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Deciphering bacterial community succession patterns and their correlations with physicochemical factors in solid-state fermentation of high-quality jujube vinegar

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Abstract

Liquid-state fermentation (LSF) with purified microbial strains is predominantly used to produce fruit vinegar, resulting in a bland flavor, poor aroma, and pungent sour aftertaste. Herein, jujube vinegar was produced using the solid-state fermentation (SSF) technology. The results showed that the concentrations of non-volatile acids, total esters, proteins, free amino acids, reducing sugars, and total flavonoids in jujube vinegar produced by SSF (SSFJV) were higher than those in jujube vinegar produced by LSF (LSFJV). Fuzzy mathematical sensory evaluation results showed that SSFJV had 'excellent' sensory quality, while LSFJV was of 'medium' quality. High-throughput sequencing identified various bacteria involved in the SSF process. Based on bacterial community succession, the SSF process can be divided into four stages, with *Staphylococcus*, *Pediococcus*, *Lactobacillus*, and *Acetobacter* as the dominant genera, respectively. Spearman's correlation analyses indicated that specific functional bacteria exhibited positive effects on the levels of non-volatile acids, total esters, proteins, free amino acids, and titratable acidity in the fermentation substrate. The results provide insight into the microbial mechanisms underpinning quality improvement of fruit vinegar by SSF, and support for artificial construction of functional bacterial agents suitable for fruit vinegar brewing.

Keywords: *Daqu*; solid-state fermentation; jujube vinegar; quality improvement; bacterial community structure; physicochemical factors.

Practical Application: This study developed a reliable method to improve the quality of jujube vinegar by SSF with *Daqu* of Shanxi aged vinegar as starter. Characterization of bacterial community related to quality of jujube vinegar produced by solid-state fermentation.

1 Introduction

Vinegar is a popular and nutritious acidic liquid condiment with a long history (Ho et al., 2017). In general, vinegar refers to brewed vinegar, which is produced by microbial fermentation using individual or mixed raw materials containing starch and sugar, or edible alcohol (Budak et al., 2014). Brewed vinegar can be classified based on raw material such as grain, fruit, or malt, and brewing technology comprising liquid-state fermentation (LSF) or solid-state fermentation (SSF). In European countries, different vinegar products, such as Italian balsamic vinegar and French sherry vinegar, are mainly produced by LSF using fruits. However, in Asian countries, many types of vinegar, including Japanese Kurozu vinegar and Chinese Shanxi aged vinegar (SAV), are brewed by SSF from raw materials comprising sorghum, wheat bran, beans, rice, or rice hulls (Xia et al., 2020).

Fruit vinegar has the nutritional and health functions of both fruit and vinegar, and this is fueling demand (Liu et al., 2011). To date, production of fruit vinegar primarily relies on LSF, and the raw materials, mainly fruit or juice, are subjected to submerged fermentation with pure microbial strains. Typically, *Saccharomyces cerevisiae* is inoculated in the alcohol fermentation (AF) stage and *Acetobacter* species are inoculated in the acetic acid fermentation (AAF) stage (Budak et al., 2014). LSF technology benefits from high production efficiency, and facile large-scale and mechanized production. However, due to its simple raw materials and microbial strains, vinegar products of LSF tend to suffer from a bland flavor, poor aroma, and pungent sour aftertaste (Tesfaye et al., 2002). Therefore, improving flavor and quality are major goals for LSF fruit vinegar production (Yao & You, 2010).

Great effort from many aspects has been expended to improve the quality of LSF fruit products. For example, some studies screened and cultivated excellent microbial strains (Chen et al., 2019b; Xing et al., 2018; Fernández-Pérez et al., 2010), while others explored mixed fermentation (Kong et al., 2019; Chen et al., 2019a; Liu et al., 2016) and SSF technology (He & Fan, 2012; Luo et al., 2016).

In China, *Daqu* (made from barley and pea by spontaneous fermentation) is an essential starter for the preparation of SSF vinegar (Zheng et al., 2014). *Daqu* contains diverse microorganisms and enzymes, amounting to ~60% of the total raw materials. In the presence of *Daqu* together with raw and auxiliary materials, the SSF substrate (called *pei* in Chinese) is in a loose solid state, making it suitable for the growth and reproduction of different microorganisms; this is also conducive to fusion reactions between multiple substances (Hutchinson et al., 2019). Therefore, vinegar produced by SSF is not only rich in nutritional and functional components, but also has superior color, flavor,

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and taste (Hugenholtz, 2013). Thus, SSF is considered to be an effective method to improving the quality of fruit vinegar. However, application of this technology in China is still limited to grain vinegar, and the microbial mechanisms by which it improves fruit vinegar quality remain unclear.

In the present study, jujube (*Zizyphus jujuba* Mill.) was used to produce fruit vinegar using SSF technology, and vinegar quality was evaluated by comparing with LSF vinegar. Changes in physicochemical factors, bacterial community succession patterns, and their correlations during the SSF process were also investigated. The results could help to identify specific bacteria potentially contributing to the high quality of SSF fruit vinegar.

2 Materials and methods

2.1 Jujube vinegar production

SSF jujube vinegar (SSFJV, referred to the brewing of SAV) and LSF jujube vinegar (LSFJV) was produced follow the flow in Figure 1. Details of the process refer to supplementary materials.

2.2 Sample collection and processing

Parallel *Daqu* and *pei* samples from three different batches (one sample per batch) were collected every day throughout the fermentation process (days 0-14), the detail steps were performed as previously described (Zhu et al, 2018). For analysis of some chemical properties, *pei* samples (10 g each) were accurately weighed into 250 mL Erlenmeyer flasks, 90 mL of distilled water was added, flasks were plugged, and samples were mixed evenly on a 120 rpm shaker for 30 min. Samples were then filtered through qualitative filter paper and filtrates were collected. In addition, SSFJV and LSFJV samples were taken after thoroughly mixing the vinegar products.

2.3 Physicochemical analysis of pei

The temperature (T) of *pei* was measured using a sterilized thermometer inserted into the center of samples every day before the *pei* was turned over. The water content (W) of *pei* samples was determined by measuring dry weight after oven-drying at 105 °C. Proteins (P) and total sugar (TS) concentrations of *pei* samples were determined by Kjeldahl nitrogen analysis (Association of Official Analytical Chemists, 2011) and the phenol-sulfuric acid colorimetric method (Tomáš et al, 2018), respectively. A Hach pH meter (Loveland, CO, USA) was used to measure the pH value of *pei* filtrates. Alcohol degree (A) of *pei* samples, titratable acidity (TA) of *pei* filtrates and the concentrations of non-volatile acids (NVA), total esters (TE), Reducing sugar (RS), and free amino acids (AA) in *pei* filtrates were determined according to the Chinese National Standard of GB 19777-2013 (Wu et al., 2012). P, TA, NVA, TE, AA, and RS of SSFJV and LSFJV samples were analyzed using the same methods as described for *pei* analysis. In addition, total flavonoid and polyphenol concentrations of

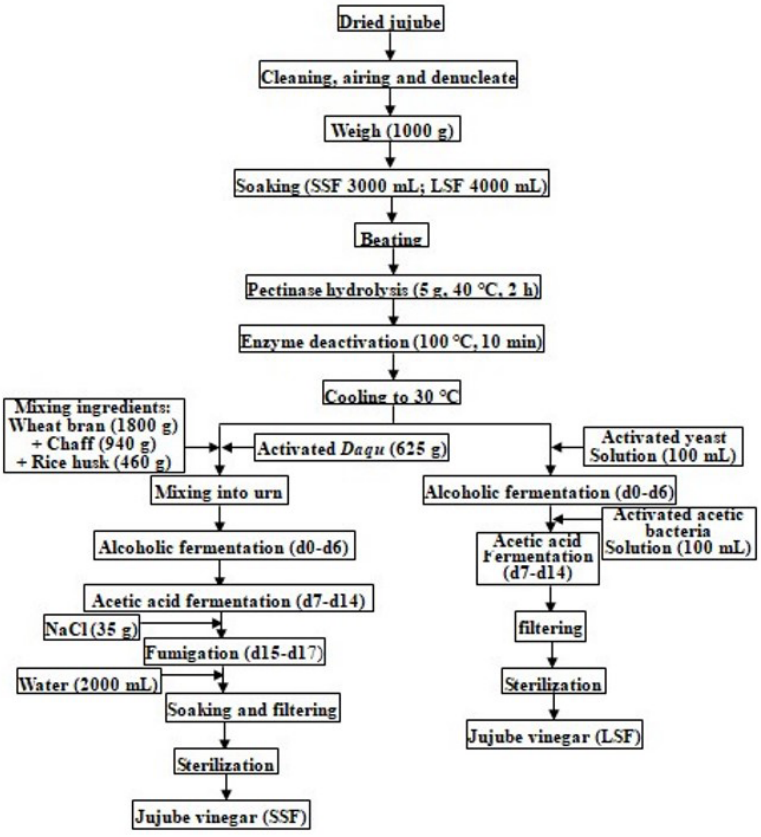

Figure 1. A flowchart of jujube vinegar production by solid-state fermentation (SSF) and liquid-state fermentation (LSF).

vinegar samples were determined by colorimetric methods of sodium nitrite-aluminum nitrate and Folin-phenol, respectively (Verzelloni et al., 2007). Vitamin C concentration of vinegar samples was determined by titration with 2,6-dichlorophenol (Li et al., 2007).

2.4 Fuzzy mathematical sensory evaluation of jujube vinegar

The sensory attributes of SSFJV and LSFJV were analyzed from the four dimensions of color, body, aroma, and taste using fuzzy mathematics sensory evaluation method, the major steps was implemented as previously described (Jiang, 2011). Details of the process refer to supplementary materials.

2.5 Microbial analysis

Samples from activated *Daqu* and jujube vinegar *pei* (days0, 2, 4, 6, 8, 10, 12, 14) were sent for bacterial diversity analysis using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) with reference to the report by (Xu et al., 2018).

2.6 Statistical analysis

Statistical significance (p <0.05) of differences between SSFJV and LSFJV were determined by one-way analysis of variance (ANOVA) with SPSS v19.0 (IBM SPSS, Somers, NY, USA). Principal component analysis (PCA) and Spearman's correlation coefficients were conducted using the ggplot2 package in R v4.0.2. Bacterial genera that differed significantly in relative abundance across stages were determined using the Kruskal-Wallis H test.

3 Results and discussion

3.1 Comparison of vinegar quality between SSFJV and LSFJV

Nutritional and functional components

Compared with LSFJV, SSFJV had significantly higher concentrations of NVA, TE, P, AA, and RS (Figure 2a), as well as total flavonoids and polyphenols (Figure 2b). The increase in nutritional and functional components in SSFJV may be attributed to the rich substrate and diverse microorganisms in the fermentation system. Auxiliary materials, such as wheat bran, rice hull, and millet chaff, are rich in minerals, proteins, polysaccharides, flavonoids, and polyphenols, all of which slowly dissolve during fermentation and eventually enter the vinegar (Ong & Lee, 2021). In addition, enzymes produced and secreted by microorganisms may synthesize-phenols, amino acids, and sugars, thereby increasing their concentrations in the vinegar (Javanmardi et al., 2003). However, the vitamin C concentrations of SSFJV were slightly, but significantly, lower than those of LSFJV. Although the TA concentrations of SSFJV were also lower than those of LSFJV, the differences were not significant (Figure 2b).

Sensory quality

Based on the weight distribution results (Table 1), the fuzzy weight set of jujube vinegar was obtained as follows: $X = (x_1, x_2, x_3, x_4) = (0.12, 0.10, 0.38, 0.40)$. The weights of color, body, aroma, and taste factors were 0.12, 0.10, 0.38, and 0.40, respectively, indicating that taste and aroma were two major sensory evaluation indicators of jujube vinegar.

A total of 10 sensory evaluators were used to evaluate jujube vinegar according to the evaluation criteria. The results (Table 2) were normalized to obtain the fuzzy relation matrices *R1* and *R2* as follows (Equation 1):

$$R1 = \begin{vmatrix} 0.6 & 0.3 & 0.1 & 0 \\ 0.4 & 0.4 & 0.1 & 0.1 \\ 0.7 & 0.2 & 0.1 & 0 \\ 0.8 & 0.1 & 0.1 & 0 \end{vmatrix} \quad R2 = \begin{vmatrix} 0.6 & 0.3 & 0.1 & 0 \\ 0.7 & 0.2 & 0.1 & 0 \\ 0.2 & 0.3 & 0.4 & 0.1 \\ 0.1 & 0.3 & 0.4 & 0.2 \end{vmatrix} \tag{1}$$

According to the principle of fuzzy matrix transformation, $Y_i = X \times R_i = |0.12, 0.10, 0.38, 0.40| \times R_i$. Therefore, the comprehensive evaluation results of Y_1 and Y_2 (after normalization) were obtained as follows (Equation 2 and 3):

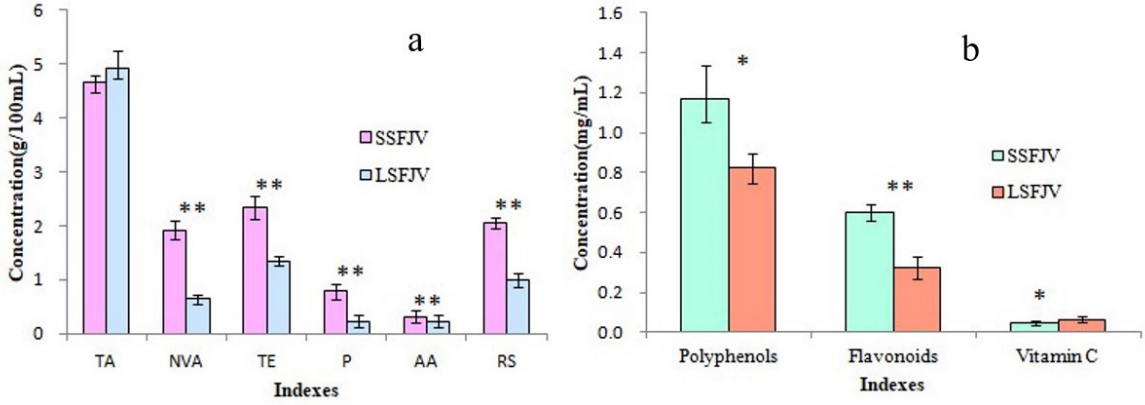

Figure 2. Comparison of (a) nutritional and (b) functional components between jujube vinegar produced by solid-state fermentation (SSFJV) and jujube vinegar produced liquid-state fermentation (LSFJV). * $P < 0.05$; ** $P < 0.01$.

Table 1. Weight distribution of sensory evaluation indicators for jujube vinegar.

Professional reviewer	Membership degree				Normalization			
	u_1	u_2	u_3	u_4	u_1	u_2	u_3	u_4
1	0.20	0.20	0.90	0.90	0.10	0.10	0.45	0.45
2	0.37	0.18	0.78	0.88	0.17	0.08	0.36	0.40
3	0.25	0.25	0.75	0.75	0.125	0.125	0.375	0.375
4	0.40	0.30	0.85	0.85	0.16	0.12	0.34	0.34
5	0.30	0.20	0.80	0.80	0.14	0.10	0.38	0.38
Mean					0.12	0.10	0.38	0.40

Table 2. The evaluation results of SSFJV (a_1) and LSFJV (a_2).

Object	Factor	Evaluation set			
		v_1	v_2	v_3	v_4
a_1	u_1	6	3	1	0
	u_2	4	4	1	1
	u_3	7	2	1	0
	u_4	8	1	1	0
a_2	u_1	6	3	1	0
	u_2	7	2	1	0
	u_3	2	3	4	1
	u_4	1	3	4	2

$$Y1 = X \times R1 = \left|0.12,\quad 0.10,\quad 0.38,\quad 0.40\right| \times \begin{vmatrix} 0.6 & 0.3 & 0.1 & 0 \\ 0.4 & 0.4 & 0.1 & 0.1 \\ 0.7 & 0.2 & 0.1 & 0 \\ 0.8 & 0.1 & 0.1 & 0 \end{vmatrix} = \left|0.698,\quad 0.192,\quad 0.100,\quad 0.010\right| \tag{2}$$

$$Y2 = X \times R2 = \left|0.12,\quad 0.10,\quad 0.38,\quad 0.40\right| \times \begin{vmatrix} 0.6 & 0.3 & 0.1 & 0 \\ 0.7 & 0.2 & 0.1 & 0.1 \\ 0.2 & 0.3 & 0.4 & 0.1 \\ 0.1 & 0.3 & 0.4 & 0.2 \end{vmatrix} = \left|0.258,\quad 0.290,\quad 0.334,\quad 0.118\right| \tag{3}$$

The total score of fuzzy evaluation was calculated (Equation 4 and 5):

$$T1 = Y1 \times V = \left|0.698,\quad 0.192,\quad 0.100,\quad 0.010\right| \times \left|90,\quad 80,\quad 70,\quad 60\right| = 85.78 \tag{4}$$

$$T2 = Y2 \times V = \left|0.258,\quad 0.290,\quad 0.334,\quad 0.118\right| \times \left|90,\quad 80,\quad 70,\quad 60\right| = 76.88 \tag{5}$$

Because $T_1 > T_2$, the sensory score of the evaluation object a_1 was higher than a_2. Thus, the fuzzy mathematical sensory score of SSFJV was higher than that of LSFJV. According to the membership function theory and the degree of maximum membership, the peak value of the comprehensive evaluation score of SSFJV was 0.698, corresponding to the 'excellent' grade. The peak value of the comprehensive evaluation score of LSFJV was only 0.334, which corresponded to the 'medium' grade. In conclusion, the results of sensory evaluation in four dimensions (color, body, aroma, and taste) indicated that the sensory quality of SSFJV reached an excellent level, while that of LSFJV only reached a medium level.

The main objective in the development and improvement of products is to meet the needs and acceptance of consumers (Costa et al., 2020). So, there is still a need for more sensory studies using consumer perception for the evaluation of SSFJV and LSFJV in the future, such as CATA test, acceptance test and hedonic test. These methods have been successfully used in studies with several foods, such as coffee (Bressani et al., 2021), juice blend (Campos et al., 2021) and sirkencubin syrup (Yikmis et al., 2020).

3.2 Changes in physiochemical factors during the SSF process

Physicochemical factors were measured during the SSF process (Figure 3). The overall changes in T values were complex. The W parameter varied minimally between 60-62.5%. TS concentrations continuously decreased with increasing fermentation time. RS concentrations dropped sharply in the first 3 days, and then increased slightly. The A parameter increased sharply from day 0 to 3 and reached a peak value (4.93%vol) on day 5. From day

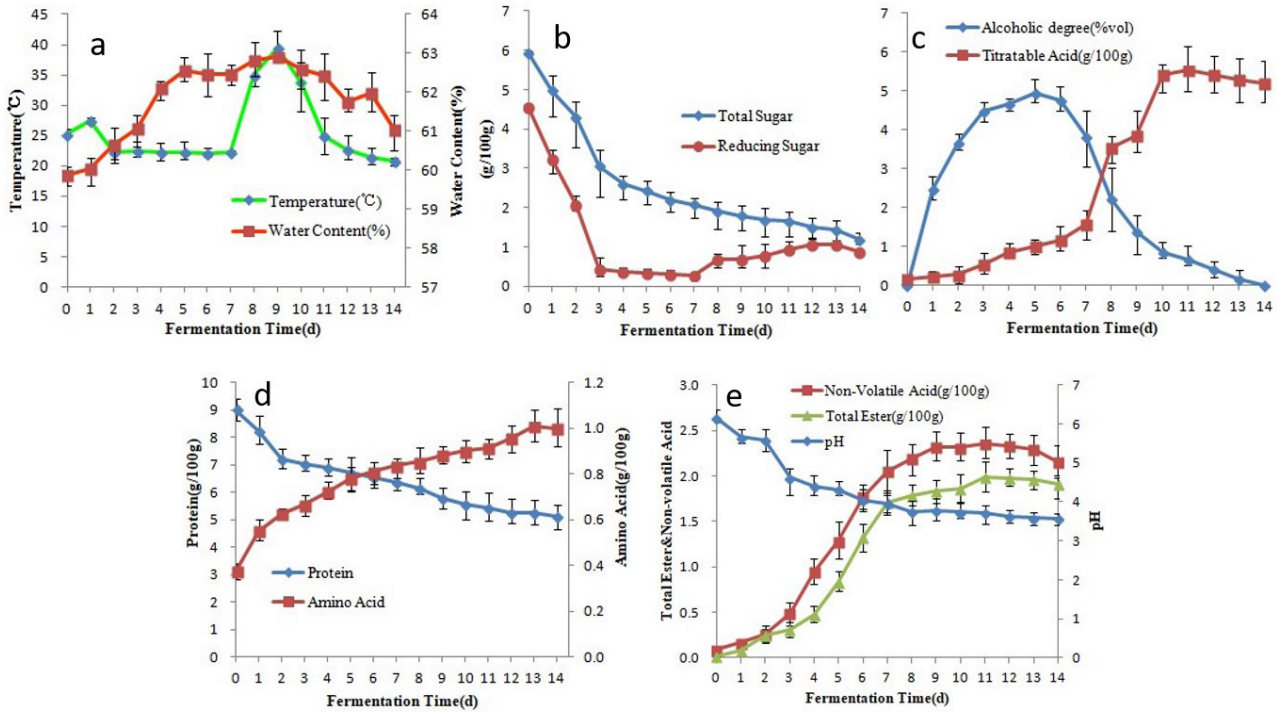

Figure 3. Changes in physicochemical factors during the SSF process. (a) T and W, (b) TS and RS concentrations, (c) A and TA, (d) P and AA concentrations, (e) TE, NVA, and pH value. Bars indicate standard deviation of the mean.

7 to the end of SSF, A values decreased rapidly. TA showed an upward trend throughout the SSF process; a slow increase occurred in the AF stage, followed by a sharp increase in the AAF stage. AA also increased with increasing fermentation time, while P showed a downward trend, indicating that the amino acids in *pei* were mainly derived from protein degradation. The pH values of *pei* decreased sharply from day 0 to 9, then leveled off between pH 3.4-3.6 due to the buffer effect of the complex system. Conversely, both NVA and TE concentrations increased continuously, and tended to level off or decrease slightly until the end of SSF, indicating continuous accumulation of these components during the SSF process.

3.3 Bacterial diversity and community succession during the SSF process

The distribution of dominant bacteria (relative abundances >0.1%) in the SSF process was analyzed at the genus level (Figure 4). More bacteria were found in *Daqu* and at the beginning (day 0) of SSF compared with other samples. The 0-day bacterial community was dominated by *Staphylococcus* (38.22%), *Saccharopolyspora* (15.73%), *Brevibacterium* (10.09%), *Streptomyces* (7.99%), *Lactobacillus* (6.45%), *Weissella* (3.38%), *Bacillus* (2.07%), most of them mainly originated from *Daqu*.

From day 2 to 6 (anaerobic, AF), the dominant genera were mainly *Pediococcus*, *Lactobacillus*, and *Staphylococcus*. *Pediococcus* was the absolute dominant genus in this stage. *Lactobacillus* abundance was low on day 0 (6.45%), then it increased sharply to 50.53% on day 6. Interestingly, *Staphylococcus* abundance gradually decreased.

From day 8 to 14 (aerobic, AAF), bacterial community diversity continued to decline. In this stage, the relative abundances of *Acetobacter*, *Lactobacillus*, and *Pediococcus* were higher than those of other genera. At the beginning of the AAF stage, *Lactobacillus* and *Pediococcus* were predominant; however, their relative abundances decreased with increasing fermentation time. In contrast, *Acetobacter* was highly enriched throughout the AAF stage and became the most dominant genus at the end of SSF. In addition, some genera with low relative abundance (only 2-3% in total during AAF), such as *Saccharopolyspora*, *Weissella*, *Kroppenstedtia*, and so on, persisted throughout the SSF process, despite their overall decreasing trends.

The diversity of the microbial community in *Daqu* and the microbial dynamics during the fermentation of SAV have been investigated, and *Acetobacter*, *Lactobacillus*, and *Komagataeibacter* are the dominant or key bacteria (Wu et al., 2012; Nie et al., 2015; Nie et al., 2017). We also found that *Lactobacillus* and *Acetobacter* were dominant, while *Kroppenstedtia* was detected at low levels during the SSF process. Unlike previous studies, *Staphylococcus* and *Pediococcus* were dominant in day 0 and day 2 to 6, respectively. *Staphylococcus* mainly came from *Daqu*, and this genus is considered part of the functional core microbiota for production of flavors in Zhenjiang aromatic vinegar (Wang et al., 2016). Accordingly, *Staphylococcus* plays a role in flavor production during SSF of jujube vinegar. *Pediococcus*

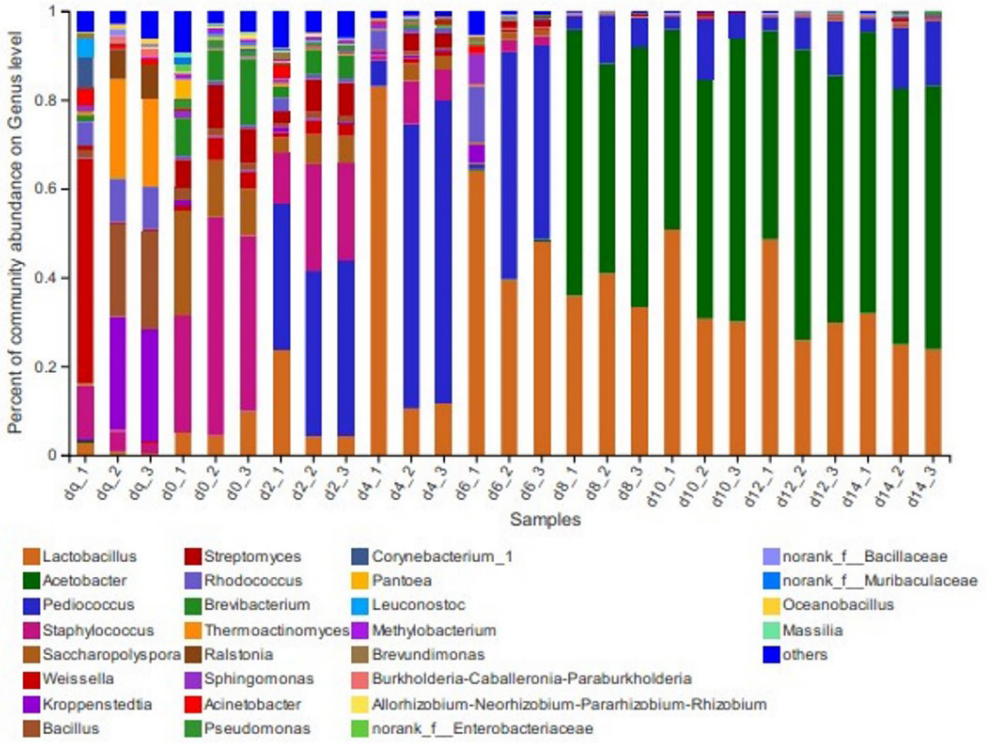

Figure 4. Distribution of bacterial community at the genus level during the SSF process. dq_1, dq_2, and dq_3 are *Daqu* samples; d0_1 to d14_3 are *pei* samples collected from day 0 to 14 of SSF (three parallel samples each).

is a group of microaerobic bacteria belonging to the family *Lactobacillaceae*. *Pediococcus* mainly ferments monosaccharides and disaccharides to produce lactic acid, and species have a strong acid production capacity (Porto et al., 2017). Unlike grain used for traditional SSF, jujube does not contain starch, but mainly contains sugars, such as glucose, fructose, sucrose, and so on (Wang et al., 2018). Thus, there is no starch saccharification stage during the SSF process of jujube vinegar, and there are high concentrations of fermentable sugars and associated derivatives at the start of SSF. These substances provide unique conditions for the proliferation of metabolically active *Pediococcus*, enabling this genus to quickly become the dominant bacteria in the AF stage. In the later SSF stages of jujube vinegar, the bacterial community essentially reaches a stable state, with little variation in structure and abundance. In short, the bacterial community structure shifted throughout the SSF process, and was dominated by *Staphylococcus*, *Pediococcus*, *Lactobacillus*, and *Acetobacter*, respectively.

3.4 Variation in bacterial community structure across different stages of SSF

Variation in bacterial community structure was analyzed by PCA at the OTU level (Figure 5a). *Daqu* samples were clustered with day 0 *pei* samples, while day 8-14 *pei* samples were close to each other. However, samples of days 2-6 were relatively scattered, indicating that the bacterial community structure shifted dynamically from the initial chaotic state to the later stable and orderly state. According to the PCA results, the SSF process of jujube vinegar could be divided into four stages: the original stage (day 0, S1); the early stage (days 2-5, S2), mainly AF; the middle stage (days 6-7, S3), which was a transition stage of AF and AAF; and the late stage (days 8-14, S4), mainly AAF. Samples from the same stage were grouped tightly.

The top 15 genera in terms of mean relative abundance showed significant differences among the four stages (p <0.05; Figure 5b). As SSF progressed, there were continuous decreases in the relative abundances of *Staphylococcus*, *Saccharopolyspora*, *Brevibacterium*, *Streptomyces*, *Weissella*, *Pantoea*, and *Bacillus* (p <0.001), as well as *Pseudomonas* (p <0.05). However, the relative abundances of *Pediococcus* and *Lactobacillus* increased (p <0.01) from S1 to S2 and S3 stages, respectively. Furthermore, the relative abundances of *Acetobacter*, *Rhodococcus*, *Sphingomonas*, *Kroppenstedtia*, and *Acinetobacter* increased in stage S4 compared with stage S3 (p <0.001).

3.5 Relationships between major bacteria and physicochemical factors

Microbial behavior contributes to the production of specific nutritional and flavor metabolites (Wang et al., 2016; Nie et al., 2013). Potential relationships between the major bacteria and physicochemical factors were discerned by a Spearman correlation heatmap (Figure 6). The results indicate that the major bacteria interacted with physicochemical factors in a complex manner across different stages of SSF.

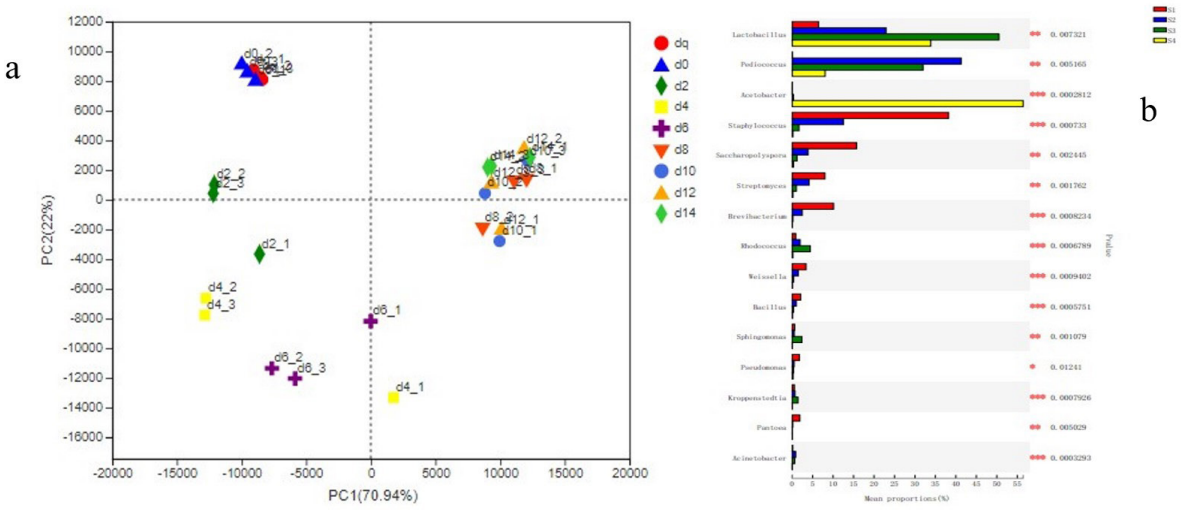

Figure 5. Bacterial community succession across different stages of the SSF process. (a) PCA biplot showing the variation in bacterial community structure (OTU level) among four SSF stages; (b) Kruskal-Wallis H test results showing the differences in bacterial abundance (genus level) among four SSF stages.

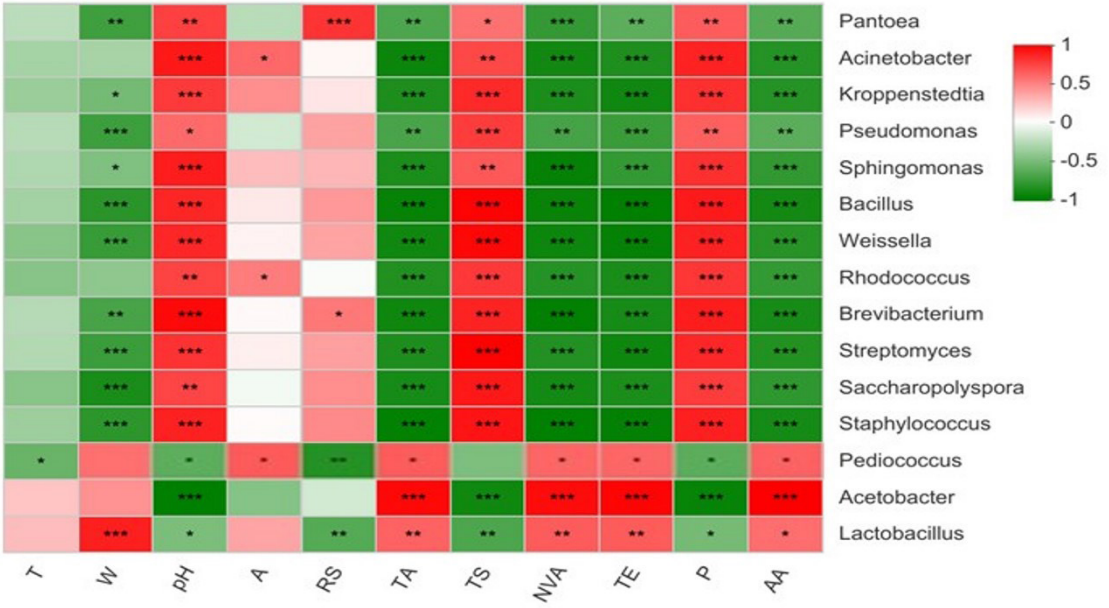

Figure 6. Spearman correlation matrix of major bacterial genera and physicochemical factors in the SSF process (top 15 genera in terms of relative abundance). Red and green colors represent positive and negative correlation, respectively. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

Staphylococcus were important bacteria in stage S1, displaying positive correlations with P, TS, and pH ($p < 0.001$). Previous study has shown that *Staphylococcus* can improving the nutritional value of fermented food and endowing products with unique flavors (Zhang et al., 2016). *Pediococcus* had a strong positive correlation with TE, TA, A, NVA, and AA ($p < 0.05$). Xing et al. (2018) isolated *Pediococcus pentosus* during SAV fermentation and used this species for *in situ* strengthening of hawthorn fruit vinegar produced by LSF, which markedly improved the vinegar flavor and quality. Therefore, *Pediococcus* could be included as one of the main components of mixed starters for fruit vinegar fermentation in order to improve vinegar quality and enhance functional attributes.

Lactobacillus was positively correlated with TE, A, and NVA ($p < 0.01$) as well as AA ($p < 0.05$), and negatively correlated with P, TS, and RS ($p < 0.01$). The main product of *Lactobacillus* is lactic acid, the most important NVA in vinegar. Lactic acid

can buffer H^+ ions in acetic acid, endow vinegar with a soft taste, and contribute to the taste of vinegar (Fang et al., 2021). Furthermore, lactic acid has inhibitory effects on the growth of other bacteria, such as *Pantoea*, *Pseudomonas*, *Methylobacterium*, and *Cladosporium* (Nie et al., 2017). This may explain why in the S2-S3 stages of SSF, the relative abundance of *Lactobacillus* increased markedly, while the relative abundances of other bacterial genera decreased sharply.

Acetobacter had a significant positive correlation with TE, TA, NVA, and AA (p <0.001). *Acetobacter* species are the main producers of acetic acid, an important volatile acid in vinegar. *Acetobacter* can also produce a small amount of NVS by oxidizing sugars to gluconic acid or glucoketo acid, then further oxygenating these intermediates to lactic acid and succinic acid (Mounir et al., 2016).

Other bacteria with low abundance also had a high correlation with physicochemical factors. For instance, *Weissella* (p <0.001) and *Bacillus* (p <0.001) were positively correlated with TS and P, while *Rhodococcus* and *Acinetobacter* were positively correlated with A (p <0.05). These non-dominant bacteria could adapt to the changing environment and exist stably in *pei*, potentially contributing to the production of flavor and functional substances. However, their roles in SSF process of jujube vinegar need to be further investigated.

4 Conclusions

This study reports a reliable method to improve the quality of fruit vinegar by SSF. The proposed method effectively improved the quality of jujube vinegar in terms of increased nutritional and functional components, and excellent sensory evaluation properties compared with LSF. Bacterial community structure shifted in response to environmental changes, being dominated successively by *Staphylococcus*, *Pediococcus*, *Lactobacillus*, and *Acetobacter*. They showed positive effects on the levels of non-volatile acids, total esters, proteins, free amino acids, and titratable acidity in the fermentation substrate. These results may prove useful for the application of microbial resources in fruit vinegar brewing. The findings may also be used to implement directional regulation of fermentation technology and establish directional targets for artificial construction of bacterial consortia suitable for SSF of fruit vinegar. Furthermore, this study provides technical support for the industrial transformation from traditional grain vinegar brewing to fruit vinegar production.

References

Association of Official Analytical Chemists – AOAC. (2011). *Official methods of analysis* (18th ed.). Washington: AOAC.

Bressani, A. P. P., Martinez, S. J., Batista, N. N., Simao, J. B. P., & Schwan, R. F. (2021). Into the minds of coffee consumers: perception, preference, and impact of information in the sensory analysis of specialty coffee. *Food Science and Technology*, 41(Suppl. 2), 667-675. http://dx.doi.org/10.1590/fst.30720.

Budak, N. H., Aykin, E., Seydim, A. C., Greene, A. K., & Guzel-Seydim, Z. B. G. (2014). Functional properties of vinegar. *Journal of Food Science*, 79(5), 757-764. http://dx.doi.org/10.1111/1750-3841.12434. PMid:24811350.

Campos, A. P. R., Chiste, R. C., & Pena, R. S. (2021). Camu-camu (*Myrciaria dubia*) and jambolan (*Syzygium cumini*) juice blend: sensory analysis and bioactive compounds stability. *Food Science and Technology*, 41(1), 82-89. http://dx.doi.org/10.1590/fst.37519.

Chen, A.-J., Fu, Y.-Y., Jiang, C., Zhao, J.-L., Liu, X.-P., Liu, L., Ma, J., Liu, X.-Y., Shen, G.-H., Li, M.-L., & Zhang, Z.-Q. (2019a). Effect of mixed fermentation (Jiuqu and *Saccharomyces cerevisiae* EC1118) on the quality improvement of kiwi wine. *CYTA: Journal of Food*, 17(1), 967-975. http://dx.doi.org/10.1080/19476337.2019.1682678.

Chen, J., Xing, X., Feng, Z., & Wang, R. (2019b). Screening and aroma characteristics of ester-producing yeasts as starter cultures for Shanxi aged vinegar. *Shipin Kexue*, 40(16), 144-151.

Costa, J. N., Brito, S. A., Leal, A. R., Rodrigues, D. C., Nascimento, L. G. L., Figueiredo, R. W., Mata, P., & Sousa, P. H. M. (2020). Sensory characteristics of structured guava (*Psidium guajava*): comparison of optimized descriptive profile, cata and sensory acceptance methods. *Food Science and Technology*, 40(Suppl. 2), 496-502. http://dx.doi.org/10.1590/fst.25819.

Fang, G. Y., Chai, L. J., Zhong, X. Z., & Jiang, Y. J. (2021). Deciphering the succession patterns of bacterial community and their correlations with environmental factors and flavor compounds during the fermentation of Zhejiang rosy vinegar. *International Journal of Food Microbiology*, 341, 109070. http://dx.doi.org/10.1016/j.ijfoodmicro.2021.109070. PMid:33503540.

Fernández-Pérez, R., Torres, C., Sanz, S., & Ruiz-Larrea, F. (2010). Strain typing of acetic acid bacteria responsible for vinegar production by the submerged elaboration method. *Food Microbiology*, 27(8), 973-978. http://dx.doi.org/10.1016/j.fm.2010.05.020. PMid:20832673.

He, J., & Fan, M. (2012). Influence of brewing method on quality of apple vinegar. *Agricultural Science and Technology*, 13(8), 1754-1756.

Ho, C. W., Lazim, A. M., Fazry, S., Zaki, U. K. H. H., & Lim, S. J. (2017). Varieties, production, composition and health benefits of vinegars: a review. *Food Chemistry*, 221, 1621-1630. http://dx.doi.org/10.1016/j.foodchem.2016.10.128. PMid:27979138.

Hugenholtz, J. (2013). Traditional biotechnology for new foods and beverages. *Current Opinion in Biotechnology*, 24(2), 155-159. http://dx.doi.org/10.1016/j.copbio.2013.01.001. PMid:23395405.

Hutchinson, U. F., Jolly, N. P., Chidi, B. S., Ngongang, M. M., & Ntwampe, S. K. O. (2019). Vinegar engineering: a bioprocess perspective. *Food Engineering Reviews*, 11(4), 290-305. http://dx.doi.org/10.1007/s12393-019-09196-x.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry*, 83(4), 547-550. http://dx.doi.org/10.1016/S0308-8146(03)00151-1.

Jiang, X. (2011). Urban ecological security evaluation and analysis based on fuzzy mathematics. *Procedia Engineering*, 15, 4451-4455. http://dx.doi.org/10.1016/j.proeng.2011.08.836.

Kong, C., Li, A., Su, J., Wang, A., Chen, C., & Tao, Y. (2019). Flavor modification of dry red wine from Chinese spine grape by mixed fermentation with *Pichia fermentans* and *S. cerevisiae*. *Lebensmittel-Wissenschaft + Technologie*, 109, 83-92. http://dx.doi.org/10.1016/j.lwt.2019.03.101.

Li, J., Fan, L., Ding, S., & Ding, X. (2007). Nutritional composition of five cultivars of Chinese jujube. *Food Chemistry*, 103(2), 454-460. http://dx.doi.org/10.1016/j.foodchem.2006.08.016.

Liu, F., He, Y., Wang, L., & Sun, G. (2011). Detection of organic acids and pH of fruit vinegars using near-infrared spectroscopy and multivariate calibration. *Food and Bioprocess Technology*, 4(8), 1331-1340. http://dx.doi.org/10.1007/s11947-009-0240-9.

Liu, P., Lu, L., Duan, C., & Yan, G. (2016). The contribution of indigenous non-*Saccharomyces*, wine yeast to improved aromatic

quality of Cabernet Sauvignon wines by spontaneous fermentation. *Lebensmittel-Wissenschaft + Technologie*, 71, 356-363. http://dx.doi.org/10.1016/j.lwt.2016.04.031.

Luo, X., Yang, Y., Lu, S., & Zhang, C. (2016). Effect of fermentation methods on sensory and nutritional quality of persimmon vinegar. *Food Science*, 37(19), 154-159. http://dx.doi.org/10.7506/spkx1002-6630-201619026.

Mounir, M., Shafiei, R., Zarmehrkhorshid, R., Hamouda, A., Alaoui, M. T., & Thonart, P. (2016). Simultaneous production of acetic and gluconic acids by a thermotolerant *Acetobacter* strain during acetous fermentation in a bioreactor. *Journal of Bioscience and Bioengineering*, 121(2), 166-171. http://dx.doi.org/10.1016/j.jbiosc.2015.06.005. PMid:26253254.

Nie, Z., Zheng, Y., Du, H., Xie, S., & Wang, M. (2015). Dynamics and diversity of microbial community succession in traditional fermentation of shanxi aged vinegar. *Food Microbiology*, 47, 62-68. http://dx.doi.org/10.1016/j.fm.2014.11.006. PMid:25583338.

Nie, Z., Zheng, Y., Wang, M., Han, Y., Wang, Y., Luo, J., & Niu, D. (2013). Exploring microbial succession and diversity during solid-state fermentation of Tianjin duliu mature vinegar. *Bioresource Technology*, 148, 325-333. http://dx.doi.org/10.1016/j.biortech.2013.08.152. PMid:24055975.

Nie, Z., Zheng, Y., Xie, S., Zhang, X., Song, J., Xia, M., & Wang, M. (2017). Unraveling the correlation between microbiota succession and metabolite changes in traditional Shanxi aged vinegar. *Scientific Reports*, 7(1), 9240. http://dx.doi.org/10.1038/s41598-017-09850-6. PMid:28835624.

Ong, A., & Lee, C. (2021). Cooperative metabolism in mixed culture solid-state fermentation. *Lebensmittel-Wissenschaft + Technologie*, 152, 112300. http://dx.doi.org/10.1016/j.lwt.2021.112300.

Porto, M. C. W., Kuniyoshi, T. M., Azevedo, P. O. S., & Oliveira, R. P. S. (2017). *Pediococcus* spp.: an important genus of lactic acid bacteria and pediocin producers. *Biotechnology Advances*, 35(3), 361-374. http://dx.doi.org/10.1016/j.biotechadv.2017.03.004. PMid:28284993.

Tesfaye, W., Morales, M. L., García-Parrilla, M. C., & Troncoso, A. M. (2002). Wine vinegar: Technology, authenticity and quality evaluation. *Trends in Food Science & Technology*, 13(1), 12-21. http://dx.doi.org/10.1016/S0924-2244(02)00023-7.

Tomáš, Z., Petra, O., Maria, A., & Jan, Č. (2018). Determination of storage (starch/glycogen) and total saccharides content in algae and cyanobacteria by a phenol-sulfuric acid method. *Bio-Protocol*, 8(15), e2966. PMid:34395769.

Verzelloni, E., Tagliazucchi, D., & Conte, A. (2007). Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsamic vinegar. *Food Chemistry*, 105(2), 564-571. http://dx.doi.org/10.1016/j.foodchem.2007.04.014.

Wang, L., Fu, H., Wang, W., Wang, Y., Zheng, F., Ni, H., & Chen, F. (2018). Analysis of reducing sugars, organic acids and minerals in 15 cultivars ofjujube (*Ziziphus jujuba* mill.) fruits in China. *Journal of Food Composition and Analysis*, 73, 10-16. http://dx.doi.org/10.1016/j.jfca.2018.07.008.

Wang, Z. M., Lu, Z. M., Shi, J. S., & Xu, Z. H. (2016). Exploring flavour-producing core microbiota in multispecies solid-state fermentation of traditional Chinese vinegar. *Scientific Reports*, 6(1), 26818. http://dx.doi.org/10.1038/srep26818. PMid:27241188.

Wu, J. J., Ma, Y. K., Zhang, F. F., & Chen, F. S. (2012). Biodiversity of yeast*s*, lactic acid bacteria and acetic acid bacteria in the fermentation of "Shanxi aged vinegar", a traditional Chinese vinegar. *Food Microbiology*, 30(1), 289-297. http://dx.doi.org/10.1016/j.fm.2011.08.010. PMid:22265314.

Xia, T., Zhang, B., Duan, W., Zhang, J., & Wang, M. (2020). Nutrients and bioactive components from vinegar: a fermented and functional food. *Journal of Functional Foods*, 64, 104681. http://dx.doi.org/10.1016/j.jff.2019.103681.

Xing, X., Wang, Y., Huo, N., & Wang, R. (2018). *Candida Ethanolica* strain Y18 enhances aroma of Shanxi aged-vinegar. *Food Science and Technology Research*, 24(6), 1069-1081. http://dx.doi.org/10.3136/fstr.24.1069.

Xu, J., Wu, H., Wang, Z., Zheng, F., Lu, X., Li, Z., & Ren, Q. (2018). Microbial dynamics and metabolite changes in Chinese Rice Wine fermentation from sorghum with different tannin content. *Scientific Reports*, 8(1), 4639. http://dx.doi.org/10.1038/s41598-018-23013-1. PMid:29545525.

Yao, C., & You, T. (2010). Research progress of producing strains and brewing technology of fruit vinegar. *Zhongguo Tiaoweipin*, 35(12), 31-34.

Yikmis, S., Özpancar, N., Bozkir, C., & Çöl, B. G. (2020). Functional sirkencubin syrup with purple basil; bioactive properties, organoleptic acceptability, and possible effects on blood pressure. *Food Science and Technology*, 40(Suppl. 2), 550-557. http://dx.doi.org/10.1590/fst.34819.

Zhang, D., Cheng, W., Li, J., & Jiang, A. (2016). Inoculation of *Staphylococcus* and *Micrococcus* to improve the oxidative stability of cantonese sausages. *Xiandai Shipin Keji*, 32(1), 218-223.

Zheng, X. W., Yan, Z., Nout, M. J., Smid, E. J., Zwietering, M. H., Boekhout, T., Han, J. S., & Han, B. Z. (2014). Microbiota dynamics related to environmental conditions during the fermentative production of *Fen-Daqu*, a Chinese industrial fermentation starter. *International Journal of Food Microbiology*, 182–183, 57-62. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.05.008. PMid:24863368.

Zhu, Y., Zhang, F., Zhang, C., Yang, L., Fan, G., Xu, Y., Sun, B., & Li, X. (2018). Dynamic microbial succession of Shanxi aged vinegar and its correlation with favor metabolites during different stages of acetic acid fermentation. *Scientific Reports*, 8(1), 8612. http://dx.doi.org/10.1038/s41598-018-26787-6. PMid:29872163.