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Disparities and correlation of bacterial communities and physicochemical properties in traditional fermented suancai from different regions

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ABSTRACT

High-throughput sequencing (HTS) and gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) were used to compare the microbiota structure and metabolic compounds of traditional suancai from Heilongjiang (HLJ), Shanxi (SX) and Qinghai (QH) in China. Besides, the physicochemical properties such as total number of colonies, pH and total acid content were determined, and the related factors of the differences were analyzed. The salinity of the 3 samples was 1.9%, 8.0% and 10.0%, respectively, and the dominant bacterial genera were *Loigolactobacillus*, *Arcobacter*, and *Marinomonas*. Meanwhile, *Loigolactobacillus* was significantly positively associated with pH and nitrite in HLJ, *Arcobacter* was inversely related to pH and nitrite, while *Marinomonas* was negatively correlated with all physicochemical properties in QH which had the highest salinity. In addition, the 5 main differential metabolites in the 3 samples were acetic acid, 4-ethylphenol, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 2,4-tert-butylphenol, and 3-butenenitrile. Among them, the ketones and acids were positively correlated with the core bacteria in HLJ with the lowest salinity, and the main genera in SX were positively associated with various alcohols, while there was a positive correlation between *Marinomonas* and butyronitrile alcohol in QH with the highest salinity. This study provided a guidance for the differences and correlations of microorganisms, flavor compounds, and quality characteristics from a regional perspective by studying the various quality characteristics of the suancai.

1. Introduction

Fermentation is a biochemical process which the nutrients in the raw materials are used to promote the growth and reproduction of microorganisms, and they will produce a variety of nutrition, metabolites, and flavor to the products[1-2]. Currently, studies on fermented vegetables mainly focus on determining the correlations of quality and microbial community. Studies have shown that the abundance of microflora increases with the increase in fermentation temperature. *Leuconostoc* held a dominant position at 10 and 15 °C, positively related to 1-hexanol, 1-octanol, linalool, and phenylethyl alcohol[3]. Besides, salinity also affected the flora and flavor of kimchi, and the low-salinity sample contained higher levels of *Leuconostoc mesenteroides* and mannitol[4]. Liu et al.[5] found that salinity and sampling location would affect the structure of

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microflora, and *Lactobacillus* was the dominant genus in jiangshui from the northeast with lower salinity (0.8–3.4 g/L), whereas *Lactobacillus* and *Pediococcus* were the main genera in the northwest suancai with higher salt concentrations (4.1–22.7 g/L). The levels of lactic acid, acetic acid, and oxalic acid were lower in jiangshui compared with suancai. Additionally, some researchers have revealed Enterobacteriaceae and Lactobacillaceae could produce *L*-lactate dehydrogenase and acetate kinase through metatranscriptomics. They were related to lactic acid, acetic acid, and malic acid metabolism, and promoted the acidification of paocai[6]. The studies have revealed how these conditions affected the flora and flavor of kimchi, and the ultimate goals were to improve the quality and provide some theoretical guidance for actual production and health benefits to human body[7].

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is widely consumed in China. It contains glucosinolates, a wholesome substance, which has antioxidant properties and a negative association with colon and rectal cancer[8]. In China, traditional suancai was made of Chinese cabbage and was widely consumed because it has unique flavor, taste, and a long storage period after fermentation. Moreover, different processing methods might affect the content of beneficial substances[9-11]. Liu et al.[12] found Harbin sauerkraut contained 88 volatile organic compounds and inoculated sauerkraut contained more alcohols, esters, and acids. Compared with sauerkraut without microbial supplementation, the product had more kinds of compounds that were closely related to the core microorganisms. A parallel study demonstrated that the key bacteria at the levels of phyla, genus, and species in paocai were Firmicutes, *Lactobacillus*, and *Lactobacillus plantarum*, respectively[13]. In addition, compared with fungi, the abundance of bacteria had a stronger correlation with physicochemical attributes. Therefore, the diversity of microbial communities affected the types of compounds, which gave suancai different physicochemical properties.

Most previous studies focused on the impact of conditions on suancai and monitored changes in physical and chemical properties during fermentation. Based on geographic and cultural differences, different regions have developed distinct eating habits, which led to different flavors and textures of suancai. However, there is still a lack of research on the comparison and the reason for the quality differences of traditional suancai in different regions of China. The study investigated suancai from 3 different regions in China, Qinghai (QH), Heilongjiang (HLJ) and Shanxi (SX), and analyzed the bacterial communities and microbial diversity *via* high-throughput sequencing (HTS), and the differential volatile components in the suancai were detected through gas chromatography time-of-flight mass spectrometry (GC-TOF-MS). The correlations among bacterial communities, physicochemical properties, and flavor substances were systematically compared to deepen the understanding and the contribution of dominant bacteria to the suancai quality.

2. Material and methods

2.1 Sample collection

The home-made suancai acquired from Xining (Qinghai, China), Jiamusi (Heilongjiang, China), and Datong (Shanxi, China), which were made of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Fresh cabbages were purchased from the local market, washed and cut into equal-sized strips, then mixed with 10.0%, 1.9% and 8.0% salt, and stored in a cool place. After around 2 months, the samples were collected and sent to the laboratory within 48 h, then were divided evenly into 2 parts, and stored at 4 ℃ for microbiological analysis or –80 ℃ for physicochemical determination (Alphavita Bio-scientific Co., Ltd., Dalian, China).

2.2 Total number of bacterial colonies

Total bacteria count was counted using dilution coating method. The 0.5 mL sample was mixed in 4.5 mL sterile saline and gradually diluted to 10^1–10^9 CFU/mL, then selected the appropriate concentration gradient for dilution coating. All plates were counted after 48 h incubation at 37 ℃ (Zhejiang Huayuan Instrument Co., Ltd., Hangzhou, China). All experiments were performed in triplicate.

2.3 Determination of physicochemical properties

pH was determined using the PB-10 pH meter (Sartorius Scientific Instruments Co., Ltd., Beijing, China) according to Stoll et al.[14]. The total acid (TTA) content was determined using the acid-base titration[15]. Nitrite (NIT) was determined *via* ultraviolet spectrophotometry (Mapped Instrument Co., Ltd., Shanghai, China)[16]. All experiments were performed in triplicate.

2.4 Analysis of volatile flavor compounds through non-targeted GC-TOF-MS

Volatile compounds were detected using the GC-MS system equipped with an Agllent DB-WAX column (30 m × 250 μm, 0.25 μm, California, USA), and 2-octanol was used as the internal standard. A total of 100 μL sample were added to a headspace bottle, and preheated at 60 ℃ for 15 min, extracted for 30 min, then purged at 3 mL/min in split-flow mode. The column oven temperature program was 40 ℃ for 4 min, then raised to 245 ℃ and held for 5 min. The MS data were acquired with the *m/z* range of 20–400 at a solvent delay of 0 min (7890B, Agilent Technologies Inc., California, USA).

2.5 Determination of bacterial community diversity

The samples were centrifuged at 8 000 × *g*, 4 ℃ for 10 min (Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd., Shanghai, China).

Table 1
Sample information.

Sample name	Raw materials	Fermentation temperature (℃)	Salt (%)	Pickling time (month)	Sampling site
QH	Chinese cabbage	15	10.0	2	Xining, Qinghai
HLJ	Chinese cabbage	14	1.9	2.5	Jiamusi, Heilongjiang
SX	Chinese cabbage	15	8.0	2	Datong, Shanxi

After centrifuging, the primer was designed according to the conserved region (F: 5′-ACTCCTACGGGAGGCAGCA-3′; R: 5′-GGACTACHVGGGTWTCTAAT-3′) (Tiangen Biotech Co., Ltd., Beijing, China). PCR was performed at the end of the primer with a sequencing adapter, and a sequencing library was formed (Beijing Bomei Fuxin Technology Co., Ltd., Beijing, China). The library was qualified and sequenced with Illumina NovaSeq 6000 (Illumina, California, USA). All experiments were performed in triplicate.

2.6 Data analysis

A total of 427 metabolites were retained after the data were normalized for the univariate and multivariate statistical analyses. SPSS statistics was used for Student's *t*-test and one-way analysis of variance (ANOVA). In SIMCA 14.1, principal component analysis (PCA) determined the differences in the characteristic volatile flavor compounds of the samples. Volatiles with variable importance in the projection (VIP) > 1 and $P < 0.05$ were selected as markers for distinguishing flavor characteristics. The corresponding orthogonal partial least squares discrimination analysis (OPLS-DA) model was used to maximize the difference in flavor compounds among the pickles.

The bioinformatics analysis was finished with the help of the BMK Cloud (Biomarker Technologies Co., Ltd., Beijing, China). *α*-Diversity was estimated using Chao1, Shannon, and Simpson, which reflected the microbial abundance and diversity. Principal coordinate analysis (PCoA) was analyzed based on *β*-diversity. Spearman correlation was used to measure the relationship of 2 ranking variables.

3. Results

3.1 Analysis of basic physical and chemical properties

The physical and chemical properties of the samples were shown in Table 2, and the total number of colonies were 4.30×10^3, 5.95×10^6, and 1.67×10^6 CFU/mL, respectively. The pH and TTA were crucial indicators of suancai. The pH value of QH, HLJ, and SX was 3.77, 5.66, and 3.71, respectively. Their corresponding nitrite contents were 0.02, 0.16, and 0.04 mg/kg, respectively.

Table 2
Determination results of physical and chemical properties of different pickles.

Sample name	Total number of bacterial (CFU/mL)	pH	TTA (g/L)	Nitrite (mg/kg)
QH	4.30×10^3	3.77 ± 0.00	47.40 ± 0.62	0.02 ± 0.00
HLJ	5.95×10^6	5.66 ± 0.01	32.41 ± 0.78	0.16 ± 0.02
SX	1.67×10^6	3.71 ± 0.00	68.04 ± 0.36	0.04 ± 0.00

3.2 Analysis of volatile compounds

3.2.1 PCA analysis

The PCA of different flavor metabolites were presented in Fig. 1A. In total 93.5% of the cumulative differences were described, including 61.4% of PC1 and 32.1% of PC2. The 3 samples were far apart in the coordinate axis, demonstrating that the volatile flavor compounds were quite different.

Figs. 1B-D presented the metabolic difference compound PCA of the 3 types of suancai. Overall, 94.6%, 93.7%, and 93.5% of the

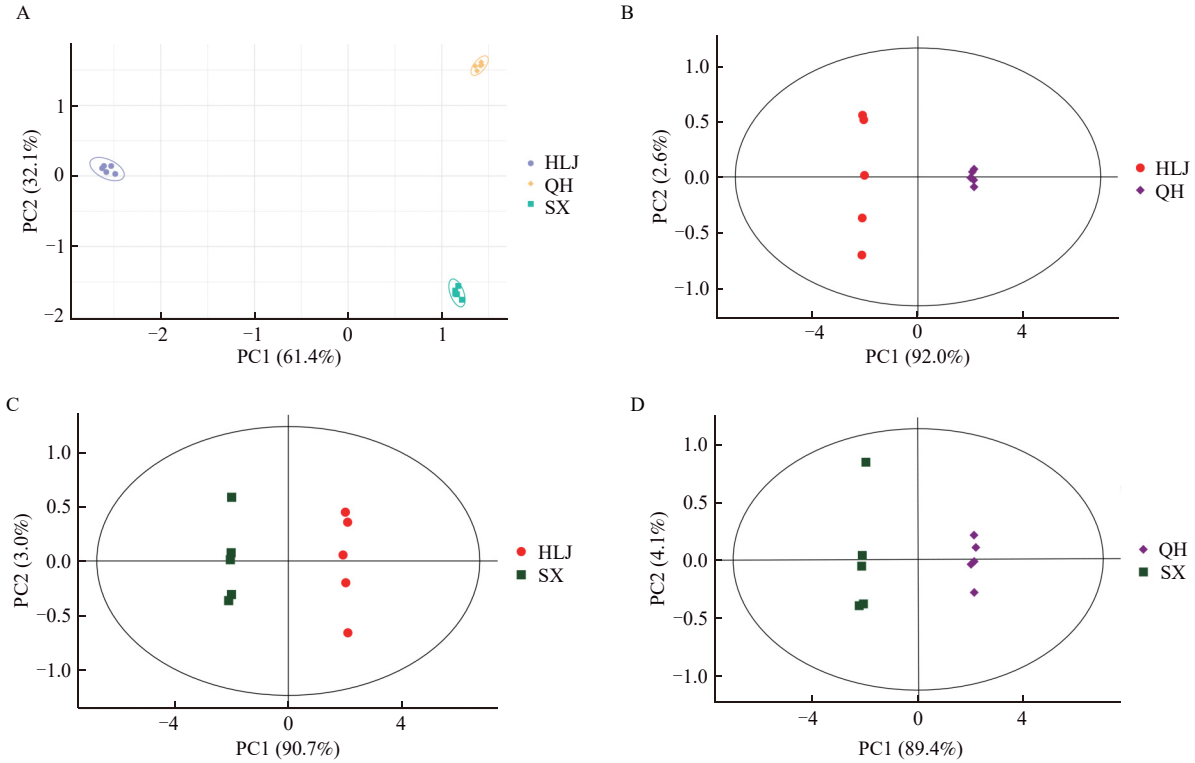

Fig. 1 PCA of flavor compounds. (A) PCA scores plot of the volatile compounds in the pickles. Score scatter plot of PCA model for group (B) HLJ *vs*. QH, (C) SX *vs*. HLJ, and (D) SX *vs*. QH.

cumulative differences were described among HLJ compared with QH, SX compared with HLJ, and SX compared with QH. All samples were in the 95% confidence interval. Two samples in the 3 groups were located in different quadrants, which means that differences were present in volatile compounds among them. These differences in distribution reflected that the differences of regions affected the types of volatile compounds.

3.2.2 OPLS-DA score analysis and permutation test

The OPLS-DA score scatter plot was shown in Figs. 2A-C. The results revealed significant differences among the 3 samples. Permutation test results showed that the model interpretation rate R^2_Y and the model prediction ability Q^2 value were all greater than 0.5, which indicated that the fitting model was reasonable and they could reflected the difference of volatile compounds (Figs. 2D-F).

3.2.3 Univariate analysis for screening differential flavor compounds and volcano maps

In total, 427 volatile components were identified through GC-TOF-MS and 34 substances with similarity greater than 900 were selected for metabolites analysis. The results were presented in Fig. 3. The marked substances were the top 20 differential compounds with the smallest P value. Differential metabolites with VIP > 1 and $P < 0.05$ were screened among the differential metabolites. The top 5 up-regulated substances among the differential metabolites compound were 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 4-ethylphenol, acetic acid, 3-butenenitrile, and 1-hexanol, which were screened from HLJ compared with QH. In total 28 differential metabolites were screened from SX compared with HLJ, among them the top 5 up-regulated compounds were methyl alcohol, 2,4-tert-butylphenol, benzenepropanenitrile, 3-methyl-1-butanol, 1-hexanol. Among the 30 differential metabolites screened from SX compared with QH, the top 5 up-regulated metabolites were 2,2,4-trimethyl-1,3-vpentanediol diisobutyrate, benzenepropanenitrile, 1-hexanol, methanethiol, and 2-butanone.

3.3 Analysis of bacterial communities

3.3.1 Taxonomic analysis of species

The abundance of the top 10 species at different levels were presented in Fig. 4. The abundance of bacterial communities varied at different levels. The microbial community in HLJ was the most abundant, and the feature number of QH and SX were less (Fig. 4A).

At the phylum level, the top 5 abundant phyla were Firmicutes, Proteobacteria, Campylobacterota, Cyanobacteria, and Bacteroidota (Fig. 4C). Firmicutes was the most important bacteria of the suancai, and the average relative abundance was 36.3%. At the class level,

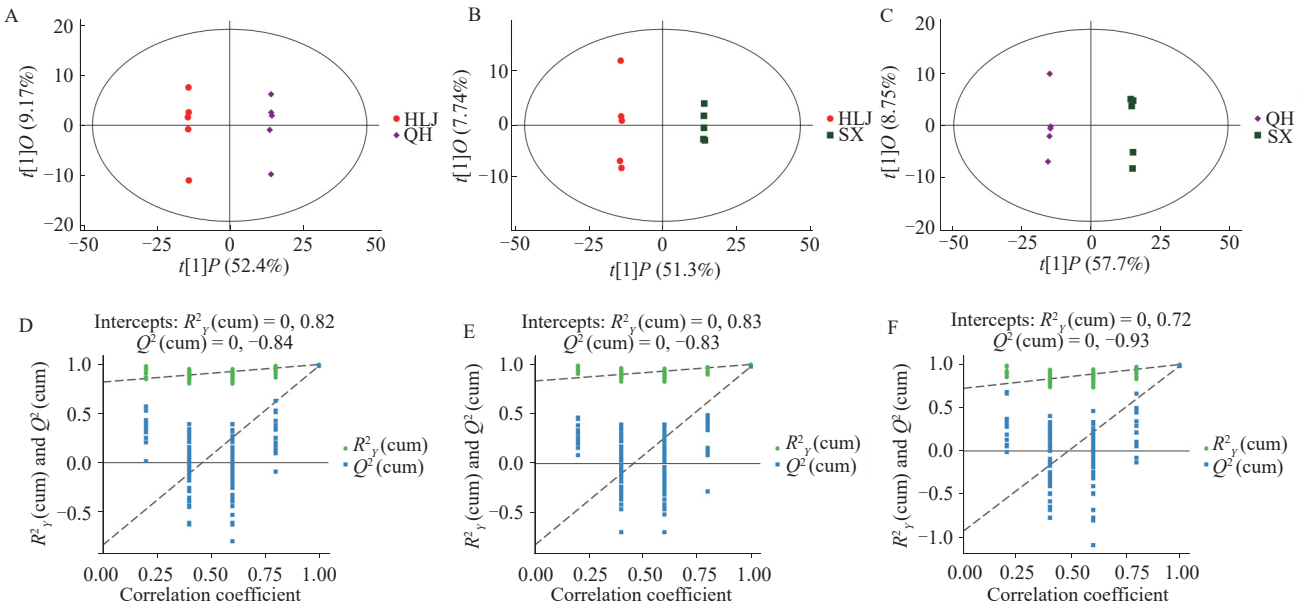

Fig. 2 Testing the model by using OPLS-DA. Score scatter plot of OPLS-DA model for group (A) HLJ *vs*. QH, (B) SX *vs*. HLJ, and (C) SX *vs*. QH. Permutation plot test of OPLS-DA model for group (D) HLJ *vs*. QH, (E) SX *vs*. HLJ, and (F) SX *vs*. QH.

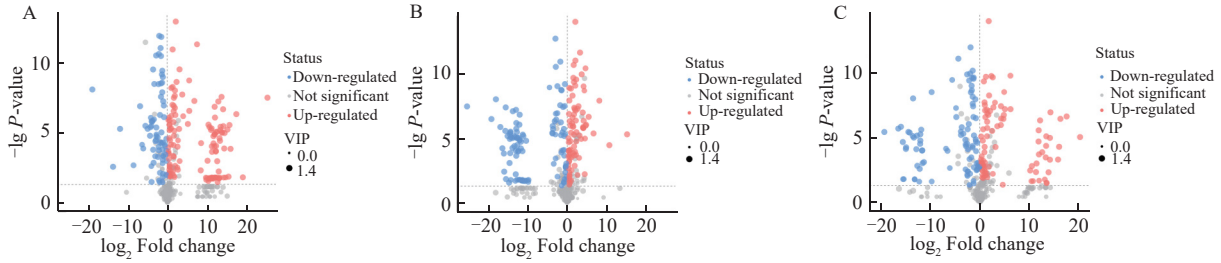

Fig. 3 Screening of differential metabolites in the samples. Volcano plot for groups of (A) HLJ *vs*. QH, (B) SX *vs*. HLJ, and (C) SX *vs*. QH.

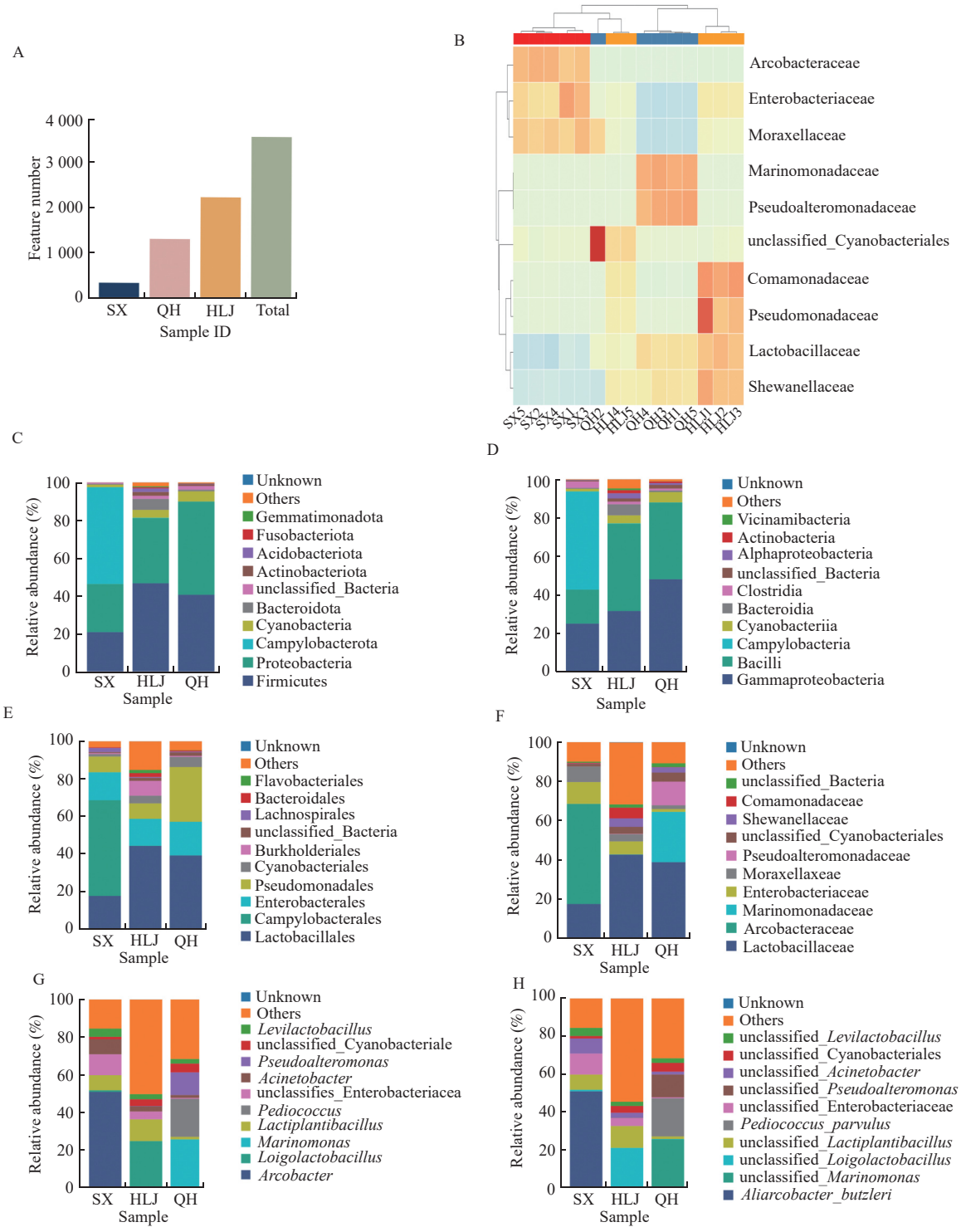

Fig. 4 Bacterial species annotation and taxonomic analysis in the samples. (A) Distribution map of characteristic number of the samples. (B) The heat map of species abundance at the genus level for each sample. Composition of bacterial communities in SX samples, HLJ samples, and QH samples at (C) phylum, (D) class, (E) order, and (F) family, (G) genus, and (H) specied level.

Gammaproteobacteria, Bacilli, Campylobacteria, Cyanobacteriia, and Bacteroidia were the top 5 abundant classes (Fig. 4D). At the order level, Lactobacillales, Campylobacterales, Enterobacterales, Pseudomonadales, and Cyanobacteriales were most dominant (Fig. 4E). Among them, Lactobacillales was the most dominant, with an average abundance of 33.7%. In addition, Lactobacillaceae (33.0%) was the dominant family. At the genus level, there were some differences in the samples. As shown in Fig. 4G, the top 3 dominant bacteria in SX were *Arcobacter*, *Lactiplantibacillus*, and *Acinetobacter*, in HLJ were *Loigolactobacillus*, *Lactiplantibacillus* and unclassified_ Enterobacteriaceae, while the main genus of QH were *Marinomonas*, *Pediococcus* and *Pseudoalteromonas*.

3.3.2 α-Diversity analysis results

Fig. 5A shows that there were 42 common characteristic flora among 3 groups. A total of 186 characteristic flora were presented between QH and HLJ, while there were 70 same characteristic flora between HLJ and SX. There were 3 577 special characteristic flora and 230 common flora among the suancai. The analysis results were consistent with the operational taxonomic unit (OTU) results.

The richness index (ACE and Chaol), bacterial diversity index (Shannon and Simpson), phylogenetic diversity index (PD whole tree), and sequencing depth index (Good's coverage) of bacterial community were calculated based on the α-diversity, and the results were shown in Table 3. The species richness were high to low in the order of HLJ, QH and SX. The Shannon and Simpson indices revealed that the species diversity of HLJ was greater than that of QH and SX, and the abundance and diversity of bacteria were varied. In addition, the coverage of all samples were close to 1, which demonstrated the probability of species detection were high.

The rarefaction curves indicated that the sequencing data of microorganisms were reasonable (Fig. 5B). The Shannon index curve indicated that the sequencing data were sufficient to reflect most of the microorganisms (Fig. 5C). The species abundance were higher and the gradual flattening of curve reflected the species distribution were relatively uniformly distributed.

Table 3
Statistical table of α-diversity index.

Sample name	Feature	ACE	Chao1	Simpson	Shannon	PD whole tree	Coverage
HLJ 1	342	342.706 1	342.019 2	0.932 6	5.490 2	32.813 4	1.000 0
HLJ 2	464	466.899 1	464.538 5	0.932 8	5.717 4	47.996 9	0.999 9
HLJ 3	511	512.790 6	511.135 1	0.938 3	5.849 0	50.594 6	0.999 9
HLJ 4	867	869.472 3	867.175 0	0.976 0	7.306 2	92.232 1	0.999 9
HLJ 5	865	868.222 0	865.324 3	0.977 4	7.379 8	89.106 4	0.999 8
QH 1	140	141.269 8	140.300 0	0.893 0	3.952 5	15.584 8	1.000 0
QH 2	1 051	1 051.890 8	1 051.073 2	0.923 6	5.920 1	125.890 1	0.999 9
QH 3	137	137.693 1	138.000 0	0.890 7	3.904 1	13.144 0	1.000 0
QH 4	175	176.978 2	175.370 4	0.892 6	3.954 5	23.914 6	0.999 9
QH 5	142	142.984 8	142.333 3	0.889 3	3.877 5	14.334 5	1.000 0
SX 1	193	193.000 0	193.000 0	0.793 0	3.851 0	35.492 8	1.000 0
SX 2	124	124.789 9	124.750 0	0.724 8	3.450 8	12.133 6	0.999 9
SX 3	117	117.000 0	117.000 0	0.768 6	3.593 5	11.331 0	1.000 0
SX 4	114	114.328 6	114.000 0	0.736 3	3.524 2	10.037 4	1.000 0
SX 5	121	121.000 0	121.000 0	0.760 7	3.606 8	10.761 8	1.000 0

3.3.3 β-Diversity analysis

PCA revealed the differences of the bacterial community (Fig. 5D). Overall, 91.0% of the cumulative differences were described. HLJ was in the third quadrant, the samples of SX were close to each other and they were near the positive *X*-axis. This demonstrated that PC2 had larger contribution to the difference of QH and SX, whereas the contribution of PC1 to HLJ was relatively small. The results of PCoA were presented in Fig. 5E. The suancai from SX, HLJ, and QH were far away from each other in the coordinate axis. This indicated differences among the 3 bacterial communities. These results reflected the bacteria composition of the suancai were quite different.

The heat map among the samples shown that HLJ and SX were clustered together in general, and they were at a relative distance from the QH (Fig. 5F). Besides, the samples of QH and SX could be clustered into one group in the degree of microbial community clustering at the genus level, and both of them could be clustered with HLJ (Fig. 5F). The bacterial community differences between QH and SX were relatively small.

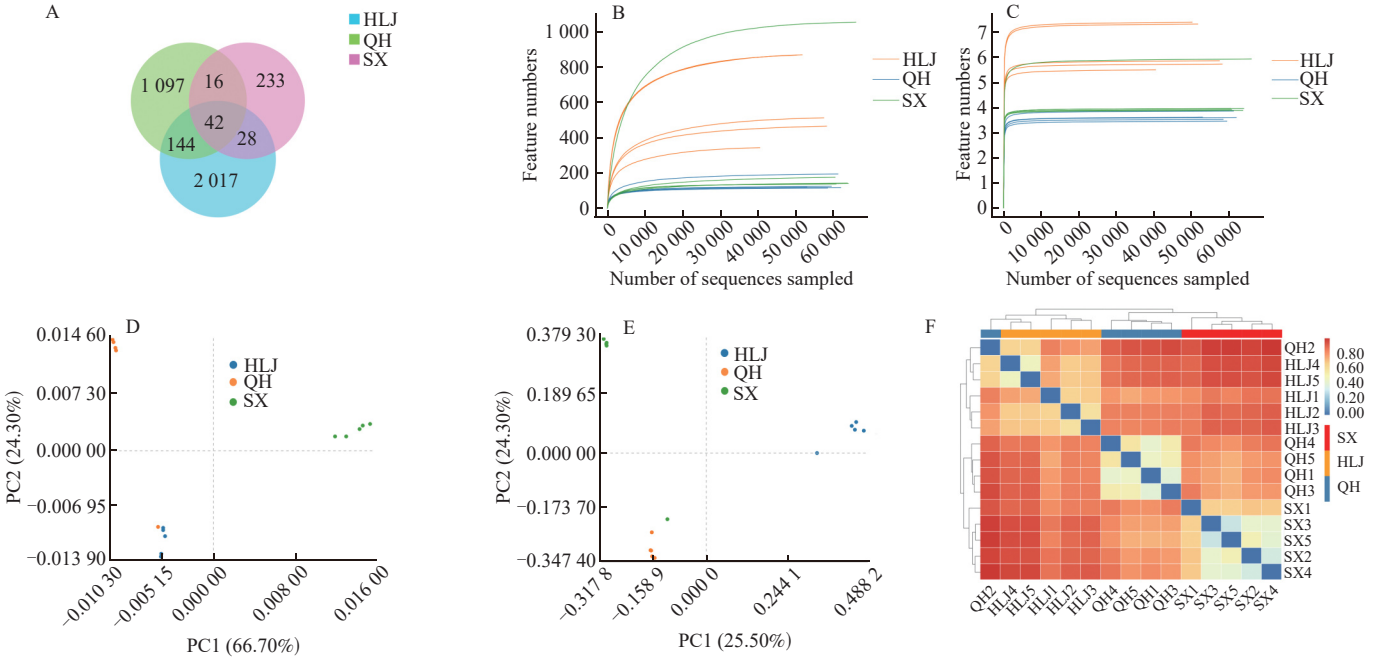

Fig. 5 Analysis of the bacterial community diversity. (A) Venn plot of features among the samples; (B) Sample dilution curve; (C) Shannon index curves; (D) PCA analysis diagram; (E) PCoA analysis diagram; (F) Heatmap of distance.

3.3.4 Significance analysis of difference among the three samples

The linear discriminant analysis effect size (LEfSe) analysis was used to reflect significant difference of bacterial community (Fig. 6A). Arcobacteraceae, *Arcobacter*, *Aliarcobacter_butzleri*, Campylobacterota, *Campylobacteria*, and Campylobacterales had a high relative abundance in SX. The relative abundance of unclassified_*Marinomonas*, Marinomonadaceae, *Marinomonas*, Proteobacteria, *Pediococcus*, and *Pediococcus_parvullus* were higher in QH. Moreover, Bacilli, Lactobacillales, Firmicutes, Lactobacillaceae, *Loigolactobacillus*, unclassified_*Loigolactobacillus*, and unclassified_*Lactiplantibacillus* were the marker microorganisms in HLJ.

The Random Forest was used to estimate the effect of species abundance on the difference of the suancai (Fig. 6B). The top 10 microorganisms that led to the variation of the bacterial composition were *Aeromonas*, *Leuconostoc*, *Stenotrophomonas*, *Pediococcus*, *Arcobacter*, unclassified_Enterobacteriaceae, *Hafnia_Obesumbacterium*, *Anaerosinus*, *Flavobacterium*, and *Pseudoalteromonas*.

3.4 Correlation analysis between physical and chemical properties, flavor, and dominant bacteria of the suancai

Redundancy analysis (RAD) was used to develop correlation of the physicochemical factors and the bacteria in the samples (Fig. 7A). As were shown the first 2 canonical axes explained 35.36% and 27.98% of the variation. The pH, TTA content and nitrite content had an effect on the bacterial community of the suancai. In HLJ, pH and nitrite content were positively correlated, and they were inversely related to QH. The correlation between TTA content and SX was strong. *Lactiplantibacillus* was positively correlated pH and nitrite. *Levilactobacillus*, *Acinetobacter*, *Arcobacter* and unclassified_Enterobacteriaceae were concentrated in the fourth quadrant, and showed a positive relationship with TTA.

Pearson correlation test reflected the interaction network among the identified microbes in these samples. The top 10 bacteria at the genus level were selected and they belonged to the top 4 species at the phylum level (Fig. 7B). Most genera affiliated to Firmicutes and Proteobacteria were negatively correlated, and Campylobacterota had a negative correlation with Firmicutes. *Latilactobacillus* was highly positive with *Pediococcus* (correlation > 0.94, $P < 0.05$, correlation was used to measure the strength and direction of the linear relationship between 2 species, which was the strength of correlation). *Loigolactobacillus* was positively correlated with *Lactiplantibacillus* and *Comamonas* (correlation > 0.90, $P < 0.05$). Besides, only *Loigolactobacillus* positively correlated with *Lactiplantibacillus*, and the correlation of them with *Marinomonas* were all negitive.

The flora with abundance greater than 1% at the genus level were defined as the core microorganism. The Spearman rank correlation between the core flora and the main differential metabolites were shown in Fig. 7C. The common differential metabolite in the comparison of suancai was 1-hexanol. 1-Hexanol was positively correlated with the core microorganisms, *Acinetobacter*, *Arcobacter* and unclassified_Enterobacteriaceae (correlation > 0.90, $P < 0.001$), while it was significantly negatively correlated with *Latilactobacillus* and *Pediococcus* (correlation < –0.80, $P < 0.001$). In the comparison

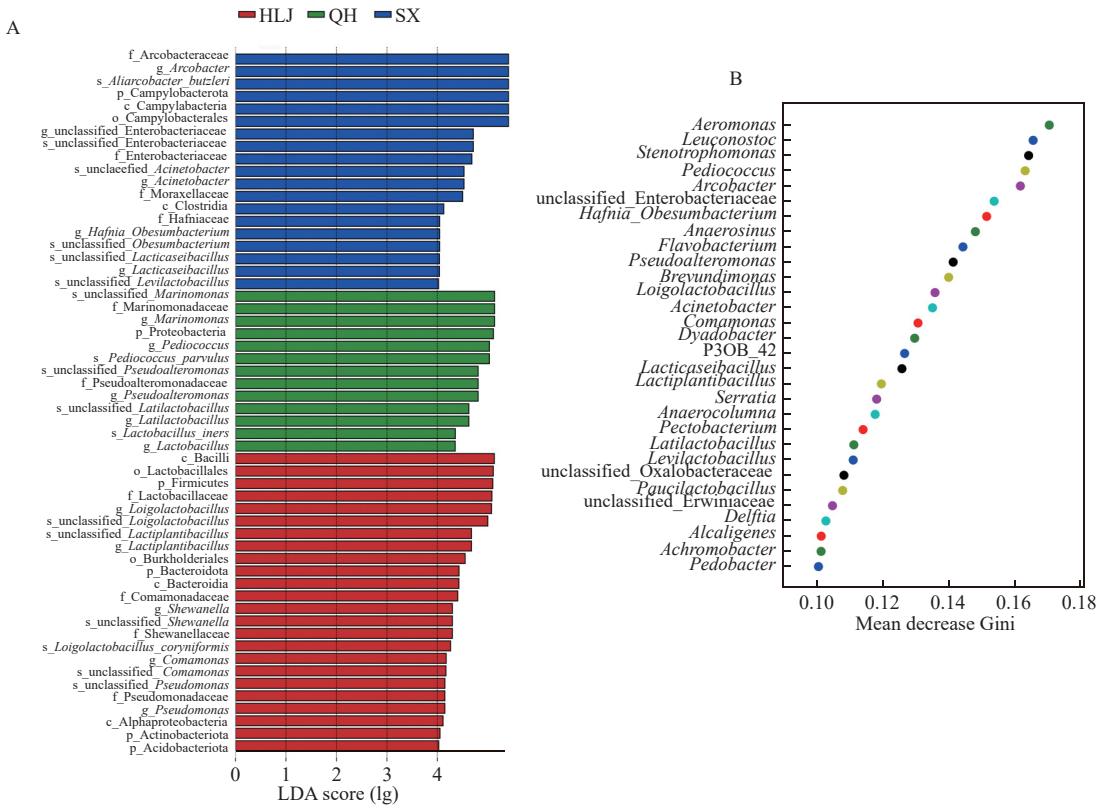

Fig. 6 Significance analysis of the difference of bacterial community. (A) LDA value distribution histogram. (B) Species importance ranking diagram between the suancai based on Random Forest analysis.

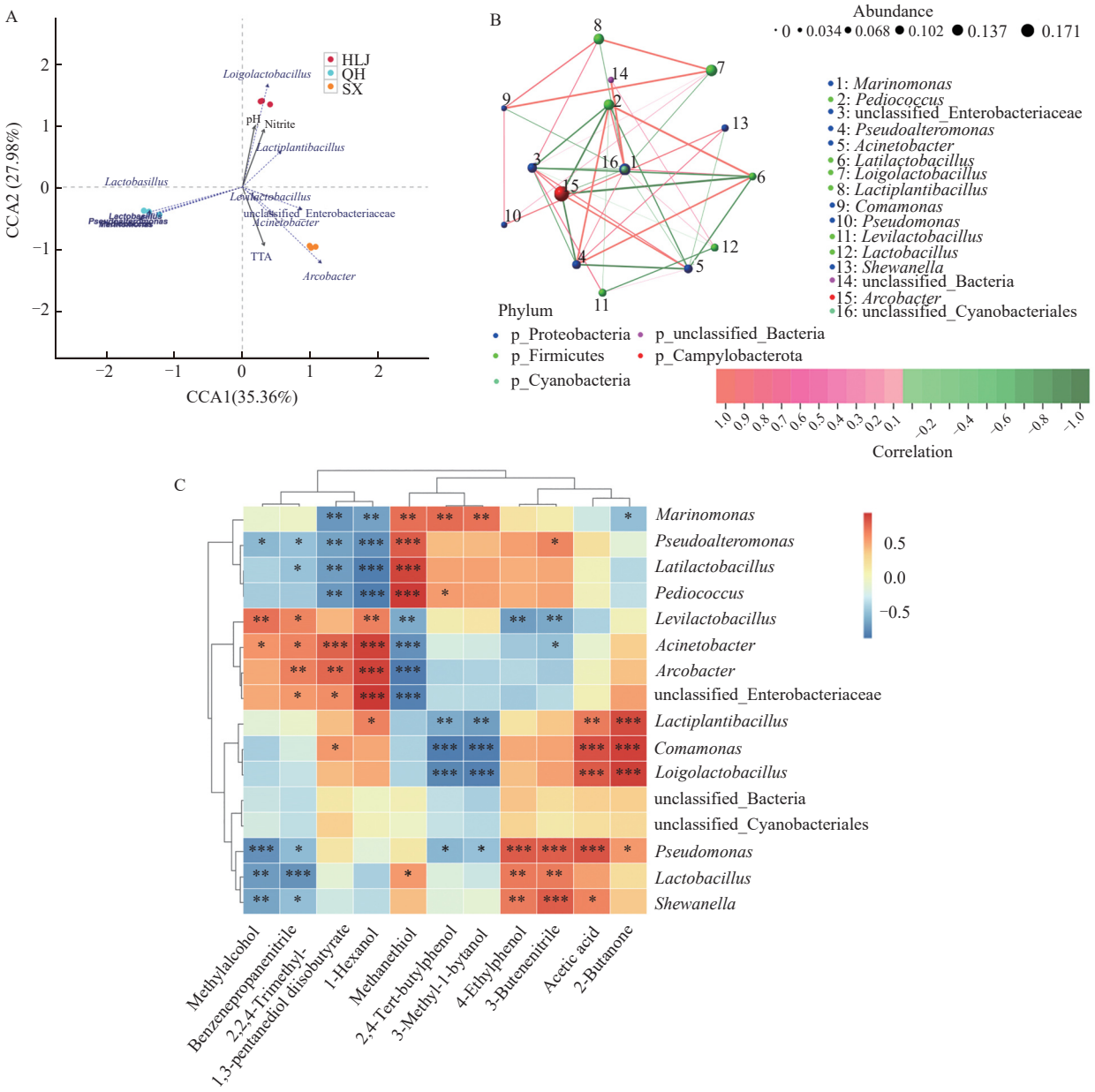

Fig. 7 Correlation analysis. (A) Correlation between the dominant bacteria and physicochemical property. (B) Correlation analysis of the core flora with species relative abundance greater than 1%. (C) Correlation analysis of the core species with the main differential metabolite. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

of SX and QH, the main differential metabolite (methanethiol) shown positive correlation with *Pediococcus* and negatively relevant with *Arcobacter* and unclassified_Enterobacteriaceae. Another differential metabolite, 2-butanone, was positively correlated with the core microorganisms *Loigolactobacillus* and *Comamonas*. The main differential metabolites in SX and HLJ were mainly negatively correlated with core microorganisms. Among them, 2,4-tert-butylphenol and 3-methyl-1-bytanol had a negative relationship with *Loigolactobacillus* and *Comamonas* (correlation < –0.80, $P < 0.001$). The varieties of volatile compounds had relationship with the differences of microorganisms and the salinity of the suancai. These metabolites contributed to different flavors of the suancai and resulted a pleasant taste.

4. Discussion

In the present study, the variances and reasons in physicochemical properties, bacterial communities, and flavor compounds were analyzed to compare the suancai which were made of the same material from QH, HLJ, and SX. The total number of microbial communities in the samples was 4.30×10^3, 5.95×10^6, and 1.67×10^6 CFU/mL, respectively. The fermentation temperatures of the samples were basically same, but there was a large difference in the amount of salt. The salt amount of HLJ was 1.9%, which was smaller than 8.0% and 10.0% in SX and QH. The total number of colonies gradually decreased with the increase of salt addition[17]. As a result, the number of bacteria in HLJ was more than the other 2 samples. The diversities of microbial communities in suancai were closely linked to types of

vegetables and the physical and chemical properties. Jeong et al.[18] reported a maximum value of dongchimi was about 6.2×10^6 CFU/mL and then decreased to 1.0×10^4 CFU/mL after 40 days. The ingredients of dongchimi were Korean leek, garlic, and ginger and the suancai were made of Chinese cabbage. This suggested that the types of raw materials led to the differences of bacterial numbers. The differences in the micronutrient elements and antimicrobial properties of raw materials would also affect the community composition of kimchi[19]. Moreover, the pH values of these samples were lower than that of suancai, while the total bacterial count was higher. The total number of bacteria varied with the decrease of pH during fermentation due to the accumulation of acidity and salinity, which inhibited the growth of many intolerant microorganisms.

The pH of the pickles ranged from 3.71 to 5.66, and TTA content ranged from 32.41 to 68.04 g/L. A pH of 4.5 indicated that the pickle was completely fermented[20]. Therefore, the pH of the HLJ was close to that indicating fermentation maturity, while the suancai from the other 2 regions did not reach this pH value. This may be because the pickling time of HLJ was longer than the other samples. The pH and TTA were different from a previous report, with the pH ranging between 2.89 and 5.12, and TTA content between 0.13 and 1.86 g/100 mL[21]. This may be related to the differences of the sample environment. Moreover, the dominant strains in previous studies were *Lactobacillus*, *Pediococcus*, and *Pichia*. Thus, the variance of the microbial community structure was also one of the crucial factors that affected physical and chemical properties of suancai.

Nitrite was related to the microbial communities as well as had a negative impact on human health[22-23]. In the present study, the nitrite of QH, HLJ, and SX was 0.02, 0.16, 0.04 mg/kg, respectively. According to China's national food standard, the nitrite content of food should not exceed 20 mg/kg. The results indicated that the nitrite content of the 3 types of suancai were all lower than the standard. Thus, the safety of home-made fermented suancai was relatively reliable.

In recent years, molecular biology techniques have been widely used for studying fermented foods. HTS technology was used to analyze and characterize the microbial community structure of fermented vegetables and explore the relation of bacteria and flavor compounds[24-25]. The results showed that the microorganisms in the suancai were various at the genus level (Fig. 4G). At the phylum level, Firmicutes, Proteobacteria, and Campylobacterota were main dominant bacteria of the three kinds of suancai, which were similar to previous reports[26-28]. The important markers in QH, HLJ, and SX were unclassified_Marinomonadaceae, *Baccilli*, and Arcobateraceae (Fig. 6A). The research of Wang et al.[29] showed that *Lactobacillus* was the main strain in southern pickles and contributed greatly to flavors. The result was same as the dominant species at family level of the present study.

In addition, it has been reported that *Halanaerobium* and *Halomonas* played a major role in the flavor of high-salinity industrial pickles[30]. These bacteria correlated with flavor and spoilage of pickles. Besides, Firmicutes, Proteobacteria and Halanaerobiaeota were the 3 dominant phyla of xuecai from Jiaxing and Ningbo, which were similar to the results of the present study (Fig. 4C)[31]. Furthermore, at the general level, the major genera in the fermented brine were *Lactobacillus* (37.53%), *Halanaerobium* (14.95%), *Halomonas* (8.37%), *Weissella* (4.00%), and *Pseudomonas* (2.85%) in xuecai with traditional technology, which were different from the suancai (Fig. 4G). The difference in the microbial structure might due to the different sampling varieties. The genera of microorganisms in fermented suancai varied, with *Lacticaseibacillus* being the predominant genus, followed by *Lactococcus*, which was inconsistent with the dominant species in this study[32]. This may be due to the differences in the diversity of the flora which were caused by the regional geographical environment and climate of suancai.

The GC-TOF-MS results revealed that there were great differences in the flavor compounds of the 3 types of suancai (Figs. 1A-D). The main differentiated compounds were 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, acetic acid, 4-ethylphenol, 2,4-tert-butylphenol, 3-butenenitrile, benzenepropanenitrile, 1-hexanol, methyl alcohol, 3-methyl-1-butanol, methanethiol, and 2-butanone. Acetic acid and 4-ethylphenol were 2 main differential metabolites. A total of 121 volatile compounds were identified from different Sichuan industrial paocai[33]. The results indicated that acetic acid contributed to sourness and 4-ethylphenol added strong sourness and fermented bamboo shoot flavor. According to Yang et al.[34], a kind of Northeast sauerkraut had higher alcohol content, mainly in the form of ethanol, phenylethanol, and 1-hexanol. Besides, 1-hexanol was also detected in the current study, and mainly added the fruity flavor. These alcohols made different contributions to the flavors of suancai. It was reported that 3-butenenitrile was the main compound in pickled sauerkraut from Jiaxing and Ningbo, Zhejiang, and it was found in high levels in modern pickled xuecai[35]. It was mainly derived from the decomposition of glucosinolates in these vegetables and were closely related to the Chinese cabbage of this study. Besides, 3-methyl-1-butanol might be produced by *Pseudomonas*, resulting in stale and putrid off-odors in chicken, seafood and other foods, so it was identified as a marker compound of food spoilage[36-37]. In the present study, it was detected as a differential metabolite, indicating that microbial species were closely related to the metabolites, and affected the quality and safety of products ultimately.

The differences in metabolic compounds in suancai were mainly related to the types of microbial communities, salinity and pH. Firstly, the differential compounds of suancai were closely related to the core microbiota. The dominant flora in HLJ with the lowest salinity of 1.9% were *Lactiplantibacillus* and *Loigolactobacillus.* They were significantly positively correlated with acetic acid, 2-butanone, and negatively correlated with 3-methyl-1-butanol and 2,4-tert-butylphenol (Fig. 7C), which were the source of delicate flavor of suancai. However, *Acinetobacter*, *Arcobacter* and unclassified_Enterobacteriaceae, the core microorganisms in SX with high salt content, were positively associated to 1-hexanol and imparted a pleasant favor to the suancai. The content of alcohols and nitriles were higher in Suancai and inoculated with *Pediococcus pentosaceus*[38]. Besides, *L. plantarum* and *P. pentosaceus* could impart more flavor compounds, such as alcohols, esters, aldehydes, hydrocarbons, and nitriles. Similarly, alcohols and nitriles could enhance the flavor of paocai after inoculation of *P. pentosaceus*[39].

In addition, difference of salinity impacted microorganisms and generated various metabolites. The fermentation time and temperature of the samples in the 3 regions were basically the same, but the salt addition of suancai from HLJ was significantly lower than other samples (Table 1). The abundance of *Latilactobacillus sakei* in HLJ was higher than other samples and inversely correlated with

metabolites glucose and fructose in the high-salinity kimchi[4]. However, in the suancai with low salinity, lactic acid and acetic acid accumulated rapidly and pH dropped fast at the early stage of fermentation. As a result, the contamination of *L. mesenteroides* was relatively high in the low-salinity suancai. These were consistent with the results that acetic acid was the main differential metabolite of the suancai at different salinity.

The correlation analysis demonstrated that there were some variances of the correlation between physicochemical properties and dominant bacteria in different suancai (Fig. 7A). The influence of pH and nitrite on bacteria were more significant than those of TTA in HLJ samples, owing to the minimal amount of salt in HLJ. In SX, the vector angles between *Levilactobacillus*, *Acinetobacter*, *Arcobacter* and TTA were less than 90° , revealing that the dominant strains became more abundant with the increase of TTA. But only the predominant bacterium in QH with the maximum salt had a lower correlation with pH, TTA and nitrite. Therefore, the difference in physicochemical property in fermented suancai was a result of interaction between multiple microorganisms[40].

5. Conclusion

In summary, this study examined the characteristics of bacterial community diversity and volatile compounds of traditional suancai from 3 regions. The dominant specie at the genus level in QH, HLJ, and SX was *Marinomonas*, *Loigolactobacillus*, and *Arcobacter*, respectively. A total of 5 representative differences were screened, 1-hexanol, the common differential metabolite was negatively correlated with *Latilactobacillus* and *Pediococcus*. *Loigolactobacillus* and *Lactiplantibacilllus*, the dominant species in HLJ were positively correlated with pH. These differences were related to the lowest salt additions, and beneficial microorganisms such as *Loigolactobacillus* were present in HLJ with 1.9% salinity. The difference in volatile flavor compounds among the suancai correlated to the interaction between various bacteria. Therefore, the findings were helpful for further understanding of the differences and relationship of flavor substances, physicochemical properties and the bacterial community of the traditional suancai, and providing a theoretical basis for strain selection and quality control. However, the follow-up studies should focus on the connection between the major bacteria and key metabolic compounds to improve the quality of suancai.

Data availability statement

Data will be made available on request.

Conflict of interest

The authors declare no competing interests.

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