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Seasonal dynamics of microbiota and physicochemical indices in the industrial-scale fermentation of Sichuan Baoning vinegar

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ABSTRACT

We investigated the impact of seasons of the year on microbiota and physicochemical indices in industrial-scale fermentation of Sichuan Baoning vinegar. Illumina HiSeq sequencing results showed significant differences (P < 0.05) between the microbiomes of vinegar *Pei* in every-two seasons, except for bacterial communities between summer and autumn. Total acid, reducing sugar, starch, and alcohol contents of vinegar *Pei* from the same sampling day of each season were measurably different. Although total acid content in vinegar *Pei* was similar at the end of fermentation (P > 0.05), the increase in total acidity was highest in the autumn. Acetic acid content in raw vinegar was highest in the autumn (3472.42 mg/100 mL), and lowest in the summer (2304.01 mg/100 mL). This study provides a theoretical basis for the production of Sichuan bran vinegar with consistent quality and provides insights into the quality control of traditional fermented foods.

Introduction

Vinegar is a nutritious acidic condiment that is widely available commercially (Ho, Lazim, Fazry, Zaki, & Lim, 2017) and has a history of over three thousand years in China. The most famous, traditionally produced vinegars are the Shanxi aged vinegar, Zhenjiang aromatic vinegar, Sichuan Baoning vinegar, and Fujian Monascus vinegar (Chen, Chen, Giudici, & Chen, 2016). The traditional vinegar brewing process can be divided into solid-state and liquid-state fermentation (Wu et al., 2019); traditional Chinese vinegar is typically brewed through solidstate fermentation, with cereal as the principal raw ingredient (Wei, 2001). In order to enhance yield, however, two-stage liquid-solid fermentation is increasingly being explored by vinegar manufacturers. Sichuan Baoning vinegar is representative of Sichuan bran vinegar, and differs from other vinegars in three main aspects: use of uncooked wheat bran as the principal ingredient and Daqu (great koji) incorporated with Chinese herbs as the saccharifying agent; complete solid-state fermentation during thirty days of production; an open fermentation process, with saccharification, alcohol fermentation, and acetic acid fermentation carried out simultaneously in the same fermentation pond. During

the production of Sichuan Baoning vinegar, raw materials are thoroughly mixed mechanically on the first and fifth days, followed by mixing every-three days thereafter, and raw vinegar was collected by leaching on day 30.

The raw materials for Sichuan Baoning vinegar production include wheat bran, water, vinegar *Pei* collected from a previous batch (also known as vinegar *Pei* residue, the remaining fermentation mixture after vinegar leaching), Daqu, and wheat flour. Vinegar brewing is a process that involves a variety of microorganisms, mainly mold, yeast, and acetic acid bacteria (Wu et al., 2021). Due to the open fermentation, seasonal dynamics on climate may introduce differences in biodiversity and richness of microorganisms in the raw materials and environment. For example, the characteristics of Shanxi mature vinegar (Kou et al., 2022), Fuyu-flavor Baijiu (Kang et al., 2022), and Chinese te-flavor baijiu Daqu (Fu et al., 2021) were reported to be affected by seasonal variation.

Ensuring the consistency of the quality of products manufactured during different seasons is essential for industrial-scale production, and the quality control of fermented foods relies heavily on stable microbiota (Lee et al., 2018; Zhang et al., 2022). Culture-dependent (Vegas

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et al., 2013) and -independent methods, including polymerase chain reaction-denaturing gradient gel electrophoresis (Milanovic et al., 2018) and high-throughput sequencing (Hutchinson et al., 2019), were extensively employed to reveal the microbial communities in vinegar. Although there have been many studies on the succession of microorganisms during the fermentation of vinegar (Huang et al., 2022; Zheng et al., 2022), few of them have focused on seasonal changes in industrialscale vinegar production. In the present study, Illumina HiSeq sequencing was employed to investigate the microbiota of industrialscale Sichuan Baoning vinegar manufactured throughout all four seasons of one year. Dynamic changes in the physicochemical indices of vinegar *Pei* were also monitored.

Materials and methods

Sampling

Sampling of the vinegar *Pei* and raw vinegar was conducted as per the method by Liu et al. (2020). Samples were collected from Sichuan Baoning Vinegar Co., ltd., (Langzhong, Sichuan, China) in the winter (Jan. 2018 to Feb. 2018), spring (Mar. 2018 to Apr. 2018), summer (Jul. 2018 to Sept. 2018), and autumn (Oct. 2018 to Nov. 2018). Three fermentation ponds (16#, 17#, and 28#) were randomly selected for sampling. Vinegar *Pei* were collected on days 1, 5, 13, 21, and 30; samples are identified based on seasons (SP = spring, SU = summer, A = autumn, W = winter) and sampling days, e.g., SP30 indicates samples collected on day 30 in the spring. The raw vinegar from each seasonal batch was also sampled, and named raw vinegar SP, raw vinegar SU, raw vinegar A, and raw vinegar W, respectively. The total acid content (calculated as acetic acid) of raw vinegar was approximately 5.0 %.

Dynamics of microbial community during fermentation

Vinegar Pei samples were sent to Novogene Co., ltd (Beijing, China) for bacterial and fungal diversity analysis using the Thermofisher Ion S5TMXL platform (Thermofisher, USA) as per the method by Wang et al. (2008) and Guan et al. (2020). Primers 314F (5'-CCTAYGGGRBGCAS-CAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with specific barcodes were used to amplify the V3-V4 regions of bacterial 16S rRNA. Additionally, primers 1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and 2043R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify fungal internal transcribed spacer (ITS) regions. Sequenced data analysis was performed through the novogene online Cloud Platform (https:// magic.novogene.com), including alpha-, and beta- diversity calculations, unweighted pair-group method with arithmetic mean (UPGMA) distance analysis with Qiime (version 1.9.1), linear discriminant analysis (LDA) effect size (LEfSe) using LEfSe software (version 1.0), as well as principal coordinates analysis (PCoA) with weighted UniFrac and heatmap using R package (version 2.15.3).

Characterization of vinegar Pei and raw vinegar

Total acid, starch, reducing sugar, and alcohol contents in vinegar Pei

The pre-analytical treatment of vinegar *Pei* samples, as well as characterization of total acid, starch, reducing sugar, and alcohol contents in vinegar *Pei* were conducted according to our previous report (Liu et al., 2021).

Lactic acid and acetic acid contents in vinegar Pei and raw vinegar

The pre-analytical treatment of vinegar *Pei* and raw vinegar samples, as well as determination of lactic acid and acetic acid contents were conducted as reported by Liu et al. (2020). Briefly, lactic acid and acetic acid contents were monitored by using HPLC (LC-10A2010C HT, Shimadzu, Japan) equipped with ReproSil-Pur 120 C18-AQ column (250 mm \times 4.6 mm, 5 µm; Dr. Maisch, Germany) and a UV detector set at 230 nm. The mobile phase was 100 % 0.2 mol/L H₃PO₄ with a flow rate of

0.5 mL/min. The injection volume was 10 μL , and the column temperature was 20 $^\circ C.$

Statistical analysis

For the determination of physicochemical indices of vinegar *Pei*, three independent replicates were set up, while two independent replicates were set up for testing of lactic acid and acetic acid contents in raw vinegar; the means and standard deviations were calculated. Statistical significance was performed using nonlinear regression analysis with SPSS 27.0 (SPSS, Chicago, IL, USA) unless otherwise specified.

Results

Microbiota analysis

Alpha-diversity and beta-diversity

Alpha diversity indices, including Shannon, Chao1, and Good's coverage, were calculated to reveal microbial diversity (Table S1). The Good's coverage index was approximately 1.0, indicating sufficiently high sequencing numbers for each sample. Shannon and Chao1 values revealed that the diversity and richness of fungal communities were higher than that of bacterial communities in each season, except on the first day of spring, contrary to the findings in Shanxi mature vinegar (Kou et al., 2022). The Venn diagrams indicated that the shared OTUs of bacterial communities (Fig. 1A) were less than that of fungal communities (Fig. 1B) across all four seasons, and the number of unique fungal OTUs was highest in the summer, while unique bacterial OTUs was highest in the spring. Meanwhile, in five samples from each season, shared OTUs of both bacterial (Fig. S1) and fungal communities (Fig. S2) were significantly higher in the autumn than in the spring. The microbial communities were significantly different (P < 0.05) in every-two seasons, except for the bacterial communities between summer and autumn (Table S2).

PCoA analysis based on weighted Unifrac metrics indicated that the first two principal components make up 65.88 % and 20.54 % of the total variance contribution ratio in the bacterial community (Fig. 1C). Vinegar *Pei* SP1 and SP30 were the most different samples from the others. Compared to samples from the spring and winter, samples from the summer and autumn were more similar. UPGMA analysis (Fig. 1E) indicated that there was little difference among day-5 vinegar *Pei* samples from different seasons. In addition, vinegar *Pei* samples from days 13 and 21 from all four seasons clustered together, except for sample SU21.

As to the fungal community, the first two principal components accounted for 41.17 % and 21.33 % of the variation in taxonomic composition (Fig. 1D). Similar to the bacterial community, vinegar *Pei* SP1 and SP30 were the most different from the others. Compared to samples from the spring and autumn, samples from the summer and winter were more similar. Notably, samples from the autumn were highly similar, indicating a stable fungal community during the whole fermentation process. Similarly, UPGMA analysis (Fig. 1F) indicated that vinegar *Pei* samples from the autumn changed little during the entire fermentation.

Composition and dynamics of microbial community

Firmicutes and Proteobacteria were the top two bacterial phyla present during vinegar fermentation in all four seasons (Fig. S3). The average relative abundance of Firmicutes was the highest in the winter (72.27 %), followed by 66.09 % in the summer, 63.05 % in the autumn, and 50.70 % in the spring. Similar to our previous report (Liu et al., 2021), Firmicutes' abundance peaked on the 5th day.

The top two bacterial genera during vinegar fermentation in the summer, autumn, and winter were *Lactobacillus* and *Acetobacter*, whereas they were *Lactobacillus* and *Bacillus* in the spring (Fig. 2). Variations in the bacterial community were observed in every season.

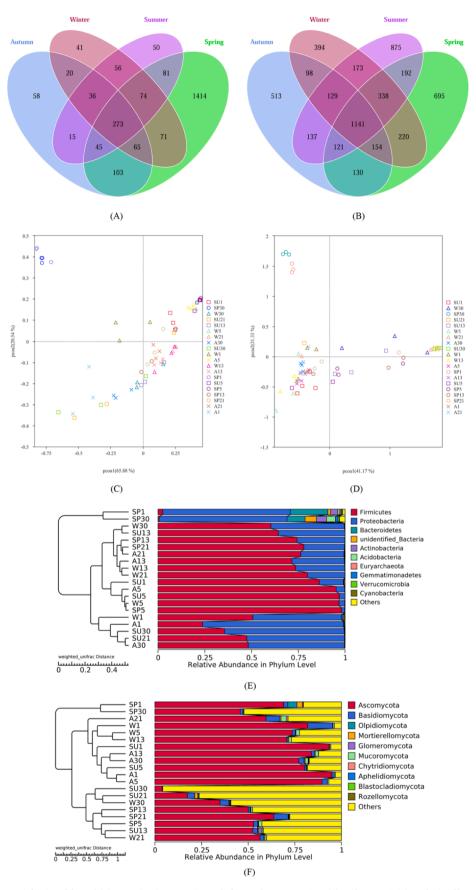


Fig. 1. Venn plots of the bacterial (A) and fungal (B) OTUs in vinegar *Pei* sampled in each season; PCoA (C) and UPGMA (E) analysis of bacterial community structure in vinegar *Pei* collected from each season at phylum level; PCoA (D) and UPGMA (F) analysis of fungal community structure in vinegar *Pei* collected from each season at phylum level; PCoA (D) and UPGMA (F) analysis of fungal community structure in vinegar *Pei* collected from each season at phylum level; PCoA (D) and UPGMA (F) analysis of fungal community structure in vinegar *Pei* collected from each season at phylum level.

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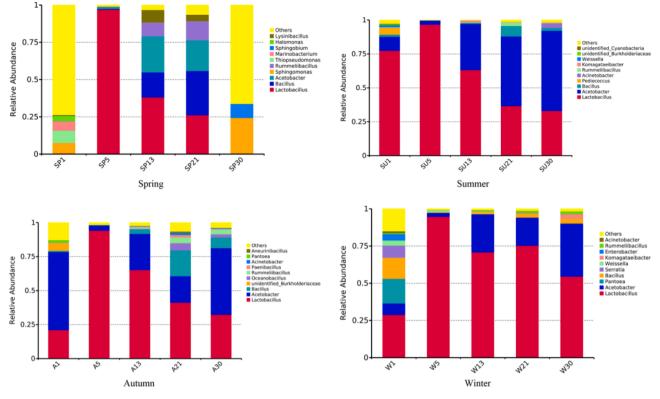


Fig. 2. Relative abundance of the bacterial community structure in vinegar Pei collected from each season at genus level.

Generally, *Lactobacillus* showed a trend of increasing, followed by a decrease in every season. The average relative abundance of *Lactobacillus* was highest in the winter (64.86 %), followed by the summer (61.41 %), autumn (50.79 %), and spring (32.30 %). The average

relative abundance of *Acetobacter* was 31.52 % in the summer, 31.27 % in the autumn, 18.10 % in the winter, and 9.24 % in the spring. These results indicated that there might be competition between *Lactobacillus* and *Acetobacter* species.

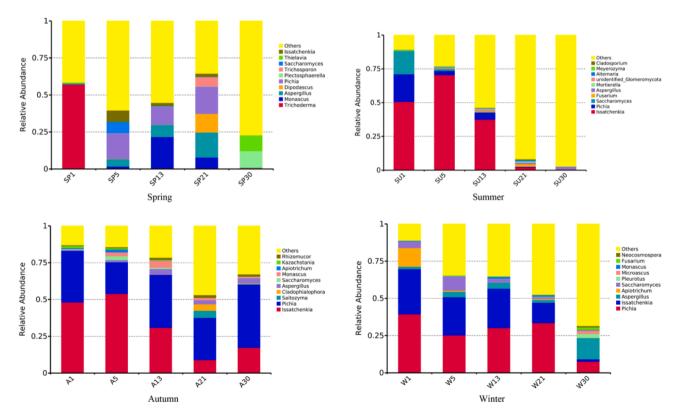


Fig. 3. Relative abundance of the fungal community structure in vinegar Pei collected from each season at genus level.

The dominant fungal phylum during vinegar fermentation in all four seasons was Ascomycota, with its highest average relative abundance in the autumn (80.99 %), followed by the winter (63.46 %), spring (56.32 %), and summer (49.48 %) (Fig. S4). Basidiomycota was the second largest phylum, and its average relative abundance was highest (4.41 %) in the winter and lowest in the summer (1.58 %).

The dominant fungal genera during vinegar fermentation in the summer, autumn, and winter were *Issatchenkia* and *Pichia*, whereas those in the spring were *Trichoderma* and *Pichia* (Fig. 3). Variations in the fungal community were also observed in every season, with the most difference found in the spring. The average relative abundance of *Issatchenkia* was highest in the summer (32.29 %), followed by the autumn (31.83 %), winter (19.55 %), and spring (2.40 %). The average relative abundance of *Pichia* was 32.85 % in the autumn, followed by the winter (27.19 %), spring (9.54 %), and summer (5.87 %).

LEfSe analysis (with LDA threshold of 4) was performed to identify the classified bacterial and fungal taxa with significant abundance differences in each season (Fig. 4). The bacterial species *Lysinibacillus macrolides*, *Acetobacter pasteurianus* and *Lactobacillus acetotolerans* were significantly enriched in the spring, summer, and winter, respectively. Meanwhile, the fungal species *Pichia mandshurica* and *Pichia membranifaciens* were significantly enriched in the autumn and winter, respectively. The genus *Issatchenkia* significantly increased in the summer, while *Trichoderma*, *Dipodascus*, and *Thielavia* significantly increased in spring.

Total acid content

Changes in total acid content of vinegar *Pei* during seasonal production of Sichuan Baoning vinegar were shown in Fig. 5A. During the 30-day fermentation, total acid content accumulated continuously, and rapidly increased in the first 21 days. After that, total acid content changed little, with no significant seasonal difference in total acid content at the end of fermentation (P > 0.05). The increase in total acidity was highest in the autumn (3.82 g/100 g), followed by winter (3.80 g/100 g), spring (3.49 g/100 g), and summer (3.40 g/100 g).

Starch content

As the fermentation proceeded, starch content decreased (Fig. 5B), and by the end of fermentation, starch content was highest in the summer (P < 0.05). Starch utilization rate was highest in the winter (88.03 %), followed by autumn (83.77 %), spring (83.35 %), and summer (66.96 %). Although starch utilization rate was not largely affected by the season during the first 5 days, it was the least in the summer by the end of fermentation. Temperature in the fermentation pond generally followed an increasing trend after each mixing, and the proliferation of starch-utilization fungi, especially *Aspergillus* and *Monascus* (Fig. 3),

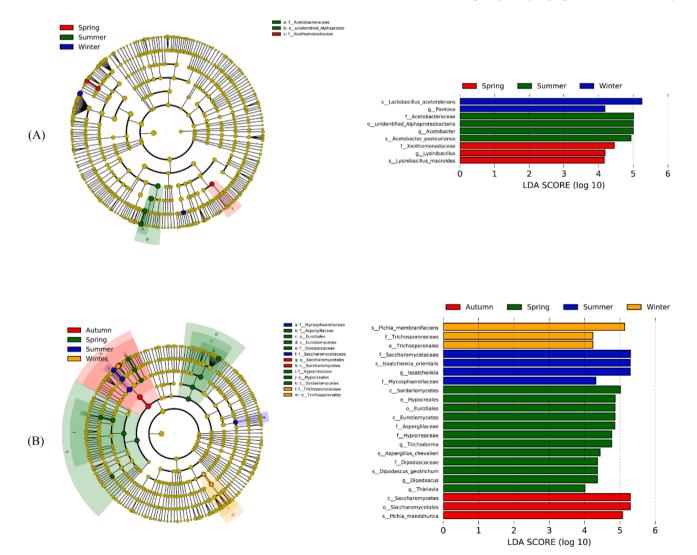


Fig. 4. The bacterial (A) and fungal (B) communities of vinegar Pei collected from each season were analyzed through LEfSe to determine the optimal characteristic taxa.

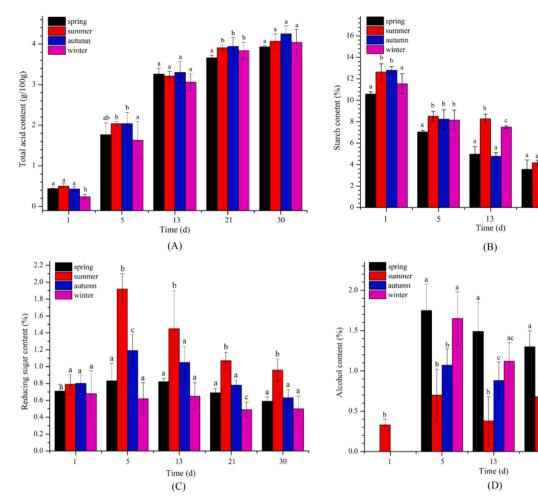


Fig. 5. Dynamics of contents of total acid (A), starch (B), reducing sugar (C), and alcohol (D) in vinegar Pei sampled in each season.

was prevented considering the higher ambient temperature. Notably, there was an obvious decrease in starch content from day 13 to 21 in the summer, which might be caused by weather changes.

Reducing sugar content

Reducing sugar content (Fig. 5C) in spring, summer, and autumn showed an overall trend of first increasing, then decreasing. Saccharification plays a major role in fermentation during the first 5 days when starch is converted into reducing sugar. Reducing sugar content in the first 5 days rose from 0.71 % to 0.83 % in the spring, 0.79 % to 1.92 % in the summer, and 0.80 % to 1.19 % in the autumn. On day 5, vinegar Pei was mixed thoroughly, which provided sufficient oxygen leading to an increase in aerobic and facultative anaerobic microorganisms, and consequently, massive consumption of reducing sugar. At the end of fermentation, the reducing sugar content was 0.59 % in the spring, 0.96 % in the summer, and 0.63 % in the autumn. Notably, reducing sugar content in the summer was highest during the whole fermentation, whereas it was lowest in the winter, changing little in the first 13 days, and declining on day 21. Considering the total acid content and starch utilization, the proliferation of reducing sugar-utilization microorganisms was also affected in the summer.

Alcohol content

Alcohol content first increased, then decreased in all seasons, except summer (Fig. 5D). Notably, alcohol content in the summer fluctuated as fermentation proceeded, which might have been caused by higher

ambient temperature in the summer. As illustrated, the alcohol content was highest on the fifth day of fermentation, with 1.75 %, 1.65 %, 1.07 %, and 0.7 % in spring, winter, autumn, and summer, respectively, which agreed with our previous report (Liu et al., 2020). In the first 5 days, yeast produced a large amount of alcohol in the anaerobic environment. Later, aerobic conditions favored the growth of acetic acid bacteria, converting alcohol into acetic acid.

Acetic acid and lactic acid contents in vinegar Pei and raw vinegar

As shown in Fig. 6, the variations in lactic acid and acetic acid contents were generally similar across the different seasons. The content of lactic acid was higher than that of acetic acid during the whole fermentation process, in contrast to the trends observed in Zhenjiang balsamic vinegar and Shanxi old aged vinegar production, where there was an increase during alcoholic fermentation, and an initial increase followed by a decrease during acetic acid fermentation (Huang et al., 2022; Nie et al., 2017; Wang et al., 2015). In samples from the same sampling day of different seasons, the lactic acid content changed relatively little, while the acetic acid content changed greatly. Acetic acid content at the end of fermentation was highest in the autumn (2775.62 mg/100 g), followed by spring (2678.50 mg/100 g), winter (1645.63 mg/100 g), and summer (1377.13 mg/100 g), whereas lactic acid content was much higher at the end of fermentation in the spring (5764.43 mg/100 g), followed by summer (5179.67 mg/100 g), winter (4806.98 mg/100 g), and autumn (4597.99 mg/100 g). The acetic acid content and lactic acid content in raw vinegar varied in different seasons. The highest acetic acid content was observed in autumn (3472.42

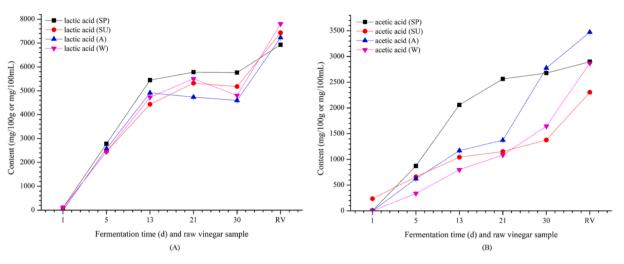


Fig. 6. Contents of lactic acid (A) and acetic acid (B) in vinegar Pei (mg/100 g) and raw vinegar (mg/100 mL) sampled in each season.

mg/100 g) and the lowest in summer (2304.01 mg/100 g), while the highest lactic acid content was in winter (7801.71 mg/100 g) and the lowest was in spring (6932.03 mg/100 g).

Discussion

Cereal vinegar is generally processed through three consecutive steps: saccharification of carbohydrates, fermentation of alcohol, and acetic acid fermentation (Ai et al., 2019; Al-Dalali et al., 2022). Among the four famous traditional vinegars produced in China, Sichuan Baoning vinegar is the only one currently employing solely solid-state fermentation throughout the entire process at industrial production scales (Liu et al., 2020). Meanwhile, due to the usage of uncooked wheat bran as the main raw material, and the open fermentation process, the composition of the microbial communities present during fermentation is complex. Adequate control over the fermentation process is highly important to maintain consistent quality in the production of traditional fermented foods, which relies heavily on stable microbiota. As shown in Fig. 5A, although there was no significant seasonal difference in total acid content at the end of fermentation (P > 0.05), the increase in total acidity was lowest in the summer. Considering the amount of vinegar Pei in each fermentation pond, the difference matters for the manufacturer in industrial-scale production.

In the present study, bacterial and fungal communities are observed to vary by season, during the production of Sichuan Baoning vinegar. Fermentation conditions are known to play an important role in shaping microbial communities, and it is challenging to ensure the stability of the microbiota throughout industrial-scale production. As shown in Fig. 2 and Fig. 3, abundant microorganisms were detected from vinegar Pei at the initial fermentation in each season, with differences observed within the microbial community. Some of the microorganisms were from Daqu, and others from the environment (Liu et al., 2004; Wang, Du, & Xu, 2017). Additionally, a large amount of vinegar Pei residue is added into the fermentation pond, which introduces even more microorganisms. Further studies are needed to uncover the seasonal differences of Daqu and vinegar Pei residue to improve our understanding of the initiation of vinegar fermentation. Furthermore, seasonal environmental factors, such as temperature, oxygen content, relative humidity, water content, and pH, are significant factors affecting the growth of microorganisms (Hu, Du, Ren, & Xu, 2016; Wang et al., 2020). And effects of temperature on the fermentation of some fermented foods were well studied. The relative abundance of Bacillus, Pediococcus, and Aspergillus decreased during Baijiu (Wang, Du, Zhang, & Xu, 2018) and Suancai (He et al., 2020) fermentation due to the increasing temperature. As shown in Fig. 5B, starch utilization was lowest in the summer,

perhaps due to higher summer temperatures preventing the growth of mold, and reflected by the lowest relative abundance of the genera *Aspergillus* in the summer (Fig. 3). In the fermentation of Shanxi aged vinegar, temperature-sensitive genus *Lactobacillus* decreased under elevated temperature, thus temperature-tolerant genus of *Acetobacter* was self-screened to dominate acid-producing (Wu et al. 2021). The observation agreed with our findings in the summer (Fig. 2). Therefore, deciphering how microbial communities respond to seasonal variation is advantageous for determining their dynamics and functions; the effects of environmental factors on microbial community dynamics during vinegar fermentation need further study.

Lactic acid content in Sichuan Baoning vinegar was higher than acetic acid content, in contrast to the other three famous traditional vinegars, which contributes to its unique flavor profile different from Shanxi aged vinegar (Li, Li, Liu, Luo, & Lin, 2016), Zhenjiang aromatic vinegar (Wang, Lu, Shi, & Xu, 2016) and Monascus vinegar (Jiang et al., 2019). As illustrated in Fig. 2, Lactobacillus dominated the entire fermentation, with highest average relative abundance in the winter (64.86 %). However, lactic acid content by the end of fermentation in the winter was not correspondingly the highest, which might be caused by the composition of lactic acid bacteria and conversion of lactic acid by other microbes present. Lactobacillus was also the predominant lactic acid bacteria in the spring and winter. Lactobacillus, Pediococcus and Weissella were the predominant lactic acid bacteria in the summer, while Lactobacillus and Weissella was predominant in the winter. Among the Lactobacillus, Lactobacillus acetotolerans was predominant in the spring, autumn, and winter, whereas the relative abundance of Lactobacillus acetotolerans was slightly lower than that of Lactobacillus amylovorus in the summer. So far, there was limited information on factors that determine the predominance of Lactobacillus over Acetobacter during Sichuan Baoning vinegar fermentation. The biodiversity and richness in sources of microorganisms were determining factors. Additionally, specific interactions between different types of strains might also matter, and competition between Lactobacillus and Acetobacter species was reported by Nie et al. (2015) in Shanxi aged vinegar. Bacillus was the dominant bacterial genus overall, regardless of the season, which was also reported in Dagu for vinegar production (Li, Aflakpui, Yu, Luo, & Lin, 2015) and Qishan vinegar (Gan et al., 2017). Bacillus oleronius was the dominant species in the spring and summer, while Bacillus thermoamylovorans was dominant in the autumn and winter. The dominant Lactobacillus sp. and Bacillus sp. observed in this study were different from those previously reported (Chai et al., 2020).

In the present study, we aim to investigate the impact of seasons of the year on microbiota and physicochemical indices of industrial-scale fermentation process of Sichuan Baoning vinegar. As indicated by our research, spring and summer might be not the optimum season considering the increase in total acidity, which is related to the economic efficiency of manufacturers. However, as a commercial product, nutritional indicators and sensory quality of vinegar also matter, and the flavor metabolite by microorganisms, amino acids as well as organic acids are important indicators that can be used to evaluate vinegar quality (Jiang et al., 2019). Acetic acid and lactic acid are the primary organic acids in vinegar (Liu et al., 2011). Acetic acid has a pungent sour taste, while lactic acid has a soft sour taste; the content and ratio of these two organic acids have an important influence on the sensory quality of vinegar (Jiang et al., 2010; Zhao et al., 2020). As shown in Fig. 6, the contents and ratio of lactic acid and acetic acid varied at the end of fermentation in each season. Additionally, variations in microbiota also bring changes in flavor metabolite and nutritional indicators (Liu et al., 2020; Liu et al., 2021), thus imposing impacts on sensory quality. The sensory quality difference of vinegar produced in four seasons needs further study to ensure the optimum season for Baoning vinegar production.

Conclusions

Overall, this study confirmed that the microbiota present during fermentation, was affected by the seasons of the year. The diversity and richness of the fungal community were higher than that of the bacterial community in each season, with significant differences (P < 0.05) observed in microbial communities between every-two seasons, except for the bacterial communities between the summer and autumn. Variations were found in the physicochemical indices of vinegar *Pei* and raw vinegar. This study provides insights into the industrial-scale seasonal production of Sichuan Baoning vinegar.

CRediT authorship contribution statement

Aiping Liu: Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. Wanshu Pan: Methodology, Data curation, Validation. Sha Li: Writing – review & editing. Jianlong Li: Writing – review & editing. Qin Li: Methodology, Writing – review & editing. Li He: Methodology, Writing – review & editing. Shujuan Chen: Methodology, Writing – review & editing. Kaidi Hu: Methodology, Writing – original draft. Xinjie Hu: Methodology, Writing – original draft. Guoquan Han: Methodology, Writing – original draft. Shuhong Li: Writing – review & editing. Jiang Zhou: Resources, Supervision. Fusheng Chen: Writing – review & editing. Shuliang Liu: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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