crucial part in the fermentation of Caishiji soybean paste (CSP). In the current study, the microbiota and volatile flavor compounds (VFCs) in CSP were identified through Illumina MiSeq sequencing and headspace gas chromatography–mass spectrometry. Five bacterial (*Bacillus*, *Tetragenococcus*, *Salinivibrio*, *Halomonas*, and *Staphylococcus*) and four fungal genera (*Aspergillus*, *Debaryomyces*, *Nigrospora*, and *Curvularia*) were revealed as dominant among the entire microbiome of CSP. More than 70 VFCs, including 8 acids, 15 esters, 8 alcohols, 14 aldehydes, 4 ketones, 5 phenols, and 20 miscellaneous VFCs were detected during the fermentation process. A total of 12 kinds of VFCs were identified in the odor activity value (OAV) analysis. The results of the correlation analysis between microbiota and VFCs indicated that *Bacillus*, *Tetragenococcus*, *Staphylococcus*, and *Aspergillus* were the main microbiota affecting the flavor of CSP. These results may serve as a reference for enhancing the quality of CSP.

**Keywords:** Caishiji soybean paste; volatile flavor compounds; Illumina MiSeq sequencing; correlations



**Citation:** Cai, J.; Han, Y.; Wu, W.; Wu, X.; Mu, D.; Jiang, S.; Li, X. Correlation Analysis of Microbiota and Volatile Flavor Compounds of Caishiji Soybean Paste. *Fermentation* **2022**, *8*, 196. <https://doi.org/10.3390/fermentation8050196>

Academic Editors: Xian Zhang and Zhiming Rao

Received: 8 April 2022

Accepted: 23 April 2022

Published: 27 April 2022

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## 1. Introduction

Soybean paste [1,2], *sufu* [3–5], soy sauce [6–8], and *douchi* [9–11] are traditional bean products consumed in China and other East Asian countries. Among them, in China, soybean paste products are indispensable condiments with unique flavors and abundant nutriment [12–14]. Soybean paste can be produced using three fermentation methods: traditional spontaneous fermentation, low-salt, solid-state fermentation, and high-salt, diluted-state fermentation. Traditional spontaneous fermentation is the common fermentation method currently adopted by most enterprises. Caishiji soybean paste (CSP) from Ma'anshan, Anhui Province, has been famous for hundred years. High-quality soybeans, cinnamon, anise, licorice, sugar, salt, and other additives are used to make traditional fermented CSP. Volatile flavor compounds (VFCs) determine the unique flavors of soybean pastes. The nutrition and flavor in the soybean paste determine its quality [15]. Many studies have performed the flavor analysis in soybean pastes. Jo et al. [16] analyzed the VFCs of Korean *doenjang* and found that the VFCs in traditional *doenjang* were mostly acids, aldehydes, phenols, pyrazines, and furans, whereas the VFCs in commercial *doenjang* were mostly ethyl esters, maltol, and ethanol. Lin et al. [17] investigated the nonvolatile organic acids and amino acids in Pixian bean paste and concluded that citric acid, glutamic acid, methionine, and proline were the main flavor compounds in the paste. Zhao et al. [18] compared the VFCs of Chinese soybean pastes and identified that there were seven kinds of VFCs in the naturally fermented sample, and these were higher than that in the inoculated samples. Using a complicated biological chain and non-biological reactions, the

macromolecular substances, such as protein, starch, and lipids, in soybean paste raw materials were decomposed to produce multifarious secondary metabolites and tiny molecules, including organic acids, amino acids, esters, alcohols, ketones, aldehydes, and acids.

The diversity of microorganisms plays a key role in soybean paste fermentation, especially in flavor formation. An analysis result of microbial proteins in *dajiang* revealed that the composition and variety of microbial proteins involved in the processes of protein synthesis, glycometabolism, and nucleic acid synthesis are different [19]. Hao et al. [20] studied the content of biogenic amines and the bacterial diversity in the naturally fermented farmhouse sauce as well as the interactions between biogenic amines and microorganisms during fermentation. The core microbiota, VFCs, and correlations between them were evaluated, and the analysis results may be used to improve the quality of *doubanjiang* [21]. Controlling the flavor is difficult due to the complexity of the microbial community throughout the fermenting procedure; thus, more attention should be paid to the correlation between the microbial community and VFCs [22].

There are few studies on the correlation between microorganisms and the flavor of soybean paste. Soybean paste types have distinct flavors, depending on the area in which they are produced and the technologies with which they are produced. In this work, in order to reveal the unique nutriment, flavors, and microbiota in CSP, the VFCs and microbiota in CSP at different fermentation stages were identified using headspace gas chromatography–mass spectrometry (HS-GC-MS) [23] and MiSeq sequencing [24], respectively. Additionally, the correlations between them were determined through correlation analysis.

## 2. Materials and Methods

### 2.1. Sample Preparation

The CSP samples were separated into six independent batches according to their fermentation stage: the beginning or the first, second, third, sixth, or eighth month of fermentation (CG0, CG1, CG2, CG3, CG6, and CG8, respectively). The samples were obtained from Anhui Ma'anshan Caishiji Foods, China. A multipoint sampling method was utilized (Figure S1), and the samples were kept at 4 °C for further analysis.

### 2.2. Identification of VFCs through HS-GC-MS

The fiber (50/30 µm DVB/CAR/PDMS) (Supelco, Inc., Bellefonte, PA, USA) was inserted into the headspace of a 20 mL glass vial containing a 2.0 g sample of CSP and 20 µL of 2,4,6-trimethylpyridine; the vial was sealed with a Teflon cover, and the sample was incubated at 70 °C for 60 min [25]. The GC-MS system (HP6890/5975C; Agilent, CA, USA) was used to analyze the VFCs according to previously reported methods with some modifications [26]. The initial oven temperature was 40 °C, which was retained for 5 min, raised by 5 °C/min to 60 °C, retained for 5 min, raised by 2 °C/min to 120 °C, retained for 5 min, raised by 10 °C/min to 250 °C, and retained for 3 min. The split ratio was 5:1. The temperature of the quadrupoles and transfer line was 150 °C and 250 °C, respectively. The energy of the electron hit was 70 eV, and the scanning range was 45–450 *m/z*.

### 2.3. Qualitative and Quantitative Analysis of VFCs

The retention index (*RI*) of C<sub>7</sub>–C<sub>40</sub> normal paraffin under the same GC-MS conditions was used to ascertain the *RI*s of all the VFCs, and they were compared with relevant reference values. Each *RI* was calculated as follows:

$$RI = 100n + 100 \times \frac{t_R - t_{Rn}}{t_{Rn+1} - t_{Rn}} \quad (1)$$

where *n* and *n* + 1 are the numbers of normal paraffin carbon atoms before and after the addition of the VFCs, respectively. *t<sub>Rn</sub>* and *t<sub>Rn+1</sub>* are *n*-alkane retention times, and *t<sub>R</sub>* is the retention time of the unknown in the chromatography (min; *t<sub>Rn</sub>* < *t<sub>R</sub>* < *t<sub>Rn+1</sub>*).

According to the internal standard content, the VFC content of each sample was computed as follows:

$$C = \frac{A_x \times C_0 \times V}{A_0 \times m \times 1000} \quad (2)$$

where  $C$  is the VFC content ( $\mu\text{g}/\text{kg}$ ),  $A_x$  is the peak area of the VFCs ( $\text{AU}\cdot\text{min}$ ),  $C_0$  is the mass concentration of the internal standard ( $0.996 \mu\text{g}/\mu\text{L}$ ),  $A_0$  is the peak area of the internal standard ( $\text{AU}\cdot\text{min}$ ),  $V$  is the injection amount of internal standard ( $\mu\text{L}$ ), and  $m$  is the quality of the sample ( $\text{g}$ ).

To evaluate the contribution of the VFCs to the flavor in CSP, the odor activity value (OAV) was computed as follows:

$$\text{OAV} = \frac{C}{T} \quad (3)$$

where  $C$  is the VFC content ( $\mu\text{g}/\text{kg}$ ), and  $T$  is the VFC detection threshold ( $\mu\text{g}/\text{kg}$ ).

#### 2.4. DNA Extraction, PCR Amplification, and Illumina MiSeq Sequencing Analysis

DNA extraction, PCR amplification, and Illumina MiSeq sequencing experiments were conducted on the basis of the previous work [27] with minor modifications (Omega Bio-tek, Norcross, GA, USA). The  $V_4$ – $V_5$  regions of bacterial 16S rRNA genes were amplified using the universal primers, 338 F and 806 R (GeneAmp 9700, Applied Biosystems, Foster City, CA, USA).

For each reaction, the following were used: a 20  $\mu\text{L}$  mixture containing 4  $\mu\text{L}$  of  $5 \times$  FastPfu Buffer, 2  $\mu\text{L}$  of 2.5-mM deoxynucleotide triphosphate, 0.8  $\mu\text{L}$  of forward primer (5  $\mu\text{M}$ ), 0.8  $\mu\text{L}$  of reverse primer (5  $\mu\text{M}$ ), 0.4  $\mu\text{L}$  of FastPfu Polymerase, 0.2  $\mu\text{L}$  of bovine serum albumin, 20  $\mu\text{L}$  of ddH<sub>2</sub>O, and 10 ng of template DNA. The response conditions were as follows: initial denaturation 95 °C for 3 min; for bacteria, 35 amplification cycles of 95 °C for 15 s; for fungus, 35 amplification cycles of 95 °C for 15 s; the last 55 °C for 30 s, 72 °C for 45 s, 72 °C for 10 min (Majorbio, Shanghai, China). The PCR products were extracted from 2% agarose gel, and amplicons were quantified using a QuantiFluor-ST fluorometer (Promega, Beijing, China). After amplification, the PCR products were sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

#### 2.5. Data Analysis

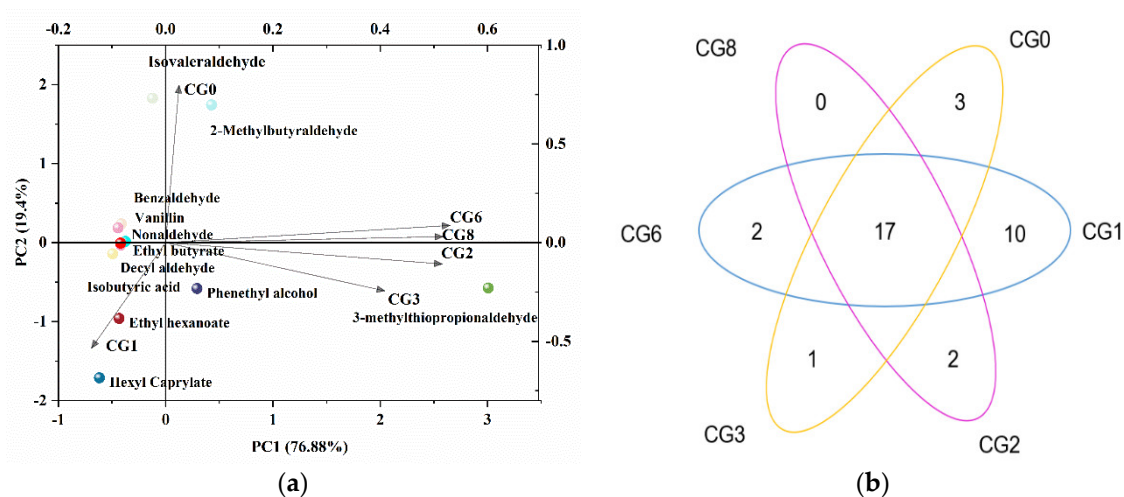
The aligned sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using the USERCH sequence analysis tool. The taxonomic classifications of the OTUs were studied by comparing the sequences with those in the SILVA and UNITE databases. The Sobs index and Circos data were calculated using Mothur software. R values were used to determine the Pearson correlation coefficients, and correlations of microbiota and VFCs were visualized by heat map. All the data are presented as the averages ( $\pm\text{SD}$ ) of three repeated tests. The SPSS software package (version 22.0; SPSS, IBM, Armonk, NY, USA) and the Origin software package (version 7.0; OriginLab, Northampton, MA, USA) were used for all statistical analyses.

### 3. Results

#### 3.1. VFC Changes during CSP Fermentation

More than 70 VFCs (including 8 acids, 15 esters, 8 alcohols, 14 aldehydes, 4 ketones, 5 phenols, and 20 miscellaneous VFCs) were detected during the CSP fermentation process (Table 1). As indicated in the Venn diagram in Figure 1a, 17 of the VFCs were common in CSP at all stages, and there were also their own unique VFCs in each stage samples (the quantity and composition of the VFCs are presented in Figures S1 and S2). Among the VFCs identified, esters, aldehydes, and alcohols were the main VFCs in CSP. All eight acids were detected in CG1, and there were only octanoic acid and nonanoic acid in CG6 and CG8. Esters, including amyl acetate,  $\alpha$ -pentyl- $\gamma$ -butyrolactone, ethyl palmitate, and diethyl phthalate, were the most abundant VFCs throughout the CSP fermentation process, whereas in the late stages of CSP fermentation, the relative abundance of the alcohols and aldehydes

was the highest. Furthermore, the analyzed VFC results revealed that aldehydes of which isovaleraldehyde, 2-methylbutyraldehyde, benzaldehyde, and phenylacetaldehyde were common in all fermentation stages, and they obviously contributed to the flavor of the CSP. In summary, all the VFCs identified in the CSP fermentation process exhibited distinct qualitative and quantitative characteristics, which may lead to diverse flavors of CSP.



**Figure 1.** Venn (a) and PCA (b) analysis of characteristic VFCs of Caishiji soybean paste in different fermentation stages. The samples were coded according to Section 2.

To assess the contribution of characteristic VFCs to the overall flavor of CSP, the OAV analysis was conducted according to the odor threshold values of the VFCs. The results revealed that CSP contains 12 kinds of VFCs: one acid, three esters, one alcohol, and seven aldehydes ( $OAV \geq 1$ , Table 2). Based on the OAV analysis results, the principal component analysis (PCA) was used to investigate the contribution rates of the characteristic VFCs to CSP flavor (Figure 1b). The two-dimension PCA model (F1 and F2) described 77.2% of the total variance, while the first and second principal components (F1 and F2) explained 57.8% and 19.4%, respectively. Isovaleraldehyde, 2-methylbutyraldehyde, and 3-methylthiopropionaldehyde greatly contributed to the flavor of CSP and were, therefore, included in the later correlation analysis.

### 3.2. Dynamics of Microbiota during CSP Fermentation

Illumina MiSeq sequencing was conducted to investigate the microbial diversity during the CSP fermentation. The aligned sequences were clustered into OTUs at a 97% similarity threshold. In total, 16 and 3 species of bacteria and fungi, respectively, were common to all stages during the CSP fermentation, and the species were most abundant in CG3 and CG6 (Figure 2 and Figure S3). Regarding the  $\alpha$ -diversity indices employed in this study, the Chao index and the Shannon index were used to quantify microbial richness and diversity, respectively. The red mark (Figure 2) indicates significant similarities and differences among the microbiota present at different stages of CSP fermentation. The samples exhibited higher microbial diversity at later stages of fermentation: bacterial diversity increased sharply in the CG3, CG6, and CG8 phases, and fungi diversity increased sharply in the CG6 and CG8 phases; the increasing trends in the other stages were relatively gently (Figure 2). Consistent with the dynamic changes in microbiota at different stages of CSP fermentation, the diversity of the bacteria was higher than that of the fungi (Tables S1 and S2).

**Table 1.** Volatile flavor compounds of Caishiji soybean paste in different fermentation stages by GC-MS.

T <sub>R</sub> (s)	Compounds	RI	RI*	Content (µg/kg)					
				CG-0	CG-1	CG-2	CG-3	CG-6	CG-8
Acids (8)									
3.223	Acetic acid		-	ND	14.50 ± 1.83	ND	ND	ND	ND
10.340	Isobutyric acid		-	ND	2.78 ± 1.67	ND	ND	ND	ND
18.196	Isovaleric acid	839.57	841	ND	3.89 ± 0.82	ND	ND	ND	ND
28.196	Hexanoic acid	972.62	967	ND	4.81 ± 0.55	ND	ND	ND	ND
40.684	Benzoic acid	1153.64	-	ND	0.70 ± 0.06	ND	ND	ND	ND
40.901	Octanoic acid	1176.25	1167	ND	2.40 ± 0.30	ND	ND	1.68 ± 0.30	7.51 ± 0.78
43.442	Phenylacetic acid	1247.63	-	ND	0.31 ± 0.08	ND	ND	ND	ND
43.859	Nonanoic acid	1275.3	1280	ND	0.57 ± 0.16	ND	ND	0.82 ± 0.27	1.19 ± 0.15
Esters (15)									
4.6465	Ethyl acetate	-	-	1.65 ± 2.00	ND	ND	7.24 ± 8.23	ND	ND
15.778	Ethyl butyrate	782.65	803	ND	ND	4.92 ± 1.61	ND	ND	ND
24.001	Amyl acetate	884.36	-	9.41 ± 1.58	0.86 ± 0.19	19.15 ± 8.75	103.82 ± 2.18	69.30 ± 33.72	22.37 ± 8.07
30.496	Ethyl hexanoate	984.83	1001	ND	6.97 ± 1.56	ND	7.45 ± 4.59	ND	ND
41.529	Ethyl benzoate	1161.25	1170	0.73 ± 0.16	ND	ND	ND	ND	ND
41.929	Butyl hexanoate	1375.85	-	ND	26.72 ± 13.98	ND	2.00 ± 0.13	0.99 ± 0.34	10.70 ± 3.64
42.096	Ethyl octoate	1203.45	-	ND	26.72 ± 13.98	ND	3.74 ± 0.50	0.99 ± 0.34	10.70 ± 3.64
46.328	α-pentyl-γ-butyrolactone	1284.99	-	1.65 ± 0.31	4.31 ± 0.58	0.67 ± 0.02	0.76 ± 0.13	2.76 ± 0.57	3.53 ± 0.43
46.535	Hexyl hexanoate	1373.11	1390	0.41 ± 0.11	7.12 ± 2.13	ND	5.77 ± 1.27	0.56 ± 0.19	4.66 ± 1.45
46.679	Ethyl caprate	-	-	ND	ND	ND	0.54 ± 0.13	ND	ND
47.174	Ethyl linoleate	1772.96	-	14.29 ± 0.67	14.15 ± 4.36	12.09 ± 0.99	ND	ND	ND
47.417	Ethyl tridecanoate	1930.57	-	7.60 ± 0.89	ND	ND	ND	ND	ND
48.672	Ethyl palmitate	1367.58	1924	45.12 ± 1.58	9.23 ± 6.27	33.39 ± 1.07	36.32 ± 5.21	41.30 ± 2.96	36.22 ± 3.62
49.614	Hexyl Caprylate	-	-	ND	2.78 ± 0.91	ND	0.92 ± 0.82	ND	ND
49.892	Diethyl phthalate	1647.27	-	10.42 ± 3.06	2.11 ± 0.78	1.07 ± 0.21	14.30 ± 0.71	24.23 ± 8.84	0.80 ± 0.26
Alcohols (8)									
11.519	Isoamyl alcohol	693.31	734	ND	ND	ND	4.09 ± 1.37	1.32 ± 0.13	0.88 ± 0.31

Table 1. Cont.

T <sub>R</sub> (s)	Compounds	RI	RI*	Content (µg/kg)					
				CG-0	CG-1	CG-2	CG-3	CG-6	CG-8
11.782	2-Methyl-1-butanol	695.63	-	ND	ND	ND	0.73 ± 0.24	0.49 ± 0.07	2.77 ± 0.25
13.552	1-Pentanol	765.98	820	ND	ND	ND	16.83 ± 5.69	0.97 ± 0.10	13.19 ± 2.75
14.703	2,3-Butanediol	746.31	-	6.91 ± 1.95	ND	ND	ND	32.20 ± 6.14	1.03 ± 0.36
29.550	1-nonen-3-ol	1054.76	-	ND	ND	ND	ND	8.01 ± 3.36	0.64 ± 0.12
38.819	Maltitol	1060.48	-	ND	ND	ND	ND	5.85 ± 0.44	ND
39.113	Phenethyl alcohol	1130.98	1121	ND	ND	ND	5.86 ± 2.09	0.95 ± 0.20	ND
46.676	1-Tetradecanol	1550.62	-	ND	10.5 ± 0.33	7.56 ± 2.34	4.94 ± 0.65	5.81 ± 0.49	6.50 ± 4.42
Aldehydes (14)									
7.102	Isovaleraldehyde	643.51	656	2.32 ± 0.11	4.13 ± 0.23	5.28 ± 0.23	15.48 ± 0.67	25.29 ± 1.77	1.22 ± 0.15
7.566	2-Methylbutyraldehyde	645.27	665	1.24 ± 0.05	2.31 ± 0.12	3.22 ± 1.17	12.46 ± 0.36	23.33 ± 1.58	16.29 ± 1.83
15.894	Hexanal	804.15	800	ND	ND	0.88 ± 0.10	0.70 ± 0.23	1.17 ± 0.40	3.26 ± 1.58
18.216	Furfural	-	-	ND	ND	4.30 ± 0.82	ND	ND	ND
23.841	3-methylthiopropionaldehyd-e	857.39	-	ND	ND	2.05 ± 0.57	2.24 ± 0.80	2.67 ± 0.22	1.76 ± 0.59
28.292	Benzaldehyde	975.41	961	7.84 ± 0.22	2.16 ± 1.04	38.45 ± 4.60	106.11 ± 40.00	15.04 ± 5.00	24.29 ± 3.67
34.439	Phenylacetaldehyde	1032.39	1045	10.79 ± 3.70	13.27 ± 0.38	12.18 ± 1.16	26.54 ± 9.60	18.02 ± 4.99	23.03 ± 1.08
36.693	Phenylglyoxal monohydrate	1217.09	-	ND	ND	ND	ND	4.87 ± 1.06	6.35 ± 1.88
38.419	Nonaldehyde	1101.97	1107	ND	ND	2.85 ± 0.31	4.64 ± 1.53	2.12 ± 0.23	9.87 ± 2.26
42.528	Decyl aldehyde	1179.84	1206	ND	ND	1.90 ± 0.24	9.30 ± 6.35	0.89 ± 0.19	3.19 ± 0.79
43.136	2,4-Dimethylbenzaldehyde	1208.59	-	ND	ND	1.18 ± 0.20	60.17 ± 15.07	1.09 ± 0.18	ND
47.087	Vanillin	1392.63	1418	1.34 ± 0.64	1.62 ± 0.54	0.81 ± 0.10	19.15 ± 0.96	ND	ND
48.035	5-methyl-2-phenyl-2-hexena-1	1505.88	-	ND	ND	ND	4.84 ± 1.57	12.00 ± 1.27	12.72 ± 4.26
50.017	FEMA 2763	1601.29	1611	ND	ND	ND	ND	0.74 ± 0.26	0.69 ± 0.23
Ketones (4)									
3.775	2-Butanone	-	-	ND	0.50 ± 1.34	ND	ND	ND	ND
23.154	(E)-hex-3-en-2-one	760.36	-	2.09 ± 0.22	ND	1.29 ± 0.42	1.00 ± 0.35	ND	ND

Table 1. Cont.

T <sub>R</sub> (s)	Compounds	RI	RI*	Content (µg/kg)					
				CG-0	CG-1	CG-2	CG-3	CG-6	CG-8
33.996	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone	-	-	ND	ND	36.85 ± 12.85	51.16 ± 9.24	50.10 ± 16.57	29.37 ± 3.87
45.340	4'-Hydroxy-3'-methylacetophenone	1363.58	-	128.97 ± 2.77	44.44 ± 8.65	43.07 ± 5.97	26.40 ± 12.09	15.64 ± 5.77	6.88 ± 1.95
Phenols (5)									
43.295	2-Naphthol	1454.96	-	ND	ND	ND	ND	0.90 ± 0.32	2.66 ± 0.83
44.479	4-Hydroxy-3-methoxystyrene	1302.59	-	ND	413.18 ± 1.54	ND	ND	ND	36.27 ± 1.80
46.021	2,6-Dimethoxyphenol	1276.32	-	ND	1.57 ± 0.13	ND	ND	ND	15.94 ± 3.88
46.175	Eugenol	1380.17	1374	ND	0.39 ± 0.10	ND	ND	ND	ND
48.665	2,4-Di-tert-butylphenol	1555.65	1513	ND	1.28 ± 0.20	ND	ND	ND	ND
Miscellaneous (20)									
4.042	2-Methylfuran	604.13	604	ND	ND	ND	ND	1.84 ± 0.62	ND
25.389	3,4-Lutidine	901.47	882	1.39 ± 0.48	1.93 ± 0.15	1.55 ± 0.51	0.76 ± 0.08	6.68 ± 0.12	3.77 ± 1.47
28.651	Mesitylene	1020.21	1013	195.72 ± 54.49	3.78 ± 0.98	146.68 ± 23.14	83.90 ± 7.92	231.64 ± 30.57	5.72 ± 0.77
29.892	cis-Anethol	-	-	6.53 ± 0.85	9.71 ± 1.18	8.28 ± 0.96	9.69 ± 1.03	4.87 ± 0.34	3.71 ± 0.29
30.161	1,2-Dimethyl-5-vinylpyrrole	945.18	-	422.06 ± 6.32	382.18 ± 14.58	465.86 ± 17.94	472.03 ± 52.02	367.79 ± 15.75	385.85 ± 15.45
30.535	2-propylpyridine	984.11	-	25.12 ± 2.41	ND	ND	ND	19.33 ± 0.31	ND
30.671	1,2,4-Trimethylenecyclohexane	921.39	-	ND	ND	575.54 ± 202.11	304.60 ± 64.54	908.03 ± 72.36	607.52 ± 55.99
31.002	4-Isopropylaniline	-	-	ND	ND	9.72 ± 3.23	ND	ND	ND
32.265	1-Isopentyl-1-cyclohexene	-	-	6.24 ± 2.58	ND	33.27 ± 5.30	ND	ND	ND
32.459	2-Isopropyltoluene	1040.66	1021	12.85 ± 4.52	ND	7.66 ± 2.51	164.24 ± 30.31	12.17 ± 3.44	59.24 ± 17.58
33.987	1-decylcyclohexene	1680.32	-	39.30 ± 12.45	7.86 ± 0.06	72.66 ± 24.57	42.54 ± 12.45	ND	5.34 ± 3.01
35.496	2-Acetyl pyrrole	1032.69	-	ND	ND	ND	ND	4.72 ± 0.52	99.31 ± 32.57
39.475	1,2,3,4-Tetramethylbenzene	1129.47	-	1.57 ± 0.10	ND	ND	8.02 ± 2.65	1.22 ± 0.31	2.73 ± 0.61
42.000	Cyclododecane	1422.4	-	ND	0.54 ± 0.12	ND	ND	0.97 ± 0.22	0.47 ± 0.16
42.361	Naphthalene	1178.73	1191	ND	0.72 ± 0.22	ND	ND	ND	ND

**Table 1.** *Cont.*

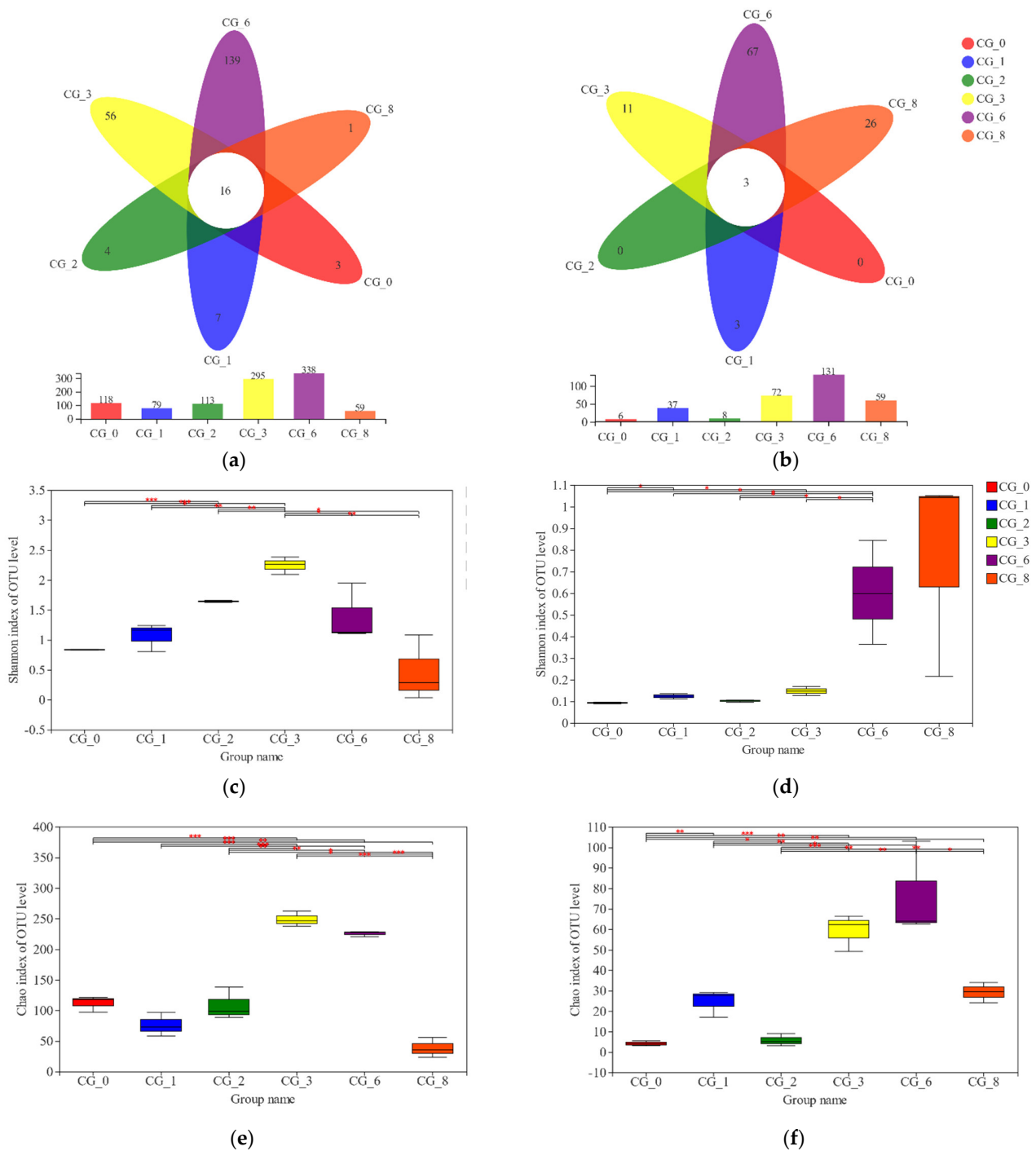
T <sub>R</sub> (s)	Compounds	RI	RI*	Content (µg/kg)					
				CG-0	CG-1	CG-2	CG-3	CG-6	CG-8
42.846	2,3-Dihydrobenzofuran	1043.32	-	45.55 ± 0.98	96.94 ± 9.18	21.19 ± 0.51	18.09 ± 4.24	7.40 ± 2.38	4.80 ± 1.90
45.081	Indole	1294.51	1294	1.40 ± 0.19	2.06 ± 0.12	ND	ND	ND	ND
46.800	Tetradecane	-	-	6.62 ± 1.27	0.69 ± 0.09	ND	16.59 ± 4.93	ND	ND
47.667	Hexadecane	-	-	3.33 ± 1.06	ND	ND	ND	ND	ND
48.419	Pentadecane	1515.22	1500	5.42 ± 0.23	5.25 ± 0.11	2.93 ± 1.39	7.14 ± 4.88	5.86 ± 1.20	6.46 ± 0.22

Values represent means ± SD. RI is the retention index obtained in this study, RI\* is the literature value; ND means the compound is not detected.

**Table 2.** Odor activity values (OAVs) of Caishiji soybean paste in different fermentation stages.

T <sub>R</sub> (s)	Compounds	Threshold (µg/kg)	Content (µg/kg)					
			CG-0	CG-1	CG-2	CG-3	CG-6	CG-8
10.340	Isobutyric acid	1.00	-	2.78 ± 1.68	-	-	-	-
15.778	Ethyl butyrate	1.00	-	-	-	4.92 ± 1.61	-	-
30.496	Ethyl hexanoate	0.50	-	13.93 ± 3.12	-	14.90 ± 9.18	-	-
49.614	Hexyl Caprylate	0.10	-	27.77 ± 9.07	-	9.24 ± 0.82	-	-
39.113	Phenethyl alcohol	0.10	-	-	-	58.63 ± 20.88	9.49 ± 2.01	-
7.102	Isovaleraldehyde	2.00	1.30 ± 0.05	2.07 ± 0.12	2.64 ± 0.12	7.74 ± 0.34	12.65 ± 0.89	0.62 ± 0.07
7.566	2-Methylbutyraldehyde	1.00	1.24 ± 0.05	2.31 ± 0.12	3.22 ± 1.17	12.46 ± 0.37	23.33 ± 1.58	16.29 ± 1.83
23.841	3-methylthiopropionaldehyde	0.04	-	-	51.25 ± 14.25	56.00 ± 19.81	66.75 ± 5.50	43.67 ± 14.25
28.292	Benzaldehyde	50.00	0.16 ± 0.01	0.04 ± 0.02	0.77 ± 0.09	2.12 ± 0.80	0.40 ± 0.10	0.49 ± 0.08
38.419	Nonaldehyde	3.50	-	-	0.81 ± 0.09	1.02 ± 0.18	0.61 ± 0.07	2.82 ± 0.65
42.528	Decyl aldehyde	5.00	-	-	0.38 ± 0.05	2.53 ± 0.64	0.18 ± 0.04	0.64 ± 0.16
47.087	Vanillin	10.00	0.13 ± 0.07	0.16 ± 0.06	0.08 ± 0.01	2.25 ± 0.61	-	-

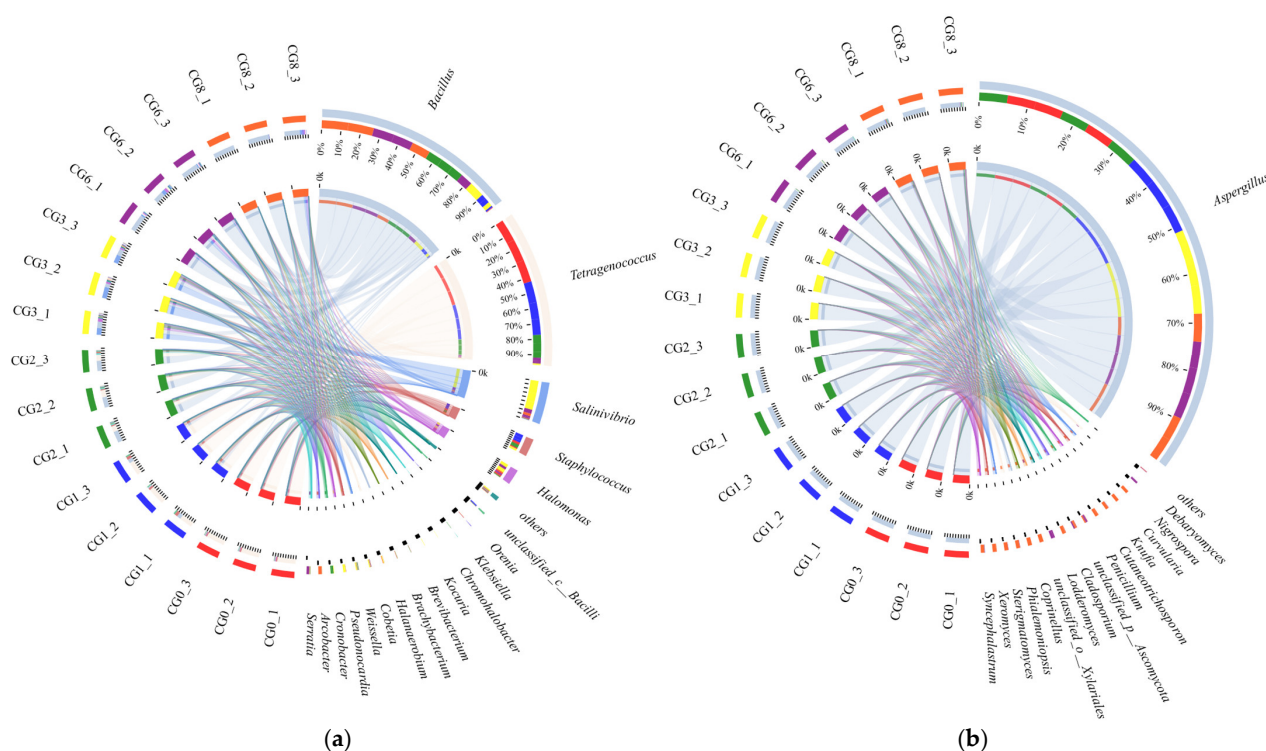




**Figure 2.**  $\alpha$ -diversity analysis of bacterial (a,c,e) and fungi (b,d,f) of Caishiji soybean paste at different fermentation stages. ( $0.01 < p \leq 0.05$  marked as \*,  $0.001 < p \leq 0.01$  marked as \*\*,  $p \leq 0.001$  marked as \*\*\*).

Circos was used to construct a circle graph to visualize the distribution of dominant species in each sample and among the different samples. The bacterial and fungal genera with the 20 highest abundances were obtained. Genera with a relative abundance higher than 4% were classified as dominant. There were five dominant bacterial genera (*Bacillus*, *Tetragenococcus*, *Salinivibrio*, *Halomonas*, and *Staphylococcus*) and four dominant fungal genera (*Aspergillus*, *Debaryomyces*, *Nigrospora*, and *Curvularia*), which were detected during the CSP fermentation process (Figure 3b). In CG0, *Tetragenococcus* (83.72%) and *Staphylococcus* (5.48%) were the dominant bacterial genera, and *Aspergillus* ( $\geq 99.9\%$ ) was the dominant

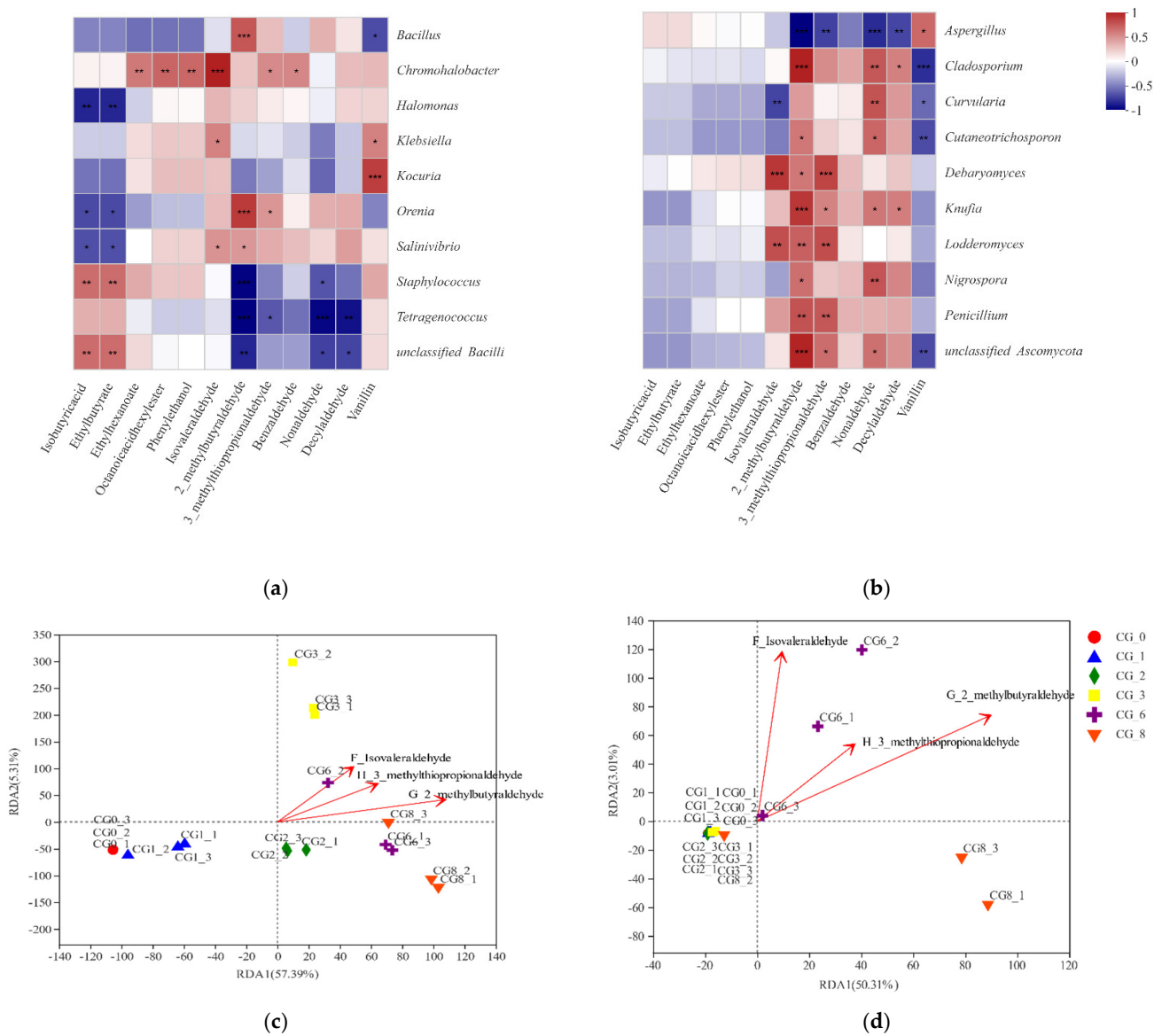
fungal genus. The relative abundances of *Tetragenococcus*, *Staphylococcus*, and *Aspergillus* in CG1 and CG2 were 29.9–67.34%, 5.32–7.38%, and 99.9%, respectively. Some emerging bacterial genera, such as *Klebsiella* (5.47%) and unclassified *Bacillus* (5.06%), were also detected in these samples. The dominant bacterial genera in CG3 and CG6 were *Bacillus* (24.97–66.79%), *Salinivibrio* (9.53–39.55%), *Halomonas* (6.36–10.50%), and *Tetragenococcus* (7.24%), while the dominant fungal genera were *Aspergillus* ( $\geq 90.0\%$ ) and *Debaryomyces* (5.37%). The relative abundance of *Bacillus*, *Salinivibrio*, and *Aspergillus* in CG8 were 88.51%, 9.99%, and 88.58%, respectively. *Orenia* (9.55%), an emerging bacterial genus, was also identified in CG8. All the results suggested that the composition and species of microbiome in CSP were changing during the fermentation process, which may affect the quality and flavor of CSP.



**Figure 3.** Circos of bacteria (a) and fungi (b) of Caishiji soybean paste in different fermentation stages. The right half circle represents the distribution of the species in different samples at the taxonomic level, the outer ribbon represents the species, the inner ribbon color represents the different groups, and the length represents the distribution of the sample in a species.

### 3.3. Correlation Analysis of Microbiota and Characteristic VFCs

In this study, *R* values and Pearson correlation coefficients were calculated to analyze the connections between microbiota and characteristic VFCs in CSP. The bacterial and fungal genera with the 10 highest abundances were included in the correlation analysis. Redundancy analysis (RDA) was conducted to evaluate the complex relationships among the microbiota, VFCs, and CSP fermentation stages. On the basis of the PCA results, isovaleraldehyde, 2-methylbutyraldehyde, and 3-methylthiopropionaldehyde were selected for the RDA. The results revealed that compared with isovaleraldehyde and 3-methylthiopropionaldehyde, the microorganisms exerted a greater effect on 2-methylbutyraldehyde in CG3, CG6, and CG8. These three characteristic VFCs were positively correlated (Figure 4c,d).



**Figure 4.** Heat-map (a,b) and RDA (c,d) correlation analysis of microbiota and characteristic VFCs of Caishiji bean paste in different fermentation stages. (*R* value was displayed in different colors in the heat-map. If the *p* value was less than 0.05, it is marked with \*, the legend on the right side was the color range of different *R* values. \* 0.01 < *p* ≤ 0.05, \*\* 0.001 < *p* ≤ 0.01, \*\*\* *p* ≤ 0.001. In RDA, the red arrow indicates the flavor substance, and the length of the arrow indicates the impact degree of the flavor substance on the species; the angle between the environmental factors arrows represents positive and negative correlation (acute angle: positive correlation; obtuse angle: negative correlation; right angle: no correlation); the distance from the projection point to the origin represents the relative impact of the flavor substance on the distribution of the sample community).

**4. Discussion**

VFCs and microbiota strongly affect the flavor of soybean paste. In this study, VFCs and microbiota in CSP at different fermentation stages (CG0, CG1, CG2, CG3, CG6, CG8) were detected to identify potential correlations between them. More than 70 types of VFCs, including 12 types of characteristic VFCs (based on OAV), were detected. Among them, esters, alcohols, and aldehydes were the most prevalent in the CSP fermentation process.

The contributions of VFCs to CSP flavor depend not only on their concentrations but also on their odor threshold values [22]. Esters (especially short-chain esters), which are

formed by a series of esterification reactions, are vital aromatic compounds in fermented foods, and it can endow fermented foods with sweetness and fruitiness. In the present study, 15 esters were detected during the CSP fermentation process. Among them, amyl acetate,  $\alpha$ -pentyl- $\gamma$ -butyrolactone, and ethyl palmitate, which have also been identified in other fermented soybean products, were common to CSP at differing fermentation stages. Based on the results of OAV analysis, ethyl butyrate, ethyl caproate, and hexyl caproate were the esters that most strongly affected the flavor of CSP. Ethyl butyrate, which produces a beany flavor [28], was detected only in CG3, and ethyl hexanoate and hexyl caprylate, which produce fruity flavors [28], were detected in CG1 and CG3. Most of the alcohols, which can endow CSP with pleasantly sweet and floral flavors, were formed in the later fermentation period. Isoamyl alcohol, 2-methyl-1-butanol, 1-pentanol, 2,3-butanediol, and 1-nonyl-3-ol were detected in CG6 and CG8. Phenethyl alcohol, which produces aromatic flavors [29], most strongly affected the flavor of CG3 according to the results of the OAV analysis. Aldehydes, including monoolefinic aldehyde, dialdehyde, and saturated aldehyde, which are mainly produced by the lipoxygenase-catalyzed decomposition of linoleic and linolenic acids, can endow fermented foods with sweet, fruity, nutty, and caramel flavors [30]. Fourteen aldehydes were detected in CSP, most of which were formed during the late stages of the fermentation process. The results of the OAV analysis indicated that seven characteristic aldehydes affected the flavor of CSP. Isovaleraldehyde, 2-methylbutyraldehyde, and benzaldehyde, which produce fruity flavors, stimulating odors, and cherry like flavors, respectively [29], were detected in each fermentation stage; 3-methylthiopropionaldehyde, which had the highest OAV value, can endow CSP with a strong roasted-potato flavor [29]. Although the other VFCs had lower thresholds, they may also endow CSP with strong flavors.

Microbiotas are the main contributors to soybean paste fermentation [31,32]. The fermentation of soybean paste involves a variety of bacteria and fungi. In addition, soybean pastes with distinct microbiota exhibit distinct flavors and qualities. In some previous studies, *Bacillus*, *Aspergillus*, *Tetragenococcus*, *Salinivibrio*, *Halomonas*, and *Staphylococcus* were the most abundant genera in CSP (Figure 3). During the fermentation process, the relative abundance of *Bacillus* increased, while that of *Staphylococcus* decreased or even disappeared completely. *Tetragenococcus* is closely related to the production of biogenic amines, which directly affect the flavor and safety of soybean paste; therefore, regulation of *Tetragenococcus* content is essential [1]. *Aspergillus* was the predominant fungal genus in CSP samples; it is found in soy sauce, douchi, and vinegar [33]. Compared with the other genera identified in this study, *Aspergillus* exhibits a higher tolerance to dryness. The analysis of microbial dynamics revealed that bacteria were involved in the decomposition of materials in the CSP fermentation, whereas most of the fungi were presented in the late stages of fermentation. The fermentation of soybean paste takes place mostly in anaerobic and high-humidity environments. Compared with bacteria, fungi may disrupt metabolic activity under dry and aerobic conditions; thus, fungal communities play a more important role in fermented bean paste.

The relationships between microbiota and VFCs in traditional fermented foods have been researched; however, such studies have produced limited relevant data. In the present study, we analyzed the complex connections between the microbiota and 12 characteristic VFCs during the CSP fermentation. As indicated in Figure 4, the Pearson's correlation coefficients ( $r$ ) and significance ( $p$ ) were calculated, and the bacteria and fungi with the 10 highest abundances were obtained. The 2-Methylbutyraldehyde was significantly positively correlated with *Bacillus* and *Orenia* ( $r > 0.7$ ) but significantly negatively correlated with *Tetragenococcus* and *Staphylococcus*. *Kocuria* had a positive correlation with vanillin, and *Halomonas* was negatively correlated with isobutyric acid and ethyl butyrate (Figure 3a). The 2-Methylbutyraldehyde, 3-methylthiopropionaldehyde, nonanaldehyde, and decyl aldehyde were significantly negatively correlated with *Aspergillus*, but they were positively correlated with other fungi, such as *Debaryomyces*, *Nigrospora*, *Curvularia*, and *Penicillium*. Vanillin was positively correlated with *Aspergillus* but negatively correlated

with *Cladosporium*, *Curvularia*, and *Cutaneotrichosporon* (Figure 3b). Through correlation analysis, microbiotas were determined to be the main factors affecting the generation of flavor compounds during the CSP fermentation. For example, *Bacillus* and *Orenia* may produce undesirable flavor compounds during CSP fermentation, whereas *Aspergillus*, *Etragenococcus*, and *Staphylococcus* may produce flavor-enhancing compounds. Through detection and analysis, the connections between microbiota and characteristic VFCs were determined. In follow-up studies, these correlations should be verified in application and potentially used to further advancements in “omics” technologies.

## 5. Conclusions

MiSeq sequencing technology is widely used, and correlation analysis of microbiota and VFCs has attracted considerable attention. In the present study, the complex influences of microbiota on the formation of VFCs during CSP fermentation were analyzed. The results indicated that *Bacillus*, *Tetragenococcus*, *Staphylococcus*, and *Aspergillus* were the main microbiota affecting the flavor of CSP. Although correlation studies involving many fermented foods have been conducted, the results of such studies still lack practical applicability. Therefore, follow-up research should focus on such practical applications to improve the sensory features of fermented soybean paste, and the pastes with consistent flavor profiles may be produced.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation8050196/s1>, Table S1: Alpha diversity index of bacteria of Caishiji soybean paste in different fermentation stages, Table S2: Alpha diversity index of fungi of Caishiji soybean paste in different fermentation stages, Figure S1: Multi-point sampling (a) and numbers of different VFCs (b) of Caishiji soybean paste in different fermentation stages, Figure S2: Contents of main volatile components of Caishiji soybean paste in different fermentation stages (a: alcohols, b: esters, c: aldehydes, d: miscellaneous), Figure S3: Simpson curves of the bacteria (a) and fungi (b) of Caishiji soybean paste in different fermentation stages.

**Author Contributions:** Conceptualization: X.L.; methodology: Y.H. and W.W.; software: J.C.; formal analysis: J.C. and Y.H.; investigation: J.C. and Y.H.; resources: X.L.; data curation: D.M.; writing (original draft preparation): J.C. and Y.H.; writing (review and editing): X.L. and X.W.; visualization: X.W. and D.M.; supervision: X.L. and S.J.; project administration: X.L.; funding acquisition: X.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by [the Project of Anhui Province] grant number [201903a06020034, 201903a06020007, 2108085MC123], and [the Fundamental Research Funds for the Central Universities of China] grant number [PA2021KCPY0048].

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The sequences from this study were deposited into the NCBI Sequence Read Archive (SRA) database (accession number:SRP255336; NCBI BioProject PRJNA623258).

**Acknowledgments:** This work was supported by the Project of Anhui Province [grant numbers 201903a06020034, 201903a06020007, 2108085MC123], and the Fundamental Research Funds for the Central Universities of China [grant number PA2021KCPY0048].

**Conflicts of Interest:** This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. The authors declare no conflict of interest.

## References

1. Byun, B.Y.; Mah, J.H. Occurrence of Biogenic Amines in *Miso*, Japanese Traditional Fermented Soybean Paste. *J. Food Sci.* **2012**, *77*, T216–T223. [[CrossRef](#)] [[PubMed](#)]
2. Kim, T.W.; Lee, J.H.; Kim, S.E.; Park, M.H.; Chang, H.C.; Kim, H.Y. Analysis of Microbial Communities in *Doenjang*, a Korean Fermented Soybean Paste, Using Nested PCR-Denaturing Gradient Gel Electrophoresis. *Int. J. Food Microbiol.* **2009**, *131*, 265–271. [[CrossRef](#)] [[PubMed](#)]
3. Chung, H.Y.; Fung, P.K.; Kim, J.S. Aroma Impact Components in Commercial Plain Sufu. *J. Agric. Food Chem.* **2005**, *53*, 1684–1691. [[CrossRef](#)]
4. Feng, Z.; Gao, W.; Ren, D.; Chen, X.; Li, J.J. Evaluation of Bacterial Flora during the Ripening of Kedong Sufu, a Typical Chinese Traditional Bacteria-Fermented Soybean Product. *J. Sci. Food Agric.* **2013**, *93*, 1471–1478. [[CrossRef](#)] [[PubMed](#)]
5. Han, B.Z.; Rombouts, F.M.; Robert Nout, M.J. A Chinese Fermented Soybean Food. *Int. J. Food Microbiol.* **2001**, *65*, 1–10. [[CrossRef](#)]
6. Lioe, H.N.; Selamat, J.; Yasuda, M. Soy Sauce and Its Umami Taste: A Link from the Past to Current Situation. *J. Food Sci.* **2010**, *75*, R71–R76. [[CrossRef](#)]
7. Wei, Q.Z.; Wang, H.B.; Chen, Z.X.; Lv, Z.J.; Xie, Y.F.; Lu, F.P. Profiling of Dynamic Changes in the Microbial Community during the Soy Sauce Fermentation Process. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9111–9119. [[CrossRef](#)]
8. Yang, Y.; Deng, Y.; Jin, Y.L.; Liu, Y.X.; Xia, B.X.; Sun, Q. Dynamics of Microbial Community during the Extremely Long-Term Fermentation Process of A Traditional Soy Sauce. *J. Sci. Food Agric.* **2017**, *97*, 3220–3227. [[CrossRef](#)]
9. Chen, T.T.; Wang, M.J.; Jiang, S.Y.; Xiong, S.Q.; Zhu, D.C.; Wei, H. Investigation of the Microbial Changes during Koji-Making Process of Douchi by Culture-Dependent Techniques and PCR-DGGE. *Int. J. Food Sci. Technol.* **2011**, *46*, 1878–1883. [[CrossRef](#)]
10. Chen, T.T.; Wang, M.J.; Li, S.J.; Wu, Q.L.; Wei, H. Molecular Identification of Microbial Community in Surface and Undersurface Douchi during Postfermentation. *J. Food Sci.* **2014**, *79*, M653–M658. [[CrossRef](#)]
11. Wang, H.; Yin, L.J.; Cheng, Y.Q.; Li, L.T. Effect of Sodium Chloride on the Color, Texture, and Sensory Attributes of Douchi during Post-Fermentation. *Int. J. Food Eng.* **2012**, *8*, 22. [[CrossRef](#)]
12. Jung, K.O.; Park, S.Y.; Park, K.Y. Longer Aging Time Increases the Anticancer and Antimetastatic Properties of *Doenjang*. *Nutrition* **2006**, *22*, 539–545. [[CrossRef](#)] [[PubMed](#)]
13. Park, K.Y.; Jung, K.O.; Rhee, S.H.; Choi, Y.H. Antimutagenic Effects of *Doenjang* (Korean Fermented Soypaste) and Its Active Compounds. *Mutat. Res. Lett.* **2003**, *523*, 43–53. [[CrossRef](#)]
14. Shin, Z.I.; Yu, R.; Park, S.A.; Chung, D.K.; Ahn, C.W.; Nam, H.S.; Kim, K.S.; Lee, H.J. His-His-Leu, An Angiotensin I Converting Enzyme Inhibitory Peptide Derived from Korean Soybean Paste, Exerts Antihypertensive Activity in Vivo. *J. Agric. Food Chem.* **2001**, *49*, 3004–3009. [[CrossRef](#)]
15. Liu, J.J.; Han, B.Z.; Deng, S.H.; Sun, S.P.; Chen, J.Y. Changes in Proteases and Chemical Compounds in the Exterior and Interior of Sufu, a Chinese Fermented Soybean Food, during Manufacture. *LWT Food Sci. Technol.* **2018**, *87*, 210–216. [[CrossRef](#)]
16. Jo, Y.J.; Cho, I.H.; Song, C.K.; Shin, H.W.; Kim, Y.S. Comparison of Fermented Soybean Paste (*Doenjang*) Prepared by Different Methods Based on Profiling of Volatile Compounds. *J. Food Sci.* **2011**, *76*, C368–C379. [[CrossRef](#)]
17. Lin, H.B.; Yu, X.Y.; Fang, J.X.; Lu, Y.H.; Liu, P.; Xing, Y.G.; Wang, Q.; Che, Z.M.; He, Q. Flavor Compounds in Pixian Broad-Bean Paste: Non-Volatile Organic Acids and Amino Acids. *Molecules* **2018**, *23*, 1299. [[CrossRef](#)]
18. Zhao, J.X.; Dai, X.J.; Liu, X.M.; Zhang, H.; Tang, J.; Chen, W. Comparison of Aroma Compounds in Naturally Fermented and Inoculated Chinese Soybean Pastes by GC-MS and GC-Olfactometry Analysis. *Food Control* **2011**, *22*, 1008–1013. [[CrossRef](#)]
19. Zhang, P.; Zhang, P.F.; Xie, M.X.; An, F.Y.; Qiu, B.S.; Wu, R. Metaproteomics of Microbiota in Naturally Fermented Soybean Paste, *Da-Jiang*. *J. Food Sci.* **2018**, *83*, 1342–1349. [[CrossRef](#)] [[PubMed](#)]
20. Hao, Y.; Sun, B. Analysis of Bacterial Diversity and Biogenic Amines Content during Fermentation of Farmhouse Sauce from Northeast China. *Food Control.* **2020**, *108*, 106861. [[CrossRef](#)]
21. Li, Z.; Dong, L.; Huang, Q.; Wang, X. Bacterial Communities and Volatile Compounds in Doubanjiang, a Chinese Traditional Red Pepper Paste. *J. Appl. Microbiol.* **2016**, *120*, 1585–1594. [[CrossRef](#)] [[PubMed](#)]
22. Xie, C.Z.; Zeng, H.Y.; Wang, C.X.; Xu, Z.M.; Qin, L.K. Volatile Flavour Components, Microbiota and Their Correlations in Different Sufu, a Chinese Fermented Soybean Food. *J. Appl. Microbiol.* **2018**, *125*, 1761–1773. [[CrossRef](#)] [[PubMed](#)]
23. Cocolin, L.; Alessandria, V.; Dolci, P.; Gorra, R.; Rantsiou, K. Culture Independent Methods to Assess the Diversity and Dynamics of Microbiota during Food Fermentation. *Int. J. Food Microbiol.* **2013**, *167*, 29–43. [[CrossRef](#)] [[PubMed](#)]
24. Rodriguez-Burruezo, A.; Kollmannsberger, H.; Carmen Gonzalez-Mas, M.; Nitz, S.; Nuez, F. HS-SPME Comparative Analysis of Genotypic Diversity in the Volatile Fraction and Aroma-Contributing Compounds of Capsicum Fruits from the *Annuum-Chinense-Frutescens* Complex. *J. Agric. Food Chem.* **2010**, *58*, 4388–4400. [[CrossRef](#)]
25. Li, X.Y.; Zhao, C.S.; Zheng, C.; Liu, J.; Vu, V.H.; Wang, X.D.; Sun, Q. Characteristics of Microbial Community and Aroma Compounds in Traditional Fermentation of Pixian Broad Bean Paste as Compared to Industrial Fermentation. *Int. J. Food Prop.* **2018**, *20*, S2520–S2531. [[CrossRef](#)]
26. Kang, K.M.; Baek, H.H. Aroma Quality Assessment of Korean Fermented Red Pepper Paste (*Gochujang*) by Aroma Extract Dilution Analysis and Headspace Solid-Phase Microextraction-Gas Chromatography-Olfactometry. *Food Chem.* **2014**, *145*, 488–495. [[CrossRef](#)]

27. Ye, J.B.; Yan, J.; Zhang, Z.; Yang, Z.C.; Liu, X.Z.; Zhou, H.; Wang, G.F.; Hao, H.; Ma, K.; Ma, Y.P.; et al. The Effects of Threshing and Redrying on Bacterial Communities that Inhabit the Surface of Tobacco Leaves. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 4279–4287. [[CrossRef](#)]
28. Moy, Y.S.; Lu, T.J.; Chou, C.C. Volatile Components of the Enzyme-Ripened Sufu, a Chinese Traditional Fermented Product of Soy Bean. *J. Biosci. Bioeng.* **2012**, *113*, 196–201. [[CrossRef](#)]
29. Beaulieu, J.C.; Stein-Chisholm, R.E. HS-GC-MS Volatile Compounds Recovered in Freshly Pressed ‘Wonderful’ Cultivar and Commercial Pomegranate Juices. *Food Chem.* **2016**, *190*, 643–656. [[CrossRef](#)]
30. Zhao, J.; Wang, M.; Xie, J.C.; Zhao, M.Y.; Hou, L.; Liang, J.J.; Wang, S.; Cheng, J. Volatile Flavor Constituents in the Pork Broth of Black-Pig. *Food Chem.* **2017**, *226*, 51–60. [[CrossRef](#)]
31. Jung, J.Y.; Lee, S.H.; Jeon, C.O. Microbial Community Dynamics during Fermentation of Doenjang-Meju, Traditional Korean Fermented Soybean. *Int. J. Food Microbiol.* **2014**, *185*, 112–120. [[CrossRef](#)] [[PubMed](#)]
32. Lee, J.H.; Kim, T.W.; Lee, H.; Chang, H.C.; Kim, H.Y. Determination of Microbial Diversity in Meju, Fermented Cooked Soya Beans, Using Nested PCR-Denaturing Gradient Gel Electrophoresis. *Lett. Appl. Microbiol.* **2010**, *51*, 388–394. [[CrossRef](#)] [[PubMed](#)]
33. Wang, Z.M.; Lu, Z.M.; Shi, J.S.; Xu, Z.H. Exploring Flavour-Producing Core Microbiota in Multispecies Solid-State Fermentation of Traditional Chinese Vinegar. *Sci. Rep.* **2016**, *6*, 26818. [[CrossRef](#)] [[PubMed](#)]