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Changes in the microbial community structure during the digitally managed fermentation of medium-temperature Daqu

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Abstract

Illumina MiSeq high-throughput sequencing technology was used to study the microbial community structure of medium-temperature Daqu produced via a digitally managed method and the traditional method. The relationship between the changes in physicochemical properties and microorganisms in Daqu during the fermentation process was analyzed. At the end of fermentation, the bacterial diversity of traditional medium-temperature Daqu was higher than that of digital medium-temperature Daqu, and there was no significant difference in the diversity of fungal communities. The dominant bacteria were *Weissella*, *Lactobacillus*, *Pediococcus* and *Saccharopolyspora*. The dominant fungi were *Thermoascus*, *Issatchenkia* and *Candia*. On the 25th day of fermentation, the bacterial community distribution in the digital Daqu was more even than that in the traditional Daqu, but the fungal community distribution was the opposite. The results of nonmetric multidimensional scale (NMDS) analysis and canonical correlation analysis (CCA) show that Daqu produced by the two fermentation methods had many similarities.

Keywords: medium-temperature Daqu; microbiology communities; digital management system; high-throughput sequencing; physiochemical properties.

Practical Application: The changes and characteristics of the microbial community structure and physicochemical properties of medium-temperature Daqu were analyzed when the digital management system was used, and the feasibility of the system in the actual production of Daqu was verified, which promoted the production of Daqu from empirical to standardized operation and reduced the labor force.

1 Introduction

Chinese Baijiu plays a critical role in traditional Chinese culture (Liu & Sun, 2018). Daqu is a fermentation starter for Chinese Baijiu production (Zhao et al., 2022). The flavor substances and precursor substances in Daqu determine the aroma of the Baijiu (He et al., 2019). Daqu can be divided into high-temperature Daqu, medium-temperature Daqu and low-temperature Daqu according to the maximum production temperature (Li et al., 2016), and different fermentation temperatures result in different microbial community structures in the Daqu. The maximum production temperature of medium-temperature Daqu is 60 °C. Higher temperature can inhibit the growth of yeast and mold, forming a microbial community structure with thermophilic bacteria as the main dominant microorganism (Gan et al., 2019; Xie et al., 2020; Yang et al., 2018). Daqu is rich in various enzymes (Fan et al., 2022), including glucoamylase, liquefaction enzymes, proteases, etc. The enzymes provided by Daqu can decompose the raw fermentation materials of Baijiu and provide nutrients for subsequent microbial fermentation. Daqu is one of the raw materials for Baijiu fermentation and can provide rich flavor substances and flavor precursor substances for fermentation products (Li et al., 2017b).

The change in microbial community structure plays a vital role in the fermentation process of Daqu. It is paramount to study the community structure of microorganisms in Daqu and understand the diversity and functions of microorganisms (Wang et al., 2011). For a long time, researchers have used cultivable methods to study microorganisms in Daqu and cultured and isolated *Bacillus thermophilus*, *cocci*, and *actinomycetes*. However, this method cannot fully detect the microbial community in Daqu. Therefore, the second-generation sequencing technology represented by the Illumina MiSeq sequencing platform has the advantages of being fast, objective and accurate and is widely used in the detection of microorganisms (Zhang et al., 2022). Currently, this technology has also been applied to the analysis of the microbial structure of Daqu (Gan et al., 2019; Fan et al., 2018; Du et al., 2019). It has been applied to the microbial community analysis of Daqu with different storage times (Fan et al., 2019), different types of Daqu and different colors of Daqu (Wang et al., 2011).

Workers produce traditional Daqu based on experience, and experience-related errors results in inconsistent Daqu quality. In addition, traditional Daqu is naturally fermented, and the quality of Daqu is greatly affected by the seasons. According to the production experience of Baijiu manufacturers, when the ambient temperature is 20-25 °C, it is the best temperature for the production of medium-temperature Daqu, and the excessive temperature and humidity difference in other seasons

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reduces the quality of medium-temperature Daqu. Therefore, Baijiu manufacturers are more inclined to explore new Daqu fermentation methods to reduce the impact of temperature and humidity on the quality of Daqu in other seasons and improve the stability of Daqu quality. In this study, sensors for parameters such as temperature, humidity, and carbon dioxide were placed in the Daqu fermentation room to monitor the environmental conditions during fermentation. The processor records real-time data and sets critical values according to the best fermentation parameters explored by the enterprise in production. When the humidity difference between inside and outside the fermentation room was greater than 20%, the computer automatically controlled the opening of the door of the fermentation room to adjust the humidity (Figure 1). Applying a digital management system to Daqu production can reduce labor costs. The sensor device is used to detect and manage various data of the fermentation environment to realize the digital management of Daqu fermentation. Many scholars have studied the microbial community structure in Daqu and have also clarified the composition of dominant microorganisms in Daqu. However, there is no systematic research on the application of digital management systems in the Daqu fermentation process.

A total of 30 samples were collected in this experiment. Illumina MiSeq high-throughput sequencing was used to analyze and compare the microbial diversity in the two types of the Daqu fermentation process to reveal the trends and differences in microbial diversity in the fermentation of medium-temperature Daqu produced via a digitally managed method (IMD) and the traditional method (TMD). This work aims to verify the feasibility of using a digital management system in Daqu production and facilitate the automated production of Daqu.

2 Materials and methods

2.1 Daqu fermentation and sample collection

The production of medium-temperature Daqu is mainly divided into three stages: molding, fermentation and maturation. In the molding stage, the raw materials of Daqu are crushed and mixed with water to form Daqu bricks. In the fermentation stage, the finished Daqu bricks are transferred to the fermentation room, covered with straw and sprinkled with water to create an environment for the growth of microorganisms. After the fermentation of Daqu was finished, the samples were transferred to the storage room for maturation for approximately six months. Samples at 0, 3, 6, 12, and 25 days were collected from the fermentation rooms. The IMD numbers are IMD0, IMD3, IMD6, IMD12 and IMD25. The traditional medium-temperature Daqu numbers are TMD0, TMD3, TMD6, TMD12 and TMD25.

The Daqu used in the experiment comes from a Baijiu company in Hubei Province. Five parts of Daqu were taken from the four corners and the middle of the fermentation room. After pulverizing and mixing in a sterile grinder, Daqu was used to analyze the physicochemical properties.

2.2 Daqu physicochemical property analysis

The retrieved Daqu samples were used to analyze the physicochemical properties. Moisture, pH, starch, fermenting activity (FA) and esterifying activity (EA) were measured according to "QB/T 4257-2011" (People's Republic of China Professional Standard, 2011). The reducing sugar (RS) content was determined by the dinitrosalicylic acid (DNS) method (Miller, 1959). Saccharifying activity (SA) and liquefying activity (LA) were also analyzed (Li et al., 2015). The physicochemical variables, enzyme activities, and alpha diversity indices were expressed as the mean ± standard deviation of three replicates.

2.3 DNA extraction and amplification

An E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA) extraction kit was used to extract microbial DNA from a 0.5 g Daqu sample, and 1% agarose gel electrophoresis was used to check the integrity of the extracted DNA. To identify bacteria, the universal primer 341F/806R was used to amplify the V3-V4 hypervariable region of 16S rRNA, and PCR was performed using TransStart FastPfu DNA polymerase. To identify the fungi in the sample, the primers ITS1F/ITS2R were used for PCR amplification and PCR using

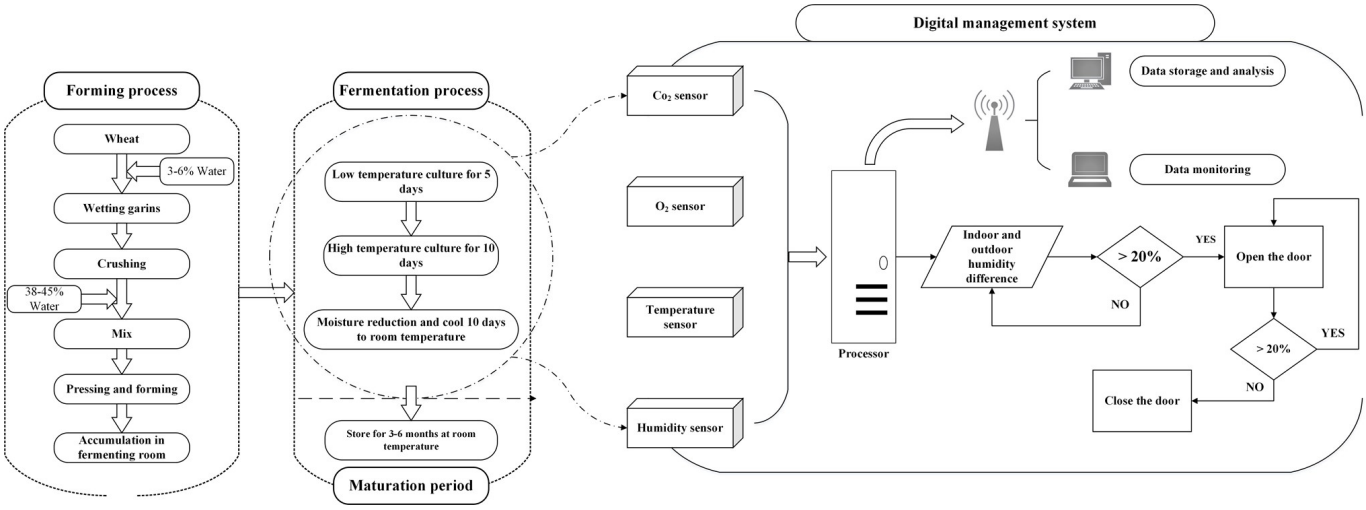

Figure 1. The production process of medium-temperature Daqu and the application of digital control system in production.

Table 1. PCR (Polymerase Chain Reaction) system.

Bacteria		Fungi	
Sample	Volume	Sample	Volume
5 × Fastpu buffer	4 μL	10 × buffer	4 μL
2.5 mM dNTPs	2 μL	2.5 mM dNTPs	2 μL
Forward Primer (5 μM)	0.8 μL	Forward Primer (5 μM)	0.8 μL
Reverse Primer (5 μM)	0.8 μL	Reverse Primer (5 μM)	0.8 μL
FastPfu Polymerase	0.4 μL	rTaq Polymerase	0.4 μL
BSA	0.2 μL	BSA	0.2 μL
DNA template	10 ng	Template DNA	10 ng
ddH_2O	Supplementary system to 20 μL	ddH_2O	Supplementary system to 20 μL

TaKaRa rTaq DNA polymerase.The total volume of the PCR system was 20 μL, as shown in Table 1.

2.4 High-throughput sequencing

The NEXTFLEX Rapid DNA-Seq Kit was used for library construction, and Illumina's MiSeq PE300 platform (Illumina, USA) was used for high-throughput sequencing of the purified PCR products, which was performed by Majorbio Inc., Shanghai, China.

2.5 Bioinformatics analyses

The QIIME (Quantitative Insights Into Microbial Ecology, v 1.17) platform was used for species analysis and diversity evaluation of retained sequences. FASTP software was used for quality control of the original sequencing sequence, and FLASH (Fast Length Adjustment of SHort reads) software was used for splicing. Using UPARSE software, nonrepetitive sequences were clustered in operational taxonomic units (OTUs) according to 97% similarity, and OTU representative sequences were obtained. Representative reads were compared with the RDP, SLIVA and Greengenes databases. Alpha diversity was evaluated through Mothur (version 1.30.1) to generate Chao1, ACE, Shannon and Simpson indices. Beta diversity was evaluated with the UniFrac method.

2.6 Statistical analysis

The physicochemical variables, enzyme activities, and alpha-diversity indices were expressed as the mean of three replicates. The statistical analysis was applied to compare significant differences in the data. Spearman's correlation of physicochemical properties and enzyme activities was conducted. Canonical correlation analysis (CCA) was employed to assess the effect of Daqu characteristics on the microbial community. Nonmetric multidimensional scale (NMDS) analysis is a data analysis method that reduces multidimensional research objects to low-dimensional space for positioning and analysis while retaining the original relationship. It was based on the microbial information contained in the sample and was reflected in the multidimensional space in the form of points. The degree of difference between samples can be expressed by the distance between points, and finally, the spatial coordinate map is obtained (Guan et al., 2021).

3 Results and discussion

3.1 The physicochemical property of Daqu

The changes in moisture, starch, pH, RS, FA and EA of Daqu are shown in Figure 2. During the fermentation process, there was no significant difference in moisture between the two types of Daqu, and both showed a gently downward trend (36.42%-11.78% for IMD and 36.48%-11.69% for TMD). The starch content also showed a trend similar to that for moisture. At the end of fermentation, the starch content was approximately 50%. During the fermentation of Daqu, the pH value was less than 7, and the pH change trends of IMD and TMD were roughly the same (Figure 2a). The EA increased significantly with increasing fermentation time, which may have been related to the accumulation of microbial metabolism. FA began to decline after reaching its peak on the third day and tended to be flat, and the FA of the last two types of Daqu was basically the same (Figure 2c). The enzyme activity of Daqu is shown in Figure 2d. SA was the highest at the beginning of fermentation. As fermentation progresses, SA gradually decreases and then starts to rise slowly after the 6th day of fermentation. LA presents a gradual upward trend, and the activity of LA reaches its peak at the end of fermentation.

3.2 Alpha diversity analysis

A total of 19 phyla, 39 classes, 107 orders, 187 families, 395 genera, and 666 species of bacteria were detected from 30 samples. A total of 6 phyla, 19 classes, 42 orders, 84 families, 144 genera, and 253 species of fungi were detected from 30 samples. The sequencing coverage of bacteria and fungi was higher than 99%, indicating that the sequencing can reflect the actual situation of the samples (Table 2). Sobs and Simpson indices were used to evaluate the abundance and diversity of Daqu microbial communities.

A high Sobs index means a large number of microorganisms in the sample, while a small Simpson index means a high diversity of microorganisms. The Sobs index of the two mesophilic Daqu bacteria showed an upward trend, reaching a peak on the 25th day. This means that as the fermentation time increases, the number of bacteria in Daqu increases. The number of species of the two types of Daqu was the highest at 25 days, and the number and diversity of microorganisms in TMD were higher than those in IMD at the end of fermentation.

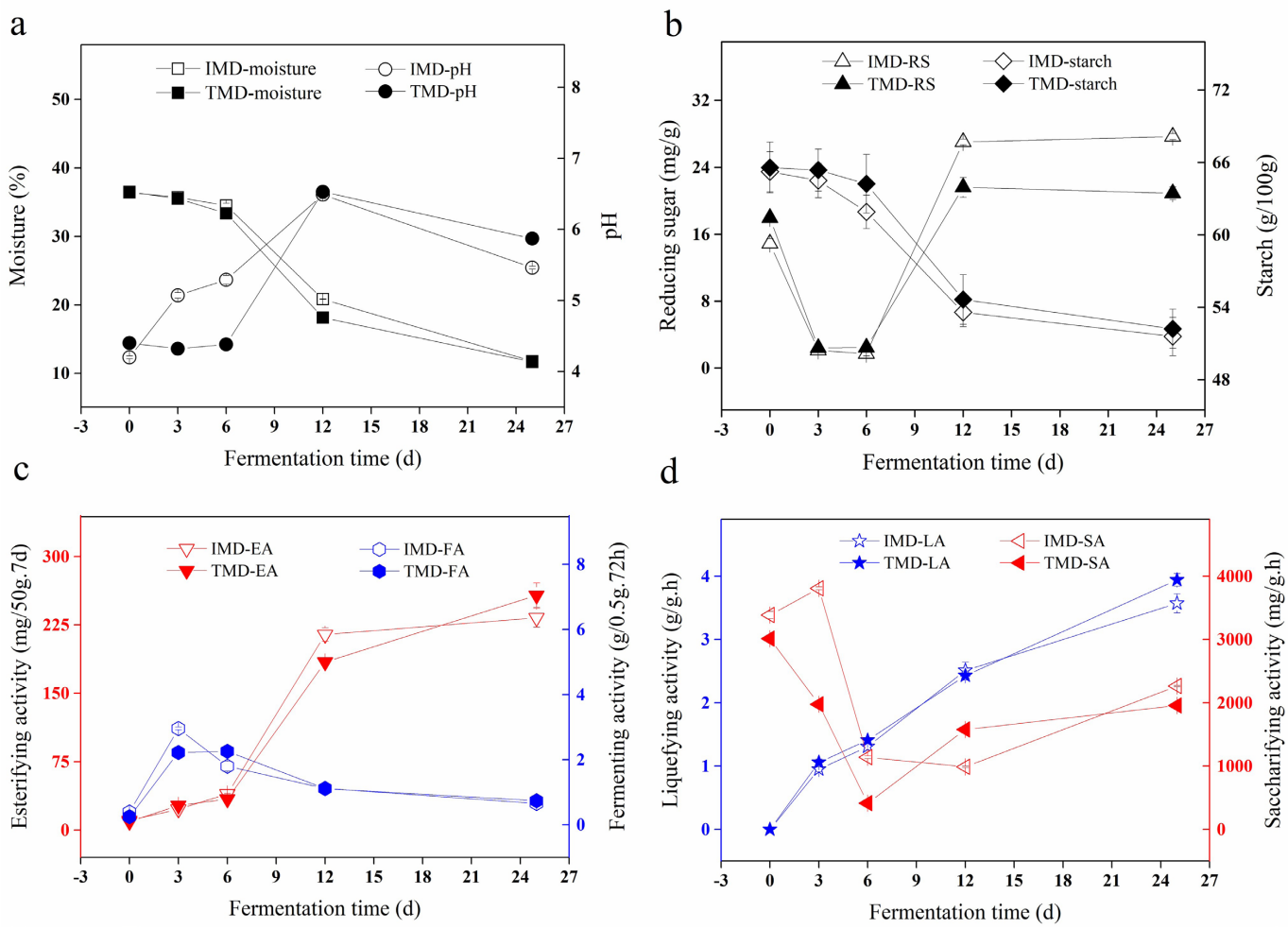

Figure 2. Changes in physicochemical and enzymatic properties during the fermentation process of medium-temperature Daqu. (a) moisture and pH; (b) reducing sugar (RS) and starch; (c) fermenting activity (FA) and esterifying activity (EA); (d) saccharifying activity (SA) and liquefying activity (LA).

Table 2. The α-diversity index in the fermentation process of medium temperature Daqu.

	Bacteria			Fungi		
	Sobs	Simpson	Coverage %	Sobs	Simpson	Coverage %
IMD0	73.67 ± 2.49	0.34 ± 0.04	99.95	112.67 ± 3.09	0.76 ± 0.04	99.95
IMD3	110.33 ± 15.58	0.28 ± 0.05	99.89	33.67 ± 4.19	0.57 ± 0.09	99.98
IMD6	95.33 ± 10.62	0.34 ± 0.01	99.92	29.33 ± 10.34	0.68 ± 0.19	99.98
IMD12	270.33 ± 21.30	0.28 ± 0.06	99.75	10.00 ± 2.45	0.99 ± 0.00	99.99
IMD25	399.67 ± 45.49	0.17 ± 0.04	99.72	103.33 ± 45.80	0.17 ± 0.16	99.98
TMD0	71.33 ± 3.86	0.35 ± 0.03	99.96	112.67 ± 3.40	0.59 ± 0.07	99.93
TMD3	95.67 ± 2.62	0.33 ± 0.05	99.92	38.67 ± 10.40	0.62 ± 0.09	99.99
TMD6	151.67 ± 10.78	0.30 ± 0.08	99.86	71.67 ± 27.01	0.46 ± 0.20	99.96
TMD12	243.67 ± 16.52	0.12 ± 0.02	99.78	11.67 ± 1.28	0.99 ± 0.00	99.99
TMD25	481.67 ± 17.91	0.059 ± 0.01	99.70	73.33 ± 28.17	0.24 ± 0.07	99.97

The Simpson index of the bacteria showed wavy ups and downs, which was the lowest on the 25th day, indicating that the diversity of the two Daqu bacteria was the highest on the 25th day. The diversity and number of bacteria in the fermentation process of IMD and TMD were similar, but the diversity and number of IMD after fermentation were lower than those of TMD. The diversity and quantity change trends of the two Daqu fungi were similar, first declining and then increasing to the level at the beginning of fermentation. After fermentation, the diversity and number of fungi in the digital medium-temperature Daqu were higher than those in the traditional medium-temperature Daqu.

The T-test results of the Shannon and Chao indices of medium-temperature Daqu are shown in Figure 3. There was a significant difference when $p < 0.05$ between two samples with the same fermentation time. The Shannon index and Chao index of the bacteria of IMD and TMD were significantly different on the 25th day, which indicates that the bacterial diversity and number of IMD25 and TMD25 were significantly different. In addition, the diversity of the two Daqu bacteria on the 6th day was similar, but the number of bacteria was significantly different (Figure 3b). Unlike bacteria, the two types of Daqu fungi had significant differences in diversity and number on day 0. On the 3rd, 6, 12th, and 25th days, there was no significant difference in the fungal diversity or number of the two types of Daqu.

3.3 Bacterial and fungal community composition

Bacteria in 30 samples belonged to 19 phyla, of which 7 phyla contained more than 1%, including Firmicutes, Proteobacteria, Actinobacteriota, Cyanobacteria, Bacteroidota, Deinococcota and Bacteria (Figure 4a). Firmicutes, Proteobacteria, and Cyanobacteria were the dominant bacterial phyla at the beginning of fermentation. As fermentation progresses, the contents of Proteobacteria and Cyanobacteria decrease, and the content of Firmicutes increases. Actinobacteria had the highest content in IMD12 and TMD12. Firmicutes was the absolute dominant phylum on the 25th day, and their content was above 90%.

The microorganisms in Daqu come from the air, raw materials and floor of the fermentation room. Daqu can accumulate microorganisms in the fermentation process and screen out microorganisms that are beneficial to fermentation from the environment (Du et al., 2019). In this study, the dominant bacteria of medium-temperature Daqu on days 3-12 of fermentation were *Weissella*, *Lactobacillus*, *Pediococcus*, *Saccharopolyspora*, *Streptomyces*, *UCG-005* and *Staphylococcus* (Figure 4). The two types of Daqu, IMD and TMD, had similarities in the types of dominant microorganisms on the 12th and 25th days, but their relative abundances were quite different. On day 25, the dominant bacteria were *Lactobacillus*, *UCG-005*, *Staphylococcus* and *Blautia*. However, it is different from the results of DU et al (Du et al., 2019). In related studies, the dominant bacteria in Daqu were *Bacillus, Lactobacillus, Lactococcus, Enterobacteriales* and *Lactobacillales*. The dominant fungal genera are *Candia, Trichoderma, Aspergillus* and *Thermomyces* (Gan et al., 2019; Jin et al., 2019; Li et al., 2017a; Wang et al., 2017). The dominant bacterial genera of different Daqu are slightly different, which

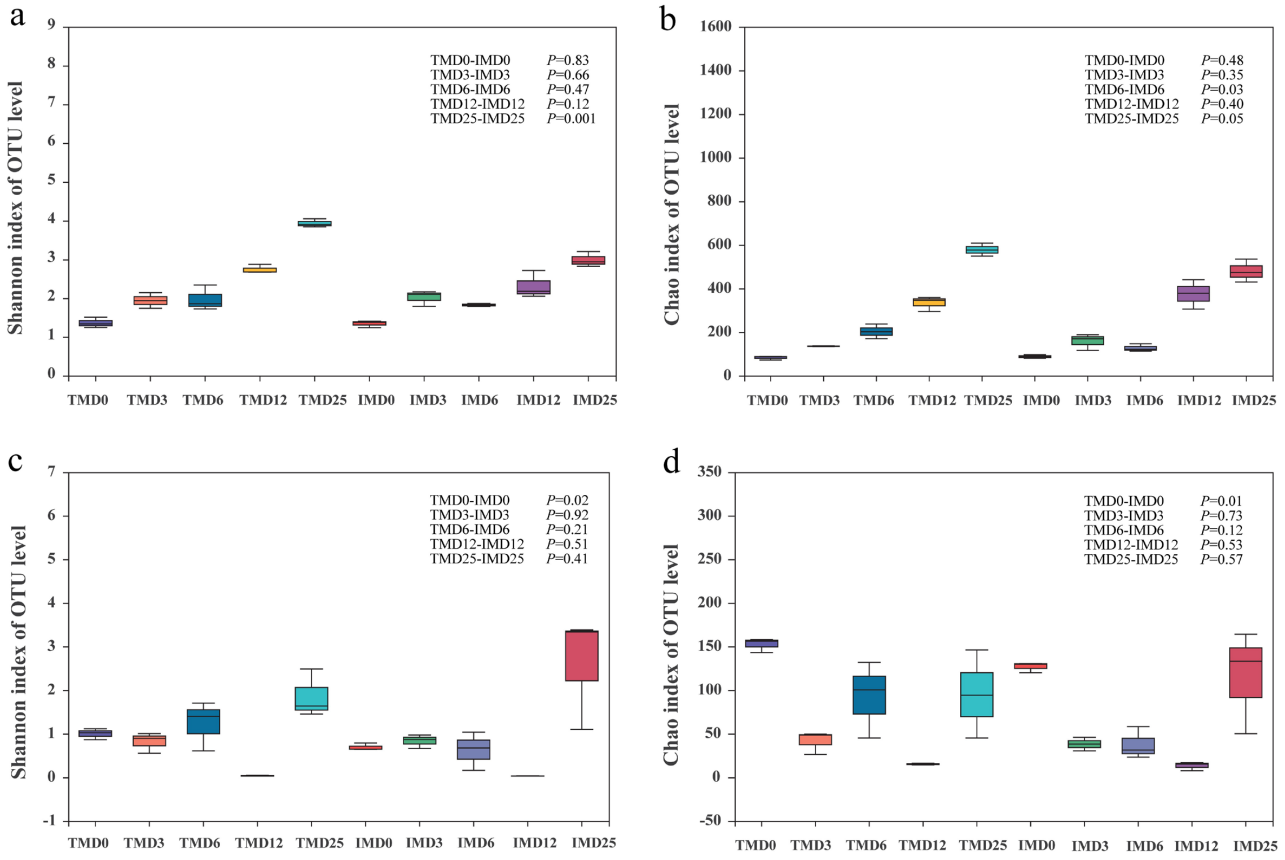

Figure 3. Significant difference test of an α-diversity index between digital Daqu and traditional Daqu. (a) Shannon index difference test of bacteria; (b) Chao index difference test of bacteria; (c) Shannon index difference test of fungi; (d) Chao index difference test of fungi.

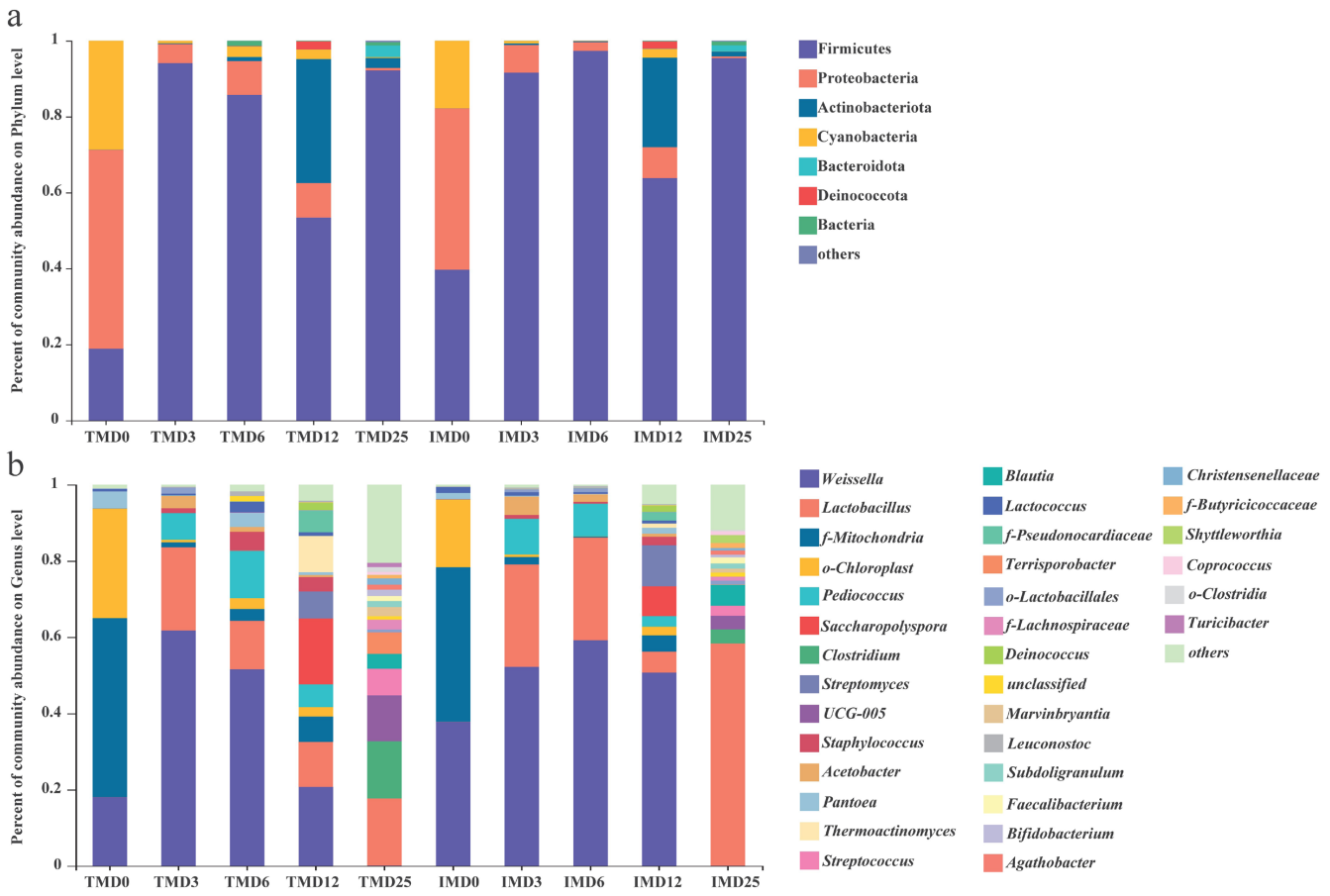

Figure 4. The relative abundance of bacterial communities at the phylum (a) and genus (b) levels.

may be related to the difference in the geographical environment and the use of raw materials (Zheng et al., 2015). Daqu produced in different regions can form a unique microbial community structure. Bacillus is the dominant bacteria in many Daqu (Gan et al., 2019; Su et al., 2015; Hu et al., 2017), but in this experiment, the content of Bacillus was very low, and it did not occupy a dominant position in the whole fermentation process. Both IMD and TMD formed a bacterial community distribution dominated by *Weissella, Lactobacillus, Pediococcus,* and *Saccharopolyspora*. *Weissella* and *Lactobacillus* are rich in Maotai-flavored and strong-flavored Daqu (Huang et al., 2017), and these bacteria have been reported in the early fermentation of medium-temperature Daqu and high-temperature Daqu (Zhang et al., 2005; Wang et al., 2011). *Lactobacillus* can convert glucose into lactic acid and provide a reactant for yeast to form ethyl lactate, and ethyl lactate is also one of the critical flavor components in Baijiu (Wang et al., 2011). *Lactobacillus* is ubiquitous in grains (Toju et al., 2012), and the content is gradually enriched as fermentation progresses. *Lactobacillus* may also affect the growth of yeast and filamentous fungi and could prevent rapid ethanol fermentation in the initial phase of the brewing process (Shoubao et al., 2019). Tao et al. (2014) found that lactic acid is a critical factor affecting community structure. In addition, a certain level of lactic acid can produce desirable flavor compounds in the Baijiu, making lactic acid bacteria critical microorganisms for Chinese Baijiu brewing (Shoubao et al., 2019). Studies have also shown that *Bacillus* has an inhibitory effect on *Lactobacillus* (Wang et al., 2017), which may also be the reason why *Lactobacillus* was the dominant genus in this study, but the content of *Bacillus* was extremely low. *Saccharopolyspora, Pantoea, Staphylococcus, Thermoactinomyces, Pediococcus, Acetobacter* and other bacterial genera detected in this experiment have also appeared in other reports (Fan et al., 2019; Su et al., 2015; Mercer et al., 2017). *Saccharopolyspora* is rich in wheat (Guan et al., 2012), which can produce heat-resistant alpha-amylase, which hydrolyzes starch to produce glucose and maltose during the fermentation process (Chakraborty et al., 2011).

In this experiment, the ITS gene was used to sequence the fungal community because many fungi are conserved in the 18S rRNA region (Li et al., 2011), and the ITS region is more variable (Martin & Rygiewicz, 2005). The dominant genus of the fungal community in the fermentation process is shown in Figure 5b. The number of fungal species is much lower than that of bacteria. A total of 4 phyla were detected from 30 samples with a content of more than 1%. Among them, Ascomycota was the absolute dominant phylum. The composition of fungi at the phylum level

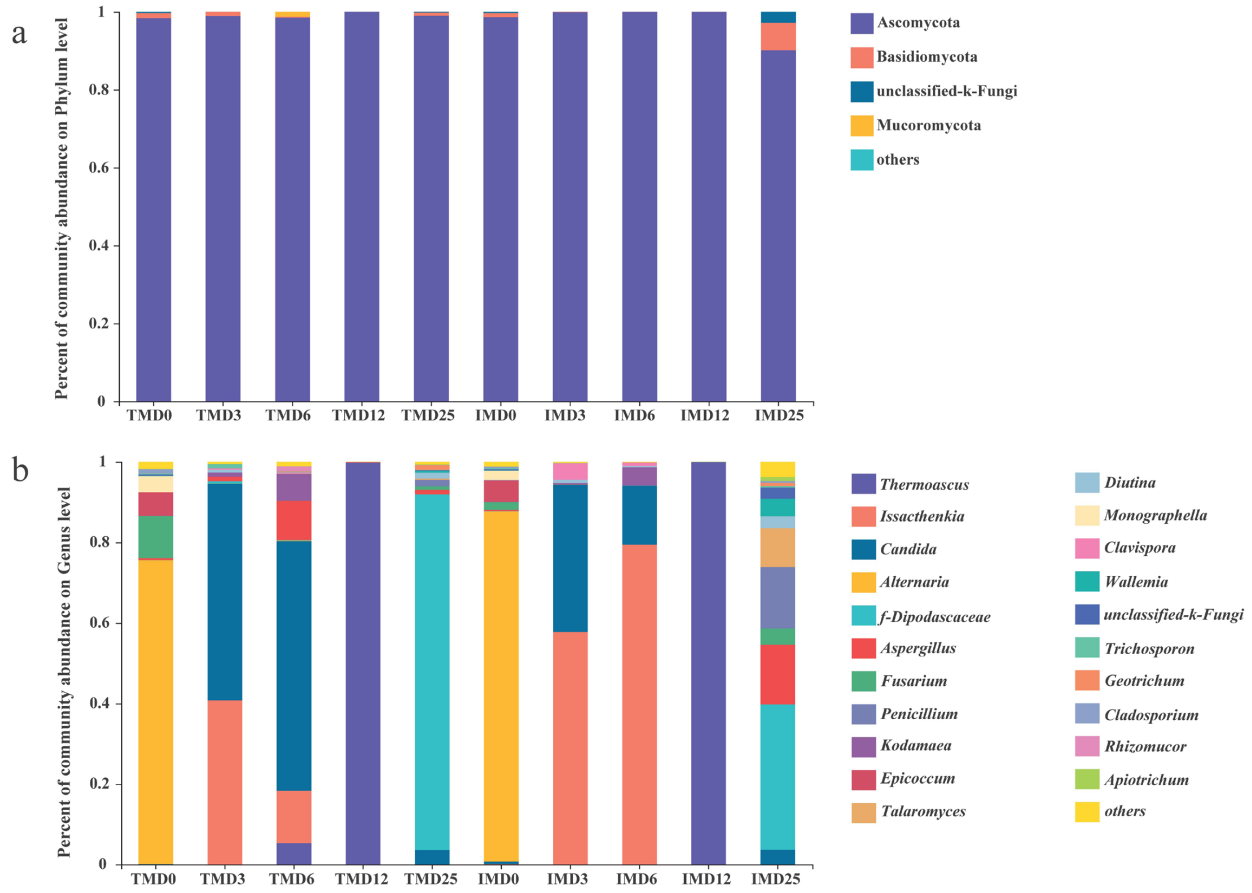

Figure 5. Relative abundance of fungal communities at phylum (a) and genus (b) levels.

hardly changed, and the content of Ascomycota in the whole fermentation process was above 90%. There were significant differences in the composition of the fungal genera over time. The highest content of IMD0 and TMD0 was *Alternaria*, but it was not detected in subsequent samples. The content of *Alternaria* in IMD0 and TMD0 was 86.96% and 75.47%, respectively. *Alternaria* exists in large quantities in the environment and in the air and can grow rapidly in grain. As fermentation progressed, *Alternaria* was not detected in subsequent samples. This result is consistent with the views in other reports (Du et al., 2019), which shows that the production of Daqu is a process of natural selection that is beneficial to the microorganisms of Baijiu brewing, and most of the microorganisms that are useless for fermentation are eliminated during the fermentation process.

IMD3 and IMD6 formed a community structure dominated by *Thermoascus* and *Issatchenkia*, but the content of *Issatchenkia* was higher than that of *Thermoascus*. The microbial composition of TMD3 and TMD6 is opposite to that of IMD3 and IMD6. IMD12 and TMD12 have a single microbial composition. Both types of Daqu form a structure with *Thermoascus* as the absolute dominant microbe, and its content reaches more than 99%. On the 12th day of fermentation, *Thermoascus* was an absolute dominant genus, with a content of more than 90%, and the temperature of Daqu was approximately 60 °C. Yeast has poor heat resistance and cannot grow above 50 °C (Hu et al., 2017). This may be the reason why thermophilic microorganisms dominate on the 12th day of fermentation.There was a difference in composition between IMD and TMD on the 25th day of fermentation. Dipodascaceae, which are unclassified at the genus level, is as high as 88.35% in TMD25 and only 36.11% in IMD25. The contents of *Aspergillus*, *Fusarium*, *Penicillium*, *Talaromyces*, and other microorganisms in IMD25 are all higher than those in TMD25. *Candida* and *Issatchenkia* dominate the abundance in the early stage of Daqu fermentation (3-6 days), but in some reports on Daqu, *Candida* and *Issatchenkia* are not the dominant bacteria (Gan et al., 2019; Jin et al., 2019; Li et al., 2017a; Wang et al., 2017), which may be due to the different types of Daqu. *Candida* and *Lactobacillus* can increase the content of esters in wine under the synergistic effect (He et al., 2019), and there is a positive correlation between the two and most of the esters in fermentation (Gan et al., 2019).

3.4 Bacterial and fungi beta-diversity

To understand the similarity of the microbial community structure, NMDS analysis was carried out based on the genus

level, and the results are shown in Figure 6. The results of bacterial community analysis are shown in Figure 6a, and the stress value was 0.062. Analysis of similarity (ANOSIM) further calculated the dispersion of bacterial communities (R = 0.8080, P = 0.001 < 0.05), and the two types of Daqu had a good correlation. The compositions of IMD0 and TMD0 are similar, and the distributions of IMD3, IMD6, TMD3 and TMD6 are relatively close. The distance between samples TMD12 and IMD12 was relatively long, indicating that the two have obvious differences in bacterial structure and composition. The bacterial composition of the samples on the 25th day was also quite different. The bacterial community composition of TMD and IMD is constantly changing during the fermentation process.

The NMDS analysis of the fungal data showed a stress value of 0.117 (Figure 6b). The change in fungal community structure was similar to that of bacteria, and the fungal community composition could also be divided into four parts. Including initial fermentation (day 0), early fermentation (day 3 and day 6), middle fermentation (day 12) and late fermentation (day 25). The similarity of the two types of Daqu (IMD and TMD) in fungal community structure was higher than that of bacteria. The results of ANOSIM (R = 0.8407, P = 0.001 < 0.05) showed that Daqu with the same fermentation time had a high similarity in microbial composition.

The results of hierarchical clustering analysis (HCA) can also explain the clustering of microorganisms. Figure 6c shows that Daqu on day 0 and day 25 had a significant difference in bacterial composition. The early and middle stages of fermentation (day 3, day 6, day 12) can be grouped, but IMD12 and TMD12 are far apart. There was a significant difference in fungal composition between IMD and TM during the fermentation process. As shown in Figure 6d, samples from the same fermentation days can be grouped into one category. The microbial community changes significantly at the beginning of fermentation and at the end of fermentation. The initial stage of fermentation (day 0), the early stage (day 3, day 6), the late stage of fermentation (day 12) and the final product can be roughly divided into 4 categories. This is similar to the result of NMDS analysis and can also corroborate the clustering of microbial composition to a certain extent.

3.5 Relationships among physicochemical properties, enzyme activities, and microbial communities

Canonical correlation analysis (CCA) based on the physicochemical properties, enzyme activity and microbial data

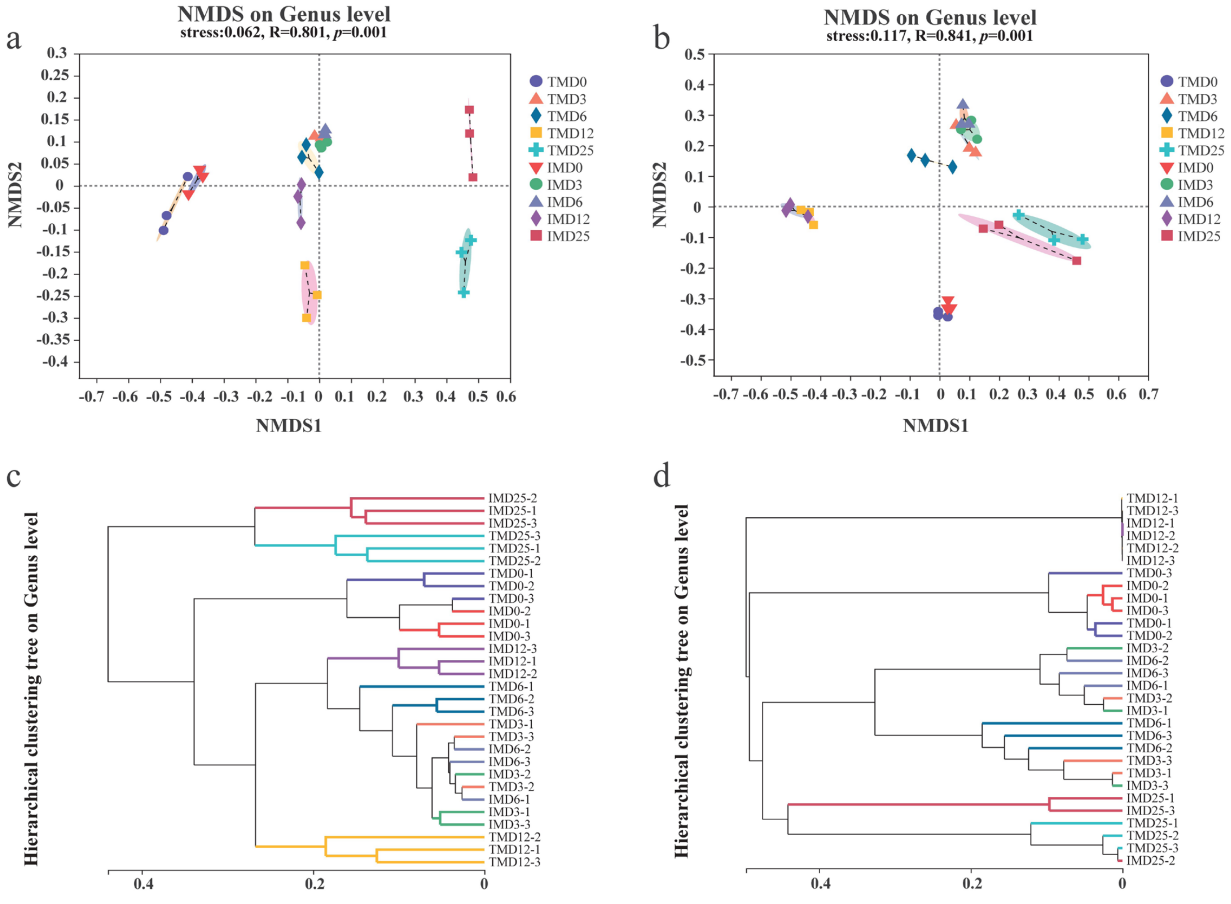

Figure 6. Non-metric multidimensional scale analysis analysis of bacteria (a) and fungi (b) communities at the genus level; hierarchical clustering analysis of bacteria (c) and fungi (d) communities at the genus level.

is shown in Figure 2. The analysis results are shown in Figure 7. Temperature had the greatest influence on the bacterial community. The microbial composition of Daqu on the 3rd, 6th and 12th days of fermentation was positively correlated with temperature and FA. Daqu on the 25th day of fermentation was negatively correlated with temperature (TE) and FA. The factor that has the greatest influence on the distribution of fungal communities is temperature. Changes in microbial community composition are related to environmental factors. Relevant studies have shown that temperature has a paramount impact on the diversity and abundance of Daqu microorganisms (Zheng et al., 2015). Tang et al. (2019) believe that environmental variables are the driving force promoting microbial changes in the fermentation process of Daqu (Tang et al., 2019). The CCA results show that the bacterial and fungal communities were most strongly affected by temperature and that the temperature changed extremely during the fermentation process, which can explain why the diversity and abundance of Daqu microorganisms changed significantly during the whole fermentation process (Figure 7). The temperature of Daqu first increased and then decreased, which may have been due to the rapid growth of microorganisms under suitable conditions, and the bioheat generated by Daqu increased the temperature of Daqu (Xiao et al., 2017). The increase in temperature inhibits the growth of most heat-labile microorganisms. Heat-resistant microorganisms, including *Weissella*, *Thermoactinomyces* and *Thermoascus,* can continue to grow.

FA was positively correlated with the samples on the 3rd and 6th days of fermentation.

The starch and water contents in Daqu showed gradually decreasing trends, and the starch was continuously consumed with the growth of microorganisms. The starch content in Daqu on the 25th day of fermentation was approximately 50 g/100 g, and the utilization rate of starch was approximately 20%. The remaining starch in Daqu would continue to be used as a raw material in the subsequent fermentation of Baijiu. Reducing sugars declined in the early stage, which may have been due to the consumption of reducing sugars by the reproduction of microorganisms. With the reproduction of microorganisms and the accumulation of metabolites, the FA of Daqu is constantly changing. Experimental results show that FA is positively correlated with *Candida*, *Issatchenkia* and *Kodamaea* (Figure 7). The FA of Daqu showed a trend of increasing first and then decreasing, and it was the highest on the 3rd and 6th days of fermentation (Figure 2). The content of yeast is higher when the fermentation temperature is lower, and a large amount of yeast is the reason for the increase in FA. The fermentation temperature rose to approximately 60°C. Too high a temperature inhibited the growth of yeast, and FA decreased. *Saccharopolyspora, Staphylococcus* and *Streptomyces* were also positively correlated with FA. Theoretically, the content of *Lactobacillus* increases, and the accumulation of lactic acid produced by metabolism should lower the pH, but the experimental results show that *Lactobacillus* is positively correlated with pH (Figure 7). This shows that a single microorganism cannot determine the physicochemical properties of Daqu but is determined by multiple microorganisms. *Lactobacillus* can metabolize esterase to promote the production of ethyl lactate from the substrate, which means that the metabolism of *Lactobacillus* can increase the EA of Daqu, which is the same as the result of CCA. The activity of the liquefaction enzyme increases as fermentation progresses. This is because the mold content in the sample at the beginning of the fermentation is extremely low, and there is no liquefaction enzyme for microbial metabolism. The contents of *Penicillium, Rhizomucor* and *Aspergillus* in the two types of Daqu were more than 1%. These microorganisms have good lignocellulose hydrolysis ability. SA is the highest in the early stage of fermentation, which may come from the raw material itself. This part of the saccharification enzyme is consumed or inactivated during the fermentation process, so there is a downward trend. The change in microbial community structure and microbial metabolism caused the activities of various enzymes in Daqu to constantly change (Figure 2).

The correlation between the Daqu microorganisms and environmental factors was calculated based on the Spearman index (Table 3). Among the more abundant fungi, only *Thermoascus* had a significant positive correlation with temperature, and most of the others showed a significant negative correlation,

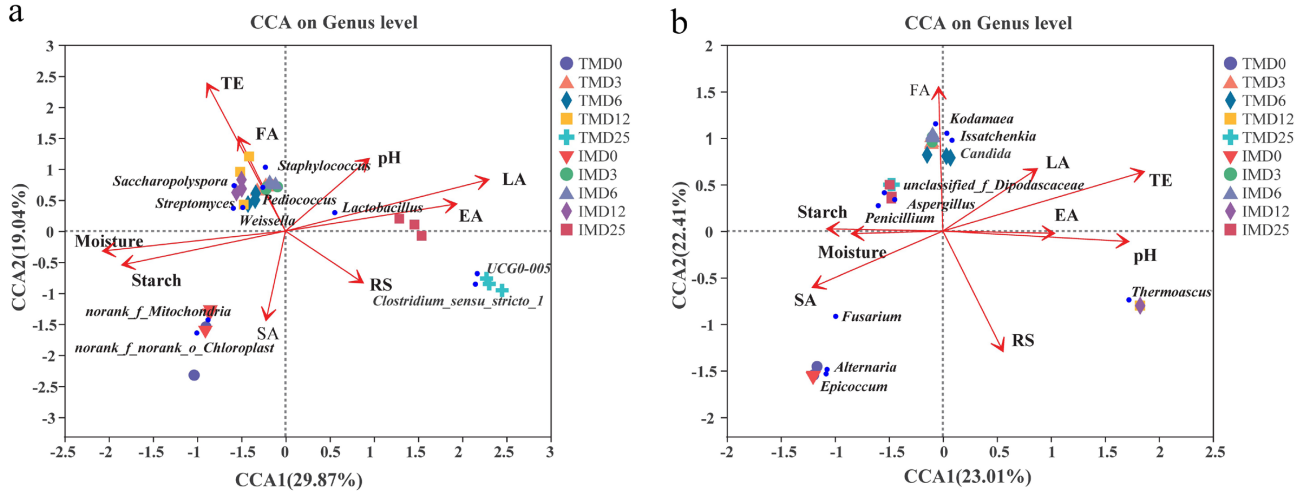

Figure 7. CCA analysis based on bacteria (a) and fungi (b) communities of two Daqu during the fermentation process (genus taxonomy).

Table 3. Correlation of dominant genera with physicochemical properties during fermentation process.

	TE	Moisture	pH	RS	Starch	EA	FA	LA	SA
Weissella	0.383[a]	0.412[a]	-0.385[a]	-0.718[c]	0.473[b]	-0.355	0.765[c]	-0.387[a]	-0.257
Lactobacillus	0.163	-0.402[a]	0.174	-0.190	-0.389[a]	0.426[a]	0.377[a]	0.378[a]	0.017
Pediococcus	0.596[c]	0.261	-0.189	-0.663[c]	0.362[a]	-0.302	0.884[c]	-0.284	-0.330
Saccharopolyspora	0.901[c]	-0.135	0.417[a]	-0.038	-0.088	0.107	0.548[b]	0.132	-0.624[c]
Clostridium_sensu_stricto_1	-0.061	-0.788[c]	0.596[c]	0.629[c]	-0.746[c]	0.763[c]	-0.565[b]	0.765[c]	-0.073
Streptomyces	0.852[c]	-0.185	0.455[a]	0.056	-0.176	0.178	0.451[a]	0.198	-0.641[c]
UCG-005	-0.269	-0.713[c]	0.420[a]	0.556[b]	-0.721[c]	0.735[c]	-0.616[c]	0.737[c]	-0.008
Staphylococcus	0.812[c]	0.044	0.062	-0.195	0.180	-0.112	0.639[c]	-0.061	-0.538[b]
Acetobacter	0.515[b]	0.326	-0.182	-0.631[c]	0.415[a]	-0.311	0.862[c]	-0.327	-0.097
Pantoea	0.083	0.546[b]	-0.178	0.012	0.468[b]	-0.563[b]	0.041	-0.531[b]	0.006
Thermoascus	0.446[a]	-0.143	0.350	0.483[b]	-0.173	0.100	-0.156	0.159	-0.520[b]
Issatchenkia	0.302	0.432[a]	-0.445[a]	-0.836[c]	0.458[a]	-0.415[a]	0.883[c]	-0.465[b]	-0.013
Candida	-0.048	0.256	-0.591[c]	-0.723[c]	0.368[a]	-0.279	0.557[b]	-0.261	0.045
Alternaria	-0.500[b]	0.723[c]	-0.765[c]	-0.266	0.663[c]	-0.759[c]	-0.038	-0.721[c]	0.394[a]
f__Dipodascaceae	-0.624[c]	-0.189	-0.288	0.150	-0.109	0.142	-0.494[b]	0.172	0.348
Aspergillus	-0.360	0.051	-0.415[a]	0.158	0.172	-0.145	-0.344	-0.068	0.123
Fusarium	-0.773[c]	0.092	-0.245	0.371[a]	-0.001	-0.122	-0.742[c]	-0.081	0.378[a]
Penicillium	-0.725[c]	-0.154	-0.154	0.378[a]	-0.218	0.140	-0.741[c]	0.151	0.317
Kodamaea	0.094	0.520[b]	-0.668[c]	-0.852[c]	0.582[c]	-0.520[b]	0.730[c]	-0.524[b]	-0.026
Epicoccum	-0.541[b]	0.727[c]	-0.715[c]	-0.209	0.712[c]	-0.769[c]	-0.172	-0.729[c]	0.400[a]

[a]Significant at the level of 0.05. [b]Significant at the level of 0.01. [c]Significant at the level of 0.001.

which indicates that the increase in temperature inhibited the fungi in Daqu. EA has a significant positive correlation with *Lactobacillus*, *Clostridium_sensu_stricto_1*, and *UCG-005* and a significant negative correlation with *Issatchenkia*, *Kodamaea*, and *Epicoccum*. LA had a significant negative correlation with most of the bacterial genera and a significant negative correlation with the fungal genera. LA and SA play a critical role in the process of Baijiu brewing; they can help the rapid decomposition of raw Baijiu fermentation materials, and LA and SA are also important indicators for evaluating the quality of Daqu. The correlation analysis result is similar to the CCA analysis result, which can further prove the relationship between the physicochemical properties and enzyme activity and the microbial community. However, the interaction mechanism between these factors needs further research.

3.6 A comparative analysis of two daqus

The microbial abundance of the two types of Daqu at the same fermentation time was slightly different, which may have been caused by the difference in the temperature and humidity of the fermentation (Figure 1). The fermentation temperature of traditional Daqu is determined by microbial metabolism and heat production and the environment. Environmental factors in different seasons have a critical impact on the production of Daqu. The Daqu used in the experiment is the best batch of Daqu fermented in a year, and the quality of the digital medium-temperature Daqu is not lower than that of the traditional medium-temperature Daqu. The Daqu used in the experiment is the best batch of Daqu fermented in a year, and the quality of the digital medium-temperature Daqu is not lower than that of the traditional medium-temperature Daqu. The digital medium-temperature Daqu is managed by the computer to control the fermentation humidity of Daqu, and the fermentation is controlled according to the external environment and set values, which can better reproduce the best production process parameters and make the quality of the produced Daqu more stable.

In summary, the microbial community of Daqu is constantly changing during the fermentation process. During the fermentation process of Daqu, the Shannon index showed significant differences among some samples (Figure 3). However, the distribution ratios and types of microbial communities (Figure 4; Figure 5) of the two kinds of Daqu had high similarities (Figure 6). The uniformity of the bacterial community of TMD was higher than that of IMD on the 25th day, but the fungal community was the opposite. From the experimental results, there was no significant difference in the microbial composition of IMD and TMD at the same time point. NMDS analysis and HCA (Figure 6) can prove this conclusion. The two kinds of Daqu have a good correlation. The experimental results show that there are some differences in the microbial community structure of the two types of Daqu, but there is no significant difference between the physicochemical properties and enzyme activity. The quality of digital medium-temperature Daqu is not lower than that of traditional Daqu. The application of a digital management system to control the fermentation of Daqu can reduce errors resulting from operator experience and provide more consistent Daqu quality.

4 Conclusion

This article aims to explore the feasibility of applying a digital management system to Daqu production. The dominant microorganisms in the early stage of fermentation were *Weissella*, *Lactobacillus*, *Pediococcus*, *Saccharopolyspora*, *Candida* and

Issatchenkia. At the end of Daqu fermentation, the dominant microorganisms were *Lactobacillus*, *UCG-005*, *Streptococcus*, *Blautia*, *Unclassified_Dipodascaceae*, *Aspergillus*, *Penicillium*, *Talaromyces*, etc. The microbial communities in IMD and TMD were similar. There was no significant difference in microbial diversity or abundance at either time point. This research applied a digital management system to the fermentation process of Daqu, and the results show that it is feasible to apply this digital management system to the fermentation process of Daqu. This research can provide a theoretical basis for the industrial production of Daqu.

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