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The study of microbial diversity and volatile compounds in Tartary buckwheat sourdoughs

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ABSTRACT

Microorganisms play an essential role in forming volatile compounds in traditional staple products. Tartary buckwheat, as a medicinal and food material, has high nutritional value but its development and utilization are seriously restricted due to its poor flavor. In this study, 16S rRNA and ITS rRNA sequencing were used to analyze the microbial diversity of Tartary buckwheat sourdoughs, while HS-SPME-GC/MS was used to identify volatile compounds during fermentation. The results showed that Lactococcus and Weissella were the dominant bacterial genus. Wickerhamomyces, Penicillium, and Aspergillus were the main fungal genera in the Tartary buckwheat sourdoughs. And the main volatile compounds in Tartary buckwheat sourdough were pyrazine compounds. After 12 h of fermentation, a large amount of alcohol and esters were produced, which endowed the sourdough with a good flavor. This suggests that sourdough fermentation could significantly improve the flavor of Tartary buckwheat.

1. Introduction

For centuries, starter cultures have been used to prepare the traditional staple food Chinese mantou (also known as steamed bread). Starter cultures endow steamed bread and other foods with a good flavor, improving the product's taste and nutritional value (Teleky, Martau, Ranga, Chetan, & Vodnar, 2020; Teleky, Martau, & Vodnar, 2020). It also can prolong the product shelf-life (Siepmann, Ripari, Waszczynskyj, & Spier, 2017). In recent years, an increasing number of researchers have focused on the microbiota of traditional starters. The diversity of microbes is considered one of the key factors influencing the final flavor of the products (Ripari, Cecchi, & Berardi, 2016). For example, Kristel Kaseleht et al., (Kaseleht, Paalme, Mihhalevski, & Sarand, 2011) compared the volatile compounds of rye sourdough made by 9 kinds of lactic acid bacteria (LAB), and the flavor substances were different due to the differences in the strains. In sourdough starters, the individual metabolic activities of LAB and yeast, or the specific interactions between strains, produce complex flavor compounds. The metabolic activities of microorganisms in sourdoughs have an important influence on the fermentation quality of the dough. These fermentation activities will generate a large number of volatile compounds and aroma precursors (Ganzle & Zheng, 2019). The diversity of microbial species leads to differences in metabolites in sourdough, which also endow the unique fermented flavor of sourdoughs (Liu et al., 2018).

A great number of microorganisms in traditional starters from all over China have been identified and separated. Combining both culture-dependent and culture-independent methods can provide a more comprehensive overview of the microbial communities of fermented dough. High-throughput sequencing has become an essential method for the investigation of microbial ecosystems. Xiaolong Xing et al. clarified the bacterial diversity in sourdough from three terrain conditions (mountain, plain, and basin) in Henan and first reported the presence of Gluconobacter oxydans (Xing, Ma, Fu, Zhao, Ai, & Suo, 2020). Biao Suo et al. (Suo, Nie, Wang, Ma, Xing, Huang, et al., 2020) compared the microbial composition of wheat sourdough in Henan and found that the abundance of microorganisms was quite different. Bowen Yan et al. investigated the microbial diversity of Jiaozi and type I sourdoughs by culture-dependent and culture-independent analysis (Bowen Yan et al., 2019). The results showed that there were differences in both microbial diversity Therefore, different regions, topography, and processing methods will affect the microbial diversity of sourdoughs.

Buckwheat is a widely consumed pseudo-cereal in Asian countries such as China, Japan, and Korea (Yoshioka, Ohmoto, Urisu, Mine, & Adachi, 2004). In recent years, the nutritional benefits of buckwheat

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have been rediscovered and people have renewed interest in the utilization of buckwheat (Wu, Gan, Dai, Cai, Corke, & Zhu, 2016). The unique chemical composition of Tartary buckwheat can be used as a food that to prevent and heal diverse diseases efficiently (Zhu, 2016). However, Tartary buckwheat has poor flavor and processing quality, which affects the development of Tartary buckwheat-related products. Nevertheless, Tartary buckwheat has a higher starch content and it is especially rich in amylopectin, which is an ideal raw material for fermentation (Nakamura, Naramoto, & Koyama, 2013). Xiao Yu Zhang et al. fermented Tartary buckwheat with Aspergillus niger CJ-1. The results showed that the antioxidant activity in Tartary buckwheat was increased (Zhang et al., 2017). Matjaž Deželak et al., (Deželak, Zarnkow, Becker, & Košir, 2014) compared gluten-free beer with buckwheat and quinoa as raw materials, evaluated their sensory characteristics, and found that the beer had good general acceptance despite its unique flavor. Blaise P. Nic Phiarais et al. (2010) developed a gluten-free beer by fermenting buckwheat malt. By altering the process conditions, the flavor and other properties can be generally improved. Therefore, fermentation can reduce the anti-nutritional factors in Tartary buckwheat, improve the nutritional content and sensory quality, and further improve the biological activity of Tartary buckwheat (Nakamura et al., 2013). So far, several studies have been conducted on the microbial diversity and characteristic flavors of various grain fermentation products (Galoburda, Straumite, Sabovics, & Kruma, 2020). In addition, fermentation has become a common method of Tartary buckwheat processing; however, there were few reports on the microbial diversity and related fermentation flavor of Tartary buckwheat sourdoughs.

To date, numerous studies have been carried out on the microbial composition in sourdoughs from many countries and regions. However, there are few studies on the microbial diversity in Tartary buckwheat sourdoughs. In this study, the microbial diversity of Tartary buckwheat sourdoughs was preliminarily investigated by high-throughput sequencing. Additionally, the volatile compounds in Tartary buckwheat sourdoughs were identified by GC–MS. This study aimed to provide a reference for the fermentation process of Tartary buckwheat.

2. Materials and methods

2.1. Tartary buckwheat sourdough preparation

Refer to the processing technology of Laomian, mix the Tartary buckwheat flour and purified water according to the mass ratio of 1:1.5. The fermentation temperature was set at 30 °C and fermented for 24 h (Corsetti, 2013). Tartary buckwheat was purchased from Yanmen Qinggao (Heifeng I), Shanxi. Purified water was drinking water that has been filtered.

2.2. pH and total titratable acidity (TTA) of fermented Tartary buckwheat sourdoughs

The pH and total titratable acidity (TTA) values of all the samples were measured as previously described (Liu et al., 2016). The pH value was determined using a pH meter (FE20, Mettler-Toledo, Mettler Toledo Instruments (Shanghai) Co., Ltd., China). The TTA value was determined by a titration method and was expressed by consuming a volume (mL) of 0.1 M NaOH standard solution when the sample's pH reached 8.6.

2.3. Microbiological analysis and counting

At 4-hours intervals during fermentation, 10 g of the samples were suspended in 90 mL of sterile 0.9% NaCl saline solution and homogenized for 2 min in a vortex oscillator. Then the suspension was diluted 10-fold in sterile saline solution. The suitable dilutions were plated on the plate count agar. For counting lactobacilli, the samples were plated on de Man, Rogosa and Sharp (MRS) agar and incubated at 37 ℃ for 48 h in an anaerobic box. At the same time, the samples were plated on potato dextrose agar (PDA) and incubated at 28 °C for 5 d to count the total yeast/mold amount (Bowen Yan et al., 2019). All agars were purchased from Hopebio Technology Co. Ltd, Qingdao, China.

2.4. Illumina sequencing

During fermentation, removed 30 g of sourdough, placed it in a 50 mL sterilized centrifuge tube, and placed it in the refrigerator at −30 °C at 4-hour intervals. Then 4 h-24 h fermented doughs were collected and then extracted DNA. The bacterial primer sets 338-F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806-R (5′-GGACTACHVGGGTWTCTAAT-3′) with specific barcodes were used to amplify the V3-V4 region of bacterial 16S rRNA genes (Wang et al., 2019). The fungal primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) with specific barcodes were used to amplify the ITS1 region of fungal ITS rRNA genes (J. Chen, Yan, Hu, Zhang, & Hu, 2019). The TruSeq ® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, USA) was used to generate the sequencing libraries of bacterial 16S rRNA genes and fungal ITS rRNA genes for high-throughput sequencing. Both libraries were then sequenced on an Illumina HiSep2500 platform (USA) by Majorbio (Shanghai, China).

2.5. Bioinformatic analysis

Flash software (Version 1.2.11) was used to merge the raw sequencing reads obtained from the Illumina platform and filtered with Quantitative Insights Into Microbial Ecology (QIIME) software (Version 1.9.1). Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off by using UPARSE (Version 7.0.1090) (Suo, et al., 2020). Bacteria used Silver (SSU 138) which is based on the RDP Classifier algorithm to annotate OTU sequences. For each representative fungal sequence, the Unite Database was used based on a BLAST algorithm calculated by QIIME software to annotate taxonomic information. The OTU abundance information was normalized by using the standard serial number corresponding to the sample with the least sequence. Subsequently, the Mothur (Version 1.30.2) and the other software were combined with relevant statistical methods to analyze the difference between the samples.

2.6. Volatile compound analysis using SPME-GC/MS

The volatile compounds were determined in Tartary buckwheat sourdoughs by using GC/MS coupled with solid-phase microextraction (SPME-GC/MS) methods. The SPME device (Supelco, Bellefonte, PA, USA) was equipped with a 75-mm divinylbenzene/ carboxen/ polydimethylsiloxane (DVS/ CAR/ PDMS fiber). 3 g of each sample were transferred to and sealed in a 20 mL SPME vial containing 20 μLof internal standard (2-octanol, 14 mg/kg). After the volatile compounds were absorbed by the fiber during a 40 min extraction at 60 ℃ (Fan et al., 2018). Chromatographic separation was achieved using an SH-Rtx-Wax analytical fused silica capillary column (30 m × 0.25 mm × 0.25 μm, Restek Corporation, Benner Circle, Bellefonte, PA, USA), and the column temperature was programmed as follows: the initial temperature was 40 ℃ for 1 min, increased to 160 ℃ at 6 ℃/min, then increased to 250 ℃ at the rate of 3 ℃/min, and then at 250 ℃ for 5 min. The mass spectrometer (MS) was operated in electron impact mode with the electron energy set at −70 eV using ion source temperatures at 250 ℃. The full-scan mode was used to detect all the compounds in the range of 33–495 *m/z*.

The acquired chromatographic and spectroscopic data were processed using the Shimadzu software (v. 4.45, GC/MS Solution, Shimadzu, Tokyo, Japan), and the volatile compounds were identified based on their retention times and mass spectra (comparison with the spectra available in the NIST14.L library) and match the quality of above 80% was needed for identification. The contents of the volatile

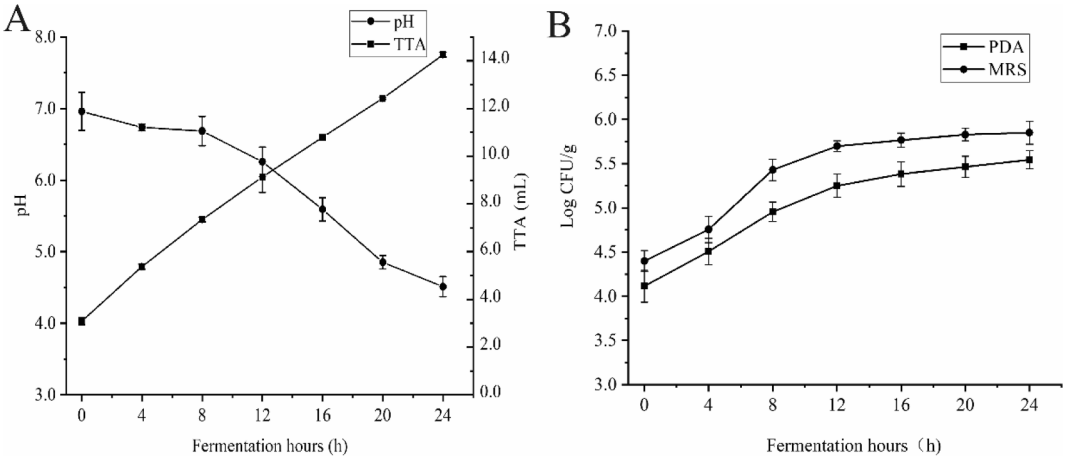

Fig. 1. The pH, TTA, and the microbial profile of Tartary Buckwheat sourdoughs.

compounds were expressed as relative peak areas (peak area of each compound/total area) × 100.

2.7. Statistical analysis

All the tests were performed in triplicate, and the figures were drawn using Origin (Version 2018). Values were expressed as mean values standard deviations. The results were subjected to a one-way ANOVA analysis and significant differences among values were calculated based on Duncan's multiple range test by SPPS (Version 24.0, SPSS Inc, Chicago, IL, USA) ($p < 0.05$).

3. Results and discussion

3.1. The pH and TTA of Tartary buckwheat sourdoughs

The changes in pH value represented the amount of strong acid produced by microbial metabolism during the fermentation of sourdoughs, while TTA represented the changes in the total acidity of the sourdoughs. The results were shown in Fig. 1A. During the fermentation process, the pH value steadily decreased, and the sourdough pH decreased to 4.510 after 24 h of fermentation. After 16 h of fermentation, the pH value of Tartary buckwheat sourdoughs was similar to that of sourdoughs reported previously (3.75–6.53) (Bowen Yan et al., 2019;

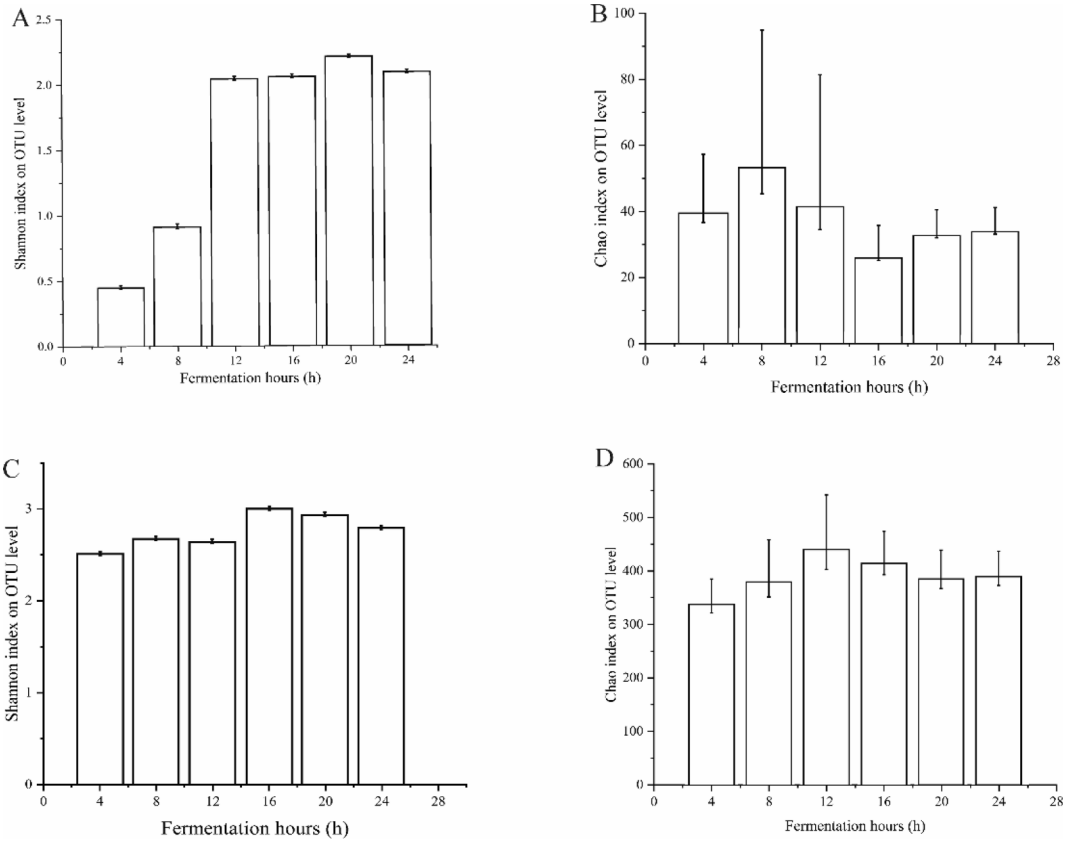

Fig. 2. The alpha diversity of Tartary buckwheat sourdoughs. A is the Shannon index of bacteria. B is the Chao index of bacteria. C is the Shannon index of fungi. D is the Chao index of fungi.

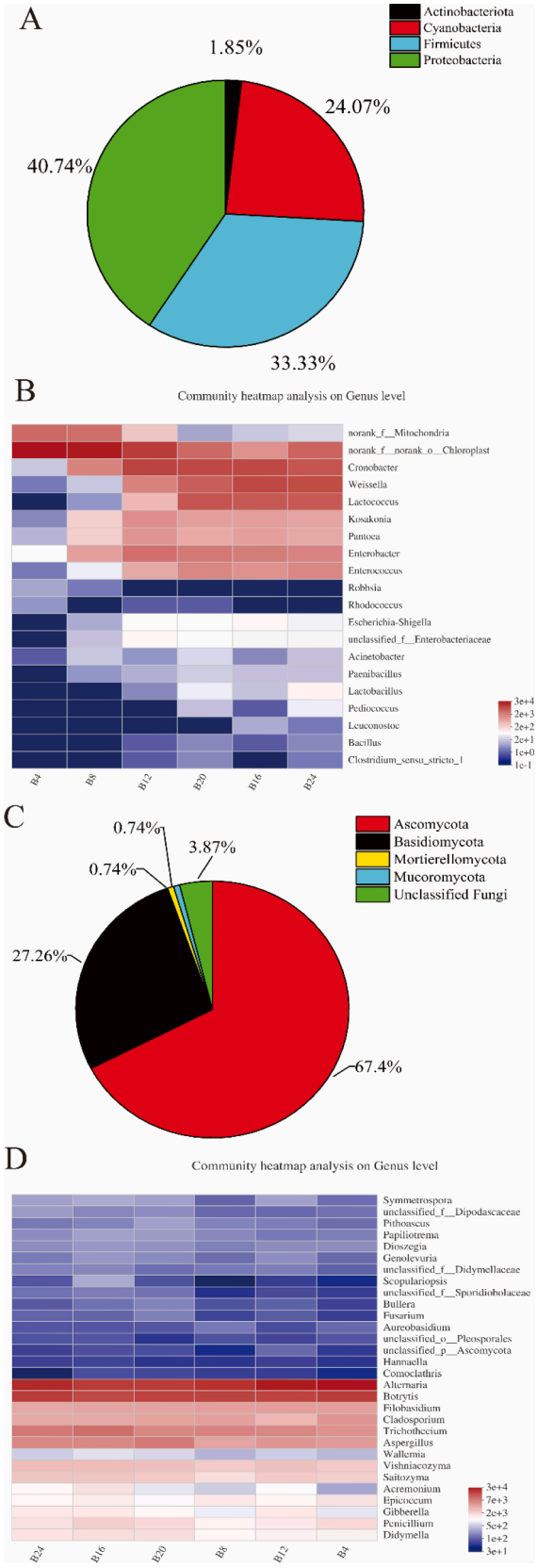

Fig. 3. Sourdoughs microbial community composition. A and C represented the community composition of bacteria and fungi at the phylum level, respectively. B and D displayed the community heat map analyses of bacteria and fungi at the genus level, respectively.

Xing et al., 2020). As it showed in Fig. 1A, the pH decreases relatively slowly in the first 8 h of fermentation and then pH decreases more rapidly.

The tendency of TTA was opposite to that of pH. With the extension of fermentation time, the TTA continued to increase from 5.3675 mL to 14.255 mL, which also showed that there were many acid-producing microorganisms in the sourdoughs.

3.2. Microbiological counting

According to recent reports, the traditional sourdoughs contained a large number of lactic acid bacteria and fungi. Some studies of wheat sourdough showed that the ratio of lactic acid bacteria and yeast was between 1:1 and 100:1 (Corsetti, 2013). In Fig. 1B, LAB and fungi grew constantly during fermentation. After 4 h, the microorganisms proliferated faster, then the rate decreased after 12 h. Moreover, the number of LAB was higher than that of fungi. This also proved that lactic acid bacteria grow easily in sourdoughs.

3.3. The alpha diversity of Tartary buckwheat sourdoughs

Alpha diversity mainly reflected the species diversity and richness in an ecosystem. In this study, the Shannon index and Chao index were selected to characterize the effect of different fermentation times on the alpha diversity of Tartary buckwheat sourdoughs. As shown in Fig. 2A, with continuous fermentation, the Shannon index of Tartary buckwheat sourdoughs increased rapidly, especially after 8-h fermentation. Shannon index represented the diversity of species in the sample. The higher Shannon index represented the higher diversity of the community. It could be inferred from Fig. 3A that there were many kinds of microorganisms when after 20 h of fermentation. The Chao index represented the richness of the microbial community. The higher the Chao index is, the greater the microbial richness is. Fig. 2B, the microbial richness was the highest in the sample after 8 h. However, the microbial richness decreased in the late stage of fermentation. This may be related to the types of microorganisms in the sample, the degree of substrate consumption, and the intermediate relationship between microorganisms. Similarly, the alpha diversity of fungi in Tartary buckwheat sourdoughs was analyzed, and the results were shown in Fig. 2C & D. Different from bacteria, the fungal diversity of all the samples was higher. The Shannon index of the sample fermented for 16 h was the highest, indicating that the community in the sample was rich. However, the community abundance of the samples fermented for 12 h was the highest. The community abundance decreased in the late stage of fermentation but did not change significantly after 20 h of fermentation. The changes in TTA and pH could also prove that Tartary buckwheat sourdoughs had accumulated a large number of acidic substances after 8 h of fermentation. As a result, the number of species and diversity of microbial communities in the dough significantly changed.

3.4. Composition of the bacterial community in Tartary buckwheat sourdoughs

The 16S rRNA gene sequencing analysis was conducted to characterize bacterial diversity (De Vuyst, Van Kerrebroeck, Harth, Huys, Daniel, & Weckx, 2014). This study mainly focused on the differences at the phylum level and genus level, as well as the changes in relative abundance with fermentation time. According to OTU clustering results, 54 kinds of microbial composition data were obtained, among which, the Proteobacteria was 21 (40.74%), the Firmicutes was 18 (33.33%), Cyanobacteria 13 (24.07%), and Actinobacteriota was 1 (1.85%). As shown in Fig. 3A, the predominant phylum was Proteobactria, followed by Firmicutes and Cyanobacteria. The top 20 species in total abundance at the genus level among all the samples were compared and a heat map analysis was performed.

According to Fig. 3B, Weisseria, Lactobacillus, Lactococcus, and

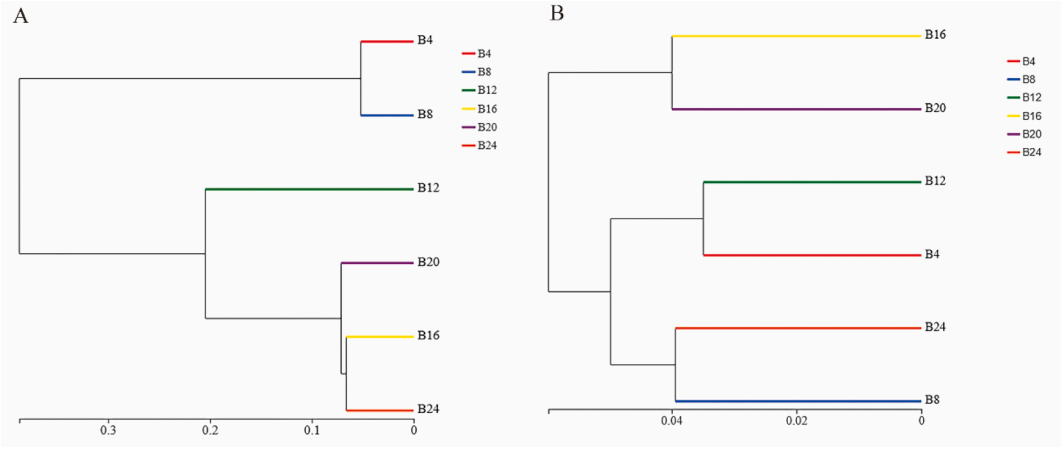

Fig. 4. Hierarchical clustering tree at the OTU level. A represented the bacteria and B represented fungi. B4, B8, B12, B16, B20, and B24 represented the Tartary buckwheat sourdoughs fermented for 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h.

Enterococcus were the main microorganisms in all the samples. These results were similar to published findings from Chinese wheat sourdoughs (X. Liu, Zhou, Jiaxin, Luo, Ye, Jiao, et al., 2018). Among them, *Weissella cibaria* was dominant. In addition, a relatively abundant strain of Lactococcus was found in these samples. The sequence was uploaded to *EzBioCloud.net* for further comparison. The results showed that the similarity of this bacterium to *Lactococcus Taiwanensis* was 99.79%, and it was speculated that this bacterium should be *Lactococcus Taiwanensis*.

Alice V. Moroni et al., (Moroni, Arendt, & Dal Bello, 2011) investigated buckwheat sourdough and identified a broad spectrum of LAB. They mainly belonged to the genera Lactobacillus, Pediococcus, and Leuconostoc. Antonio Alfonzo identified lactic acid bacteria in sourdoughs from Sicily (southern Italy), *Weissella cibaria*, *Lactobacillus Plantarum*, *Leuconostoc pseudomesenteroides*, and *Leuconostoc citreum* were the most prevalent species (Alfonzo, Ventimiglia, Corona, Di Gerlando, Gaglio, Francesca, et al., 2013). Bowen Yan et al. (2019) investigated Jiaozi (Henan China) and found *Lactobacillus Plantarum* and *Weissella cibaria* were the most dominant. J.O. Adepehin et al. analyzed the bacterial diversity of three sourdoughs produced from composite gluten-free flours and found *Pediococcus pentosaceus* was the most abundant species (Adepehin, Enujiugha, Badejo, Young, Odeny, & Wu, 2018). After investigating the bacterial diversity of sourdoughs in different regions of France, Lhomme et al. found that *Lactobacillus sanfranciscensis* was the dominant species, while *Lactobacillus Plantarum* and *Lactobacillus curvatus* were the main microorganisms (Lhomme, Orain, Courcoux, Onno, & Dousset, 2015). This indicated that *Weissella cibaria* was a relatively dominant microorganism in sourdoughs. Although *Pediococcus pentosaceus* and *Lactobacillus curvatus* were identified, their relative abundances were low. Interestingly, no biological information about *Lactobacillus Plantarum* was detected in this experiment. Similarly, Cyanobacteria were also detected in this sample and were dominant in the early stage of fermentation, which was similar to the research of bio Suo et al. (Suo, et al., 2020). *Lactococcus Taiwanensis* was first found in local fermented food (pobuzi) in Taiwan, and it was isolated from fresh cummingcordia (Chen et al., 2013). There were few reports on the application of *Lactococcus Taiwanensis* and the application in sourdough was even rarer. We are currently conducting a further study of this discovery.

It could be found that there was a succession of microbial communities during the fermentation by comparing the microbial composition of LAB in all samples. In this study, there were few microorganisms in the early stage of Tartary buckwheat sourdough fermentation, and the relative abundance was low. Enterococcus was the most abundant LAB. As the pH of Tartary buckwheat sourdoughs decreased, the types of bacteria became more varied. The relative abundance of Weissneria and Lactococcus increased, and they gradually became the dominant microorganisms. This was similar to the results of "three-phase evolution," which has been discussed in recent studies. Regardless of the type of flour, naturally fermented sourdoughs go through three stages in the fermentation process, which were comprised of "the three-phase evolution." They are (i) dominance of LAB species belonging to the genera Enterococcus, Lactococcus, and Leuconostoc; (ii) increasingly important presence of sourdough-specific LAB, such as Pediococcus and Weissella; and (iii) dominance of well-adapted sourdough strains, including heterofermentative species (Minervini, De Angelis, Di Cagno, & Gobbetti, 2014). The microbial community changes of LAB were mainly related to the degree of microbial tolerance to acidic environments and the way the microorganisms used carbohydrates and nitrogen sources in the fermentation substrate (Ganzle, Vermeulen, & Vogel, 2007).

3.5. Composition of fungal community in Tartary buckwheat sourdoughs

The obtained ITS sequences were compared with the database from sourdoughs. As shown in Fig. 3C, most reads were assigned to Ascomycota (67.4%) and Basidiomycota (27.26%). The rests were Mortierellomycota (0.74%) and Mucoromycota (0.74%) and the part without accurate classification information. As the result of fungal diversity is more complicated, the species with the top 50 genus abundance levels were selected for community heat map analysis. At the genus level, Aspergillaceae, Wickhamomyces, and Penicillium were identified (Fig. 3D). Similarly, harmful microorganisms, such as Alternaria, were identified in relatively high abundance. Li et al. collected 22 kinds of wheat grains and found that most of the samples were infected by Alternaria (F.-q. Li & Yoshizawa, 2000). This showed that Alternaria was derived from the raw materials used in fermentation.

Currently, the research on yeast in sourdoughs mainly focused on Saccharomyces, Candida, Pichia, Kazachstania, Torulaspora, Wickerhamomyces, Kluyveromyces, and Meyerozyma (De Vuyst, Van Kerrebroeck, Harth, Huys, Daniel, & Weckx, 2014). In this study, we obtained a large amount of information on yeasts including Candida, Pichia, Wickerhamomyces, etc. Research showed that the most common yeast in sourdough was *Saccharomyces cerevisiae.* (De Vuyst, Van Kerrebroeck, Harth, Huys, Daniel, & Weckx, 2014; Minervini et al., 2012). But there was no relevant biological information about *Saccharomyces cerevisiae* in this study. It could be due to the fermentation environment, process, or substrate, among other things. Li et al. found that Wickhamomyces was common yeast in Jiaozi in Henan Province, and this is directly related to the flour used (Z. Li, Li, & Bian, 2016). Gino vrancken et al. came up with a similar conclusion and found that Wickhamomyces could be more tolerant of acidic environments and more competitive than *Saccharomyces cerevisiae* (Vrancken, De Vuyst, Van der Meulen, Huys, Vandamme, & Daniel, 2010). Probably due to differences in the

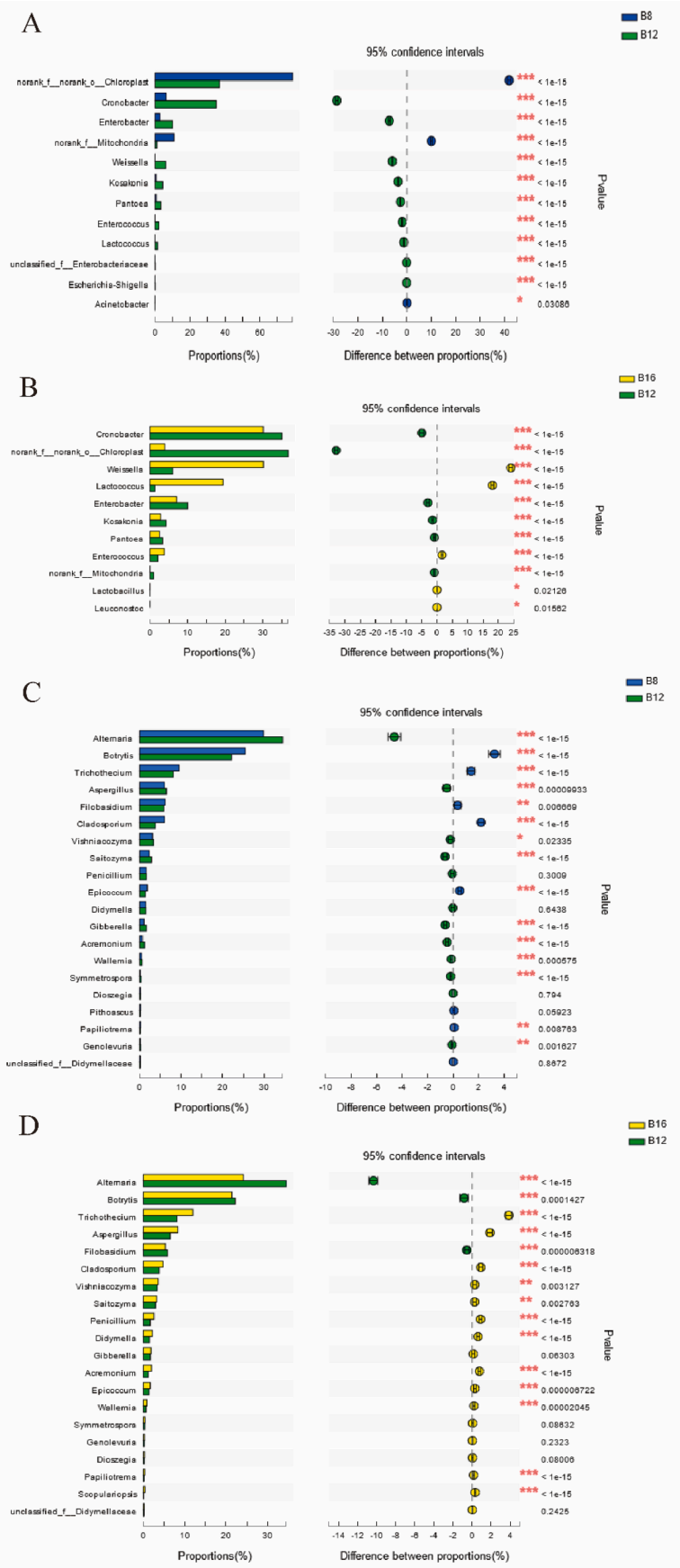

Fig. 5. Comparison of differences between two samples at the genus level by Fisher's exact test. A&B represents bacterial samples and C&D represents fungal samples. B8, B12, and B16 represent the Tartary buckwheat doughs fermented for 8 h, 12 hand16 h.

Table 1
Volatile components of Tartary buckwheat fermented doughs.

Category	NO.	Volatile components	RT (min)	Relative content (mg/kg) BF	NB	B4	B8	B12	B16	B20	B24
Ketones (2)	1	2-octanone	7.40	397.49 ± 1.78^{a}	108.30 ± 2.93^{b}	33.38 ± 11.34^{c}	11.72 ± 1.34^{c}	5.85 ± 0.32^{c}	8.15 ± 0.15^{c}	—	—
	2	acetoin	7.78	—	—	—	—	587.50 ± 3.72^{b}	903.30 ± 4.19^{a}	16.43 ± 0.26^{c}	—
Hydrocarbons (5)	3	undecane	2.55	1675.65 ± 19.48^{a}	—	13.19 ± 0.56^{b}	—	—	—	—	—
	4	3-methylundecane	5.59	285.34 ± 22.07^{a}	—	—	—	—	—	—	—
	5	indane	9.72	1430.84 ± 56.57^{a}	—	53.05 ± 0.19^{b}	29.64 ± 0.29^{b}	—	39.77 ± 3.04^{b}	14.20 ± 0.01^{b}	40.04 ± 0.11^{b}
	6	tetradecane	10.89	2686.23 ± 19.73^{a}	114.77 ± 3.44^{b}	—	—	—	—	—	—
	7	Azulene	17.44	—	—	103.41 ± 8.69^{a}	16.03 ± 1.32^{b}	24.92 ± 1.56^{b}	28.34 ± 0.96^{b}	—	—
Alcohols (8)	8	pentan-1-ol	6.29	—	—	—	35.09 ± 7.01^{b}	0.00	19.69 ± 1.54^{d}	25.63 ± 0.89^{c}	48.25 ± 1.94^{a}
	9	isoamylol	6.33	—	—	—	—	175.79 ± 14.42^{b}	116.14 ± 3.88^{d}	160.15 ± 2.47^{c}	388.05 ± 1.13^{a}
	10	oct-1-en-3-ol	11.62	439.14 ± 4.70^{a}	32.82 ± 0.98^{e}	—	41.23 ± 0.297^{e}	—	252.33 ± 15.86^{b}	136.76 ± 1.05^{d}	188.14 ± 1.09^{c}
	11	1-Heptanol	11.73	—	—	—	—	—	16.47 ± 0.49^{c}	100.92 ± 11.75^{b}	247.10 ± 1.47^{a}
	12	butane-2,3-diol	14.28	—	—	—	—	150.16 ± 4.50^{d}	448.42 ± 5.41^{c}	631.04 ± 5.42^{b}	942.83 ± 3.87^{a}
	13	(R, R)-butane-2,3-diol	14.29	—	—	—	—	104.23 ± 3.12^{c}	790.83 ± 7.61^{a}	648.66 ± 1.91^{b}	63.99 ± 1.95^{d}
	14	benzyl alcohol	20.07	—	—	—	—	102.73 ± 3.08^{d}	139.09 ± 4.17^{c}	260.89 ± 7.75^{b}	354.66 ± 5.42^{a}
	15	phenethyl alcohol	20.71	102.17 ± 6.60 ^{h}	193.57 ± 2.62^{f}	173.73 ± 7.29 ^{g}	203.16 ± 1.24^{e}	319.75 ± 1.02^{d}	397.20 ± 1.72^{a}	356.46 ± 1.23^{c}	363.01 ± 1.36^{b}
Aldehydes (5)	16	hexanal	3.70	498.55 ± 3.79^{a}	91.47 ± 1.76^{d}	214.60 ± 1.25^{b}	108.16 ± 3.59^{c}	—	—	—	—
	17	benzaldehyde	13.02	1283.06 ± 8.81^{b}	917.17 ± 5.73^{d}	1826.89 ± 13.83^{a}	1124.20 ± 8.57^{c}	93.67 ± 2.81 ^{h}	147.70 ± 1.76 ^{g}	375.52 ± 2.27^{f}	588.13 ± 3.79^{e}
	18	*trans*-2-Nonenal	13.47	—	62.58 ± 2.05^{a}	52.01 ± 1.56^{a}	—	—	—	—	—
	19	5-methyl furfural	14.11	—	115.12 ± 4.61^{a}	74.85 ± 2.24^{b}	—	—	—	—	—
	20	phenylacetaldehyde	15.52	—	278.59 ± 11.99^{b}	170.70 ± 5.12^{c}	351.89 ± 3.27^{a}	13.35 ± 0.40^{d}	25.94 ± 0.77^{d}	—	—
Esters (8)	21	*n*-hexyl formate	9.55	515.01 ± 1.63^{ab}	208.33 ± 7.97^{e}	156.33 ± 9.56^{f}	153.89 ± 1.02^{f}	260.82 ± 11.01^{d}	395.73 ± 13.40^{c}	535.23 ± 1.77^{b}	1086.58 ± 5.36^{a}
	22	Hexyl formate	11.86	—	—	—	—	—	135.80 ± 4.92^{a}	67.36 ± 0.20^{b}	—
	23	octyl formate	14.07	—	—	—	—	—	—	49.76 ± 7.20^{b}	102.79 ± 2.17^{a}
	24	methyl salicylate	18.16	—	311.76 ± 14.58^{a}	16.73 ± 5.02^{d}	34.50 ± 2.50^{c}	18.01 ± 0.54^{d}	47.32 ± 2.15^{b}	42.45 ± 6.14^{b}	43.78 ± 2.74^{b}
	25	4-Hydroxyphenylphosphonic acid	22.42	24.50 ± 7.35 ^{h}	92.41 ± 2.29^{f}	78.52 ± 2.34 ^{g}	483.81 ± 3.47^{e}	11443.02 ± 44.71^{b}	13275.78 ± 50.18^{a}	10124.46 ± 39.60^{c}	9907.41 ± 45.52^{d}
	26	Ethyl palmitate	28.40	—	—	—	—	—	147.91 ± 2.12^{b}	177.24 ± 8.83^{a}	118.18 ± 063^{c}
	27	diethyl phthalate	30.47	112.80 ± 3.38^{b}	41.59 ± 1.25^{d}	31.42 ± 0.94^{e}	—	60.41 ± 1.81^{c}	—	—	122.37 ± 1.71^{a}
	28	elaidic acid ethyl ester	33.90	—	—	—	—	31.56 ± 9.47^{d}	150.90 ± 4.14^{b}	236.33 ± 2.18^{a}	130.56 ± 1.54^{c}
Heterocyclic (9)	29	2-Pentylfuran	6.86	250.14 ± 7.65^{a}	—	—	—	—	—	—	—
	30	2-Methylpyrazine	7.41	133.09 ± 7.60^{b}	164.50 ± 9.54^{a}	87.76 ± 5.01^{e}	93.36 ± 0.62^{d}	109.39 ± 5.91^{c}	76.59 ± 2.85^{f}	65.08 ± 1.70 ^{g}	50.86 ± 1.29 ^{h}
	31	2,5-Dimethyl pyrazine	8.64	—	214.81 ± 1.92^{b}	113.14 ± 8.91^{c}	247.09 ± 2.46^{a}	93.01 ± 5.56^{d}	59.72 ± 2.27^{e}	40.30 ± 3.13 ^{g}	49.33 ± 1.08^{f}
	32	ethylpyrazine	8.92	—	26.25 ± 1.27^{d}	31.56 ± 1.27^{c}	48.62 ± 5.75^{a}	39.28 ± 1.91^{b}	5.55 ± 0.19^{f}	11.56 ± 0.35^{e}	—
	33	2-Ethyl-5-methylpyrazine	9.69	—	117.08 ± 6.23^{b}	70.91 ± 0.166^{c}	136.84 ± 13.57^{a}	137.00 ± 9.91^{a}	29.80 ± 2.12^{e}	33.59 ± 3.68^{d}	7.32 ± 0.21^{f}
	34	2,3-Dimethyl-5-ethylpyrazine	11.43	—	—	228.67 ± 6.87^{a}	129.20 ± 7.09^{b}	86.68 ± 6.31^{c}	83.67 ± 5.84^{d}	20.69 ± 0.62^{f}	63.22 ± 3.95^{e}
	35	2,6-diethylpyrazine	11.47	—	196.43 ± 5.75^{b}	146.14 ± 4.37^{c}	228.67 ± 1.67^{a}	55.68 ± 1.67^{e}	62.43 ± 1.87^{d}	42.06 ± 1.26^{f}	—

(*continued on next page*)

Table 1 (*continued*)

Category	NO.	Volatile components	RT (min)	Relative content (mg/kg) BF	NB	B4	B8	B12	B16	B20	B24
	36	Glycolophenone	15.67	—	77.62 ± 5.92^{a}	—	—	—	—	—	—
	37	Acetophenone	15.69	—	—	—	118.24 ± 10.30^{a}	—	8.34 ± 0.26^{b}	—	—
	38	2-Hydroxy-5-methyl acetophenone	26.36	—	—	—	—	93.86 ± 2.61^{b}	162.83 ± 5.34^{a}	—	39.22 ± 1.17^{c}
	39	2,3-dihydro benzofuran	30.94	—	—	—	—	383.82 ± 4.09^{c}	804.96 ± 42.29^{a}	532.03 ± 28.34^{b}	319.40 ± 16.98^{d}
Benzenes (5)	40	Toluene	2.98	294.91 ± 1.08^{a}	—	91.44 ± 16.24^{b}	—	—	—	—	—
	41	Ethylbenzene	4.48	230.20 ± 8.04^{a}	—	—	—	—	—	—	—
	42	o-Xylene	4.72	1070.43 ± 60.33^{a}	—	20.70 ± 0.62^{b}	—	—	—	—	—
	43	Naphthalene	17.41	460.09 ± 26.24^{a}	157.39 ± 3.60^{b}	88.15 ± 2.64^{f}	118.45 ± 18.58^{d}	114.14 ± 11.40^{d}	135.46 ± 6.72^{c}	119.80 ± 5.81^{d}	106.23 ± 5.54^{e}
	44	4-Methoxyphenol	19.75	—	—	40.32 ± 1.21^{e}	40.32 ± 2.64^{e}	7947.24 ± 28.19^{b}	9395.32 ± 35.29^{a}	6791.95 ± 28.08^{c}	6778.11 ± 31.64^{d}
Organic acids (1)	45	acetic acid	11.58	—	—	—	—	—	831.09 ± 29.85^{c}	1995.52 ± 10.68^{b}	2477.97 ± 11.96^{a}

Note: BF represented Tartary buckwheat flour. NB represented Tartary buckwheat unfermented doughs. B4, B8, B12, B16, B20, and B24 represented the Tartary buckwheat sourdoughs fermented for 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h. There were significant differences in the content of volatile compounds between different samples ($p < 0.05$).

fermentation environment and fermentation substrate, the identification results did not show *Saccharomyces cerevisiae*.

3.6. Analysis of community differences among samples

To understand the influence of fermentation time on the microbial community structure in sourdough samples, cluster analysis was carried out on the distance matrix of the sample community, and a hierarchical clustering tree of each sample at the OTU level was constructed. As shown in Fig. 4A, the samples were divided into two clusters at the genus level. Furthermore, the samples fermented for 12, 16, 20, and 24 h were divided into two groups. This indicated that fermentation time affected the bacterial community composition of sourdoughs. Samples fermented for 12 h were distant from other samples, indicating the reduced similarity of samples fermented for 12 h to the remaining samples. In terms of fungi (Fig. 4B), the samples were divided into two groups. The samples fermented for 16 h and 20 h were combined into one group, while the remaining samples were further divided into two groups. Because of the distance between samples, the similarity between samples was low, which also proves the complexity of fungal diversity. These differences could be due to the following reasons. Fermentation was a complex biochemical reaction, and the raw materials, geographical region, and processing parameters will cause differences in community composition.

To further analyze the sample composition, the differences between the two samples were analyzed according to the clustering results. Therefore, the samples fermented for 8 h and 16 h were compared with the sample fermented for 12 h. The results were shown in Fig. 5A & B. The relative abundance at the genus level was analyzed by Fisher exact test to compare the species composition differences between the two samples (confidence interval 95%, $P < 0.05$). The relative abundance of Cronobacter was higher in the early stage of fermentation, and the relative abundance of Weissella was lower. After 16 h of fermentation, the relative abundance of Weisslla was higher, while that of Cronobacter decreased. Similarly, the relative abundance of Lactococcus and other microorganisms changed with fermentation time, which indicated that the microbial community in the sample was in succession, and the dominant microorganisms were constantly changing. However, the acid environment is more suitable for the growth of LAB, due to the continuous decline of pH in sourdoughs.

Similarly, according to the clustering results, samples fermented for 16 h and 12 h, as well as 8 h and 12 h, were compared. As shown in Fig. 5C&D, with continuous fermentation, the relative abundance of different fungi changed significantly. In this study, the top 20 fungi with relative abundances were selected for comparison. As shown in Fig. 5C&D, the relative abundance of Alternaria increased first and then decreased, while the relative abundance of other fungi continued to increase, indicating that the fungal community was in succession and the core microorganisms were gradually changing.

3.7. Composition of volatile compounds in fermented Tartary buckwheat sourdoughs

Microbial diversity was one of the key factors influencing the flavor of sourdoughs and related products. Studies have shown that the flavor of sourdoughs prepared by homogenous LAB and heterogenous LAB is quite different. Hansen et al. compared the types and contents of bread aroma prepared by LAB and yeast and found that fermentation for more than 12 h could produce a large number of volatile substances to endow the product with a good flavor (Hansen & Schieberle, 2005). To clarify the effect of fermentation on the flavor profile of Tartary buckwheat sourdoughs, the differences in volatile compounds among Tartary buckwheat flour, unfermented Tartary buckwheat doughs, and fermented Tartary buckwheat sourdoughs were compared.

In this experiment, a total of 45 volatile compounds were identified, including 9 pyrazines, 8 alcohols and esters each, and 5 hydrocarbon compounds, aromatic compounds, and aldehydes each. The rests were furan and ketone compounds and 1 organic acid. The results were summarized in Table 1.

From Table 1, the main volatile compounds in Tartary buckwheat flour were hydrocarbon compounds. The hydrocarbon compounds were mainly derived from the homolysis of fatty acid alkoxy radicals, but due to their generally high thresholds, it could be considered that their contribution to the flavor of Tartary buckwheat was small. Pyrazine compounds appeared in almost all the Tartary buckwheat sourdoughs, and they were the dominant volatile compounds in Tartary buckwheat sourdoughs.

After 12 h fermentation, the content of alcohols in Tartary buckwheat sourdoughs increased. It mainly originates from the primary metabolism of microorganisms in the doughs or the corresponding carbonyl compounds produced by reduction reactions. For example, heterofermentative LAB in the fermentation process of sourdoughs could reduce part of the lipid oxidation products in the doughs to the corresponding alcohol compounds (Vermeulen, Czerny, Gänzle, Schieberle, & Vogel, 2007). The results of high-throughput sequencing showed that

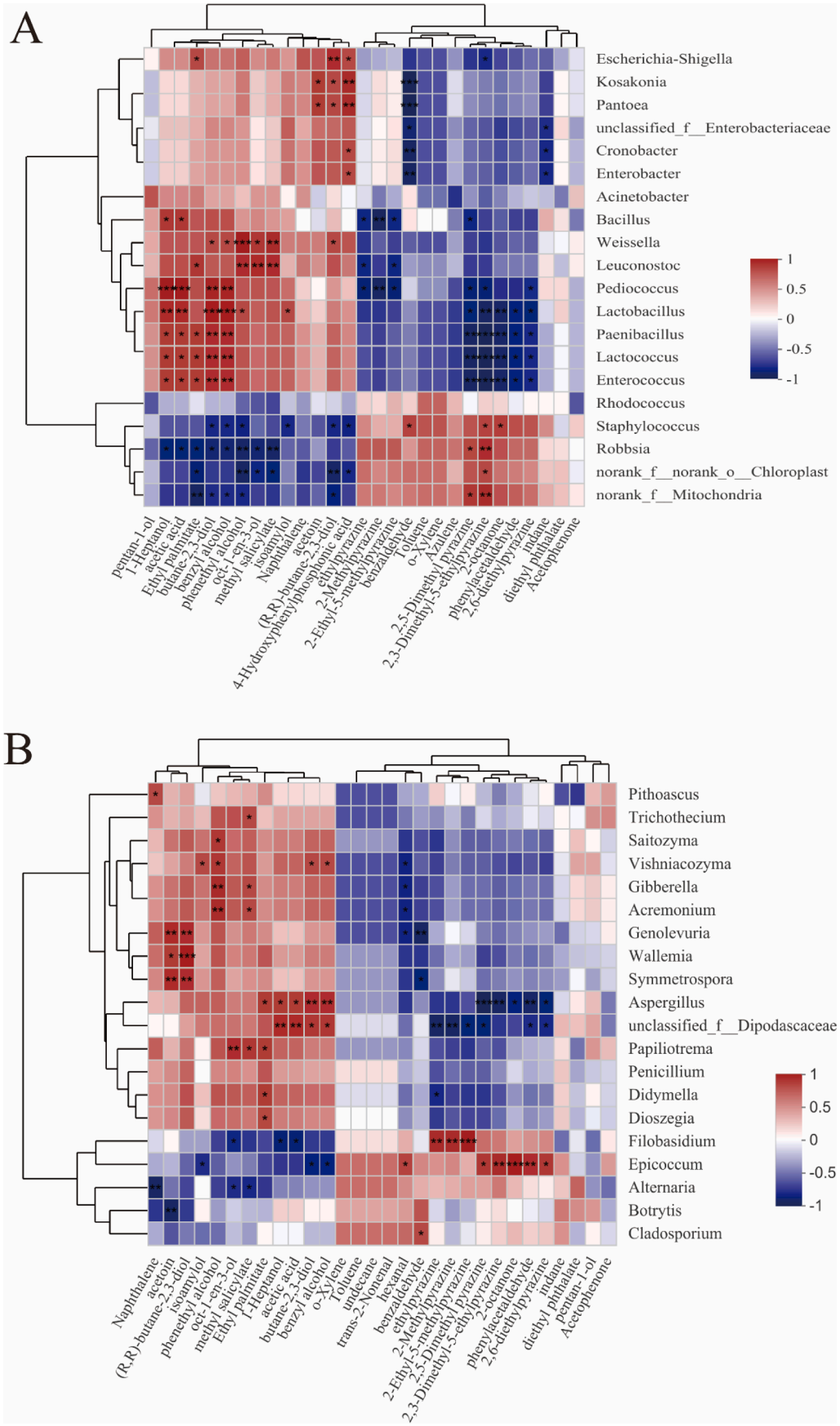

Fig. 6. Spearman correlation heatmap on genus level. A represented the bacteria and B represented fungi. (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

the number of heterogenous LAB increased in the middle and late fermentation, which was one of the reasons for the increase of alcohol content in sourdoughs, such as 1-Heptanol. 1-Heptanol has a fruit aroma and flowery flavor. In addition, benzyl alcohol and phenethyl alcohol were also detected. These compounds were usually generated by amino acid metabolism, which contributed to producing taste and flavor compounds (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). Phenylalanine in plants was initiated by microorganisms to produce benzyl alcohol via transamination reaction (Groot & Bont, 1998). Since sourdough is rich in a variety of enzymes (proteases), the fermentation and acidification process of LAB will activate these proteases to produce more amino acids and form the precursors of flavor substances. Similarly, phenethyl alcohol is usually derived from phenylalanine in flour (Pico, Bernal, & Gomez, 2015). It has a rose-honey-like odor, which usually impacts a positive role in improving the product's aroma.

Acetic acid is mainly produced by LAB in sourdoughs, which has an important influence on the flavor of many products made from sourdough. Acidification was also one of the important characteristics of sourdoughs. In addition to the metabolic activities of microorganisms in the doughs, the content of fermentable carbohydrates in the doughs was also one of the important factors (Ganzle, Vermeulen, & Vogel, 2007).

Esters were extremely important to the flavor of fermented foods, mainly from the primary metabolism of microorganisms. During the fermentation process of sourdoughs, the lipid metabolism of yeast and the sugar metabolism of LAB provided a large amount of alcohol and acid, and a variety of ester compounds could be generated under the catalytic reaction conditions of enzymes (Birch, Petersen, & Hansen, 2014). It had been shown in Table 1 that the content of ester compounds varies with different fermentation times. This also confirmed that the accumulation rate of esters was related to the rate of microbial metabolism.

The formation of aldehydes and ketones was mainly related to the endogenous lipid oxidation during the fermentation and curing process of dough (Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013). The content of substances, such as 2-octanone and hexanal, was higher in unfermented Tartary buckwheat doughs. In addition, heterofermentative LAB could participate in metabolism by utilizing electron acceptors to reduce aldehydes in the dough to corresponding alcohols. This was also one of the reasons why the aldehydes and ketones of Tartary buckwheat sourdoughs decrease and the increase of the alcohol in the middle and late fermentation.

Therefore, fermentation had an important influence on the flavor of Tartary buckwheat and it could produce many substances to improve the flavor of Tartary buckwheat. As time goes on, the metabolic activities of microorganisms in sourdoughs and related biochemical reactions produce new volatile compounds. This was also the main reason for the flavor change of Tartary buckwheat sourdoughs.

3.8. Correlation analysis between microbial diversity and volatile compounds in Tartary buckwheat sourdough

The top 20 microorganisms with a relative abundance and volatile compounds (OAV greater than 1) were screened to calculate the Pearson coefficient between them to further evaluate the effect of fermentation on volatile compounds in Tartary buckwheat sourdough. The heatmap depicted correlations and differences in microbial relative abundance and volatile compound concentrations. In the heatmap, the R-value was displayed in various colors: red indicated a positive correlation, blue indicated a negative correlation, and the darker the color, the stronger the correlation.

As shown in Fig. 6A, Weissella and Leuconostoc had a strong positive correlation with phenyl ethyl alcohol, methyl salicylate, oct-1-en-3-ol so far, and there were significant differences. Pediococcus and Lactobacillus belonged to 1-heptanol, acetic acid, etc., showing a strong positive affinity and having significant differences. The relationship between the fungus and volatile compounds was also investigated with the results depicted in Fig. 6B. According to Fig. 6 B, phenethyl alcohol and (R, R)-butane-2,3-diol had a positive correlation with Genolevuria, Wallemia, and Symmetrospora. There were also positive correlations between benzyl alcohol, butane-2,3-diol, and Aspergillus. The effect of fungal diversity on Tartary buckwheat sourdough was weaker than that of bacteria when compared to the results in Fig. 6A.

As previously stated, the presence of these compounds was considered to endow the sourdough with a pleasant flavor. These microorganisms could be found not only in wheat sourdoughs but also in Tartary buckwheat sourdoughs. After fermentation, the content of some inferior flavors in the Tartary buckwheat flour, such as alkanes, decreased. This indicates that fermenting Tartary buckwheat improved its flavor. In addition, a significant negative correlation between most pyrazine compounds and microbial diversity was discovered. These findings could help with targeted microorganism screening in Tartary sourdough based on actual needs.

4. Conclusion

In this study, the microbial diversity in Tartary buckwheat sourdoughs was preliminarily analyzed by high-throughput sequencing and culture-dependent methods. Moreover, GC–MS was used to dynamically monitor the volatile compounds produced during fermentation. The results showed that the dominant microorganisms in the Tartary buckwheat sourdough were Lactococcus and Weissella at the genus level. Then, the yeast identified at the genus level included Pichia and Wickerhamomyces. The microbial community succession in Tartary buckwheat sourdoughs as fermentation continued. The results of GC–MS showed that the content of pyrazine compounds in the fermented Tartary buckwheat sourdough was relatively high. The presence of large amounts of compounds like phenethyl alcohol in the later stages of fermentation endowed the sourdough with a good flavor. At the same time, there was a strong link between the volatile compounds of Tartary buckwheat sourdough and bacterial diversity. This could be used as a theoretical foundation for sourdough microorganism screening.

Declaration of Competing Interest

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

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References

Adepehin, J. O., Enujiugha, V. N., Badejo, A. A., Young, G. M., Odeny, D. A., & Wu, F. (2018). Bacterial ecology and rheological parameters of multigrain gluten-free sourdoughs. *LWT- Food Science and Technology, 96*, 344–349. https://doi.org/10.1016/j.lwt.2018.05.052

Alfonzo, A., Ventimiglia, G., Corona, O., Di Gerlando, R., Gaglio, R., Francesca, N., … Settanni, L. (2013). Diversity and technological potential of lactic acid bacteria of wheat flours. *Food Microbiology, 36*(2), 343–354. https://doi.org/10.1016/j.fm.2013.07.003

Birch, A. N., & M. A. P., Åse Solvej Hansen. (2014). The aroma of wheat bread crumb. *Cereal Chemistry, 91*(2), 105–114. https://doi.org/10.1094/CCHEM-06-13-0121-RW

Blaise P. Nic Phiarais, A. M., Beatus D. Schehl, Martin Zarnkow, Martina Gastl, Markus Herrmann, Emanuele Zannini Elke K. Arendt. (2010). Processing of a Top Fermented Beer Brewed from 100% Buckwheat Malt with Sensory and Analytical Characterisation. *Journal of the Institute of Brewing, 116*(3), 265-274. https://doi.org/10.1002/j.2050-0416.2010.tb00430.x.

Nierop Groot, M. N., & de Bont, J. A. M. (1998). Conversion of phenylalanine to benzaldehyde initiated by aminotransferase in Lactobacillus plantarum. *Applied and*

Environmental Microbiology, 64, 3009–3013. https://doi.org/10.1128/AEM.64.8.3009-3013.1998

Yan, Bowen, Sadiq, Faizan A., Cai, Yijie, Fan, Daming, Chen, Wei, Zhang, Hao, & Zhao, Jianxin (2019). Microbial diversity in traditional type I sourdough and jiaozi and its influence on volatiles in Chinese steamed bread. *LWT - Food Science and Technology, 101*, 764–773. https://doi.org/10.1016/j.lwt.2018.12.004

Chen, Junran, Yan, Ruixiang, Hu, Yunfeng, Zhang, Na, & Hu, Hanyan (2019). Compositional shifts in the fungal diversity of garlic scapes during postharvest transportation and cold storage. *LWT - Food Science and Technology, 115*, 108453. https://doi.org/10.1016/j.lwt.2019.108453

Chen, Y. S., Chang, C. H., Pan, S. F., Wang, L. T., Chang, Y. C., Wu, H. C., & Yanagida, F. (2013). Lactococcus taiwanensis sp. nov., a lactic acid bacterium isolated from fresh cummingcordia. *International Journal of Systematic and Evolutionary Microbiology, 63*(7), 2405–2409. https://doi.org/10.1099/ijs.0.045757-0

Corsetti, Aldo (2013). In *Handbook on Sourdough Biotechnology* (pp. 85–103). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4614-5425-0_4.

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H. M., & Weckx, S. (2014). Microbial ecology of sourdough fermentations: Diverse or uniform? *Food Microbiology, 37*, 11–29. https://doi.org/10.1016/j.fm.2013.06.002

Deželak, Matjaž, Zarnkow, Martin, Becker, Thomas, & Košir, Iztok Jože (2014). Processing of bottom-fermented gluten-free beer-like beverages based on buckwheat and quinoa malt with chemical and sensory characterization. *Journal of the Institute of Brewing*, n/a–n/a. https://doi.org/10.1002/jib.166

Fan, H., Zheng, X., Ai, Z., Liu, C., Li, R., & Bian, K. (2018). Analysis of volatile aroma components from Mantou fermented by different starters. *Journal of Food Processing and Preservation, 42*(6). https://doi.org/10.1111/jfpp.13627

Galoburda, Ruta, Straumite, Evita, Sabovics, Martins, & Kruma, Zanda (2020). Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods, 9*(12), 1837. https://doi.org/10.3390/foods9121837

Ganzle, M. G., Vermeulen, N., & Vogel, R. F. (2007). Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiology, 24*(2), 128–138. https://doi.org/10.1016/j.fm.2006.07.006

Ganzle, M. G., & Zheng, J. (2019). Lifestyles of sourdough lactobacilli - Do they matter for microbial ecology and bread quality? *International Journal of Food Microbiology, 302*, 15–23. https://doi.org/10.1016/j.ijfoodmicro.2018.08.019

Hansen, A., & Schieberle, P. (2005). Generation of aroma compounds during sourdough fermentation: Applied and fundamental aspects. *Trends in Food Science & Technology, 16*(1–3), 85–94. https://doi.org/10.1016/j.tifs.2004.03.007

Hazelwood, L. A., Daran, J. M., van Maris, A. J., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on Saccharomyces cerevisiae metabolism. *Applied and Environmental Microbiology, 74*(8), 2259–2266. https://doi.org/10.1128/AEM.02625-07

Kaseleht, K., Paalme, T., Mihhalevski, A., & Sarand, I. (2011). Analysis of volatile compounds produced by different species of lactobacilli in rye sourdough using multiple headspace extraction. *International Journal of Food Science & Technology, 46*(9), 1940–1946. https://doi.org/10.1111/j.1365-2621.2011.02705.x

Lhomme, E., Orain, S., Courcoux, P., Onno, B., & Dousset, X. (2015). The predominance of Lactobacillus sanfranciscensis in French organic sourdoughs and its impact on related bread characteristics. *International Journal of Food Microbiology, 213*, 40–48. https://doi.org/10.1016/j.ijfoodmicro.2015.05.010

Li, F.-Q., & Yoshizawa, T. (2000). Alternaria Mycotoxins in Weathered Wheat from China. *Journal of Agricultural and Food Chemistry, 48*(7), 2920–2924. https://doi.org/10.1021/jf0000171

Li, Z., Li, H., & Bian, K. (2016). Microbiological characterization of traditional dough fermentation starter (Jiaozi) for steamed bread making by culture-dependent and culture-independent methods. *International Journal of Food Microbiology, 234*, 9–14. https://doi.org/10.1016/j.ijfoodmicro.2016.06.024

Liu, T., Li, Y., Chen, J., Sadiq, F. A., Zhang, G., Li, Y., & He, G. (2016). Prevalence and diversity of lactic acid bacteria in Chinese traditional sourdough revealed by culture dependent and pyrosequencing approaches. *LWT - Food Science and Technology, 68*, 91–97. https://doi.org/10.1016/j.lwt.2015.12.025

Liu, Xiaojiao, Zhou, Man, Jiaxin, Chen, Luo, Ying, Ye, Fuzhou, Jiao, Shuo, … Lü, Xin (2018). Bacterial diversity in traditional sourdough from different regions in China. *LWT - Food Science and Technology, 96*, 251–259.

Maire, M., Rega, B., Cuvelier, M. E., Soto, P., & Giampaoli, P. (2013). Lipid oxidation in baked products: Impact of formula and process on the generation of volatile compounds. *Food Chemistry, 141*(4), 3510–3518. https://doi.org/10.1016/j.foodchem.2013.06.039

Minervini, F., De Angelis, M., Di Cagno, R., & Gobbetti, M. (2014). Ecological parameters influencing microbial diversity and stability of traditional sourdough. *International Journal of Food Microbiology, 171*, 136–146. https://doi.org/10.1016/j.ijfoodmicro.2013.11.021

Minervini, Fabio, Di Cagno, Raffaella, Lattanzi, Anna, De Angelis, Maria, Antonielli, Livio, Cardinali, Gianluigi, … Gobbetti, Marco (2012). Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical italian breads: Interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology, 78*(4), 1251–1264. https://doi.org/10.1128/AEM.07721-11

Moroni, A. V., Arendt, E. K., & Dal Bello, F. (2011). Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. *Food Microbiology, 28*(3), 497–502. https://doi.org/10.1016/j.fm.2010.10.016

Nakamura, K., Naramoto, K., & Koyama, M. (2013). Blood-pressure-lowering effect of fermented buckwheat sprouts in spontaneously hypertensive rats. *Journal of Functional Foods, 5*(1), 406–415. https://doi.org/10.1016/j.jff.2012.11.013

Pico, J., Bernal, J., & Gomez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International, 75*, 200–215. https://doi.org/10.1016/j.foodres.2015.05.051

Ripari, V., Cecchi, T., & Berardi, E. (2016). Microbiological characterisation and volatiles profile of model, ex-novo, and traditional Italian white wheat sourdoughs. *Food Chemistry, 205*, 297–307. https://doi.org/10.1016/j.foodchem.2016.02.150

Siepmann, F. B., Ripari, V., Waszczynskyj, N., & Spier, M. R. (2017). Overview of Sourdough Technology: From Production to Marketing. *Food and Bioprocess Technology, 11*(2), 242–270. https://doi.org/10.1007/s11947-017-1968-2

Suo, Biao, Nie, Wenjing, Wang, Yuexia, Ma, Jingyi, Xing, Xiaolong, Huang, Zhongmin, … Ai, Zhilu (2020). Microbial diversity of fermented dough and volatile compounds in steamed bread prepared with traditional Chinese starters. *LWT - Food Science and Technology, 126*, 109350. https://doi.org/10.1016/j.lwt.2020.109350

Teleky, B. E., Martău, A. G., Ranga, F., Chețan, F., & Vodnar, D. C. (2020). Exploitation of Lactic Acid Bacteria and Baker's Yeast as Single or Multiple Starter Cultures of Wheat Flour Dough Enriched with Soy Flour. *Biomolecules, 10*(5), 778. https://doi.org/10.3390/biom10050778

Teleky, B. E., Martău, G. A., & Vodnar, D. C. (2020). Physicochemical Effects of Lactobacillus plantarum and Lactobacillus casei Cocultures on Soy-Wheat Flour Dough Fermentation. *Foods, 9*(12), 1894. https://doi.org/10.3390/foods9121894

Vermeulen, N., Czerny, M., Gänzle, M. G., Schieberle, P., & Vogel, R. F. (2007). Reduction of (E)-2-nonenal and (E, E)-2,4-decadienal during sourdough fermentation. *Journal of Cereal Science, 45*(1), 78–87. https://doi.org/10.1016/j.jcs.2006.07.002

Vrancken, G., De Vuyst, L., Van der Meulen, R., Huys, G., Vandamme, P., & Daniel, H.-M. (2010). Yeast species composition differs between artisan bakery and spontaneous laboratory sourdoughs. *FEMS Yeast Research, 10*(4), 471–481. https://doi.org/10.1111/j.1567-1364.2010.00621.x %J FEMS Yeast Research

Wang, H., Qin, X., Mi, S., Li, X., Wang, X., Yan, W., & Zhang, C. (2019). Contamination of yellow-feathered broiler carcasses: Microbial diversity and succession during processing. *Food Microbiology, 83*, 18–26. https://doi.org/10.1016/j.fm.2019.04.006

Wu, Kao, Gan, Renyou, Dai, Shuhong, Cai, Yi-Zhong, Corke, Harold, & Zhu, Fan (2016). Buckwheat and Millet Affect Thermal, Rheological, and Gelling Properties of Wheat Flour. *Journal of Food Science, 81*(3), E627–E636. https://doi.org/10.1111/jfds.2016.81.issue-310.1111/1750-3841.13240

Xing, Xiaolong, Ma, Jingyi, Fu, Zhongjun, Zhao, Yirui, Ai, Zhilu, & Suo, Biao (2020). Diversity of bacterial communities in traditional sourdough derived from three terrain conditions (mountain, plain and basin) in Henan Province, China. *Food Research International, 133*, 109139. https://doi.org/10.1016/j.foodres.2020.109139

Yoshioka, H., Ohmoto, T., Urisu, A., Mine, Y., & Adachi, T. (2004). Expression and epitope analysis of the major allergenic protein Fag e 1 from buckwheat. *J Plant Physiol, 161*(7), 761–767. https://doi.org/10.1016/j.jplph.2004.01.010

Zhang, X.-Y., Chen, J., Li, X.-L., Yi, K., Ye, Y., Liu, G., Wang, S.-F., Hu, H.-L., Zou, L., & Wang, Z.-G. (2017). Dynamic changes in antioxidant activity and biochemical composition of tartary buckwheat leaves during Aspergillus niger fermentation. *Journal of Functional Foods, 32*, 375–381. https://doi.org/10.1016/j.jff.2017.03.022

Zhu, F. (2016). Chemical composition and health effects of Tartary buckwheat. *Food Chemistry, 203*, 231–245. https://doi.org/10.1016/j.foodchem.2016.02.050