RESEARCH ARTICLE

Analysis of Sourdough's Microbial Community and Flavor Components in Traditional Chinese Steamed Bread

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Abstract

This study aims to figure out the structure of the microbial community in traditional sourdough and the different flavor components of Chinese steamed bread(CSB) made with it. The sourdough samples were selected from Shanxi, Henan and Shandong provinces. The microbial community composition of the sourdoughs was analyzed by a culture-dependent method and high-throughput sequencing, and the flavor compounds of the steamed breads were determined by the solid-phase micro extraction (SPME) method coupled with gas chromatography/mass spectroscopy (GC/MS). The results showed that: in the 28 samples collected, the pH ranged from 3.50 to 4.91, and the total titratable acid (TTA) values ranged from 2.50 to 7.80 mL of NaOH (1 M), while the total number of lactic acid bacteria (LAB) colonies ranged from 10^4 to 10^7 CFU/mL. Lactobacillus, Lactococcus, and Weissella were the dominant genera, as determined by pyrosequencing. A total of 180 LAB strains were isolated and identified using the 16S rRNA gene; the results indicate that Lactobacillus plantarum, Lactobacillus fermentum, and Weissella confusa were isolated with a relatively high frequency. By GC/MS, a total of 24 volatile compounds were detected, including hydrocarbons, aromatic compounds, alcohols, esters, aldehydes, acids, etc. Benzeneethanol and benzaldehyde were abundant in all five samples detected, while benzene-propoxyethyl was not detected in the control group.The flavor compounds contained in sourdough fermented CSBs, both in terms of quantity and concentration, are higher than those in yeast-fermented CSBs. This study provides a useful base for further exploring the deep connections between microbial communities and food flavors.

Keywords: sourdough, Chinese steamed bread, microbiota, lactic acid bacteria, flavor component.

1 Introduction

Chinese steamed bread (CSB), one of the most popular staple foods in China, plays an important role in the

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dietary structure and accounts for about 60% of the total wheat consumption in northern China. During the process of fermentation, many types of starter cultures are used, such as traditional sourdough, active dry yeast, fresh yeast, baking powder, and so on. Among them, the traditional sourdough is the most widely used and historically significant, with a history of use spanning thousands of years. It is similar to European type I sourdough, named "Lao-mian" and "Mian qi-zi" [1–4]. Compared with dough fermented with yeast, the sourdough fermentation has a profound impact on the physicochemical, rheological, morphological, and nutritional characteristics of CSB, such as G'and G" are decreased by 18% and 7%, respectively, the content of the total amino acid in CSB is decreased, the protein digestibility of CSB fermented by sourdough (91.12%) is 10% higher than that of yeast-fermented dough [5].

The microbiota of sourdough includes a large amount of species diversity and is mainly composed of yeasts and lactic acid bacteria (LAB) [6, 7]. More than 50 species of LAB, such as the genera Lactobacillus, Leuconostoc, Pediococcus, Weissella, and more than 20 species of yeasts, mostly belonging to the genus Saccharomyces and Candida, were found during sourdough propagation in the process of making traditional leavened baked goods [8–10]. In mature sourdough breads, the LAB count is greater than 108 CFU/g, which is one order of magnitude higher than the number of yeasts at least [11]. The representative LAB in sourdough monoculture or co-culture with yeasts has different effects on dough characteristics: the co-culture groups increased proteolysis and enhanced nutritional properties, yeast enhanced the protein metabolic activity of LAB, and bacteria-mediated consumption of proteins and increased proteolysis in co-culture groups may underlie the improved digestibility and nutritional effects of sourdough fermented products [12]. The role of microbes has been detected in conferring aromatic properties to fermented cereal products and the metabolites and enzymes produced by LAB in sourdough help to reduce postprandial hyperglycemia and phytate level [13].

Ten years ago, numerous scholars had already been researching the complex microbial ecology and their functions of CSBs' sourdough [2, 3, 14]. In the sourdoughs of CSB from Xinxiang and Heze cities, Lactobacillus and Pediococcus predominated in both fermented doughs, the species and content of acid-producing bacteria in the Heze dough were greater than those in the Xinxiang dough [15]. Seven spontaneous Lactobacillus plantarum mutants, isolated from CSB's sourdough had high phage-resistance stabilities [16]. High-throughput sequencing revealed: Lactobacillus and Bacillus are the dominant strains of different CSBs; Over two-thirds of the aroma compounds showed correlations with microorganisms [18]. The quantitative descriptive and luster analysis indicated that CSBs from eight regions were divided into four categories: CSBs made from Shangqiu, Tanghe and Xi' an sourdoughs had a unique property of sour, CSBs fermented by Heze and Zhenping sourdoughs had rich sensory attributes, CSBs made with Minqin, Taian, Zhumadian, Weinan and Nanyang sourdoughs had bland flavor, and CSB fermented by Angel yeast (a commercialized strain) had a prominent characteristic of sweet [19]. Lactobacillus paracasei AH2 isolated from traditionally homemade CSBs' sourdough showed the potential to reduce the immune reactivity of wheat protein by in vitro evaluation; it could suppress anaphylaxis symptoms, gluten-specific immunoglobulin E, histamine and interleukin-4, and increased the relative abundance of Lactobacillus and short-chain fatty acids producers (Faecalibaculum, Alloprevotella and Bacteroides genera) [17].

Because of the huge consumer market of CSB in daily life, the aromatic compounds of CSB are paid more and more attention, which play a vital role in CSBs' flavor. The key aroma profiles of CSBs fermented by different types or origins of sourdough are significantly different. In an analysis of eight CSBs, the odor activity values of (E,E)-2,4-decadienal, naphthalene, 1-pentanol, 1-heptanol, 2-pentylfuran, 1-octen-3-ol were high, and these compounds provided CSBs with the green, floral, fruity, alcoholic, nutty, sweet, fatty and mushroom odor, which made different CSBs have their own unique aroma characteristics. The regional characteristics of CSBs' flavor are also evident: CSBs from Shandong province exhibited a winelike character with a weak sweet aftertaste, whereas CSBs from Shanxi province had a distinct sour attribute and a less prominent floury taste; 2,3-butanediol, decanal, methyl isobutenyl ketone, gamma-nonanolactone, ethylcaprate, 2-ethylhexyl acetate, vanillin, and indole contributed significantly to the CSBs' diverse aroma profiles [18].

In this study, we analyzed the LAB's community structure of 28 traditional sourdoughs and the flavor components of several typical CSBs, attempting to find the underlying connections.

2 Materals and Methods

2.1 Collection and chemical analysis of sourdough samples

Twenty-eight sourdoughs of traditional CSB (referred to as SD-1, SD-2, SD-3…and SD-28) were studied, which were sourced from individual workshop in central China (Table 1). They were kindly supplied by the producers of the steamed bread and propagated through traditional protocols, without using commercial yeast or starter cultures. All samples were taken immediately at the end of the last back slopping and were stored at 4°C and -80°C, respectively. Samples stored at 4°C were used to select LAB, while the purpose of storing samples at -80°C was to examine biodiversity.

Table 1. Results of pH and TTA of Chinese traditional sourdough samples.

Source	No.	pH	TTA (ml)
Linfen, Shanxi Province	SD-1	3.67	3.65
	SD-2	3.71	4.10
	SD-3	3.76	4.50
	SD-4	3.53	5.45
	SD-5	3.96	3.60
Yuncheng, Shanxi Province	SD-6	4.42	2.70
	SD-7	4.19	3.55
	SD-8	3.78	4.00
Pingyao County, Shanxi Province	SD-9	3.7	4.00
Taiyuan, Shanxi Province	SD-10	3.61	4.45
	SD-11	4.91	7.80
	SD-12	3.77	4.05
Yanan, Shanxi Province	SD-13	3.50	4.70
	SD-14	3.88	3.85
	SD-15	3.99	3.60
Xianyang, Shanxi Province	SD-16	3.73	3.80
	SD-17	3.66	3.85
	SD-18	3.69	3.90
Xian, Shanxi Province	SD-19	3.7	4.00
	SD-20	3.74	3.75
	SD-21	3.72	3.45
	SD-22	3.7	4.05
Zhenzhou, Henan Province	SD-23	3.58	4.00
	SD-24	3.73	3.90
	SD-25	4.30	2.50
	SD-26	3.98	3.50
Liaocheng, Shandong Province	SD-27	3.90	3.30
Jinan, Shandong Province	SD-28	3.94	3.40

The potentiometric measurement of pH was carried out with a pin electrode of a pH meter by standard methods. For each sample, three independent measurements were performed, and then the means were calculated. The total titratable acidity (TTA) was expressed as the volume of 1M NaOH (mL). The process was as follows: 10 g of sourdough sample was homogenized with 90 mL of distilled water and the solution was titrated with NaOH 1M at pH 8.5, under shaking conditions. For each sample, three independent measurements were performed [20].

2.2 Enumeration and isolation of LAB

10 g of each sourdough sample was homogenized with 90 mL of distilled water, and 1 mL of each homogenized sample was diluted 1:10 with 9 mL of NaCl (0.9% [wt/vol]). LAB were enumerated and isolated by performing serial dilutions in sterile saline and plating on MRS agar. The plates were incubated at 30°C for 48 h. For each sample, plates with colonies between 30 and 300 were randomly selected, and the strains were purified by successive streaking on the same medium at 30°C. These colonies were subcultured and stored at -80°C in 50% (vol/vol) glycerol. The number of yeasts was estimated at 30°C for 48 h on rose bengal agar medium.

2.3 Genotypic identification of LAB by 16S rRNA sequencing

The LAB strains were identified using primer 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) to amplify the 16S rRNA gene fragment. The extraction of total DNA from presumptive LAB isolates was performed using 2 mL samples that were cultured overnight at 30°C in MRS broth.The DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) was used for microbial DNA extraction according to the manufacturer's protocols. Amplification was carried out as follows: first, denaturation at 94°C for 4 min; then, 30 cycles at 94°C for 45 s, 55°C for 45 s; final, extension at 72°C for 1 min. Additionally, the final extension at 72°C for 10 min was included in the protocol. The separation of PCR products was completed by electrophoresis on 1% (w/v) agarose gels. DNA sequencing reactions were carried out by Sangon Biotech Co., Ltd. (Shanghai, China) using both forward and reverse primers. The BLAST database provided the sequences of each isolate. The taxonomic identification of strains was completed by comparing these sequences.

2.4 The analysis of bacteria microbial diversity

The DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) was used to extract the microbial DNA from sourdough samples according to manufacturer's protocols. The 467 bp of the V3-V4 region of the bacterial 16S's ribosomal RNA (rRNA) gene were amplified by PCR using primers 338F 5′-ACTCCTACGGGAGGCAGCAG)-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′. PCR reactions

were performed in triplicate with a 20 μL mixture containing 4 μL of 5×FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

The PCR products were sent to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for sequencing on an Illumina MiSeq platform. The raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) with some criteria. Operational Units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1[1]). Chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier[2] using a confidence threshold of 70% [26].

2.5 Volatile compounds detection of steamed bread by Gas Chromatography-Mass Spectrometer (GC-MS)

Four samples (SD-8, SD-10, SD-18 and SD-20) were randomly studied. For the fermentation of steamed bread using sourdough, 200 g wheat flour was mixed with each 20 g of traditional sourdough sample and 90 ml distilled/deionized (dd) water for 5-10 min. 0.6 g of baking soda was added to the dough after it had fermented at 3°C and 85% relative humidity in a fermenting box for 4 hours. Then the dough was rolled into round-shaped manually and fermented at 37°C and 85% relative humidity for 30 min in a fermentation box. Finally, the proofed dough samples were steamed for 30 min at 100°C. The control sample was prepared by adding 1.2 g of instant yeast and fermenting at 37°C for 40 min.

Solid-phase microextraction (SPME) method was used to extract the volatile compounds. The optimal parameters were as follows: 2.0 g of steamed bread crumbs was placed in 15-ml vials for each analysis. SPME was performed with the SPME fiber assembly carboxen/Divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS, 50/30 μm, 1cm) mounted on an SPME manual holder assembly (Supelco, Bellefonte, USA). The vials which contained samples were placed in a water bath (80 °C) for 20 min to equilibrate, and then the SPME needle septum the pierced so that the fiber could exposed to the headspace of the sample for 30 min. The fiber was retracted into the needle and transferred immediately into the injection port of a gas chromatograph and desorbed for 5 min at 250 °C. The fiber should be aged for 15 min at 250°C before the next sample is analyzed.

A GC apparatus (7890A, Agilent, Palo Alto, CA, USA) coupled to a PolarisQ GC-IT-MS (ThermoFisher USA) was employed to analyses volatiles. The carried gas was ultrahigh-purity helium, and the flow rate was 1.0 ml/min. The oven initial temperature was set to 50 °C for 2 min, then increased to 90 °C at 2 °C/min and held for 3 min, then further increased to 220 °C at 5 °C/min, where it was held for 5 min. Mass spectra were acquired over the range of 40 to 550 m/z, and the ion source temperature was 250 °C. All experiments, including the GC-IT-MS analyses, were performed in triplicate.

2.6 Results

2.7 Enumeration, pH, and total titratable acidity (TTA) measurements

The results of the microbiological analysis and acidity measurements are shown in Figure 1 and Table 1. The yeast and LAB counts were comparable; Yeasts were present at levels of 10^2-10^5 CFU/mL while LAB was counted at 10^4-10^7 CFU/mL. There is no doubt that the ratio of yeasts to LAB affects the ecological system of sourdoughs. The ratio ranged from 1:10,000 to 1:1 between yeasts and LAB of the 28 sourdoughs, except for SD-16 (1:10^5) and SD-21 (1:10^6) sourdoughs. However, the ratio of yeasts to LAB in sourdoughs ranged from 1:1000 to 1:100 in [3], while the sourdoughs in [2] presented a much higher yeasts to LAB ratio, ranging from 1:67 to 1:4.

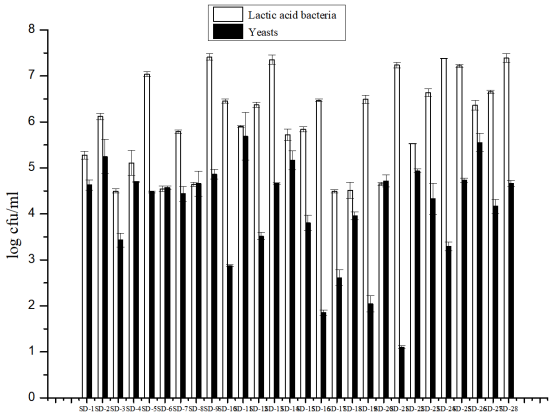

Figure 1. Histogram representations of cell density (log CFU/mL) of presumptive LAB and yeasts in Chinese traditional sourdough samples.

In this study, the sourdoughs under consideration showed values of pH, which ranged from 3.50 (SD-13) to 4.91 (SD-11), which were similar to those reported in the literature [9]. The lowest TTA value was 2.5

[1] http://drive5.com/uparse/
[2] http://rdp.cme.msu.edu/

mL of NaOH (1 M) for sample SD-25, which also had the highest pH value of 4.3. It was clear that the more acids produced by the LAB, the lower the pH. However, sample SD-11 had a pH value as high as 4.91 and a TTA value as high as 7.8 mL, which was surprising. It is presumed that the TTA values provide an indication on the total acidity found in the sourdough, whereas the pH values indicate the amount of strong acid produced by bacteria in the sourdough [21].

2.8 Isolation and 16S rRNA sequence analysis

By using partial sequence analysis of 16S rRNA, a total of 180 strains were identified. With the exception of samples SD-5, SD-12, and SD-24, Lactobacillus plantarum was identified in all the sourdoughs, and 51% of the LAB isolated from sourdoughs was allotted to this species (Figure 2). L. plantarum is the key LAB in most of the sourdoughs, which is in agreement with other studies [22]. The dominance of L. plantarum may be ascribed to the parameters in the manufacturing operation, such as the type of flour used, storage temperature, leavening, back-slopping practices, and/or pH of the dough [23]. The intrinsic capacity of adaptation to the sourdough environment may also play important roles. However, Lactobacillus sanfranciscensis is the key LAB in some foreign sourdoughs [24].

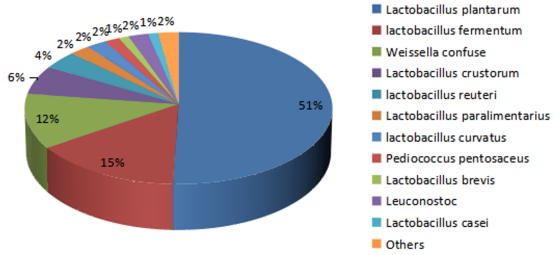

Figure 2. Distribution of all the LAB species in different sourdough samples. Other species included Lactobacillus pontis, Lactobacillus rossiae, and Lactobacillus farciminis.

Other LAB identified among the 180 strains were: Lactobacillus fermentum (15%), Weissella confusa (12%), Lactobacillus crustorum (6%), Lactobacillus reuteri (4%), and these strains had all been isolated in previous studies [24, 25]. Lactobacillus paralimentarius, Lactobacillus curvatus, Pediococcus pentosaceus, and Leuconostoc were the less dominant LAB, accounting for 2% respectively. Lactobacillus brevis and Lactobacillus casei accounted for only 1%. The last 2% was comprised of other species, including Lactobacillus pontis, Lactobacillus rossiae, and Lactobacillus farciminis.

2.9 Identification and estimates of microbial diversity of bacteria

Microbial diversity of bacteria was assessed using pyrosequencing analysis, yielding a large number of reads. The sequence reads per sample ranged from 20,048 to 37,568, with 20,048 reads from each sample further analyzed. Among all samples, the Good's coverage values at the 3% dissimilarity level were 99.9%, which indicated that most species were included in the sourdoughs.

The structure of community in sourdough samples at the genus level was shown in Figure 3. We only considered the sequences with a minimum threshold of 1%. It can be seen that the bacterial community was different for different samples. The distinct and dominant genera were Lactobacillus and Weissella, which agreed with the results of culture-dependent methods. Lactobacillus was the major common genus and appeared in the all samples. Sourdough SD-1, SD-10, SD-12- SD-15, SD-17, SD-20, SD-21, SD-27, SD-28, SD-3,SD-5, and SD-7, SD-9 harbored Lactobacillus as the most abundant bacterial species (>50%), while Weissella was the major genus in other samples, except for SD-23. Chloroplast norank and Lactococcus also appeared in all samples, but accounted for a lesser percentage, with the exception that Lactococcus was the major genus in SD-23.

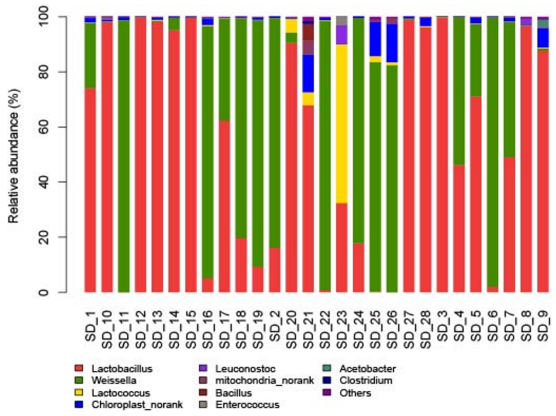

Figure 3. Phylogenetic taxonomic structure of different traditional sourdough samples. 16S amplicon pyrosequencing analysis was classified to the genus level using the BLASTN sever based on the 16S rRNA database to the genus level.

An in-depth taxonomic analysis at the genus level was conducted. The heat map which targeted the top 33 genera basically showed that many minor genera exhibited different distributions (Figure 4). For example, the abundance of Leuconostoc and Enterococcus was higher in SD-23, while Weissella

displayed opposite distributions. Remarkably, the microbiota of SD-24, SD-25, and SD-26 was similar to the samples collected from Henan in Zhenzhou Province. Otherwise, relative abundance of Thauera, Dechloromonas, and Bacteria unclassified was higher in SD-22. In previous studies, 35 genera were identified by using pyrosequencing analysis [2], while only 3 genera (Lactobacillus, Leuconostoc, and Weissella) were found in traditional sourdough samples and 4 genera (Lactobacillus, Leuconostoc, Lactococcus, and Weissella) were found by denaturing gradient gel electrophoresis (DGGE) methodology [3].

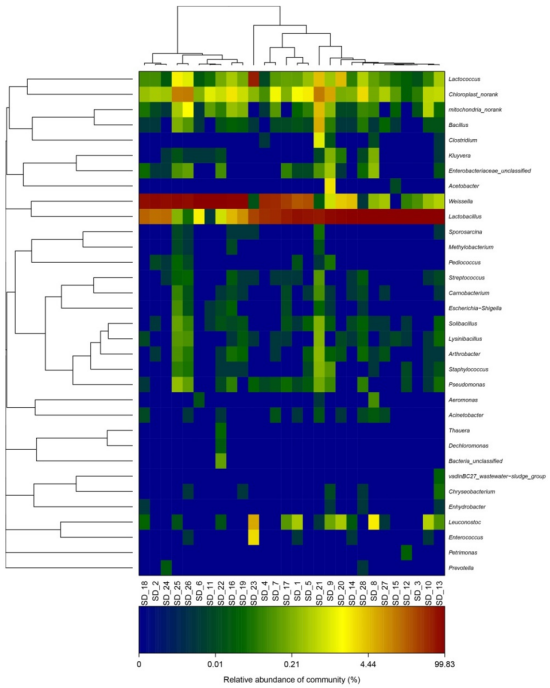

Figure 4. Double hierarchical dendrogram showing the bacterial distribution among the twenty-eight traditional sourdough samples. The bacterial phylogenetic tree was calculated using the neighbor-joining method, and the relationship among samples was determined by Bray distance and the complete clustering method. The heat map plot depicts the relative percentage of each bacterial family shown in the legend indicated at the bottom of the figure. Clusters along the X-axis and the bacterial families along the Y-axis are indicated in the upper and left portions of the figure, respectively.

Furthermore, we had explored the correlation between different samples of the microbial communities by principal component analysis (PCA). The first and second principal components (PCs) could explain 67.28% and 16.01% of the total variation, respectively (Figure 5). The plot area was roughly divided into three parts. One group formed by sourdoughs SD-3, SD-17, SD-1, SD-20, SD-13, SD-27, SD-15, SD-8, SD-14, SD-10, SD-28, was positioned in the upper left half of the plane."This position may indicate that Lactobacillus contributed to the differences when compared with other samples, as shown in Figure 4. From Figure 4 we can see that SD-18, SD-22, SD-11, SD-2, SD-16, SD-6, SD-19, SD-24, SD-25, SD-26, SD-4, had higher level of Weissella, positioned in the upper right part of the plane in Figure 5. The remaining area, formed by SD-23, SD-7, SD-5, SD-9, SD-21, SD-12, was characterized by higher Lactococcus levels in SD-23 and a 1:1 ratio of Lactobacillus to Weissella in SD-7, although it still had more Lactobacillus. The results show that the predominant genera differentiate all the samples, but there were still other factors influence the distribution of samples.

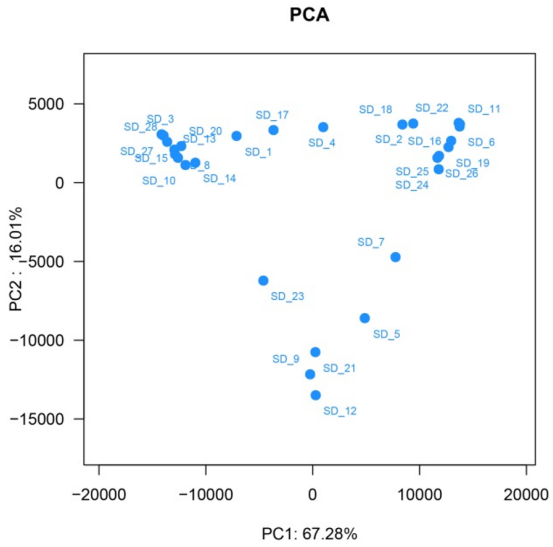

Figure 5. Principal component analysis of bacterial classes abundance.

2.10 SPME/GC-MS analysis

There was variation among the five steamed breads in the category and content of the volatile compounds. A total of 24 volatile compounds were identified (Table 2). Besides two specific compounds (benzaldehyde and o-hydroxybenzoic acid), 20 other volatile compounds were identified, categorized as hydrocarbons (10), aromatic compounds (6), alcohols (3), and esters (3). 11 compounds were identified in the control group, while 19 were in the SD-8 sample, and 17, 15, 16 were in SD-18, SD-20 and SD-10 samples, respectively. In sourdough steamed bread samples, benzeneethanol and benzaldehyde were abundant, as previously reported in sourdough breads. Generally, n-tetradecane, vinyl ethyl furan, benzene-propoxyethyl and n-hexyl alcohol were much more abundant than other compounds. 1-Methylnaphthalene, identified in steamed breads, had a relative proportion ranging

Table 2. GC-MS identification of traditional sourdough steamed bread.

Compound		Molecular formula	Formula weight	Relative peak area (%)				
				Control	SD-8	SD-10	SD-18	SD-20
Hydrocarbon	n-pentane	C_5H_{12}	72	–	1.066	1.43	1.505	1.535
	Cyclopentene-1-butyl	C_9H_{16}	124	–	2.392	1.347	1.999	–
	1-octylene	C_8H_{16}	112	–	–	1.314	1.152	1.612
	1-heptylene	C_7H_{14}	102	–	2.49	–	–	–
	n-tetradecane	$C_{14}H_{30}$	198	4.339	4.841	–	3.071	3.056
	Pentadecyl-1,3,5,8-tetraene	$C_{15}H_{24}$	204	19.408	–	1.799	–	–
	n-cetane	$C_{16}H_{34}$	226	–	1.36	1.031	0.804	0.826
	n-heptadecane	$C_{17}H_{36}$	240	–	–	–	0.414	–
	1-hexene	C_6H_{12}	84	1.879	1.439	–	–	–
	1-heptadecene	$C_{17}H_{34}$	238	–	–	0.723	–	–
	Total			25.626	13.588	7.644	8.945	7.029
Aromatic compounds	Propyl benzene	C_9H_{12}	120	–	–	3.242	0.942	1.729
	1-methylnaphthalene	$C_{11}H_{10}$	142	1.342	3.824	0.998	2.418	1.753
	Butylated hydroxytoluene	$C_{15}H_{24}O$	220	–	1.926	–	–	–
	Benzocyclohexane	$C_{10}H_{12}$	132	–	1.967	–	–	–
	Vinyl ethyl furan	$C_8H_{10}O$	138	7.242	13.244	6.389	12.684	14.765
	Benzene -propoxyethyl	$C_{11}H_{16}O$	164	–	6.345	3.646	5.795	5.576
	Total			8.584	27.306	14.275	21.839	23.823
Alcohols	n-hexyl alcohol	$C_6H_{14}O$	102	7.466	3.939	7.093	9.352	9.129
	Benzeneethanol	$C_8H_{10}O$	122	10.574	5.14	36.868	8.319	–
	Allyl alcohol	C_3H_6O	58	1.665	–	–	–	4.969
	Total			19.705	9.079	43.961	17.671	14.098
Esters	1, 2, phthalate butene 2 - sec-butyl			–	1.977	–	0.97	0.835
	Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.544	0.62	1.499	1.592	0.839
	Ethyl linoleate	$C_{20}H_{36}O_2$	308	–	0.669	1.605	2.776	0.959
	Total			0.544	3.266	3.104	5.338	2.633
Aldehydes	Benzaldehyde	C_7H_6O	106	2.894	4.064	3.343	3.221	3.569
	Total			2.894	4.064	3.343	3.221	3.569
Acids	o-hydroxybenzoic acid	$C_7H_6O_3$	138	–	1.176	–	–	–
	Total			0	1.176	0	0	0

from 0.998% in the SD-10 sample to 3.824% in the SD-8 sample [30]. In control steamed bread, the relative proportion of aromatic compounds and esters (8.584% and 0.544%) was much lower compared to sourdough breads. Ethyl linoleate and benzene-propoxyethyl were detected in tested samples but not detected in control steamed bread.

3 Discussion

CSB has become a staple food with industrialized production methods in China. Fermentation method is one crucial step during the processing of final products, and it is closely linked to the quality of CSB. Sourdough fermentation, not only positively affects the CSB' texture and flavor, but also improves nutritional value, shelf-life, and is effective in reducing the increment of postprandial glycemia [36–40]. These functions are likely closely related to its complex microbial ecosystems. Currently, yeast fermentation, being the main method of dough processing, is considered the reason traditional flavors have not been preserved. Moreover, traditional sourdough is easily accessible, but mainly in artisanal bakeries due to the difficulties and costs of maintaining a live microbial culture in industries. In the future, to reduce the need for sourdough starter maintenance, drying techniques (such as freeze-drying and spray-drying) is also an ideal way to preserve high fermentation capacity and cell viability after reconstitution [41].

This study first conducts a scanning analysis of some basic parameters of traditional CSBs from different regions that are representative. The range of pH value (3.5-4.91) in this investigation was larger than that found in Italian sourdoughs (3.70–4.28) [25]. Additionally, the pH value reached as high as 5.51. There is no doubt that pH values are mainly caused by differences in microorganisms in sourdoughs, especially the LAB contained. According to the culture-dependent result, L. plantarum was the dominant species. This result was in agreement with isolated results performed on traditional sourdough samples gathered from the western region of Inner Mongolia [3] and Italy [20]. In other studies, Ricciardi et al. [29] baked traditional durum wheat breads using L. plantarum as the main microorganism. L. sanfranciscensis was proved to be the dominant species in sourdoughs which are used for the manufacture of traditional Italian leavened baked products [27] and in French organic sourdoughs [24]. L. sanfranciscensis may be the most suitable species and thus is regarded

as the autochthonous primary organism in type-I sourdoughs [28]. However, we did not detected L. sanfranciscensis in our study.

The pyrosequencing analysis and culture-dependent method can offer a complementary protocol to describe the sourdough microbiota diversity. Lactobacillus and Weissella were the predominant species among bacterial communities, which is in agreement with the result of previous studies [3, 20]. We also observed that some species (Bacillus, Enterococcus and Acetobacter) were not found in culture-dependent methods. This could be as a result of sampling and sample handling techniques, such as aerobic or anaerobic storage, transport, or refrigeration procedures, which may affect the microbial population by increasing or decreasing the occurrence and number of species to be detected in sourdough.

In recent decades, most research focused on the investigation of the metabolism of carbohydrate and nitrogen in the yeasts and LAB as well as the flavor generated during fermentation [27]. SPME, namely solid-phase microextraction, is a powerful sample preparation tool for the analysis of flavor compounds. Its greatest advantage is that it allows sampling, extraction, and concentration in a single solvent-free step and is commonly combined with gas chromatography/mass spectrometry (GC/MS) for use [31]. For instance, Zhang et al. [3] studied the aromatic compounds of CSB using three different extraction methods: SPME, P&T, and SDE. Wu et al. [30] extracted the aromatic compounds of spontaneously fermented and LAB-fermented steamed breads by using SPME coupled with GC/MS. Similarly, the volatile profiles of breads have long been studied in foreign countries. It is well acknowledged that sourdough affects the generation of aromatic compounds in wheat bread [35]. Bread from spontaneously fermented sourdoughs and mixed cultures of yeasts and LAB has also been studied [32–34]. As shown in the experimental results, it is obvious that the metabolic activities of LAB influence the generation of volatile compounds and the relatively high content of aromatic compounds reported during the microbial fermentation of sourdoughs. Moreover, Benzene-propoxyethyl may be produced by LAB, yeasts and certain molds during fermentation, influencing the flavor of traditional fermented products through its aromatic properties. Future research could employ metabolomics to analyze its specific biosynthetic pathways and regulatory mechanisms, thereby optimizing the flavor quality of CSBs.

This study found that the bacterial community and LAB diversity of the traditional CSBs have been revealed, which will provide us with important insights to understand their functions in sourdoughs; The flavor compounds contained in sourdough fermented CSBs, both in terms of quantity and concentration, are higher than those in yeast-fermented CSBs. Obviously, microbial diversities, especially LAB, affect the flavor generation during fermentation. The present work has laid the foundation for further exploring the deep connections between microbial communities and food flavors, as well as developing novel starter. For instance, starter cultures can be developed using the predominant microbes (1-2 species/genera) in CSBs' sourdough, with the levels of 1-2 key flavor compounds serving as benchmarks to replicate its traditional flavor profile.

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

Xiaomin Li is an employee of COFCO Nutrition and Health Institute, Beijing 102209, China.

Ethical Approval and Consent to Participate

Not applicable.

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