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The amino acids, bacterial communities, and their correlations in *Wuliangye-flavour* liquor production

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ABSTRACT

With the enhancement of people's awareness of drinking health, the health factors in *Wuliangye-flavour* liquor is worth our attention. Bacterial communities in 4 layers of Zaopei from the same fermentation pit and amino acids as major health factors in 4 liquors directly related Zaopeis were investigated by Illumina MiSeq sequencing and liquid chromatography mass spectrometry, respectively. Results indicated that 18 amino acids were detected and 8 dominant bacteria (genus level) were observed. Meanwhile, total amino acids, 11 amino acids (Glu, Asp, Val, etc), bacterial diversity, and the percentages of *Lactobacillus* and *Pseudomonas* increased with the increase of Zaopei's depth; 5 amino acids (Pro, Ser, Phe, etc) and the percentages of *Pediococcus* and *Bacteroides* first increased and then decreased with the increase of Zaopei's

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depth. Moreover, 11 amino acids were significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) positively correlated with *Lactobacillus* and *Pseudomonas* numbers.

KEYWORDS

***Wuliangye-flavour* liquor, amino acids, bacterial communities, correlation**

1. INTRODUCTION

Wuliangye-flavour liquor is one of strong-flavour liquor (Baijiu) in China famous for its century old brewing workshop (Kim, 2009; Fan et al., 2021; Wang et al., 2021). During 70–160 days of solid-state fermentation in a fermentation pit for *Wuliangye-flavour* liquor production, the five fermented grains (Sorghum, glutinous rice, rice, wheat, and corn), namely Zaopei, are not only the substrates for microbial metabolism, but also the direct sources of flavour substances and health factors (Zheng et al., 2018; You et al., 2021). Similarly to other strong-flavour liquors, fermentation pit is one of the necessary facilities for *Wuliangye-flavour* liquor production, and the pit must be continuously used for over 10 years to produce high quality *Wuliangye-flavour* liquor (Zhang et al., 2015), this suggests that the microbes in the pit play a key role in liquor brewing (Hu et al., 2021).

At present, nutrition, delicacy, and health have become widely concerning topics in Baijiu industry (Huo et al., 2020). Many components (health factors) in Baijiu are found to be beneficial to human health, such as amino acids, phenols, acids, pyrazine, peptides, etc., which have anti-oxidation, anti-inflammatory, anti-cancer characteristics, with the functions of promoting ethanol metabolism, improving the comfort after drinking, prevention and treatment of cardiovascular diseases (Wu et al., 2017; Liu et al., 2020). Meanwhile, some genera of bacteria, including *Lactobacillus* and *Bacillus*, contribute to the formation of strong-flavour liquor quality and health factors during the brewing process (Yang et al., 2017; Zou et al., 2018). In addition, *Lactobacillus* can provide other microorganisms with amino acids and vitamins that can be used for growth and reproduction (Xie et al., 2008); *Bacillus* can increase the production of tetramethylpyrazine enriching Baijiu (Xu et al., 2018). Thus, we need to support the corresponding functional microorganisms in Baijiu by establishing a high-throughput screening method based on health factors, and further investigating the fermentation characteristics and fermentation conditions of the microorganisms.

Therefore, the main objective of this study was to investigate the amino acids as major health factors in 4 liquors from the same fermentation pit aged over 50 years by liquid chromatography mass spectrometry (LC-MS) and bacterial communities in various Zaopeis directly corresponding to liquors by Illumina MiSeq sequencing. Furthermore, multivariate statistical techniques were used to investigate amino acids, bacterial communities, and their correlations in *Wuliangye-flavour* liquor production, so as to offer a guidance for increasing amino acid content in *Wuliangye-flavour* liquor.

2. MATERIALS AND METHODS

2.1. Materials

Liquors and directly related Zaopeis were simultaneously collected in the same fermentation pit (same batch) from a *Wuliangye-flavour* liquor producing company in Yibin, Sichuan, China

(May 2020). 4 Zaopeis were respectively taken from the bottom layer (BO), middle layer (MI), upper layer (UP), and top layer (TO) of the fermentation pit aged over 50 years (Fig. 1). The sampling method of Zaopeis was carried out according to the 5-point sampling method (Wang et al., 2021), and then the samples were respectively put into sterilising bags with a mark and stored at –80 °C for amplicon sequencing analysis. Meanwhile, 4 liquors directly related to the 4 Zaopeis were also collected with distillation time of 0.5–10.0 min.

Citric acid, sodium citrate, chloroform, acetone, toluene, acetic acid, potassium hydroxide, trichloroacetic acid, methanol, acetonitrile, and tetrahydrofuran were purchased from Kelon Chemical Reagent Factory, Chengdu, China. The standards of glutamic acid (Glu), aspartic acid (Asp), citrulline (Cit), threonine (Thr), glycine (Gly), arginine (Arg), serine (Ser), methionine (Met), leucine (Leu), proline (Pro), isoleucine (Ile), alanine (Ala), tyrosine (Tyr), cysteine (Cys), valine (Val), histidine (His), phenylalanine (Phe), lysine (Lys), *o*-phthalaldehyde (OPA), 9-fluorenylmethylchloroformate (FMOC), and triethylamine (chromatographic pure) was purchased from Sigma-Aldrich (Shanghai, China).

2.2. Illumina MiSeq sequencing

To analyse the taxonomic composition of the bacterial communities in Zaopeis, the universal primer pairs 515F and 806R, which incorporate Illumina adapters and barcode sequences, were used to amplify the V4 hypervariable region of the 16S rRNA gene, using a two-step amplification procedure. DNA extraction, polymerase chain reaction (PCR), and Illumina MiSeq sequencing (2-by 150-bp reads) were performed by Wuhan Biotechnology Co., Ltd. (Wuhan, China), as described previously (Huang et al., 2021). Each sample was extracted twice and each extraction was analysed three times. Data preprocessing was performed mainly using QIIME (Quantitative Insights Into microbiota, V1.8.0). Chimeric sequences were excluded with default parameters and sequences with similarities >97% were clustered into one operational taxonomic unit (OTU) using QIIME. The taxonomical assignment of each OTU was performed using the Greengenes database (https://greengenes.secondgenome.com) at a 90% confidence level (Li et al., 2016; Wang et al., 2018b). After calculating the OTU matrix, statistical analysis was applied using alpha indices (Shannon, Simpson, Chao 1 and ACE) calculated by using QIIME.

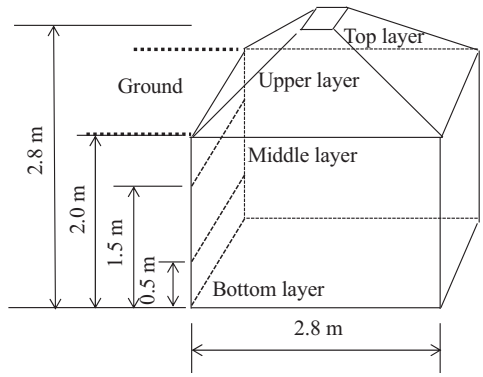

Fig. 1. Sampling sites of top layer (TO), upper layer (UP), middle layer (MI), and bottom layer (BO) in fermentation pit aged over 50 years for *Wuliangye-flavour* liquor production

2.3. Identification and quantification of amino acids

One mL *Wuliangye-flavour* liquor was mixed with 10% trichloroacetic acid solution in equal volume and centrifuged at 10,000 r.p.m. for 15 min. After filtration by aqueous phase filter membrane (0.45 μm), 500 μL sample was collected for testing. Then, the amino acids in liquor were determined by OPA-FMOC precolumn derivatisation, while, the retention time was used for qualitative analysis and the peak area was quantified by external standard method (Mattivi et al., 2000).

2.4. Statistical analysis

One-way analysis of variance (ANOVA) with the least-significant difference (LSD) method (P <0.05) was applied to compare the alpha indices between different Zaopeis. Principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and cluster analysis were performed using SIMCA 14.1 software and R Project 3.5, respectively. Meanwhile, to establish the OPLS-DA model, a permutation analysis was carried out on the data, with the number of tests set to 200; the differences between the two groups of data were analysed as a whole, to obtain the volcano plots and variable importance in projection (VIP) prediction value distribution. The amino acids with VIP >1, $P < 0.05$ and fold change (FC) >2 or <0.5 were designated as significantly changed amino acids. Pearson correlation analysis was completed by SPSS 22.0 software, and visual network analysis was completed using Cytoscape 3.7 software.

3. RESULTS AND DISCUSSION

3.1. Bacterial communities in 4 Zaopeis

After filtering out the low-quality reads and chimeras, 20,480 to 36,123 sequences were obtained and 279 to 386 OTUs were generated. These OTUs were classified into 37 phyla and unclassified bacteria. With the increase of Zaopei's depth, bacterial richness and diversity increased significantly ($P < 0.05$, Table 1).

Due to the changes of environmental conditions (temperature, humidity, oxygen content, and pH) in the fermentation pit (Tao et al., 2014; Hu et al., 2016), the bacterial communities of Zaopeis changed dynamically with the increase of depth (Fig. 2). The dominant bacteria (at genus level) in Zaopeis were *Lactobacillus* (35.74–53.37%), *Pediococcus* (12.46–23.57%), *Bacillus* (6.40–22.88%), *Bacteroides* (2.49–7.82%), *Clostridium* (1.43–4.69%), *Proteiniclasticum* (1.81–4.41%),

Table 1. Bacterial diversity indices calculated based on the cutoff of 97% identity of 16S rRNA gene region

Name	Community richness		Community diversity	
	ACE	Chao1	Shannon	Simpson
TO	$2019.68^{d} \pm 149.42$	$2136.36^{d} \pm 134.95$	$5.14^{d} \pm 0.15$	$0.66^{d} \pm 0.02$
UP	$2401.09^{c} \pm 113.01$	$2613.28^{c} \pm 113.17$	$6.36^{c} \pm 0.24$	$0.72^{c} \pm 0.02$
MI	$3153.14^{b} \pm 66.99$	$3444.37^{b} \pm 61.03$	$7.38^{b} \pm 0.25$	$0.78^{b} \pm 0.03$
BO	$3777.89^{a} \pm 82.71$	$4070.41^{a} \pm 82.89$	$8.26^{a} \pm 0.12$	$0.84^{a} \pm 0.01$

Data are presented as means ± standard deviations ($n = 3$), values with different letters within a column are significantly different ($P < 0.05$). BO: bottom layer; MI: middle layer, UP: upper layer; TO: top layer in fermentation pit.

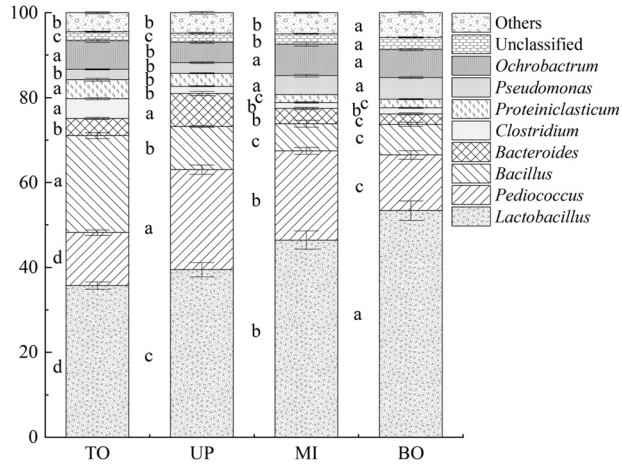

Fig. 2. Bacterial communities at the genus level in four Zaopeis from the same fermentation pit aged over 50 years for *Wuliangye-flavour* liquor production. Values with different letters for the same bacteria are significantly different ($P < 0.05$)

Pseudomonas (2.44–5.07%), and *Ochrobactrum* (4.80–7.45%). Meanwhile, the percentages of *Lactobacillus* and *Pseudomonas* increased with the increase of Zaopei's depth; the percentages of *Clostridium* showed the opposite trend. Due to *Lactobacillus* are anaerobic bacteria and prefer acidic environment (Yang et al., 2020; da Silva et al., 2021), the content of oxygen and pH decrease with the increase of Zaopei's depth (Zhang et al., 2017a; Zhao et al., 2020), the percentages of *Lactobacillus* increased significantly ($P < 0.05$, Fig. 2). In contrast, *Clostridium* was negatively related with the occurrence of *Lactobacillus* and in agreement with previous report (Zhang et al., 2017b; Wang et al., 2018a). Moreover, the percentages of *Pediococcus* and *Bacteroides* first increased significantly ($P < 0.05$) and then decreased with the increase of Zaopei's depth (Fig. 2). *Pediococcus* spp. are lactic acid bacteria that are widely described as probiotics (Porto et al., 2017), although the percentages of *Pediococcus* began to decline from UP, they were still the dominant bacteria in Zaopeis.

3.2. Amino acids in 4 liquors

In this study, amino acids in 4 liquors were quantified using liquid chromatography mass spectrometry (LC-MS) approach. A total of 18 amino acids, including 8 essential amino acids (Thr, Met, Leu, Ile, Val, His, Phe, and Lys), were detected in liquors. Of them, 17 amino acids were detected in each sample, and His was only detected in TO (Fig. 3). Meanwhile, PCA showed that the amino acids composition of liquors in the same fermentation pit changed significantly and 4 clusters were respectively formed (Fig. 3A).

The total content of amino acids in TO, UP, MI, and BO were 18.27 mg L^{-1}, 29.41 mg L^{-1}, 35.54 mg L^{-1}, and 38.03 mg L^{-1}, respectively. While, the contents of 11 amino acids (Glu, Asp, Val, Ile, Cys, Lys, Arg, Gly, Ala, Tyr, and Thr) increased with the Zaopeis from TO to BO; the contents of 5 amino acids (Pro, Ser, Phe, Cit, and Leu) first increased and then decreased with the increase of Zaopei's depth (Fig. 3B). Furthermore, 3 amino acids with the highest average

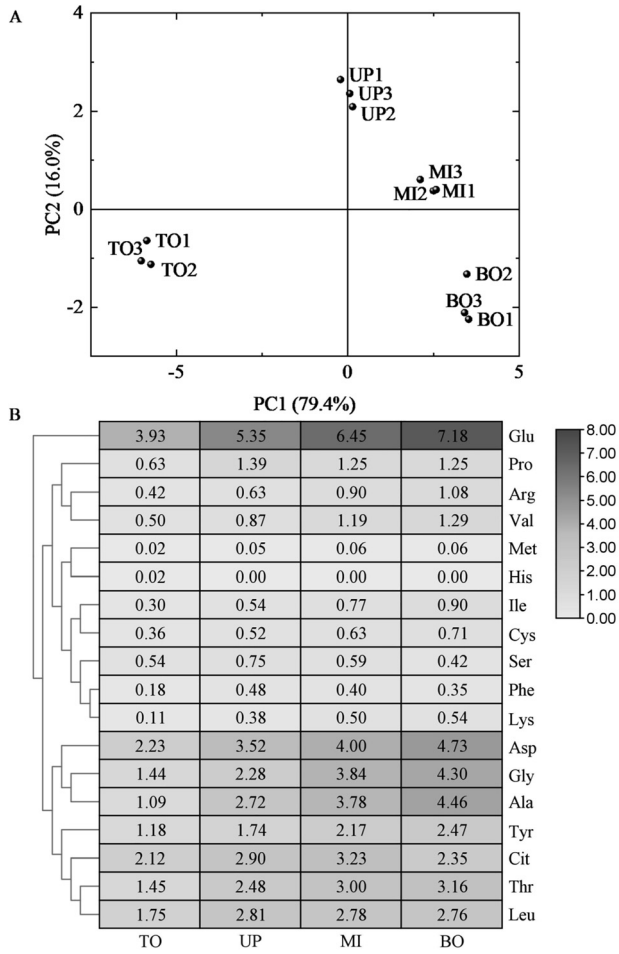

Fig. 3. PCA (A) and cluster analysis (B) of amino acids in four liquors from the same fermentation pit aged over 50 years for *Wuliangye-flavour* liquor production

content in 4 liquors were Glu (3.93–7.18 mg L^{-1}), Asp (2.23–4.73 mg L^{-1}), and Ala (1.09–4.46 mg L^{-1}). In order to further investigate the differences in amino acids between samples, OPLS-DA model was established to observe the differential amino acids between 4 liquors. As shown in Fig. 4, volcano plots were drawn based on OPLS-DA model. Among them, OPLS-DA identified His in UP with significantly decreased relative levels [VIP > 1.0, $P < 0.05$, FC (UP/TO) < 0.5] comparing TO (Fig. 4A); conversely, 5 amino acids (Pro, Phe, Lys, Met, and Ala) increased significantly [VIP > 1.0, $P < 0.05$, FC (UP/TO) > 2]. It can be seen from Fig. 4B that His, Gly, Phe, Lys, Ile, Val, Thr, Ala, Met, and Arg were the significantly different amino acids [VIP > 1.0, $P < 0.05$, FC (MI/TO) < 0.5 or >2] in TO and MI. As shown in Fig. 4C, His in BO decreased significantly [VIP > 1.0, $P < 0.05$, FC (BO/TO) <0.5] compared to TO, and 11 amino acids (Gly, Cys, Tyr, Asp, Lys, Ile, Val, Thr, Ala, Met, and Arg) increased significantly [VIP > 1.0,

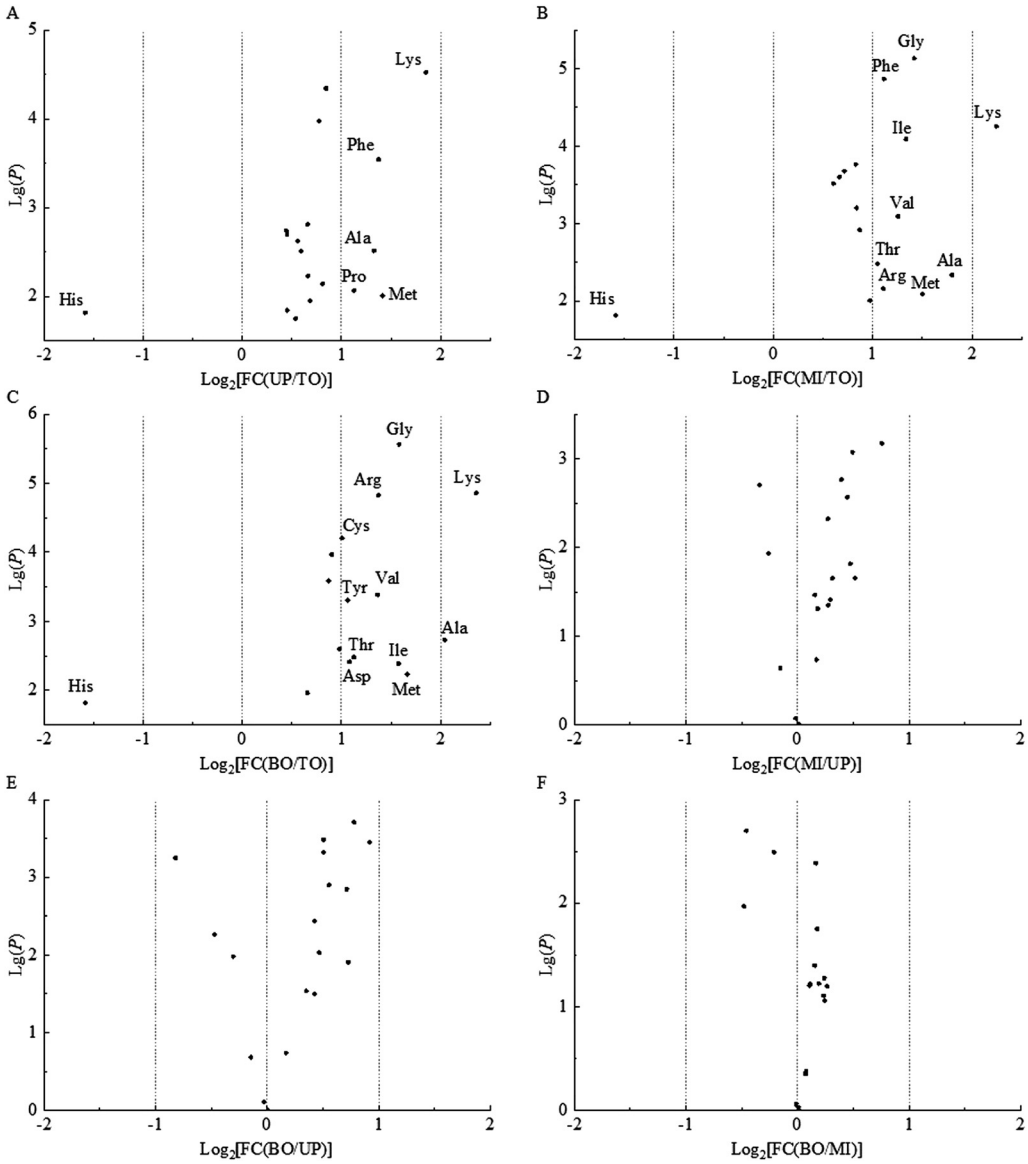

Fig. 4. Volcano plot of the contribution of amino acids in TO and UP (A), TO and MI (B), TO and BO (C), UP and MI (D), UP and BO (E), MI and BO (F) using OPLS-DA

$P < 0.05$, FC (BO/TO) >2]. Furthermore, there were no significantly different amino acids between UP, MI, and BO (Fig. 4D, E, F). There is a trace of oxygen in TO and the oxygen concentration in the fermentation pit decreases gradually from TO to BO (Porto et al., 2017), the bacterial communities of Zaopeis changed dynamically with the increase of depth, and the chemical compounds produced by the bacterial communities also changed dramatically.

3.3. Correlation analysis between amino acids and bacterial communities

Pearson correlation analysis between 18 amino acids and 8 dominant bacteria was carried out, aiming to obtain more useful information by clarifying their relationship. As demonstrated in Fig. 5, 11 amino acids were significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) positively correlated with *Lactobacillus* and *Pseudomonas* (Solid lines). Thus, *Lactobacillus* and *Pseudomonas* can be beneficial to form Glu, Asp, Thr, Gly, Arg, Ile, Ala, Tyr, Cys, Val, Lys; and this result verified that *Lactobacillus* and *Pseudomonas* had positive effects on the quality of strong-flavour liquor (Yang et al., 2017; Wang et al., 2017, 2019; Liu et al., 2019).

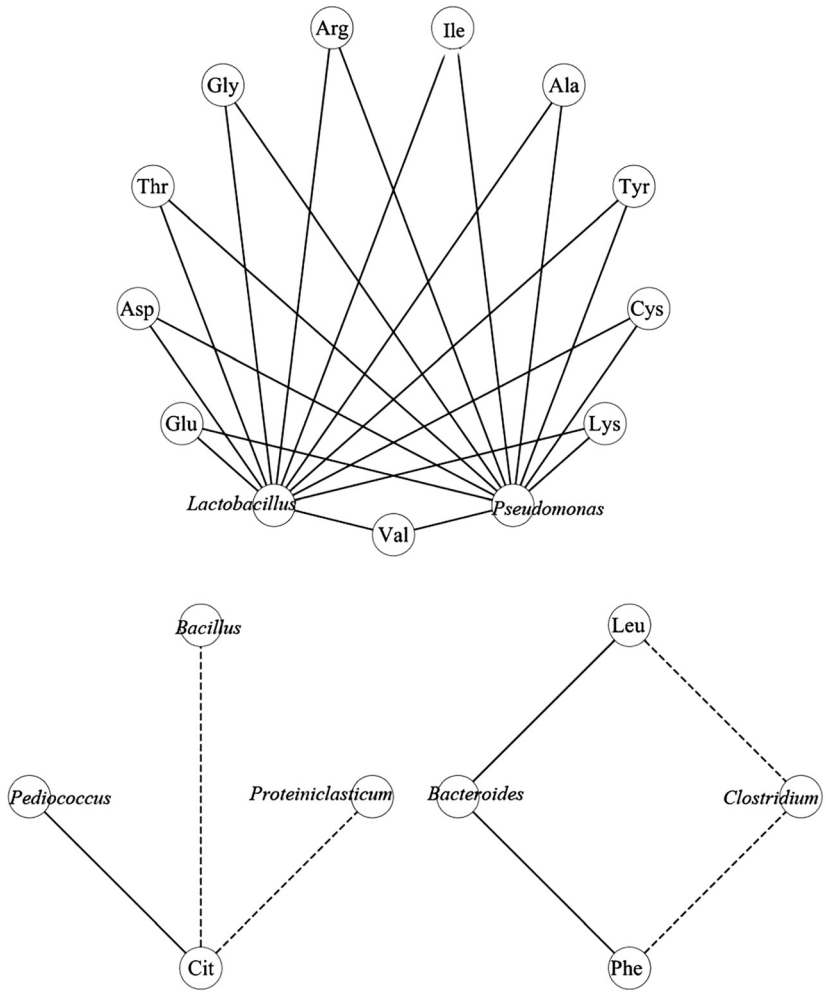

Fig. 5. Interaction of microbes and amino acids on the basis of the Pearson correlation analysis in samples from the same fermentation pit aged over 50 years for *Wuliangye-flavour* liquor production. The connection indicates a statistically significant ($P < 0.01$) strongly positive (Solid lines) or negative (Dotted lines) correlation with Spearman's $|\rho| > 0.8$

Meanwhile, Cit was significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) positively correlated with *Pediococcus*, but significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) negatively correlated with *Bacillus* and *Proteiniclasticum* (Dotted lines). Although *Bacillus* had a negative effect on the formation of Cit, *Bacillus* is the dominant bacterium that forms the aroma components of Baijiu (Zhao et al., 2019), which is consistent with the conclusion that *Bacillus* was the dominant bacterium (6.40–22.88%) in 4 layers of Zaopei from the same fermentation pit. Furthermore, Leu and Phe significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) positively correlated with *Bacteroides*, but significantly ($P < 0.01$) and strongly ($|\rho| > 0.8$) negatively correlated with *Clostridium*.

4. CONCLUSIONS

Lactobacillus and *Pseudomonas*, forming 11 amino acids (Glu, Asp, Val, Ile, Cys, Lys, Arg, Gly, Ala, Tyr, Thr), can be beneficial in solid-state fermentation of *Wuliangye-flavour* liquor. Consumers' perception is of great importance, as Baijiu with health factors has increasingly been valued on the market. Furthermore, these results can be used as reference to understand the relationship between amino acids and bacteria in *Wuliangye-flavour* liquor brewing process, and can provide relevant data for increasing the amino acid content in *Wuliangye-flavour* liquor.

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REFERENCES

Fan, W., Zhao, X., Du, G., Chen, J., Li, J., Zheng, J., Qiao, Z., and Zhao, D. (2021). Metaproteomic analysis of enzymatic composition in Baobaoqu fermentation starter for Wuliangye baijiu. *International Journal of Food Science and Technology*, 56(8): 4170–4181.

Hu, X., Du, H., Ren, C., and Xu, Y. (2016). Illuminating anaerobic microbial community and cooccurrence patterns across a quality gradient in Chinese liquor fermentation pit muds. *Applied and Environmental Microbiology*, 82(8): 2506–2515.

Hu, X.L., Tian, R.J., Wang, K.L., Cao, Z.H., Yan, P.X., Li, F.Q., Li, X.S., Li, S.L., and He, P. X. (2021). The prokaryotic community, physicochemical properties and flavors dynamics and their correlations in fermented grains for Chinese strong-flavor Baijiu production. *Food Research International*, 148: 110626.

Huang, C.Y., Ying, H.Q., Yang, X.B., Gao, Y., Li, T., Wu, B., Ren, M., Zhang, Z.X., Ding, J., Gao, J., Wen, D., Ye, X., Liu, L., Wang, H., Sun, G., Zou, Y., Chen, N., and Wang, L. (2021). The *Cardamine enshiensis* genome reveals whole genome duplication and insight into selenium hyperaccumulation and tolerance. *Cell Discovery*, 7(1): 62.

Huo, J., Luo, X., Huang, M., Wu, J., Zhang, J., Liu, X., Li, H., and Sun, X. (2020). Identification and antioxidant activity of a novel peptide from Baijiu. *International Journal of Peptide Research and Therapeutics*, 26(3): 1199–1210.

Kim, J. (2009). Comparison of the volatile components in two Chinese wines, Moutai and Wuliangye. *Journal of the Korean Society for Applied Biological Chemistry*, 52(3): 275–282.

Li, P., Lin, W., Liu, X., Wang, X., and Luo, L. (2016). Environmental factors affecting microbiota dynamics during traditional solid-state fermentation of Chinese Daqu starter. *Frontiers in Microbiology*, 7: 1237.

Liu, C.C., Feng, S.B., Wu, Q., Huang, H.Q., Chen, Z.X., Li, S.W., and Xu, Y. (2019). Raw material regulates flavor formation via driving microbiota in Chinese liquor fermentation. *Frontiers in Microbiology*, 10: 1520.

Liu, J., Wang, H.L., Liu, X.Y., Zhang, G.H., and Liu, Z.G. (2020). Chinese liquor extract attenuates oxidative damage in HepG2 cells and extends lifespan of *Caenorhabditis elegans*. *Food Science and Nutrition*, 8(7): 3164–3172.

Mattivi, F., Monetti, A., Vrhovsek, U., Tonon, D., and Andres-Lacueva, C. (2000). High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. *Journal of Chromatography A*, 888(1–2): 121–127.

Porto, M.C.W., Kuniyoshi, T.M., Azevedo, P.O.S., Vitolo, M., and Oliveira, R.P.S. (2017). *Pediococcus* spp.: an important genus of lactic acid bacteria and pediocin producers. *Biotechnology Advances*, 35(3): 361–374.

da Silva, B.L., Pereira, P.V., Bertoli, L.D., Silveira, D.L., Batista, N.N., Pinheiro, P.F., de Souza Carneiro, J., Schwan, R.F., de Assis Silva, S., Coelho, J.M., and Bernardes, P.C. (2021). Fermentation of *Coffea canephora* inoculated with yeasts: microbiological, chemical, and sensory characteristics. *Food Microbiology*, 98: 103786.

Tao, Y., Li, J., Rui, J., Xu, Z., Zhou, Y., Hu, X., Wang, X., Liu, M., Li, D., and Li, X. (2014). Prokaryotic communities in pit mud from different-aged cellars used for the production of Chinese strong-flavored liquor. *Applied and Environmental Microbiology*, 80(7): 2254–2260.

Wang, Q., Liu, K.Y., Liu, L.L., Zheng, J., Chen, T., Chen, F., Li, P.P., Zhang, M., and Shen, X.J. (2021). Correlation analysis between aroma components and microbial communities in Wuliangye-flavor raw liquor based on HS-SPME/LLME-GC-MS and PLFA. *Food Research International*, 140: 109995.

Wang, S.L., Wu, Q., Nie, Y., Wu, J.F., Xu, Y., and Björkroth, J. (2019). Construction of synthetic microbiota for reproducible flavor compound metabolism in Chinese light-aroma-type liquor produced by solid-state fermentation. *Applied and Environmental Microbiology*, 85(10): e3090–18.

Wang, X., Ren, H., and Zhan, Y. (2018b). Characterization of microbial community composition and pathogens risk assessment in typical Italian-style salami by high-throughput sequencing technology. *Food Science and Biotechnology*, 27(1): 241–249.

Wang, X.S., Du, H., and Xu, Y. (2017). Source tracking of prokaryotic communities in fermented grain of Chinese strong-flavor liquor. *International Journal of Food Microbiology*, 244: 27–35.

Wang, X.S., Du, H., Zhang, Y., and Xu, Y. (2018a). Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Applied and Environmental Microbiology*, 84(4): e02369–17.

Wu, J., Huo, J., Huang, M., Zhao, M., Luo, X., and Sun, B. (2017). Structural characterization of a tetrapeptide from sesame flavor-type Baijiu and its preventive effects against AAPH-induced oxidative stress in HepG2 cells. *Journal of Agricultural and Food Chemistry*, 65(48): 10495–10504.

Xie, Y.Q., Zhong, Y., Xie, X., and Wang, W.D. (2008). Roles and functions of lactic acid bacteria in the production of liquor by solid fermentation. (In Chinese with English abstract). *Liquor-making Science and Technology*, 11(11): 83–86.

Xu, Y., Xu, C., Li, X., Sun, B., Eldin, A.A., and Jia, Y. (2018). A combinational optimization method for efficient synthesis of tetramethylpyrazine by the recombinant *Escherichia coli*. *Biochemical Engineering Journal*, 129: 33–43.

Yang, F., Zhang, Q., Liu, Y.F., Li, J.H., Wang, L., and Chen, J. (2020). Lactic acid biosynthesis pathways and important genes of *Lactobacillus panis* L7 isolated from the Chinese liquor brewing microbiome. *Food Bioscience*, 36: 100627.

Yang, X.P., Teng, K.L., Zhang, J., Wang, F.F., Zhang, T., Ai, G.M., Han, P.J., Bai, F.Y., and Zhong, J. (2017). Transcriptome responses of *Lactobacillus acetotolerans* F28 to a short and long term ethanol stress. *Scientific Reports*, 7(1): 2650.

You, L., Zhao, D., Zhou, R., Tan, Y., Wang, T., and Zheng, J. (2021). Distribution and function of dominant yeast species in the fermentation of strong-flavor Baijiu. *World Journal of Microbiology and Biotechnology*, 37(2): 26.

Zhang, L., Zhou, R., Niu, M., Zheng, J., and Wu, C. (2015). Difference of microbial community stressed in artificial pit muds for Luzhou-flavour liquor brewing revealed by multiphase culture-independent technology. *Journal of Applied Microbiology*, 119(5): 1345–1356.

Zhang, Q.Y., Yuan, Y.J., Zeng, L.Y., Wang, S., Tang, Q.L., Wu, Z.Y., and Zhang, W.X. (2017a). Discrimination of Luzhou-flavoured fresh raw liquor distilled from Zaopei fermented in new, trend to-be aged and aged pit mud based on their aroma and flavour compounds. *Journal of the Institute of Brewing*, 123(2): 242–251.

Zhang, Y.Y., Zhu, X.Y., Li, X.Z., Tao, Y., Jia, J., and He, X.H. (2017b). The process-related dynamics of microbial community during a simulated fermentation of Chinese strong-flavored liquor. *BMC Microbiology*, 17: 196.

Zhao, Q.S., Yang, J.G., Zhang, K.Z., Wang, M.Y., Zhao, X. X., Su, C., and Cao, X.Z. (2020). Lactic acid bacteria in the brewing of traditional Daqu liquor. *Journal of the Institute of Brewing*, 126(1): 14–23.

Zhao, X.X., Liu, Y.H., Shu, L., and He, Y.G. (2019). Study on metabolites of *Bacillus* producing soy sauce-like aroma in Jiang-flavor Chinese spirits. *Food Science and Nutrition*, 8(1): 97–103.

Zheng, J., Zhao, D., Peng, Z., Yang, K., Zhang, Q., and Zhang, Y. (2018). Variation of aroma profile in fermentation process of Wuliangye baobaoqu starter. *Food Research International*, 114: 64–71.

Zou, W., Zhao, C., and Luo, H. (2018). Diversity and function of microbial community in Chinese strong-flavor Baijiu ecosystem: a review. *Frontiers in Microbiology*, 9: 671.