Received: 10/04/2022 Revised: 12/12/2022 Accepted: 25/01/2023 Published: 14/03/2023

ORIGINAL ARTICLE
DOI 10.58430/jib.v129i1.12

INSTITUTE OF BREWING & DISTILLING

The microbial diversity and flavour metabolism of Chinese strong flavour Baijiu: a review

- Wei Cheng [1,2]
- Xuefeng Chen [1,3] ✉
- Yuxi Guo [1]
- Duan Zhou [1,3]
- Huawei Zeng [4]
- Huan Fu [1]

[1] School of Food and Biological Engineering, Shaanxi University of Science & Technology, Xi'an 710021, China.
[2] Jinzhongzi Distillery Co., Ltd., FuYang 236023, China.
[3] Shaanxi Research Institute of Agricultural Product Processing Technology, Xi'an 710021, China.
[4]School of Life Sciences, Huaibei Normal University, Huaibei 235000, China

✉ chenxf201693@163.com

Abstract

Strong flavour Baijiu is widely consumed in China and is produced by the fermentation of grains using microbial starters. However, a comprehensive understanding of the diversity and metabolic characteristics of microbial communities involved in the solid-state fermentation of Baijiu is important for determining the relationship between microbial composition, flavour metabolism and understanding Baijiu fermentation conditions. Although studies have examined the metabolic pathways and major processes of flavour compounds in strong flavour Baijiu, aspects of the fermentation process remain unexplored. In this review, methods are discussed the optimisation of microbial diversity in strong flavour Baijiu and effects on the flavour of Baijiu. In particular, recent studies are reviewed on starters (Daqu), fermented grains (Jiupei), and pit mud together with the effects of microbial composition on the quality of strong flavour Baijiu. The challenges of strong flavour Baijiu research and production are discussed, including the role of the microbial diversity of Daqu and Jiupei in the flavour composition of strong flavour Baijiu. This review contributes to the current understanding of processing for strong flavour Baijiu and serves as a reference for screening flavour-related microorganisms, which is valuable for improving the quality of strong flavour Baijiu.

Keywords:
strong flavour Baijiu; microbial diversity; flavour metabolism

Introduction

Chinese Baijiu is brewed from raw materials (sorghum, rice, and other grains) and a saccharifying starter (Daqu, Xiaoqu, or Fuqu). Baijiu brewing involves complex enzymatic reactions and microbial processes. Of the various types of Baijiu, strong flavour Baijiu is the most popular in China and is produced by solid-state fermentation using medium temperature Daqu as a starter (Jin et al, 2017; Zou et al, 2018a). The type and quality of strong flavour Baijiu are related to the different areas of production characterised by different brewing environments, microbial diversity, and process. The main production areas of strong flavour Baijiu are concentrated in Chinese provinces with varying climatic conditions including Sichuan, Anhui, Shandong, Jiangsu, and Henan Province. The different regions are characterised by their own microbiome that imparts individual characteristics to the Baijiu (Wang et al, 2015). Furthermore, the microbial community simultaneously determines the yield and quality of Baijiu based on its diversity and stability.

The distillation process for strong flavour Baijiu is divided into three steps: steaming of grains, distillation of Jiupei, and mixed distillation of Jiupei and grains (Figure 1). The mixing of Jiupei with sorghum, rice or other cereals, and steamed rice hulls, followed by distillation, is referred to as the mixed distillation of Jiupei and grains. After distillation and steaming, the mixture containing the grains is cooled and mixed with Daqu powder to obtain Liangpei. Daqu is the saccharifying and fermentation agent in the production of strong flavour Baijiu; Daqu is produced by solid-state fermentation of wheat, barley, or peas, and involves grinding, mixing, shaping, incubation, and maturation (Gou et al, 2015; Zheng et al, 2011; Zheng et al, 2012). The Liangpei is then transferred into a mud pit and covered with Mianzao (Diuzao is often used), which covers fermenting grains in the pit. Jiaopi-mud then covers the Mianzao to maintain an anaerobic environment. The Liangpei is then fermented in the pit for 60–90 days. The fermented Liangpei is called Jiupei and the different layers are harvested and distilled to produce strong flavour Baijiu. Generally, the quality of Jiupei at the bottom of the pit is better than that of the other layers for distilling strong flavour Baijiu. Moreover, the liquid seepage sinks to the bottom and accumulates in the pit, in contact with the mud (Hu et al, 2021a; Qian et al, 2021; Gao et al, 2020). Distilled Jiupei from the upper part of the pit is called Diuzao (which is not used in later fermentation processes) and distilled Jiupei from other parts of the pit is called Huizao, which is used in further fermentation processes. Huizao is mixed with cereals and subjected to another cycle of fermentation to produce strong flavour Baijiu.

The flavour and taste of strong flavour Baijiu can be attributed to the the raw materials, the microbial activity of Daqu, the flavour of the Jiupei, and the microbial processes occurring in the pit mud. Of these factors, the composition of the microbial community and associated processes are important factors in the production of strong flavour Baijiu (Wu et al, 2016). However, little is known about the microbial composition, associated enzymes and metabolic activities, and flavour metabolism of strong flavour Baijiu, especially, the microbial pathways involved. Therefore, in this review, the literature on the microbial diversity and flavour metabolism of strong flavour Baijiu is critically analysed and summarised.

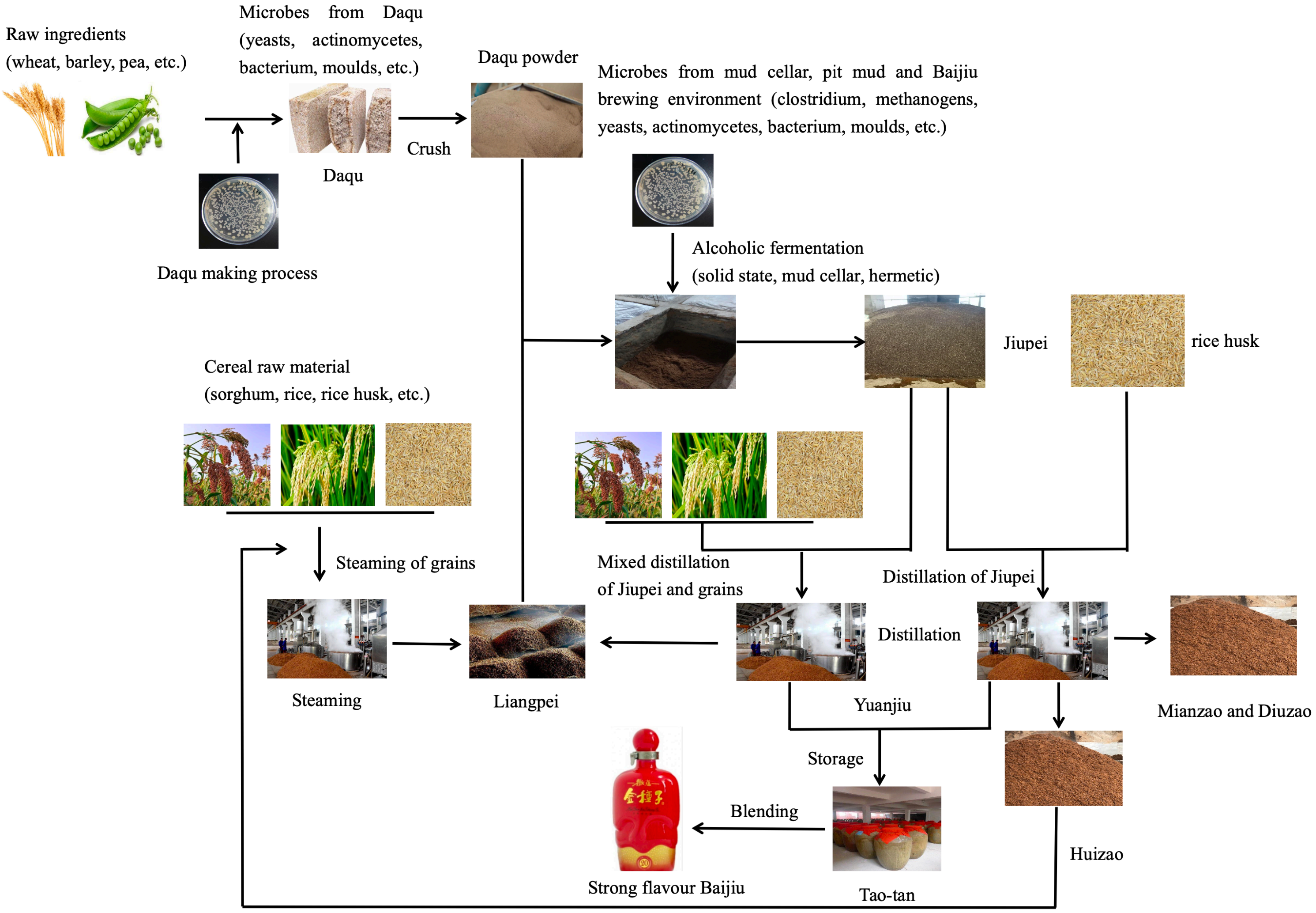
Studies on the microbial diversity of strong flavour Baijiu

Research methods, classification, and comparison

The main sources of microorganisms in the fermentation of strong flavour Baijiu are the Daqu, grains, mud pit for fermentation, and the environment (Figure 1). Several culture-dependent and culture-independent methods are used in the identification of microorganisms. Culture-independent methods are used to identify specific non-culturable microbes in a mixed population. These methods include microscopy for identifying microorganisms based on visual appearance; which can also used with VBNC (viable but non culturable) organisms (Manafi, 1996). Culture-dependent methods are used to isolate and culture

Figure 1.

Processes and sources of microorganisms in the fermentation of strong flavour Baijiu (Wu et al, 2016; Jin et al, 2017; Xu et al, 2017; Zou et al, 2018b).



microorganisms based on their phenotypic characteristics (Ramamurthy et al, 2014; Li et al, 2014). Several biochemical and molecular methods have been used to study microbial diversity during Baijiu brewing (Table 1). The biochemical methods include plate counting (PC), community level physiological fingerprinting (CLPF), phospholipid-derived fatty acids spectrum analysis (PLFA), and fatty acid methyl ester spectrum analysis (FAME). The molecular methods include terminal restriction fragment length polymorphism (T-RFLP), single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis, or temperature gradient gel electrophoresis (DGGE/TGGE), and ribosomal intergenic spacer analysis (RISA) (Franco-Duarte et al, 2019).

Traditional techniques are plate culture, colony counting, and physiological and biochemical experiments, which are mainly culture-dependent methods and facilitate the morphological, physiological, chemical, and biochemical characterisation of microorganisms. These methods enable studies on the distribution, growth of microorganisms and the influence of the ecological environment on the quality of strong flavour Baijiu. The time needed for studying microbial diversity using these methods is 2–5 days or longer. In contrast, molecular techniques, which may be supplemented with molecular fingerprinting, reduce this time significantly. Currently, fingerprinting methods based on PCR amplification are used to evaluate the diversity of microbial communities, however, these methods can only be used to assess the relative abundance of different species in microbial communities and provide little or no information on cellular metabolism and ecological functions. However, some novel techniques, such as gene chip analysis (Hu et al, 2020b), high-throughput sequencing (Chen et al, 2020; Tan et al, 2020), and the use of stable isotopes (Zachleder et al, 2018), provide improved procedures for studying microbial diversity during Baijiu brewing.

Compared with more traditional sequencing methods, high-throughput sequencing facilitates a more detailed and comprehensive analysis of genes and genomes. High-throughput sequencing technologies include amplicon sequencing, metagenomic sequencing, and metatranscriptome sequencing. Amplicon sequencing is based on the sequencing and classification of characteristic sequences in microbial genomes for taxonomic identification and determination of the relative abundance of a species. Metagenome sequencing does not requirem amplification, and directly sequences all the DNA present in the sample to enable species identification and analysis of gene function. Metatranscriptome sequencing differs from metagenomics and focuses on both DNA and RNA in a sample; it also identifies the active species, active genes, and active functions (Hu et al, 2020a).

Microbial diversity of materials used in strong flavour Baijiu

Microbial diversity of Daqu (saccharifying starter)

Daqu plays an important role in the yield, quality, and flavour of Baijiu (Yang et al, 2021). A fermentation starter for strong flavour Baijiu production, Daqu provides the microorganisms, flavour precursors, and enzymes for the process. Recent studies have examined the microbial structure, genetic diversity, and phylogenetic status of Daqu (Chen et al, 2021; Yang et al, 2018). The main microbial communities in Daqu include bacteria, moulds, yeasts, and actinomycetes, whose metabolic activity affects the flavour of Baijiu. The microorganisms isolated from various strong flavour Daqu (SFD) samples are reported in Table 2. The microbial diversity of Daqu is crucial for Baijiu production and has been studied in detail (Li et al, 2017).

Modern molecular technologies have been used to examine the functional microorganisms in Daqu during the solid-state fermentation of strong flavour Baijiu (Yang et al, 2017a). The microbial communities of Daqu vary between layers, with moulds predominant at the surface, bacteria predominant in the deeper layer, and yeasts present across all layers, but at a low abundance. Moreover, the different microbial distribution between the surface and inner layers of Daqu significantly influence its physiochemistry (Chen et al, 2021). The microflora of Daqu is largely dependent on the weather and geographical factors, as well as the manufacturing process (Xu et al, 2017). The Daqu microbiota

Table 1.

Classification and comparison of research methods for determining microbial diversity in strong flavour Baijiu.

Method	Classification	Merits	Shortcomings
Biochemical	Plate counting (PC)	Equipment is simple. Easy operation. Low technical barriers. Low cost.	Tedious. Lengthy incubation times. Low efficiency. Not applicable to all microflora (Wilkinson, 2018).
	Community level physiology fingerprinting (CLPF)	High degree of automation. Rapid detection.	Sample handling, culture conditions. Microplate application contributes to assessment of microbial diversity (Gałązka and Furtak, 2019).
	Phospholipid derived fatty acids spectrum analysis (PLFA)	Direct information on microbial community. Assessment of dynamic changes. Experimental conditions are straight forward. Relatively cheap. Objective and reliable results.	PLFA maps cannot be used to analyse archaea. PLFA often overlaps different species. Environmental conditions can influence the microbial community PLFA pattern (Twining et al, 2020).
	Fatty acid methyl ester spectrum analysis (FAME)	Suitable for tracking changes in the community. Storage time of samples is not high. Provides information of all microorganisms in the sample Easy to operate, relatively low cost. Suitable for the total microbial community, rather than specific microbial species (Haack et al, 1994; Schutter and Dick, 2000; Torres et al, 2020).	Fatty acids must be exhaustively extracted. It is difficult to identify microorganisms that are distant from each other by phospholipid fatty acid composition. Archaea cannot be analysed using FAME spectrograms (Torres et al, 2020).
Molecular	Terminal restriction fragment length polymorphism (T-RFLP)	High throughput, simple operation. High sensitivity (Franco-Duarte et al, 2019; Kari et al, 2019).	Inability to perform hybridisation, direct cloning and sequencing analysis. Inability to determine the microbe; qualitative information is insufficient (Franco-Duarte et al, 2019; Kari et al, 2019).
	PCR-denaturing gradient gel electrophoresis (DGGE)	Suitable for the detection of difficult microorganisms. High detection sensitivity (1% of the total community). Fast detection and low cost. Bands in the fingerprint are sequenced (Franco-Duarte et al, 2019).	Only small fragments (< 500 bp) isolated. A single band in the map does not always represent a single strain; DGGE usually indicates the dominant species in the community. Gene sequence information in the database is limiting. Special equipment is required; Gradient gels contain toxic formamide. Not possible to identify mutations (Franco-Duarte et al, 2019).
	PCR-single-strand conformation polymorphism (SSCP)	High sensitivity and operability. Suitable for large number of samples. Sequence of DNA fragments is not required. No need for special instrumentation. Easy-to-master technology. Low requirements for raw materials Low cost, short cycle. It is a simple and sensitive method for detecting genomic mutations (Hashim and Al-Shuhaib, 2019).	Sensitivity is low in DNA fragments with > 300 bp (Ushijima et al, 1995). False negative results.
	Fluorescence quantitative PCR	Fast and efficient. High specificity, high throughput. Viable but non-culturable (VBNC) microorganisms can be detected (Agusti et al, 2018; Sunyer-Figueres et al, 2018; Zhong and Zhao, 2017). The composition of the microbial community is determined. The functional microbial group is quantified (Crevecoeur et al, 2017; Reumer et al, 2018; Tajima et al, 2001).	False positive results.
	Ribosomal intergenic spacer analysis (RISA)	Good repeatability. Good analysis for special strip sequencing, Simple operation.	Difficult to distinguish DNA fragments with little difference. Some diversity information is lost due to restriction to the dominant bacterial population in the environment

Table 2.

Microorganisms isolated from strong flavour Daqu (SFD).

Samples	Source of samples	Isolated species/genus	Reference
Mature SFD	Wuling/Deshan/Baisha, Hunan/different strong flavour Baijiu brewing companies	*Bacillus licheniformis*, *Bacillus subtilis Bacillus amyloliquefaciens*, and *Bacillus cereus*	Li et al, 2014
Mature SFD	Luzhou, Sichuan/a strong flavour Baijiu brewing companies	*Candida sp.*, *Hansenula sp.*, *Brettanomyces sp.*, *Dekkera sp.*	Xu 2016
SFD fermented for 5, 7, 10, 25, and 90 days	Luzhou, Sichuan/ Luzhou Laojiao Co. Ltd.	*Bacillus subtilis* subsp. *inaquosorum*, *Saccharomycopsis fibuligera*, *Lichtheimia ramosa, Mucor circinelloides,* F. *circinelloides*, *Rhizopus oryzae*	Yang et al, 2017b
Mature SFD	Luyi, Henan/ Songhe Distillery Co., Ltd	Actinomycete species: *Streptomyces lividans*, *Nocardiopsis dassonvillei*, *Streptomyces azureus*, *Streptomyces xiamenensis*, *Streptomyces cacaoi subsp.Cacaoi*	Hou et al, 2019
SFD	Different strong flavour Baijiu brewing companies: Wuliangye, Gujing, Yanghe etc	High yield ethyl caproate producing yeast (YX3307): *Clavispora lusitaniae*	Fan et al, 2021
Fortified SFD	Luan, Anhui/ Yingjia Distillery Co., Ltd.	Yeasts: *Saccharomyces cerevisiae*, *Hanseniaspora vineae*, *Pichia kluyveri*, *Trichosporon asahii*, *Wickerhamomyces anomalus*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Wickerhamomyces mori*, *Galactomyces geotrichum*, *Dabaryomyces hansenii*, and *Saccharomyces kudriavzevii.*	Pu et al, 2021
SFD	Ningling, Henan/ Zhanggong Distillery Co., Ltd	Protease producing bacteria: *Bacillus velezensis*	Liu et al, 2022

is known to exhibit species-specific and cyclical succession characteristics during fermentation (Yang et al, 2021; Xiao et al, 2021).

The microbiota of newly prepared Daqu is diverse and can result in a rapid increase in temperature and excessive production of total acids in fermented grains (Li et al, 2022), which negatively affect strong flavour Baijiu fermentation. Therefore, it is important to mature the Daqu during storage and before its use in brewing. Several biochemical reactions, including changes in enzyme, substrate, and bacterial systems, occur during Daqu production and storage (Yang et al, 2017a). Liang et al (2017) found that *Firmicutes* was the dominant phylum in old Daqu, accounting for 62.7 and 63.5% of the relative species abundance in three and six month old Daqu. In nine month old Daqu, *Proteobacteria* accounted for 80.9% species abundance. Further analysis showed that three to six month old Daqu improved the quality and flavour of Baijiu (Liang et al, 2017). Notably, the microbial composition varies among different Daqu reflecting differences in the composition of raw materials, production technique, and climate (Xu et al, 2017).

Microbial diversity of fermented grains (Jiupei)

During the fermentation of grains, microorganisms, metabolites and enzymes, trigger biochemical reactions which determine the flavour of strong flavour Baijiu. The microbiota of Jiupei comprises bacteria, yeasts, and moulds. Saccharifying enzymes are mainly produced by moulds, such as *Rhizopus*, *Aspergillus*, and *Monascus*. *Saccharomyces cerevisiae* is the most common yeast found in grain fermentations. Bacteria, including *Bacillus licheniformis* and caproic acid producing bacteria, are the primary producers of protease, esterase, and other enzymes during the fermentation process. However, during the fermentation process, changes in the microbiota occur driven by shifts in oxygen, ethanol concentration, and acidity (Wu et al, 2021).

Tan et al (2019) determined that lactobacilli reflect the rate of microbial succession at different stages of the process and that fermentation parameters affect the rate. Shi et al (2011) compared the fungal communities of multiple grain and single grain Zaopei using traditional identification methods and denaturing gradient gel electrophoresis (DGGE) and found that raw materials and fermentation location can affect the composition of the fungal community. Further, Song et al (2017) identified thermophilic fungi flora - *Thermomyces lanuginosus* and *Thermoactinomyces* - in Jiupei using high-throughput amplicon and metatranscriptomics sequencing.

Microbial diversity of pit mud

Fermentations are performed in mud pits where the rich microbial community can influence the flavour of strong flavour Baijiu. Analysis of the microbial community in pit mud can provide new insights into factors influencing the quality of strong flavour Baijiu. Indeed, the variety and abundance of microorganisms in pit mud, the mutual influence of different microflora, and the mechanisms by which metabolic activities affect flavour would benefit from further study.

The microbial composition of pit mud includes anaerobic bacteria, such as Methanobacteria and *Clostridium* species, lactic acid, sulphate-reducing, and nitrate-reducing bacteria. In pit mud used for three types of strong flavour Baijiu, Hu et al (2015) identified a total of 28 closely related bacteria, including 15 *Clostridium* and eight *Bacillus* species. They found that *C. kluyveri* N6 could produce caproic, butyric, and octanoic acids, which can react with ethanol to produce the corresponding ethyl esters. Additionally, the microbial community in pit mud is influenced by multiple stressors including low oxygen concentration, low pH, and high alcohol concentration (Zhao et al, 2012). Tao et al (2017) found that prokaryotes were the predominant microbial population in pit mud, accounting for 95.3% of the total metagenomic reads, whereas the abundance of the eukaryotic population was 1.8%.

Furthermore, the microbial diversity of pit mud is influenced by the age of the pit mud, with a stable community structure improving the quality of pit mud (Chen et al, 2020). Archaea communities, consisting primarily of Methanobacteria aged more than 30 years have been reported to be abundant in pit muds (Xu et al, 2017). Methanobacteria promote the growth of caproic acid-producing bacteria such as Clostridia. This may explain why high quality strong flavour Baijiu is produced in aged pit muds with a high Methanobacteria population (Ding et al, 2014). Species belonging to the order Bacillales, especially those belonging to the genus *Bacillus* are dominant in young pit muds (Liang et al, 2016); *Virgibacillus* has also been detected in young pit muds (Ding et al, 2014). In contrast, Chloroflexi, Synergistetes, and Actinobacteria are only detected in aged pit muds (Luo et al, 2014a; Luo et al, 2014b).

Other contributors to microbial diversity

The environment is an important source of fermentation microbiota and can drive microbial succession and alteration of metabolic profiles during the fermentation of strong flavour Baijiu. However, the mechanisms by which microbes in the environment influence the quality of strong flavour Baijiu are unclear. Wu et al (2016) reported that Baijiu flavour improved by perturbing the intrinsic microbial metabolism with extrinsic microbes. Further, Wu et al (2019b) confirmed the relationship between the quality index and the bacterial community of strong flavour Daqu. Therefore, analysis of microbial communities in the target environment and their dynamics could help optimise community structure, regulate community function, and identify important microbial resources for Baijiu brewing (Wang et al, 2019).

Moreover, as the flavour of strong flavour Baijiu is directly influenced by the enzymes produced during fermentation, it is important to determine the microbial species present during the different fermentation stages and to assess the production of corresponding enzymes to modulate their levels for regulating and improving the quality of strong flavour Baijiu.

Microbial supplementation with fortified Daqu, artificial pit mud, and simulated microbial communities

Fortified Daqu

The dominant genera in different types of Daqu are *Bacillus, Lactobacillus, Thermoactinomyces,* *Saccharomyces, Aspergillus* and *Thermomyces* (Gou et al, 2015; Jin et al, 2019). These genera produce various hydrolytic enzymes and flavour compounds, which contribute to the quality of Daqu. The application of fortified Daqu is an important factor, which can be regulated to produce high quality strong flavour Baijiu. Exogenous application of selected strains can increase the levels of enzymes and flavour compounds in Daqu, which may help diversify the indigenous microbial community (Wang et al, 2017). He et al (2019) found that bioaugmentation using *B. velezensis* and *B. subtilis* altered the microbial community and metabolic activity during fermentation and improved the flavour of Daqu. Further, Wang et al (2017) reported that the addition of *B. licheniformis* increased the abundance of *Bacillus, Clavispora,* and *Aspergillus* and decreased the abundance of *Pichia* and *Saccharomycopsis*, which resulted in increased aromatic compounds possibly because of changes in the metabolic activity of native microflora. Li et al (2020) also found that the composition of the microbial community and metabolic activity of Daqu can be influenced by fortification with aroma producing yeast strains.

Artificial pit mud

Mature pit mud older than 20 years is important for brewing high quality strong flavour Baijiu because of the unique microbial community. Accordingly, the pit mud used for fermentation in high quality strong flavour Baijiu production is typically over 20 years old (Tao et al, 2014). Artificial pit muds have been formulated to simulate mature pit mud based on the microbiology of natural pit mud. The approach may reduce the time required for pit mud maturation. *Clostridium* and *Hydrogenispora* are predominant in natural mature pit mud. Further, Bacillus is the predominant species in immature pit mud and the clostridia/bacilli ratio is positively correlated with pit mud quality (Chen et al, 2020). Such findings provide a basis for producing suitable artificial pit mud. *Clostridium*, the dominant bacterial genus in aged pits, can be inoculated into new or young fermentation pits to produce artificial pit mud with increased caproic acid content (Zou et al, 2018b). Liu et al (2019) studied the of artificial pit mud maturation and its connection with high quality Baijiu and found that the relative abundance of Clostridia increased, whereas bacilli decreased, exhibiting characteristics like those of natural pit mud. In summary, pit mud maturation can be reduced through the application of artificial pit mud to produce high quality strong flavour Baijiu.

Simulating microbial communities

Constructing functional microbial models and simulating microbial communities is necessary for the development of artificially controlled fermentation. The construction of simulated microbial communities aims to utilise the individual capabilities of microorganisms and their interactions to achieve specific goals, such as improved productivity, stability and metabolism (Bernstein and Carlson, 2012). Currently, this involves the identification of core microorganisms at the system level and the construction of simulated microbial communities to study the community performance and stability (Wang et al, 2018a). A mixture of *S. cerevisiae* and *Wickerhamomyces anomalus* has been found to improve the metabolism of yeast, promote the production of ethanol and ethyl acetate and to improve Baijiu quality (Zha et al, 2018).

Microbial flavour metabolism

Microbial flavour metabolism in solid-state fermentation is a complex mixture of microorganisms, trace compounds, and enzymes, which is influenced by raw materials, pit mud, and environmental factors (natural microflora, air, and water) (Jin et al, 2017). Several studies have investigated the minor compounds in Baijiu, and have identified over 2,000 acids, esters, alcohols, aldehydes, pyrazines, and terpenes (Sun et al, 2021; Wu et al, 2019a; Yao et al, 2015). Several of these compounds are beneficial to health (Yao et al, 2015); microbial metabolites are related to the trace compounds of strong flavour Baijiu (Table 3). Metabolite analysis of dominant fermentation microorganisms has confirmed that each is intricately involved in the formation of flavour compounds during Baijiu fermentation and contributes to the aromatic profile of Baijiu (Wang et al, 2022; Wang et al, 2019).

Sample pre-treatment for the detection of flavour compounds

The types and quantities of flavour compounds vary in strong flavour Baijiu (Huang et al, 2020; Jia et al, 2020). Conventional chemical analysis is used to determine the content of acids, esters, and aldehydes in strong flavour Baijiu through sample pre-treatment, detection, identification, data processing, and analysis.

Raw materials including Daqu, distiller's grains, pit mud, original strong flavour Baijiu, and yellow pulp water, can be used to determine the flavour compounds produced by different microflora during Baijiu fermentation. The pre-treatment methods include direct sampling (DI), solid-phase microextraction (SPME), liquid-liquid extraction (LLE), simultaneous distillation and extraction (SDE), stir bar sorptive extraction (SBSE), thermal desorption extraction (TDE), and supercritical

Table 3.

Classification and comparison of the microbial diversity and flavour metabolism of strong flavour Baijiu.

Raw material	**Main fermentation microorganism**	**Major volatile flavour substances**	**Major metabolic pathway or substances**
Daqu: barley, wheat, peas, etc. Strong flavour Baijiu-brewing: sorghum, barley, rice, glutinous rice, corn, etc.	Yeast: *Saccharomyces cerevisiae, Schizosachaeomyces, Saccharomycopsis*, *Pichia*, etc. (You et al, 2021)	Esters: ethyl caproate, ethyl lactate, ethyl acetate, ethyl butyrate, ethyl valerate, heptanoic acid ethyl ester, and octanoic acid ethyl ester (Liu et al, 2019). Acids: acetic acid, butyric acid, and caproic acid (Zhong and Zhao, 2017; You et al, 2021). Alcohols: butanol, isoamyl alcohol, octanol, and heptanol, benzene ethanol (Wu et al, 2021). Aldehydes: aldehyde and furfural.	• Under hypoxic conditions or in the presence of fermentable sugars, saccharides are to produce pyruvate. • Pyruvate is converted to ethanol by acetaldehyde dehydrogenase and ethanol dehydrogenase (Wang et al, 2017). • *Saccharomyces cerevisiae* is mainly responsible for ethanol production. • Non-*Saccharomyces* interact with esterases to convert sugar, aldehydes, organic acids, salts, and other substances, which are used as substrates to synthesise esters, the major flavour compounds of strong flavour Baijiu (Jin et al, 2017; Liu et al, 2019). • Main metabolites: film yeasts → ester, Baijiu brewing yeasts → alcohol; enzyme; the production of acetic acid and caproic acid is related to yeast (Zhang et al, 2011).
	Bacteria: *Lactobacillus, Bacillus, Petrimonas, Caproiciproducens, Proteiniphilum, Christensenellaceae, Caldicoprobacter, olsenella, Pediococcus, Acidithiobacillus, Syntrophomonas, Sed-imentibacter,* and *Aminobacterium* (Sun et al, 2016)	Others: beta tetramethyl pyrazine, three methyl pyrazine, dimethyl sulphur and other metabolites and their precursor.	• Bacteria metabolise and secrete saccharifying enzymes and proteases, which hydrolyse starch and protein in fermentation materials, and the products serve as precursors of aromatic substances (Li et al, 2011). • Main metabolites: aromatic substances or their precursors and enzymes (Zheng et al, 2013).
	Mould: *Rhizopus, Aspergillus, Mucor, Absidia, Rhizomucor* and *Penicillium* (Chen et al, 2021; Liu et al, 2019)		• Mainly produce hydrolytic enzymes, including α-amylase and glucoamylase. • Moulds decompose raw materials containing starch into reducing sugars via saccharification (Wang et al, 2018a). • Moulds promote the decomposition and transformation of starch, proteins, and other substances in the fermentation via liquefaction and esterification. • Main metabolites: sugars, enzymes and amino acids, flavour compounds, including esters, alcohols, and acids, etc. (Kari et al, 2019; Wu et al, 2019).
	Actinomyces: *Streptomyces*, Massilia, Nocardiopsis, and *Ermoactinomyces sanguinis* (Zhang et al, 2012)		• Actinomycetes produce enzymes, which degrade starch, cellulose, and other polymers (Wang et al, 2019). • Produce esters, acids, aldehydes, ketones, and alcohols as well as other important aroma and flavour compounds. • Other metabolites: antibiotics.

carbon dioxide extraction (SCDE) (Guo et al, 2022). As shown in Figure 2, the pre-treatment methods for SPME, SBSE, and LLE can be combined with GC-MS/IMS and GC-O to determine the flavour compounds in strong flavour Baijiu. Although several pre-treatment methods can be used for Baijiu flavour detection, their application is limited owing to cost, complex operation, and inability to perform multiple analyses simultaneously.

Modern methods for detecting flavour compounds in strong flavour Baijiu include mass spectrometry (MS) and nuclear magnetic resonance (NMR). NMR techniques allow the rapid, accurate, and non-invasive high throughput analysis of compounds and provides a metabolic map. MS is usually applied for the measurement of biological macromolecules, characterisation of trace compounds, and analysis of trace molecular bioactive substances present in complex systems. Chromatography combined with organic mass spectrometry can be divided into gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and gas/liquid chromatography-quadrupole mass spectrometry (GC/LC-Q-MS) (Table 4). Overall, it is important to select and combine appropriate analysis techniques to detect flavour compounds.

Microbial flavour metabolism of Daqu, Jiupei and pit mud

Microbial flavour metabolism of Daqu

Daqu is produced by solid-state fermentation of grains by environmental inoculation. Owing to thermal and microbial effects, different classes of volatile compounds are generated during Daqu fermentation, and the changing dynamics of microorganisms and flavour substances during fermentation impact on the quality of Baijiu (Mao et al, 2022; Yan et al, 2019). However, research on the olfactory substances in Daqu is lacking.

Zhang et al (2011) characterised 75 volatile compounds in different Daqu samples using HS-SPME-GC-MS and classified 10 types of Daqu samples into three groups by principal component analysis (PCA) according to their origin and production. Further, Zheng et al. (2018) quantified 50 flavour components and four organic acids in Wuliangye baobaoqu using an internal standard curve method and benzyl ester derivatisation. Of these compounds, hexanal, phenylacetaldehyde, and 4-ethyl guaiacol were regarded as pivotal volatile active compounds in Daqu (Zhang et al, 2012).

The production process of Daqu includes fermentation for one month and storage for three to six months. During storage, the flavour compounds of Daqu are influenced by changes in microbial composition and activity (Fan et al, 2019a; Liang et al, 2017). Fan et al (2019a) detected 72 classes of volatile compounds in Daqu and found that the quantity of these compounds changed considerably but then stabilised during storage. Similarly, Guan et al (2021) reported changes in microbial communities, physicochemical properties, and enzymatic indices of Daqu with increasing storage, and suggested that these factors may bring about changes in microbial flavour metabolism.

Microbial flavour metabolism of fermented grains (Jiupei)

The quality of strong flavour Baijiu is determined by the content and proportion of flavour compounds, which are related to the variety and abundance of the microorganisms in Jiupei. Changes in the microbial community during the fermentation of grains can affect the degradation of macromolecular compounds and formation of flavour metabolites and enzymes by moulds, yeasts, and bacteria (Li et al, 2011; Xu et al, 2022). During the fermentation of grains, enzymes including fibrinolytic enzymes, thermostable amylases, proteases, and cellulases are produced by actinomycetes leading to degradation products.

The degradation products of grains are used by yeasts and bacteria to produce low molecular substances, including ethanol, acetic acid, ethyl acetate, peptides, and amino acids, which can support the growth of caproic acid producing bacteria (Wang 2022a; Jin et al, 2017). The high alcohol levels in Jiupei can also influence the flavour of Baijiu, acting as a bridge for the conversion of flavour substances such as acids, and esters

Figure 2.

Methods used for the pre-treatment and analysis of flavour compounds in Baijiu.
(**a**) SPME (Solid-phase microextraction)
(**b**) SBSE (Stir bar sorptive extraction)
(**c**) LLE (Liquid-liquid extraction)

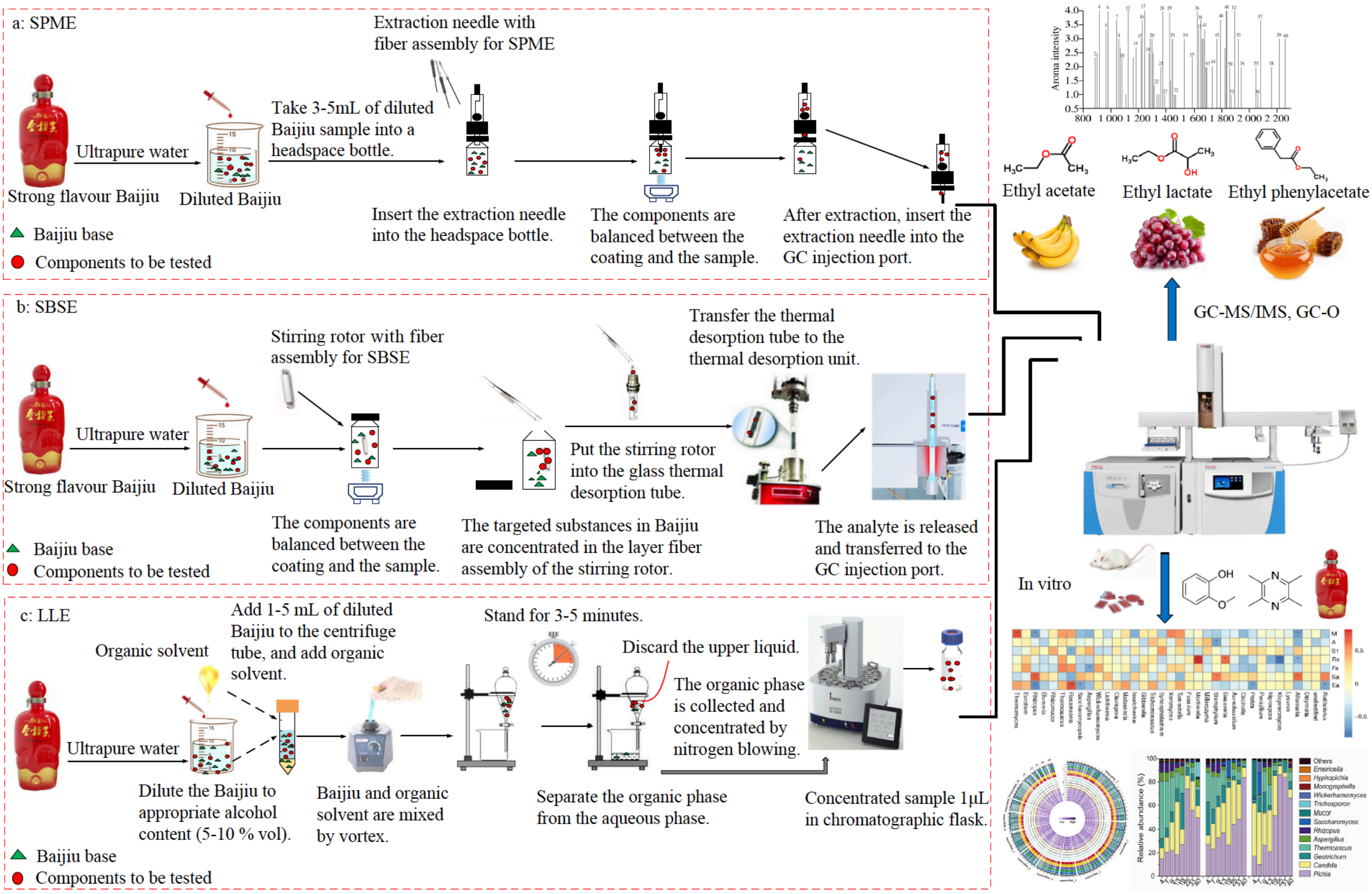


Table 3.

Comparison of NMR and MS in the qualitative analysis of flavour substances in strong flavour Baijiu.

Method	Classification	Merits	Shortcomings
Nuclear magnetic resonance (NMR) (Xiong et al, 2021; Tabago et al, 2021)	^{1}H NMR	• Highly sensitive.	• The chemical environment of organic compounds containing more carbon is similar to alkane hydrogen.
	^{13}C NMR	• No radioactive chemicals. • No environmental contamination. • Sensitive. • Can compensate for the inability of ^{1}H NMR to detect hydrogen free functional groups and to distinguish between alkane like hydrogen in the chemical environment of organic compounds.	• ^{13}C naturally exists at low concentrations and requires a long scanning time.
Mass spectrometry technique (Xiong et al, 2014; Picariello et al, 2011; Koeberl et al, 2014)	Gas chromatography (GC)-MS	• The relative price of the equipment is low. • Rich database for qualitative analysis.	• Quantitative deviation caused by sample derivation. • Temperature and other factors affect column efficiency and cause signal loss. • Long determination time.
	Liquid chromatography (LC)-MS	• Higher sensitivity than NMR, without sample derivatisation.	• Spectral library data is limited.
	Direct infusion (DI)-MS	• The sample has high flux and a relatively simple spectrogram • No sample retention	• Ion inhibition is indistinguishable from the plasmid nucleus ratio of the same metabolite.

(Avalos and Fink, 2013; Hu et al, 2021b). Yeasts are the main producers of ethanol during grain fermentation, and some other species play pivotal roles in the production of strong flavour Baijiu (You et al, 2021). Sun et al (2016) found that *Lactobacillus*, the dominant bacterial genus in Jiupei during the brewing of strong flavour Baijiu, produce lactic acid, acetic acid, and other organic acids. Further, Zou et al (2018a) reported that the precursors of esters, alcohols, and short chain fatty acids are produced by *Clostridium* in Jiupei.

Microbial flavour metabolism in pit mud (Jiaoni)

Mud cellars are the fermentation 'vessels' for the production of strong flavour Baijiu and the species, quantity, and metabolism of microbial flora in Jiaoni determine the quality of strong flavour Baijiu (Chai et al, 2021; Hu et al, 2016). Esters are the main aroma compounds formed by microorganisms in Baijiu, with ethyl hexanoate the main aromatic compound in strong flavour Baijiu (Xu et al, 2017). *Clostridium*, whose main metabolites are caproic acid, butyric acid, and hydrogen, also plays an important role in the production of ethyl hexanoate. Tao et al (2014) found that high quality Jiaoni contains more *Clostridium* than low quality. Further, lactobacilli and methanogens can metabolise and synthesise caproic acid, which can be esterified to form ethyl caproate. Microbes in pit mud produce various organic acids, such as caproic, butyric, acetic, and lactic acids, which are the main precursors of flavour esters (Hu et al, 2015).

Zou et al (2018b) reviewed the research on *Clostridium* in strong flavour Baijiu production, including species diversity, physiological metabolism, and interspecies interactions. Understanding the role of *Clostridium* is helpful for understanding the mechanism of the formation of flavour compounds. Understanding the formation of flavour compounds may help improve the quality of Baijiu (Xu et al, 2017; Xu et al, 2022). A lactate utilising and butyrate producing bacterium termed BPY5 has been isolated from an aged fermentation pit and can be used in the production of butyrate or for the recovery of butyrate from wastewater containing lactate (Tao et al, 2016).

Generally, aged pit muds of high quality are maintained for at least 20 years with continuous use. The prokaryotic community in aged pit muds produce high levels of caproic acid, whichis necessary for the synthesis of ethyl caproate, an important flavour component in strong flavour Baijiu (Tao et al, 2014; Zheng et al, 2013).

Metabolism of unpleasant aroma compounds

During the fermentation of Baijiu, some unpleasant aroma compounds are produced due to bacterial contamination (e.g., *C. aminovalericum* and *C. ultunense*) or poor process management. For example, *C. purinilyticum* produces 4-methylphenol which has an unpleasant aroma (Liu et al, 2017). The most common unpleasant smell is a musty odour. Du et al (2011) speculated that geosmin, which causes an earthy odour, may be a metabolite from microorganisms such as *Streptomyces*.

Research on Baijiu composition is focused on identifying and characterising flavour components that have positive effects on flavour (Jia et al, 2020; Xu et al, 2022). However, tracking the source and changes in unpleasant aromas and flavours in the raw materials, fermentation processes, and during microbial metabolism is also of value (Jin et al, 2017; Liu et al, 2018). However, only a few studies have investigated the unpleasant aroma and flavours in strong flavour Baijiu (Du et al, 2017; Fan et al, 2019b; Sun et al, 2015). Du et al (2017) found a positive correlation between *Streptomyces* and the odours produced by Daqu during storage and found that a storage time of Daqu for about three months is conducive to the reduction of unpleasant aromas.

Other factors affecting flavour

In addition to Daqu, Jiupei, and pit mud, brewing parameters are a major factor influencing the flavour of strong flavour Baijiu. These include water quality, acidity, starch content, amount of rice husk, and fermentation time, which also affect the production of flavour substances by affecting the microbial metabolism in Jiupei and pit mud during fermentation.

During fermentation, the degradation of starch and other macromolecules can result in the accumulation of yellow pulp water in the fermentation pit. Yellow pulp water contains microorganisms and metabolites, including lactic acid, ethanol, and acetic acid (Fang et al, 2017; Gao et al, 2020). These organic acids, ethanol, and other flavour substances permeate the Jiupei and are distilled in the Baijiu using solid-state distillation. This is an important step for developing the characteristic flavour of Baijiu. The microbial metabolites in yellow pulp water can be metabolised by pit mud microbiota (Chai et al, 2021; Gao et al, 2020; Wang et al, 2021). Moreover, microorganisms present in yellow pulp water play important roles in grain fermentation and pit mud ageing (Kang et al, 2022).

The fermentation of strong flavour Baijiu involves metabolism by bacteria, yeasts, and moulds from the environment. Previous studies have focused on the influence of the environment on the succession of the microbial community (Wang et al, 2018b; Sun et al, 2016). However, there is a lack of systematic studies on the effect of environmental microbial factors on microbial succession and metabolism (Wang, 2022). In addition, biofilms and cross-feeding play important roles in microbial flavour metabolism. However, there is a lack of direct evidence of the formation of multispecies biofilms and associated microbial interactions and communication. Only a few studies have investigated biofilms formed by functional microorganisms during food fermentation (Yao et al, 2022).

Flavour metabolism - data and correlation analysis

Owing to advances in modern analytical methods, the chemical nature of key flavour components, their formation, and pathways, with implications for Baijiu have been identified (Tang et al, 2020). Recent research has focused on ways to extract information from complex flavour metabolite data and to screen potential biomarkers. The collection, storage, mining, and exploitation of big data on flavour metabolites are key issues underlying microbiome development (Tang et al, 2020).

Statistical analyses, such as dimension reduction, PCA, cluster analysis (CA), and Procrustes analysis, can be used to determine the characteristics of flavour compounds. PCA transforms the analysis of component data into comprehensive indicators through dimensionality reduction and determines weight according to the correlation and degree of variation to determine key components (Meira et al, 2020).

Cluster analysis classifies indicators through multi-factor analysis, according to the similarity of quality characteristics and aggregates multiple varieties according to the comprehensive properties of categories (Gong et al, 2014). PCA and CA can be used to identify the flavour type, origin, and brewing process of Baijiu. Further, Procrustes analysis can be used to evaluate the differences between two sets of geometric structures. Together with optimal sorting by rotation, translation, image, and scaling, to evaluate differences between two groups. The sorting methods include PCA, principal co-ordinates analysis and non-metric multidimensional scaling (Guo et al, 2022).

Potential relationships between the prokaryotic community and flavour compounds in strong flavour Baijiu fermentation can be determined by correlation analysis using a correlation heat map, correlation scatter plot, redundancy analysis, ROC curve analysis, and chord map. Pearson's correlation coefficient is widely used to measure the strength of linear correlation between two variables. Based on the Pearson's correlation coefficient, the degree of association between species diversity and metabolites in environmental samples can be measured (Wang et al, 2020). The correlation coefficient can be expressed as a heat map reflecting the correlation between each flavour metabolite and microbial classification using a colour gradient (Wu et al, 2016).

The relationship between microbial colonies and flavour compounds in strong flavour Baijiu has been reported (Tan et al, 2019; Yang et al, 2021). Wang et al (2021) reported that the aromatic components of Wuliangye Baijiu were positively correlated with Gram-negative bacteria, anaerobic bacteria, and fungi, but were negatively correlated with Gram-positive bacteria and aerobic bacteria. Importantly, there are potential and complex metabolic correlations among multi-species during strong flavour Baijiu fermentation, and their relationships should be examined in future studies (Hu et al, 2021a; Jin et al, 2017; Xu et al, 2022).

Flavour metabolism - pathway analysis

Although many microbial species have been identified in Baijiu fermentation, little is known about the compounds and their roles produced during fermentation (Wang et al, 2018a; Wang et al, 2019). Further, the interaction and molecular mechanisms of these microorganisms during fermentation remains unclear (Wang et al, 2019). Currently, studies on the pathways and mechanisms of flavour compound production are limited (Xu et al, 2021; Xu et al, 2022). Therefore, it would be valuable to unravel the diversity of flavour compounds and perform metabolomic analysis. Esters, alcohols, aldehydes, and acids are the four major types of flavour compounds in strong flavour Baijiu. The metabolic pathways for the major alcohols, acids, and nine flavour esters are shown in Figure 3 (Xu et al, 2021; Xu et al, 2022).

Microbial synthesis is recognised as the main source of ester formation in Baijiu (Li et al, 2018a; Xu et al, 2022). Esterification in the fermentation of strong flavour Baijiu is catalysed by three types of enzymes - lipases, alcohol dehydrogenases, and alcohol acyltransferases (Tao et al, 2017; Xu et al, 2022).

During fermentation, ethanol is formed under anaerobic conditions by yeasts such as *S. cerevisiae.* Glucose in converted to pyruvic acid via glycolysis which is decarboxylated to acetaldehyde, which is reduced to ethanol (Xu et al, 2021). Studies involving the knockdown or overexpression of genes involved in alcohol synthesis in *S. cerevisiae* have shown that overexpression of the BAT2 gene significantly reduced the output of higher alcohols, such as isoamyl alcohol and isobutanol, but significantly increased the output of ethyl esters (Wang et al, 2018b).

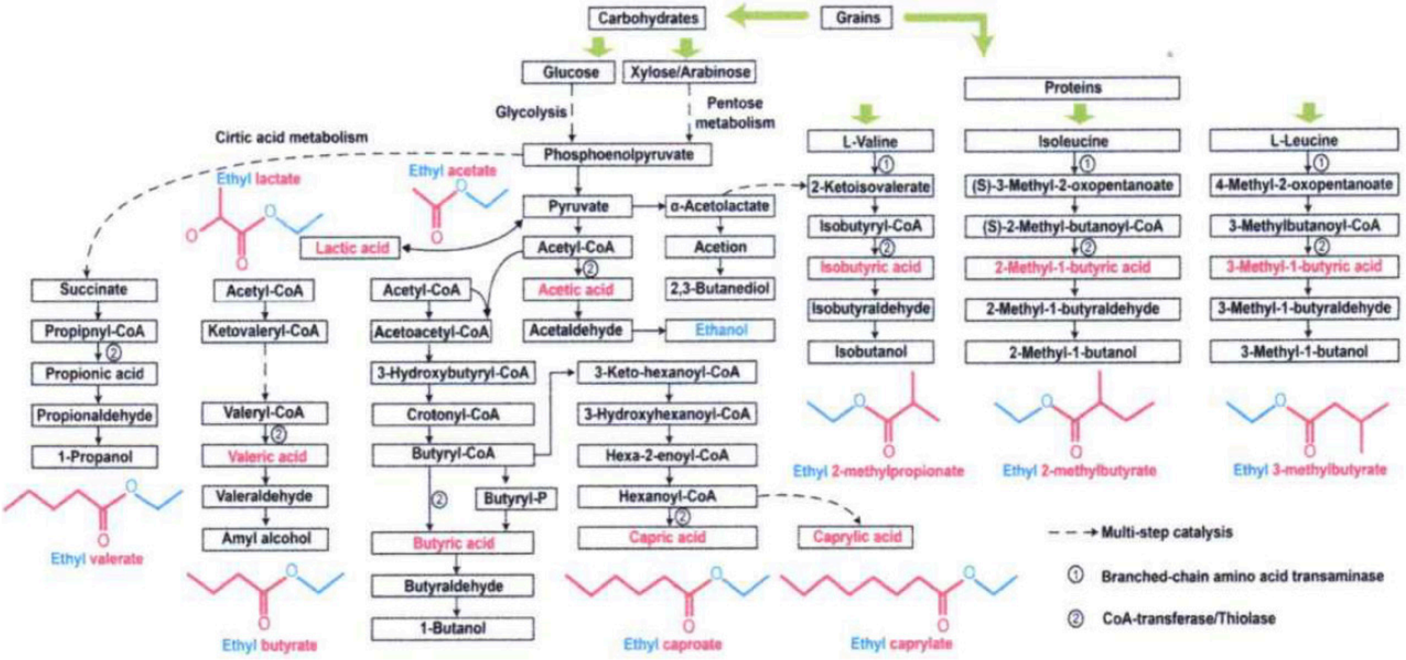
The aldehydes in strong flavour Baijiu mainly include formaldehyde, acetaldehyde, furfural, butyraldehyde, valeraldehyde, and acetal. Most of these are produced from the oxidation of corresponding alcohols during fermentation (Fan et al, 2019b; Sun et al, 2015). Acetaldehyde and acetal are important aromatic compounds in Baijiu (Fan et al, 2019b). Acetal which is formed from the condensation of acetaldehyde and ethanol contributes a pleasant fragrance and taste to Baijiu (Wang et al, 2022). Some aldehydes in Baijiu are bioactive with strong antioxidant, anti-colitic, anti-mutation, and analgesic properties (Tai et al, 2011).

Acids constitute an important class of flavour chemicals in strong flavour Baijiu. Isobutyric acid and isovaleric acid are produced by several species of bacteria under anaerobic conditions (Li et al, 2018b). Additionally, lactic acid can be synthesised by microorganisms during the fermentation of Baijiu (Li et al, 2018c; Chen et al, 2022).

Moreover, acetic acid and lactic acid can serve as substrates for the synthesis of butyric acid and caproic acid (Sifeeldein et al, 2019). The main pathway of butyric acid formation involves butyryl-CoA from reverse β-oxidation from ethanol and acetic acid. Further, organic acids such as caproic acid, butyric acid, acetic acid, and lactic acid, can react with ethanol through the action of esterification enzymes and be converted to acid ethyl esters. Similarly, octanoic acids can react with ethanol, and be converted to octanoic acid ethyl ester (Xu et al, 2022; Nan et al, 2021).

Figure 3.

Metabolic pathways of alcohols, acids, and esters during the fermentation of strong flavour Baijiu (Xu et al, 2021; Xu et al, 2022).



Conclusions and perspectives

In summary, numerous studies have examined the microbial diversity and flavour metabolism to optimise the quality of strong flavour Baijiu. However, the following issues remain to be addressed:

(**1**) Despite decades of research on the microbial composition and structure of strong flavour Baijiu, the relationship between microbial diversity, metabolism, flavours and aroma remains unclear. Further, the impact of physical and chemical factors on microbial metabolism requires further study.

(**2**) Little is known about the mechanisms by which microorganisms interact with each other and the metabolic pathways by which functional microflora produce flavour and aroma compounds in strong flavour Baijiu fermentation.

(**3**) The metabolic pathways of the microorganisms responsible for unpleasant aromas and flavours remains unclear.

(**4**) Studies on metabolic and enzymatic pathways during fermentation, such as the synthesis of esters and alcohols, may help clarify the mechanisms of flavour metabolism in strong flavour Baijiu.

Further, the application of genetic techniques will be necessary to identify and screen functional strains of microorganisms. This will be important for manipulating the quality and flavour of strong flavour Baijiu. Finally, the targeted regulation and application of microorganisms and their flavour metabolites in Baijiu brewing should be examined in future work.

Author Contributions

Wei Cheng: Conceptualisation, writing (original draft).
Xuefeng Chen and Huawei Zeng: Supervision.
Huan Fu: Data curation.
Xuefeng Chen, Yuxi Guo, and Duan Zhou: Writing (review and editing).

Acknowledgments

We thank Shaanxi University of Science and Technology, Jinzhongzi Distillery Co., Ltd., for their support of this review. Additionally, the review was funded by the Province Science and Technology Major Special Program Project of Anhui (No.201903a06020054), and the Science and Technology Program in Fuyang city, Anhui (No. FK2020-81136). We would like to thank Editage (www.editage.cn) for English language editing.

Conflicts of interest

The authors declare no conflicts of interest.

References

Agusti G, Fittipaldi M, Codony F. 2018. Optimization of a viability PCR method for the detection of *Listeria monocytogenes* in food samples. *Curr Microbiol* 75:779-85. https://doi.org/10.1007/s00284-018-1448-6

Avalos JL, Fink GR. 2013. Stephanopoulos, G. Compartmentalization of metabolic pathways in yeast mitochondria improves the production of branched-chain alcohols. *Nat Biotechnol* 31:335-45. https://doi.org/10.1038/nbt.2509

Bernstein HC, Carlson RP. 2012. Microbial consortia engineering for cellular factories: In vitro to *in silico* systems. *Comput Struct Biotechnol* J 3:e201210017. https://doi.org/10.5936/csbj.201210017

Chai L, Qian W, Zhong X, Zhang X, Lu Z, Zhang S, Wang S, Shen C, Shi J, Xu Z. 2021. Mining the factors driving the evolution of the pit mud microbiome under the impact of long-term production of strong flavour baijiu. *Appl Environ Microbiol* 87:1-9. https://doi.org/10.1128/AEM.00885-21

Chen L, Li Y, Jin L, He L, Ao X, Liu S, Yang Y, Liu A, Chen S, Zou L. 2020. Analyzing bacterial community in pit mud of Yibin Baijiu in China using high throughput sequencing. *PeerJ* 8:e9122. https://doi.org/10.7717/peerj.9122

Chen Y, Li K, Liu T, Li R, Fu G, Wan Y, Zheng F. 2021. Analysis of difference in microbial community and physicochemical indices between surface and central parts of Chinese special-flavour baijiu Daqu. *Front Microbiol* 11:1-9. https://doi.org/10.3389/fmicb.2020.592421

Chen C, Xiong Y, Xie Y, Zhang H, Jiang K, Pang X, Huang M. 2022. Metabolic characteristics of lactic acid bacteria and interaction with yeast isolated from light-flavor Baijiu fermentation. *Food Biosci* 50: 102102. https://doi.org/10.1016/j.fbio.2022.102102

Crevecoeur S, Vincent WF, Comte J, Matveev A, Lovejoy C. 2017. Diversity and potential activity of methanotrophs in high methane-emitting permafrost thaw ponds. *PLOS One* 12:1-11. https://doi.org/10.1371/journal.pone.0188223

Ding X, Wu C, Zhang L, Zheng J, Zhou R. 2014. Characterization of eubacterial and archaeal community diversity in the pit mud of Chinese Luzhou-flavour liquor by nested PCR-DGGE. *World J Microbiol Biotechnol* 30:605-12. https://doi.org/10.1007/s11274-013-1472-4

Du H, Du X, Zhao J, Zhang X, Xu Y. 2017. Prokaryotic microbial diversity and geosmin content in the storage process of Daqu. *F&FI* 43:1-6. https://doi.org/10.13995/J.cnki.11-1802/ts.201704001

Du H, Fan W, Xu Y. 2011. Characterization of geosmin as source of earthy odor in different aroma type Chinese liquors. *J Agric Food Chem* 59:8331-7. https://doi.org/10.1021/jf201171b

Fan G, Fu Z, Sun B, Zhang Y, Wang X, Xia Y, Huang M, Li X. 2019a. Roles of aging in the production of light-flavoured Daqu. *J Biosci Bioeng* 127:309-17. https://doi.org/10.1016/j.jbiosc.2018.08.005

Fan G, Liu P, Chang X, Yin H, Cheng L, Teng C, Gong Y, Li X. 2021. Isolation and identification of a high-yield ethyl caproate-producing yeast from daqu and optimization of its fermentation. *Front Microbiol* 12: 663744. https://doi.org/10.3389/fmicb.2021.663744

Fan W, Xu Y, Qian M. 2019b. Current practice and future trends of aroma and flavor research in Chinese baijiu. *In* Guthrie B, Beauchamp JD, Buettner A, Toth S, Qian MC, (eds). *Sex, Smoke, and Spirits: The Role of Chemistry* (pp. 145–175). Washington, DC: American Chemical Society. https://doi.org/10.1021/bk-2019-1321.ch012

Fang X, Deng J, Xie J, Wei C, Luo H, Huang Z, Wang W, Li M. 2017. Brief analysis on current situation of comprehensive utilization of by-products yellow water from Baijiu-making. *China Brew* 36:6-9. https://doi.org/10.11882/j.issn.0254-5071.2017.02.002

Franco-Duarte R, Černáková L, Kadam S, Kaushik KS, Salehi B, Bevilacqua A, Corbo MR, Antolak H, Dybka-Stępień K, Leszczewicz M, Relison Tintino S. 2019. Advances in chemical and biological methods to identify microorganisms-from past to present. Microorganisms 7:30. https://doi:10.3390/microorganisms7050130

Gałązka A, Furtak K. 2019. Functional microbial diversity in context to agriculture. p 347-358. *In* Das S, Dash HR (eds), *Microbial Diversity in the Genomic Era*, Academic Press. https://doi.org/10.1016/B978-0-12-814849-5.00020-4

Gao Z, Wu Z, Zhang W. 2020. Effect of pit mud on bacterial community and aroma components in yellow water and their changes during the fermentation of Chinese strong flavour liquor. *Foods* 9:372. https://doi.org/10.3390/foods9030372

Gou M, Wang H, Yuan H, Zhang W, Tang Y, Kida K. 2015. Characterization of the microbial community in three types of fermentation starters used for Chinese liquor production. *J Inst Brew* 121:620-7. https://doi.org/10.1002/jib.272

Gong L, Meng X, Liu N, Bi J. 2014. Evaluation of apple quality based on principal component and hierarchical cluster analysis. *Trans Chin Soc Agric Eng* 30:276-85. https://doi.org/10.3969/j.issn.1002-6819.2014.13.034

Guan T, Yang H, Ou M, Zhang J. 2021. Storage period affecting dynamic succession of microbiota and quality changes of strong flavour baijiu Daqu. *LWT-Food Sci Technol* 139:1-9. https://doi.org/10.1016/j.lwt.2020.110544

Guo Y, Cheng W, Chen X, Zhou D, Li N, Chen X. 2022. Application of modern instrumental analysis technology in the study of Baijiu flavoromics. *J Food Saf Qual* 13:5218-5226. https://doi.org/10.19812/j.cnki.jfsq11-5956/ts.2022.16.021

Haack SK, Garchow H, Odelson DA, Forney LJ, Klug MJ. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Appl Environ Microbiol* 60:2483-93. https://doi.org/10.1128/aem.60.7.2483-2493.1994

Hashim HO, Al-Shuhaib MBS. 2019. Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: A review. *J Appl Biotechnol Rep* 6:137-44. https://doi.org/10.29252/JABR.06.04.02

He G, Dong Y, Huang J, Wang X, Zhang S, Wu C, Jin Y, Zhou R. 2019. Alteration of microbial community for improving flavour character of Daqu by inoculation with *Bacillus velezensis* and *Bacillus subtilis*. *LWT-Food Sci Technol* 111:1-8. https://doi.org/10.1016/j.lwt.2019.04.098

Hou X, Sun Z, Li X, Chu C, Pan Q, Chen H, Chen Y, Yuan J. 2019. Isolation, identification and enzyme-production of culturable Actinomycetes from Daqu applied in making strong-flavor liquor. *Food Sci Technol* 44:28–35. https://doi.org/10.13684/j.cnki.spkj.2019.12.006

Hu X, Du H, Ren C, Xu Y. 2016. Illuminating anaerobic microbial community and cooccurrence patterns across a quality gradient in Chinese liquor fermentation pit muds. *Appl Environ Microbio*l 82:2506–2515. https://doi.org/10.1128/AEM.03409-15.

Hu X, Wang K, Chen M, Fan J, Han S, Hou J, Chi L, Liu Y, Wei T. 2020a. Profiling the composition and metabolic activities of microbial community in fermented grain for the Chinese strong-flavor Baijiu production by using the metatranscriptome, high-throughput 16S rRNA and ITS gene sequencings. *Food Res Int* 138:109765. https://doi.org/10.1016/j.foodres.2020.109765

Hu X, Du H, Xu Y. 2015. Identification and quantification of the caproic acid-producing bacterium *Clostridium kluyveri* in the fermentation of pit mud used for Chinese strong-aroma type liquor production. *Int J Food Microbiol* 214:116-22. https://doi.org/10.1016/j.ijfoodmicro.2015.07.032

Hu W, Feng K, Jiang A, Xiu Z, Lao Y, Li Y, Long Y. 2020b. An in situ-synthesized gene chip for the detection of food-borne pathogens on fresh-cut cantaloupe and lettuce. *Front Microbiol* 10:1-11. https://doi.org/10.3389/fmicb.2019.03089

Hu X, Tian R, Wang K, Cao Z, Yan P, Li F, Li X, Li S, He P. 2021a. The prokaryotic community, physicochemical properties and flavours dynamics and their correlations in fermented grains for Chinese strong flavour baijiu production. *Food Res Int* 148:1-9. https://doi.org/10.1016/j.foodres.2021.110626

Hu Y, Yang Q, Chen D, Fu B, Zhang Y, Zhang Y, Xia X, Peng N, Liang Y, Zhao S. 2021b. Study on microbial communities and higher alcohol formations in the fermentation of Chinese Xiaoqu baijiu produced by traditional and new mechanical technologies. *Food Res Int* 140:1-9. https://doi.org/10.1016/j.foodres.2020.109876

Huang Z, Zeng Y, Liu W, Wang S, Shen C, Shi B. 2020. Effects of metals released in strong flavour baijiu on the evolution of aroma compounds during storage. *Food Sci Nutr* 8:1904-13. https://doi.org/10.1002/fsn3.1475

Jia W, Fan Z, Du A, Li Y, Zhang R, Shi Q, Shi L, Chu X. 2020. Recent advances in baijiu analysis by chromatography-based technology - a review. *Food Chem* 324:1-11. https://doi.org/10.1016/j.foodchem.2020.126899

Jin G, Zhu Y, Xu Y. 2017. Mystery behind Chinese liquor fermentation. *Trends Food Sci Tech* 63:18-28. https://doi.org/10.1016/j.tifs.2017.02016

Jin Y, Li D, Ai M, Tang Q, Huang J, Ding X, Wu C, Zhou R. 2019. Correlation between volatile profiles and microbial communities: A metabonomic approach to study jiang-flavour liquor Daqu. *Food Res Int* 121:422-32. https://doi.org/10.1016/j.foodres.2019.03.021

Kang J, Sun Y, Huang X, Ye L, Chen Y, Chen X, Zheng X, Han B. 2022. Unravelling the microbial compositions, metabolic functions, and antibacterial properties of Huangshui, a by-product of Baijiu fermentation. *Food Res Int* 157:111320. https://doi.org/10.1016/j.foodres.2022.111320

Kari A, Nagymáté Z, Romsics C, Vajna B, Kutasi J, Puspán I, Kárpáti É, Kovács R, Márialigeti K. 2019. Monitoring of soil microbial inoculants and their impact on maize (*Zea mays* L.) rhizosphere using T-RFLP molecular fingerprint method. *Appl Soil Ecol* 138:233-44. https://doi.org/10.1016/j.apsoil.2019.03.010

Koeberl M, Clarke D, Lopata AL. 2014. Next generation of food allergen quantification using mass spectrometric systems. *J Proteome Res* 13:3499-509. https://doi.org/10.1021/pr500247r

Li P, Lin W, Liu X, Wang X, Gan X, Luo L, Lin W. 2017. Effect of bioaugmented inoculation on microbiota dynamics during solid-state fermentation of Daqu starter using autochthonous of *Bacillus, Pediococcus, Wickerhamomyces* and *Saccharomycopsis*. *Food Microbiol* 61:83-92. https://doi.org/10.1016/j.fm.2016.09.004

Li R, Zheng X, Zhang X, Yan Z, Wang X, Han B. 2018a. Characterization of bacteria and yeasts isolated from traditional fermentation starter (fen-Daqu) through a H-1 NMR-based metabolomics approach. *Food Microbiol* 76:11-20. https://doi.org/10.1016/j.fm.2018.03.015

Li W, Chen S, Wang J, Zhang C, Shi Y, Guo X, Chen F, Xiao D. 2018b. Genetic engineering to alter carbon flux for various higher alcohol productions by *Saccharomyces cerevisiae* for Chinese baijiu fermentation. *Appl Microbiol Biotechnol* 102:1783-95. https://doi.org/10.1007/s00253-017-8715-5

Li W, Cui D, Wang J, Liu X, Xu J, Zhou Z, Zhang C, Chen Y, Xiao D. 2018c. Overexpression of different alcohol acetyltransferase genes with bat2 deletion in *Saccharomyces cerevisiae* affects acetate esters and higher alcohols. *Eur Food Res Technol* 244:555-64. https://doi.org/10.1007/s00217-017-2977-0

Li W, Fan G, Fu Z, Wang W, Xu Y, Teng C, Zhang C, Yang R, Sun B, Li X. 2020. Effects of fortification of Daqu with various yeasts on microbial community structure and flavour metabolism. *Food Res Int* 129:1-9. https://doi.org/10.1016/j.foodres.2019.108837

Li X, Ma E, Yan L, Meng H, Du X, Zhang S, Quan Z. 2011. Bacterial and fungal diversity in the traditional Chinese liquor fermentation process. *Int J Food Microbiol* 146:31-7. https://doi.org/10.1016/j.ijfoodmicro.2011.01.030

Li Z, Fan Y, Huang X, Han B. 2022. Microbial diversity and metabolites dynamic of light-flavor baijiu with stacking process. *Fermentation* 8:67. https://doi.org/10.3390/fermentation 8020067

Li Z, Bai Z, Wang D, Zhang W, Zhang M, Lin F, Gao L, Hui B, Zhang H. 2014. Cultivable bacterial diversity and amylase production in three typical Daqus of Chinese spirits. *Int J Food Sci Technol* 49:776–786. https://doi.org/10.1111/ijfs.12365

Liang C, Du H, Xu Y. 2017. The succession of procaryotic microbial community and the flavor components in the storage process of Daqu. *Microbiology China* 44:384-93. https://doi.org/10.13344/j.microbiol.China.160163

Liang H, Luo Q, Zhang A, Wu Z, Zhang W. 2016. Comparison of bacterial community in matured and degenerated pit mud from Chinese Luzhou-flavour liquor distillery in different regions. *J Inst Brew* 122:48-54. https://doi.org/10.1002/jib.296

Liu B, Du H, Wang X, Xu Y. 2017. Detecting source of p-cresol in strong flavour Chinese liquor by high throughput sequencing. *Microbiology China* 44:108-17. https://doi.org/10.13344/j.microbiol.China.160087

Liu H, Sun B. 2018. Effect of fermentation processing on the flavor of Baijiu. *J Agric Food Chem* 66:5425-5432. https://doi.org/10.1021/acs.jafc.8b00692

Liu M, Tang Y, Zhao K, Liu Y, Guo X, Tian X, Ren D, Yao W. 2019. Contrasting bacterial community structure in artificial pit mud-starter cultures of different qualities: A complex biological mixture for Chinese strong flavour baijiu production. *3 Biotech* 9:1-9. https://doi.org/10.1007/s13205-019-1622-y

Liu Y, Fu J, Wang L, Zhao Z, Wang H, Han S, Sun S, Pan C. 2022. Isolation, identification, and whole-genome sequencing of high-yield protease bacteria from Daqu of Zhang Gong Lao jiu. *PLOS One* 17: e0264677. https://doi.org/10.1371/journal.pone.0264677

Luo Q, Liu C, Li W, Wu Z, Zhang W. 2014a. Comparison between bacterial diversity of aged and aging pit mud from Luzhou-flavour liquor distillery. *Food Sci Technol Res* 20:867-73. https://doi.org/10.3136/fstr.20.867

Luo Q, Liu C, Wu Z, Wang H, Li W, Zhang K, Huang D, Zhang J, Zhang W. 2014b. Monitoring of the prokaryotic diversity in pit mud from a Luzhou-flavour liquor distillery and evaluation of two predominant archaea using qPCR assays. *J Inst Brew* 120:253-61. https://doi.org/10.1002/jib.132

Mao J, Liu X, Gao T, Gu S, Wu Y, Zhao L, Ma J, Li X, Zhang J. 2022. Unravelling the correlations between bacterial diversity, physicochemical properties, and bacterial community succession during the fermentation of traditional Chinese strong flavour Daqu. *LWT-Food Sci Technol* 154:1-9. https://doi.org/10.1016/j.lwt.2021.112764

Manafi M. 1996. Fluorogenic and chromogenic substrates in culture media and identification tests. *Int J Food Microbiol* 31:45-58. https://doi.org/10.1016/0168-1605(96)00963-4

Meira CLC, Novaes CG, Novais FC, Jesus VDS, Oliveira DM, Aguiar RM. 2020. Application of principal component analysis for the evaluation of the chemical constituents of *Mimosa tenuiflora* methanolic extract by DLLME/GC–MS. *Microchem J* 152:104284. https://doi.org/10.1016/j.microc.2019.104284

Nan L, Liu L, Li Y, Huang J, Wang Y, Wang C, Wang Z, Xu C. 2021. Comparison of aroma compounds in Cabernet Sauvignon red wines from five growing regions in Xinjiang in China. *J Food Qual* Article ID: 5562518. https://doi.org/10.1155/2021/5562518

Picariello G, Mamone G, Addeo F, Ferranti P. 2011. The frontiers of mass spectrometry-based techniques in food allergenomics. *J Chromatogr A* 1218:7386-98. https://doi.org/10.1016/j.chroma.2011.06.033

Pu S, Zhang Y, Lu N, Shi C, & Yan S. 2021. Yeasts from Chinese strong flavour Daqu samples: isolation and evaluation of their potential for fortified Daqu production. *AMB Express* 11:176. https://doi.org/10.1186/s13568-021-01337-y

Qian W, Lu Z, Chai L, Zhang X, Li Q, Wang S, Shen C, Shi J, Xu Z. 2021. Cooperation within the microbial consortia of fermented grains and pit mud drives organic acid synthesis in strong-flavor Baijiu production. *Food Res Int* 147:110449. https://doi.org/10.1016/j.foodres.2021.110449

Ramamurthy T, Ghosh A, Pazhani GP, Shinoda S. 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Front Public Health* 2:103. https://doi.org/10.3389/fpubh.2014.00103

Reumer M, Harnisz M, Lee HJ, Reim A, Grunert O, Putkinen A, Fritze H, Bodelier PLE, Ho A. 2018. Impact of peat mining and restoration on methane turnover potential and methane-cycling microorganisms in a northern bog. *Appl Environ Microbiol* 84:1-9. https://doi.org/10.1128/aem.02218-17

Schutter ME, Dick RP. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Sci Soc Am J* 64:1659-68. https://doi.org/10.2136/sssaj2000.6451659x

Shi S, Zhang L, Wu Z, Zhang W, Deng Y, Zhong F, Li J. 2011. Analysis of the fungi community in multiple- and single-grains Zaopei from a Luzhou-flavour liquor distillery in western China. *World J Microbiol Biotechnol* 27:1869-74. https://doi.org/10.1007/s11274-010-0645-7

Sifeeldein A, Wang S, Li J, Dong Z, Chen L, Kaka NA, Shao T. 2019. Phylogenetic identification of lactic acid bacteria isolates and their effects on the fermentation quality of sweet sorghum (*Sorghum bicolor*) silage. *J Appl Microbio*l 126:718-29. https://doi.org/10.1111/jam.14123

Song Z, Du H, Zhang Y, Xu Y. 2007. Unraveling core functional microbiota in traditional solid-state fermentation by high-throughput amplicons and metatranscriptomics sequencing. *Front Microbiol* 8:1-12. https://doi.org/10.3389/fmicb.2017.01294

Sun B, Huang M, Wang J. 2021. Research progress on flavour chemistry and healthy function of baijiu. *J Chin Inst Food Sci Technol* 21:1-13. https://doi.org/10.1007/s13213-016-1223-5

Sun W, Xiao H, Peng Q, Zhang Q, Li X, Han Y. 2016. Analysis of bacterial diversity of Chinese Luzhou-flavour liquor brewed in different seasons by Illumina Miseq sequencing. *Ann Microbiol* 66:1293-301. https://doi.org/10.1007/s13213-016-1223-5

Sunyer-Figueres M, Wang C, Mas A. 2018. Analysis of ribosomal RNA stability in dead cells of wine yeast by quantitative PCR. *Int J Food Microbiol* 270:1-4. https://doi.org/10.1016/j.ijfoodmicro.2018.01.020

Tabago MKAG, Calingacion MN, Garcia J. 2021. Recent advances in NMR-based metabolomics of alcoholic beverages. *Food Chem Mol Sci* 2:100009. https://doi.org/10.1016/j.fochms.2020.100009.

Tai A, Sawano T, Yazama F, Ito H. 2011. Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays. *Biochim Biophys Acta Gen Subj* 1810:170-7. https://doi.org/10.1016/j.bbagen.2010.11.004

Tajima K, Aminov RI, Nagamine T, Matsui H, Nakamura M, Benno Y. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl Environ Microbiol* 67:2766-74. https://doi.org/10.1128/aem.67.6.2766-2774.2001

Tan G, Hu Y, Huang Y, Liu H, Dong W, Li J, Liu J, Peng N, Liang Y, Zhao S. 2020. Analysis of bacterial communities in pit mud from Zhijiang Baijiu distillery using denaturing gradient gel electrophoresis and high-throughput sequencing. *J Inst Brew* 126:90-7. https://doi.org/10.1002/jib.595

Tang P, Shan Q, Wang L, Wang F, Li C, Lu J. 2020. A review of research methods in Baijiu flavor chemistry and recent progress in the flavor chemistry of Maotai-flavored Baijiu. *Food Sci* 41:315-324. https://doi.org/10.7506/spkx1002-6630-20190822-228.

Tan Y, Zhong H, Zhao D, Du H, Xu Y. 2019. Succession rate of microbial community causes flavour difference in strong-aroma baijiu making process. *Int J Food Microbiol* 311:1-9. https://doi.org/10.1016/j.ijfoodmicro.2019.108350

Tao Y, Hu X, Zhu X, Jin H, Xu Z, Tang Q, Li X. 2016. Production of butyrate from lactate by a newly isolated *Clostridium* sp. BPY5. *Appl Biochem Biotechnol* 179:361-74. https://doi.org/10.1007/s12010-016-1999-6

Tao Y, Li J, Rui J, Xu Z, Zhou Y, Hu X, Wang X, Liu M, Li D, Li X. 2014. Prokaryotic communities in pit mud from different-aged cellars used for the production of Chinese strong flavoured liquor. *Appl Environ Microbiol* 80:2254-60. https://doi.org/10.1128/aem.04070-13

Tao Y, Wang X, Li X, Wei N, Jin H, Xu Z, Tang Q, Zhu X. 2017. The functional potential and active populations of the pit mud microbiome for the production of Chinese strong flavour liquor. *Microb Biotechnol* 10:1603-15. https://doi.org/10.1111/1751-7915.12729

Torres A, Fuentes B, Rodríguez KE, Brito A, Díaz L. 2020. Analysis of the content of fatty acid methyl esters in biodiesel by Fourier-transform infrared spectroscopy: method and comparison with gas chromatography. *J Am Oil Chem Soc* 97:651-61. https://doi.org/10.1002/aocs.12350

Twining CW, Taipale SJ, Ruess L, Bec A, Martin-Creuzburg D, Kainz MJ. 2020. Stable isotopes of fatty acids: current and future perspectives for advancing trophic ecology. *Philos Trans R Soc B* 375:20190641. https://doi.org/10.1098/rstb.2019.0641

Ushijima T, Hosoya Y, Suzuki T, Sofuni T, Sugimura T, Nagao M. 1995. A rapid method for detection of mutations in the lacI gene using PCR-single strand conformation polymorphism analysis: Demonstration of its high sensitivity. *Mutat Res-Environ Mutagen Relat Subj* 334:283-92. https://doi.org/10.1016/0165-1161(95)90065-9

Wang L. 2022a. Research trends in Jiang-flavor baijiu fermentation: From fermentation microecology to environmental ecology. *J Food Sci* 87:1362-74. https://doi.org/10.1111/1750-3841.16092

Wang M, Yang J, Zhao Q, Zhang K, Su C. 2019. Research progress on flavour compounds and microorganisms of Maotai flavour baijiu. *J Food Sci* 84:6-18. https://doi.org/10.1111/1750-3841.14409

Wang P, Wu Q, Jiang X, Wang Z, Tang J, Xu Y. 2017. Bacillus licheniformis affects the microbial community and metabolic profile in the spontaneous fermentation of Daqu starter for Chinese liquor making. *Int J Food Microbiol* 250:59-67. https://doi.org/10.1016/j.ijfoodmicro.2017.03.010

Wang P, Wu Q, Xu Y. 2018a. Core microbiota in Chinese liquor fermentation and associations with environmental factors. *Acta Microbiol Sin* 58:142-53. https://doi.org/10.13343/j.cnki.wsxb.20170060.

Wang P, Li Z, Qi T, Li X, Pan S. 2015. Development of a method for identification and accurate quantitation of aroma compounds in Chinese Daohuaxiang liquors based on SPME using a sol-gel fibre. *Food Chem* 169: 230-40. https://doi.org/10.1016/j.foodchem.2014.07.150

Wang Q, Liu K, Liu L, Zheng J, Chen T, Chen F, Li P, Zhang M, Shen X. 2021. Correlation analysis between aroma components and microbial communities in Wuliangye-flavour raw liquor based on HS-SPME/LLME-GC-MS and PLFA. *Food Res Int* 140:1-9. https://doi.org/10.1016/j.foodres.2020.109995.

Wang W, Fan G, Li X, Fu Z, Liang X, Sun B. 2020. Application of Wickerhamomyces anomalus in simulated solid-state fermentation for Baijiu production: changes of microbial community structure and flavor metabolism. *Front Microbiol* 11, 598758. https://doi.org/10.3389/fmicb.2020.598758

Wang X, Du H, Zhang Y, Xu Y. 2018b. Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Appl Environ Microbiol* 84:1-9. https://doi.org/10.1128/aem.02369-17

Wang Z, Wang Y, Zhu T, Wang J, Huang M, Wei J, Ye H, Wu J, Zhang J, Meng N. 2022b. Characterization of the key odorants and their content variation in Niulanshan Baijiu with different storage years using flavor sensory omics analysis. *Food Chem* 376:131851. https://doi.org/10.1016/j.foodchem.2021.131851

Wilkinson MG. 2018. Flow cytometry as a potential method of measuring bacterial viability in probiotic products: A review. *Trends Food Sci Technol* 78:1–10. https://doi.org/10.1016/j.tifs.2018.05.006

Wu J, Huang M, Zheng F, Sun J, Sun X, Li H, Sun B. 2019a. Research progress of healthy baijiu. *J Food Sci Technol* 37:17-23. https://doi.org/10.3969/j.issn.2095-6002.2019.02.003

Wu Q, Kong Y, Xu Y. 2016. Favour profile of Chinese liquor is altered by interactions of intrinsic and extrinsic microbes. *Appl Environ Microbiol* 82:422-30. https://doi.org/10.1128/aem.02518-15

Wu Q, Zhu Y, Fang C, Wijffels RH, Xu Y. 2021. Can we control microbiota in spontaneous food fermentation? Chinese liquor as a case example. *Trends Food Sci Technol* 110:321-31. https://doi.org/10.1016/j.tifs.2021.02.011

Wu S, Xie J, Cheng T, Wei C, Huang Z, Deng J. 2019b. Correlation between quality index and bacterial community of Luzhou flavor Daqu. Food Res Dev 40(4):158-64.

Xiao C, Yang Y, Lu Z, Chai L, Zhang X, Wang S, Shen C, Shi J, Xu Z. 2021. Daqu microbiota exhibits species-specific and periodic succession features in Chinese baijiu fermentation process. *Food Microbiol* 98:103766. https://doi.org/10.1016/j.fm.2021.103766

Xiong L, Tong P, Xiao N, Chen H. 2014. Progress in detection of food allergens based on liquid chromatography coupled with mass spectrometry. *Food Sci* 35:274-8. https://doi.org/10.7506/spkxl002·6630-201421054

Xiong Q, Li Y, Xu K, Deng P, Hou X. 2021. An overview of alcoholic beverages discrimination and a study on identification of bland Chinese liquors by 13C-NMR and 1H-NMR spectra. *Appl Spectrosc Rev* 58:252-270. https://doi.org/10.1080/05704928.2021.1988960

Xu L. 2016. Screening and identification of ester-producing yeast in Luzhou Daqu and optimization of ester production conditions. *China Brew* 35:74-78. https://doi.org/10.11882/j.issn.0254-5071.2016.02.017

Xu Y, Sun B, Fan G, Teng C, Xiong K, Zhu Y, Li J, Li X. 2017. The brewing process and microbial diversity of strong flavour Chinese spirits: a review. *J Inst Brew* 123:5-12. https://doi.org/10.1002/jib.404

Xu Y, Wang X, Liu X, Li X, Zhang C, Li W, Sun X, Wang W, Sun B. 2021. Discovery and development of a novel short-chain fatty acid ester synthetic biocatalyst under aqueous phase from *Monascus purpureus* isolated from baijiu. *Food Chem* 338:1-9. https://doi.org/10.1016/j.foodchem.2020.128025

Xu Y, Zhao J, Liu X, Zhang C, Zhao Z, Li X, Sun B. 2022. Flavour mystery of Chinese traditional fermented baijiu: The great contribution of ester compounds. *Food Chem* 369:1-9. https://doi.org/10.1016/j.foodchem.2021.130920

Yan S, Tong Q, Guang J. 2019. Yeast dynamics and changes in volatile compounds during the fermentation of the traditional Chinese strong flavour Daqu. *LWT-Food Sci Technol* 106:57-63. https://doi.org/10.1016/j.lwt.2019.02.058

Yang J, Dou X, Han P, Bai F, Zhou J, Zhang S, Qin H, Ma Y. 2017a. Microbial diversity in Daqu during production of Luzhou-flavored liquor. *J Am Soc Brew Chem* 75:136-44. https://doi.org/10.1094/asbcj-2017-2879-01

Yang J, Dou X, Ma Y. 2018. Diversity and dynamic succession of microorganisms during Daqu preparation for Luzhou-flavour liquor using second-generation sequencing technology. *J Inst Brew* 124:498-507. https://doi.org/10.1002/jib.528

Yang J, Dou X, Han P, Bai F, Zhou J, Zhang S, Qin H, Ma Y. 2017b. Microbial diversity in Daqu during production of Luzhou-flavored liquor. *J Am Soc Brew Chem* 75: 136–144. https://doi.org/10.1094/ASBCJ-2017-2879-01

Yang Y, Wang S, Lu Z, Zhang X, Chai L, Shen C, Shi J, Xu Z. 2021. Metagenomics unveils microbial roles involved in metabolic network of flavour development in medium-temperature Daqu starter. *Food Res Int* 140:1-8. https://doi.org/10.1016/j.foodres.2020.110037

Yao F, Yi B, Shen C, Tao F, Liu Y, Lin Z, Xu P. 2015. Chemical analysis of the Chinese liquor Luzhoulaojiao by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *Sci Rep* 5:1-11. https://doi.org/10.1038/srep09553

Yao S, Hao L, Zhou R, Jin Y, Huang J, Wu C. 2022. Multispecies biofilms in fermentation: Biofilm formation, microbial interactions, and communication. *Compr Rev Food Sci Food Saf* 21:3346-75. https://doi.org/10.1111/1541-4337.12991

You L, Zhao D, Zhou R, Tan Y, Wang T, Zheng J. 2021. Distribution and function of dominant yeast species in the fermentation of strong flavour baijiu. *World J Microbiol Biotechnol* 37:1-9. https://doi.org/10.1007/s11274-020-02988-y

Zachleder V, Vitova M, Hlavova M, Moudrikova S, Mojzes P, Heumann H, Becher JR, Bisova K. 2018. Stable isotope compounds - production, detection, and application. *Biotechnol Adv* 36:784-97. https://doi.org/10.1016/j.biotechadv.2018.01.010

Zha M, Sun B, Wu Y, Yin S, Wang C. 2018. Improving flavour metabolism of *Saccharomyces cerevisiae* by mixed culture with *Wickerhamomyces anomalus* for Chinese baijiu making. *J Biosci Bioeng* 126:189-95. https://doi.org/10.1016/j.jbiosc.2018.02.010

Zhang C, Ao Z, Chui W, Shen C, Tao W, Zhang S. 2011. Characterization of volatile compounds from Daqu-a traditional Chinese liquor fermentation starter. *Int J Food Sci Technol* 46:1591-9. https://doi.org/10.1111/j.1365-2621.2011.02660.x

Zhang C, Ao Z, Chui W, Shen C, Tao W, Zhang S. 2012. Characterization of the aroma-active compounds in Daqu: A tradition Chinese liquor starter. *Eur Food Res Technol* 234:69-76. https://doi.org/10.1007/s00217-011-1616-4

Zhao J, Zheng J, Zhou R, Shi B. 2012. Microbial community structure of pit mud in a Chinese strong aromatic liquor fermentation pit. *J Inst Brew* 118:356-60. https://doi.org/10.1002/jib.52

Zheng J, Liang R, Zhang L, Wu C, Zhou R, Liao X. 2013. Characterization of microbial communities in strong aromatic liquor fermentation pit muds of different ages assessed by combined DGGE and PLFA analyses. *Food Res Int* 54:660-6. https://doi.org/10.1016/j.foodres.2013.07.058

Zheng J, Zhao D, Peng Z, Yang K, Zhang Q, Zhang Y. 2018. Variation of aroma profile in fermentation process of Wuliangye baobaoqu starter. *Food Res Int* 114:64-71. https://doi.org/10.1016/j.foodres.2018.07.060

Zheng X, Tabrizi MR, Nout MJR, Han B. 2011. Daqu - a traditional Chinese liquor fermentation starter. *J Inst Brew* 117:82-90. https://doi.org/10.1002/j.2050-0416.2011.tb00447.x

Zheng X, Yan Z, Han B, Zwietering MH, Samson RA, Boekhout T, Nout MJR. 2012. Complex microbiota of a Chinese “fen” liquor fermentation starter (fen-Daqu), revealed by culture-dependent and culture-independent methods. *Food Microbiol* 31:293-300. https://doi.org/10.1016/j.fm.2012.03.008

Zhong J, Zhao X. 2017. Detection of viable but non-culturable *Escherichia coli* o157:H7 by PCR in combination with propidium monoazide. *3 Biotech* 8:1-9. https://doi.org/10.1007/s13205-017-1052-7.

Zhou W, Liao Z, Wu Z, Suyama T, Zhang W. 2021. Analysis of the difference between aged and degenerated pit mud microbiome in fermentation cellars for Chinese Luzhou-flavour baijiu by metatranscriptomics. *J Sci Food Agric* 101:4621-31. https://doi.org/10.1002/jsfa.11105

Zou W, Zhao C, Luo H. 2018a. Diversity and function of microbial community in Chinese strong-flavor baijiu ecosystem: a review. *Front Microbiol* 9:671. https://doi.org/10.3389/fmicb.2018.00671

Zou W, Ye G, Zhang K. 2018b. Diversity, function, and application of *Clostridium* in Chinese strong flavour baijiu ecosystem: A review. *J Food Sci* 83:1193-9. https://doi.org/10.1111/1750-3841.14134