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Effect of the Different Composition of Wheat, Rice, Buckwheat, Sweet Potato and Corn on Sourdough Characteristics and Sensory of Mantou

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Abstract

To investigate the impact of different matrixes on the microbiota in Chinese traditional sourdoughs and the sensory of Mantou, the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), viable cell counting and sensory evaluation methods were applied in this study. The viable cell counting results showed that the microbes on YPD plate was sensitive to the sourdough matrixes and received a dramatic reduction in samples 100% wheat (W), 50% wheat + 50% corn (W+C), and 50% wheat + 50% rice (W+R), and the DGGE results indicated that the sourdough matrixes changed the microbial population and 8 lactobacilli and 2 yeasts were identified in 5 different sourdough samples, of which the Lactobacillus acetotolerans was identified as the dominant bacteria during sourdough fermentation. In addition, the different matrixes greatly changed the specific volume, exterior, color, structure, elasticity, stickness and flavour of Mantou. So, the matrixes played a key role in microbiota of sourdoughs and sensory of Mantou.

Keywords: PCR-DGGE; Sourdough; Yeasts; Lactic acid bacteria

Introduction

Mantou (also named as Chinese steamed breads) were originated in 2nd century, and the people in China had used it as part of their diets long before 17th century [1]. Previously, Mantou was mainly consumed in Northwestern region in China because of its dainty taste and rich nutrition. Now, Mantou had been served as one of the important staple foods in the whole China as a result of population flow and diet diversification, and Mantou was no longer solely made using wheat, but also adding some other matrixes (e.g. rice, buckwheat, sweet potato and corn) based on the traditional dietary habits of people in different regions of China.

Previous studies indicated that the combinatorial associations of lactic acid bacteria (LABs) and yeasts were responsible for the sourdough quality, which indirectly influenced the flavour, storage, safety of Mantou. Many studies had been carried out to reveal the microbial roles during fermentation process [2,3]. However, most of these studies only focused on the single matrix considering the lifestyle of local people and diet structure, and few studies were carried out to investigate the effect of different matrix combinations on the microbial community of sourdoughs during fermentation process and the sensory of the final products.

Although culture–dependent approach was still proven to be useful and quite successful in a wide range of microbiological applications [4], the culture–dependent approach had limitations in terms of recovery rate and reproducibility, and the recovered isolates of microorganisms might not always truly reflect the microbial composition of the actual situation [5,6]. As a power culture-independent tool, the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) is based on direct analysis of the extract of DNA from the microbial environment and does not require cell cultivation [7]. Therefore, there is an obvious advantage to combine both methods to obtain a comprehensive insight into the variations of microbial community during sourdough fermentation. Till now, the culture-dependent and -independent methods had already been used to analyze the microbial diversity of sourdoughs [8,9], and some microbes, e.g. *Lactobacillus rossiae, L. hammesii, L. siliginis, L. nantensis, L. secaliphilus, L. fermentum, Weissella cibaria, L. lactis, and L. crustorum* had been identified from the sourdoughs [10,11]. However, little work was done to reveal the microbial diversity in sourdoughs of Mantou. Furthermore, the sensory indexes of the specific volume, exterior (appearance), color, structure, elasticity, stickness and flavour of Mantou were evaluated based on the Ministry of Commerce of PR China standard (SB/T10139-93) [12].

In the present study, the impacts of 5 different matrixes on population of LABs and yeasts during the sourdough fermentation and the sensory of Mantou were investigated, providing useful microbial information on sourdough fermentation and data on favour, nutrition, safety and storage of fermented foods.

Materials and Methods

Sourdough fermentations and sampling

Doughs were prepared by mixing water and 100% wheat (W), 50% wheat + 50% rice (W+R), 50% wheat + 50% buckwheat (W+B), 50%

wheat + 50% sweet potato (W+S) or 50% wheat + 50% corn (W+C). Fermentations were started by adding a commercial starter mixture (AQ), and developed at 25°C by back-slopping of 10% of the ripe sourdoughs every 24 h for 12 days. At each refreshment step, samples were taken from the ripe sourdough and used for the further study. Three replications were done for each group and the samples from the same group were mixed for the culture –dependent and –independent experiments.

The pH and the total titratable acids (TTA) were determined using 10 g of sourdough suspended in 100 mL of distilled water. The TTA value is expressed as the amount (in milliliters) of 0.1 M NaOH needed to achieve a final pH of 8.5 [13]. For microbial counting, samples were serially diluted 1:10 with saline-tryptonediluent (containing, per liter, 8.5 g of NaCl and 1.0 g of tryptone [pH 6.0]) and plated on MRS agar (Difco, Detroit, MI, USA) and YPD agar (Difco, Detroit, MI, USA), and incubated at 37 or 25°C for 24-72 h.

DNA extraction and PCR amplification

DNA was extracted and purified according to a bead-beating method [14,15]. After phenol-chloroform extraction, DNA was precipitated with ethanol and suspended in 50 μL of TE buffer. Primers lac1 (F) (5′- AGCAGTAGGGAATCTTCCA-3′) and lac2 (R) (5′- ATTYCACCGCTACACATG-3′) were used to amplify the 16S rRNA of LABs, and FF390(F) (5′- CGATAACGAACGAGACCT -3′) and FR1 (R) (5′- CCGAICCATTCAATCGGTAIT -3′) were used to amplify the 23S rRNA of fungi [16,17], a GC clamp in primer was used to create PCR products suitable for separation by DGGE [18]. PCR was performed with the Taq DNA polymerase kit from Life Technologies (Takara, Shanghai, China) according to the manufacturer's instruction. The PCR reaction mixture (25 μL) consisted of 0.125 μL of Taq DNA polymerase (1.25 U), 0.5 μL of each primer (10 μM), 1 μL of ten-fold diluted DNA template (approximately 1 ng), 2.5 μL of 10 × PCR buffer, 1.5 μL of $MgCl_2$ (50 mM), and 18.75 μL of UV-sterile water. DNA was amplified in a Biosci thermal cycler with 30 cycles of 94°C for 30 s, 56°C for 30 s, and a final extension at 72°C for 5 min. 5 μL of the PCR mixtures were analyzed by electrophoresis on an agarose gel (1%) with ethidium promide staining and visualized under UV light to check the size and amounts of the amplicons.

PCR-DGGE and DNA sequencing

DGGE analysis of PCR amplicons was performed according to the method described by Nicolaisen and Ramsing [19,20]. Briefly, after PCR reaction, the PCR products (3.5 μL, 360 bp for *Lactobacillus* and 389 bp for Fungi) were separated on a 35-65% denaturant polyacrylamide gradient gel by electrophoresis. The gel was then stained with silver. The obtained DGGE fingerprints were subsequently normalized and analyzed with the Bio Numeric software version 2.0 (Applied Math, Lon- don, England). During the analysis, different lanes were defined and the background was subtracted. The intensity differences of DGGE lanes were compensated during normalization and the correlation matrix was calculated. Clustering was done with Pearson correlation and the UPGMA method. Bands of interest were then excised from the gel using a sterile blade and incubated overnight at 4°C in Tris–EDTA buffer (pH 8.0). The eluted DNA was then used for DNA sequencing with an automated DNA sequencer (Applied Biosystems) [21]. Bacterial species identification was performed by searching the GenBank using BLAST [22].

Mantou preparation and sensory evoluation

Mantou were prepared according to the method of Huang et al. with simple modification [23]. The commercial starter mixture (AQ, 1.3 g) was dispersed in 38°C water, and mixed with 130 g different matrixes (W, W+R, W+B, W+S, and W+ C) for 7 min to make the dough. Then the dough was fermented for 45 min at 38°C and 85% RH, The dough was divided into two pieces and pressed for 20 min and shaped to a round dough piece with a smooth surface using hands. The dough pieces were proofed for 15 min and steamed for 20 min in a steamer, after resting in the air for 15 min, the Mantou were cooled in the air for 40 min.

The Mantou was evaluated according to SB/T10139-93. Specific volume, exterior, color, structure, elasticity, stickness and flavour were scored by 6 experts, and the best scores were 20, 15, 10, 15, 20, 15 and 5, respectively [23,12].

Statistical analysis

Data was submitted to a variance analysis (ANOVA, means ± SD), and then significant differences between means were determined by the nonparametric Student–Newman–Keulsseparation test with significance level ($P<0.05$) or very significance level ($P<0.01$).

Results and Discussions

Microbial counts during the sourdough fermentation

The fluctuations of the microbial count on MRS plate and YPD plate in 5 different kinds of sourdoughs were counted during the fermentation process. Throughout the fermentative process, the microbial count on MRS reached 1010 CFU/mL within 1 day in 4 kinds of sourdoughs (W, W+R, W+B, W+S), and received the maximum of 1011 CFU/mL on 5th day (Figure 1a). However, the microbial count on YPD was quite different in these 5 sourdoughs, the microbial amounts of samples W, W+C, W+R reduced to 102 CFU/mL, and samples W+S, W+B also received a 10-100-fold reduction on 7th day (Figure 1b). For lactobacillus, the bacterial number in W+B was significantly lower ($p<0.01$) than that in Won first day; for fungus, the microbial number in also received a significant reduction ($p<0.01$) on first day, then it received a dramatically increase and its biomass on day 5, 7, 9 and 11 was significantly higher than that in W. It suggested that the matrixes had seriously influenced the microbial counting, especially for the fungi, whose counts were unable to detect in samples W, W+C, and W+R since 5 days. Moreover, though the presence of buckwheat had severely inhibited all the microbes in the initial stage of fermentation, the addition of rice/corn only inhibited the growth of fungi and posed little effect on the growth of lactobacilli (Figure 1a and 1b).

To further investigate the effect of matrixes on the fermentation, the pH and the TTA values were evaluated each day during the fermentation. Interestingly, though the pH values quickly reduced to 3.4 in all 5 kinds of sourdoughs on 3rd (W+R, W+B), 5th (W), or 7th days (W+S, W+C) (Figure 2a), the TTA values of sample W+B sharply increased to 25 mL on 3rd day while the others only reached the highest value of 10-14 mL since 11th day, which suggested that the presence of buckwheat in sourdough had significantly enhanced total acid the of sourdough compared with wheat group ($p<0.01$).

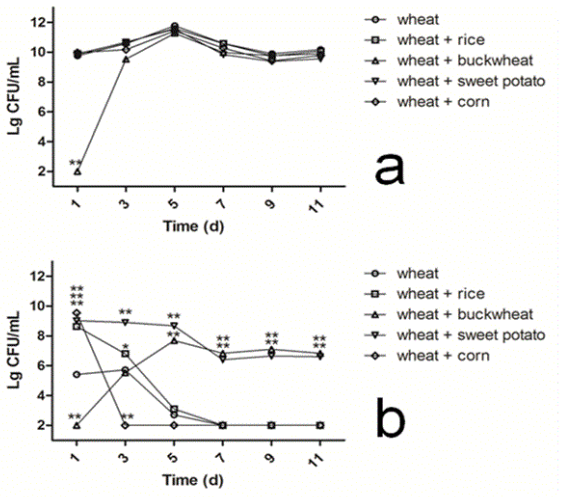

Figure 1: Fluctuations of the lactobacillus and fungus counts in sourdoughs wheat, wheat + rice, wheat + buckwheat, wheat + sweet potato, wheat + corn, measured at progressive days of back-slopping. (a) fluctuation of the lactobacillus counts, (b) fluctuation of the fungus counts. (*p<0.05, **p<0.01).

The high surviving amount of yeasts in sample W+B during the later stage of fermentation with a low pH and high concentration of TTA, suggested that the additive of buckwheat was benefit to the survive of yeasts and promoted the production of lactic and acetic acids. Though some researchers suggested that the LAB multiplied and produced more slowly in mixtures with yeasts than in pure culture [24,25], it seemed here that the yields of fatty acids for LABs were strongly influenced by the matrices used for dough fermentation (Figure 2).

LABs population dynamics in sourdough

To gain insight of the changing of LABs communities in the different matrixes of sourdough during fermentation process, the sourdough samples were monitored by PCR-DGGE with the primers Lac1 and Lac2GC. Eight LABs were identified from 5 sourdough samples, and the dominant LABs in different sourdough samples were various (Figure 3 and Table 1). In the sample W, *L. acetotolerans* and *L. plantarum* occupied the dominant positions from 1st day to 5th day, and then replaced by *L. helveticus*, *L. brevis* and *L. reuteri* from 5th day to 11th day (Figure 3a and Table 1). When different matrixes were mixed with wheat, the DGGE patterns received obvious changes compared to the wheat group. In sample W+R, *L. acetotolerans*, *L. plantarum* and *L. fermentum* were detected as the dominant LABs throughout the fermentation process (Figure 3b and Table 1). And in sample W+B, the LAB communities (*L. acetotolerans*, *L. plantarum*, *L. helveticus*, *Weissella cibaria* and *Weissella confuse*) were stable during the fermentation process for 11 days (Figure 3c and Table 1), suggesting that the additive of rice or buckwheat in sourdoughs might be benefit to the stability of LAB communities of sourdough during the fermentation process. In addition, the additive of sweet potato made the *L. brevis* and *L. fermentum* to be the dominant strains on 7th day (Figure 3d and Table 1).

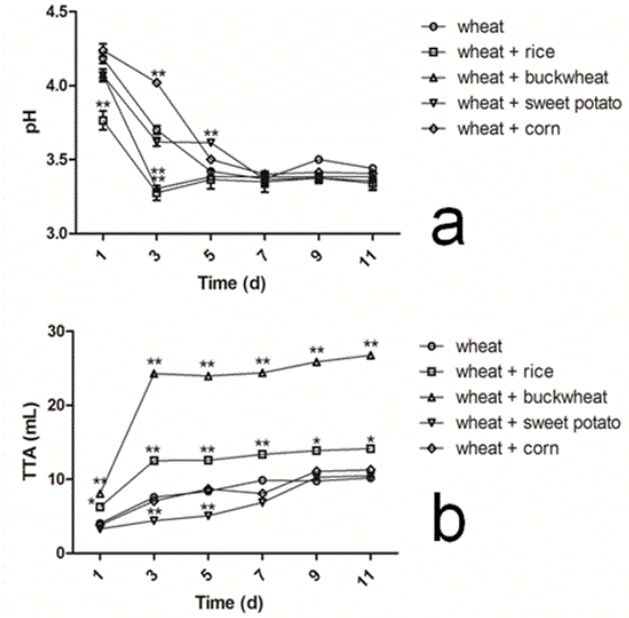

Figure 2: Fluctuations of the pH and TTA values in sourdoughs wheat, wheat + rice, wheat + buckwheat, wheat + sweet potato, wheat + corn, measured at progressive days of back-slopping. a: pH; b: TTA . (*p<0.05, **p<0.01).

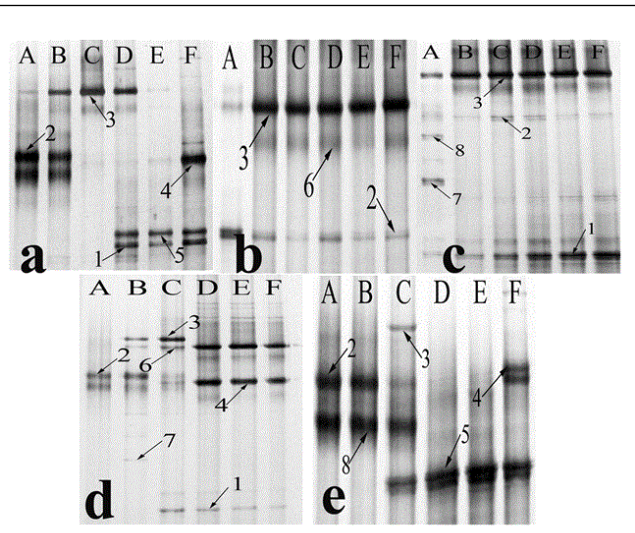

Figure 3: DGGE profiles obtained with lactobacillus primers. (a-e) sourdoughs wheat, wheat + rice, wheat + buckwheat, wheat + sweet potato, wheat + corn; A-F: fermented for 1d, 3d, 5d, 7d, 9d and 11d. The identification of the bands is reported in Table 1, bands indicated by numbers were excised, and, after re-amplification and cloning, they were subjected to sequencing.

When added 50% corn, 50% sweet potato and 50% buckwheat, *W. cibaria* and *W. confusa* occurred in the early sourdough fermentation stage and then disappeared (Figure 3 and Table 1), paving the way for

subsequent growth of sourdough-specific aciduric *lactobacilli* [26,27]. In the present study, *L. plantarum* and *L. acetotolerans* were identified in all sourdough samples during the fermentation process, ofwhich the *L. plantarum* had been previously identified as the dominant strains during fermentation of wheat, buckwheat and teff flour [28,29]. However, the *L. acetotolerans*, which rarely occurred in the fermentative food production, was first identified as the dominant bacteria during sourdough fermentation.

Strain No.	Closest relatives	Similarity (%)	GenBank No.
Wheat			
1	*Lactobacillus helveticus*	99	HM218769.1
2	*Lactobacillus acetotolerans*	100	HM218496.1
3	*Lactobacillus plantarum*	100	HM218730.1
4	*Lactobacillus brevis*	99	AB617645.1
5	*Lactobacillus reuteri*	100	JF339967.1
Wheat + Rice			
2	*Lactobacillus acetotolerans*	100	HM218496.1
3	*Lactobacillus plantarum*	100	HM218730.1
6	*Lactobacillus fermentum*	100	HM218434.1
Wheat + Buckwheat			
1	*Lactobacillus helveticus*	99	HM218769.1
2	*Lactobacillus acetotolerans*	100	HM218496.1
3	*Lactobacillus plantarum*	100	HM218730.1
7	*Weissella cibaria*	99	AB593355.1
8	*Weissella confusa*	100	GU369775.1
Wheat + Sweet potato			
1	*Lactobacillus helveticus*	99	HM218769.1
2	*Lactobacillus acetotolerans*	100	HM218496.1
3	*Lactobacillus plantarum*	100	HM218730.1
4	*Lactobacillus brevis*	99	AB617645.1
6	*Lactobacillus fermentum*	100	HM218434.1
7	*Weissella cibaria*	99	AB593355.1
Wheat + Corn			
2	*Lactobacillus acetotolerans*	100	HM218496.1
3	*Lactobacillus plantarum*	100	HM218730.1
4	*Lactobacillus brevis*	99	AB617645.1
5	*Lactobacillus reuteri*	100	JF339967.1
8	*Weissella confusa*	100	GU369775.1

Table 1: Identification of bacillus species by 16S rRNA fragment sequencing results of the selected bands from the DGGE fingerprint.

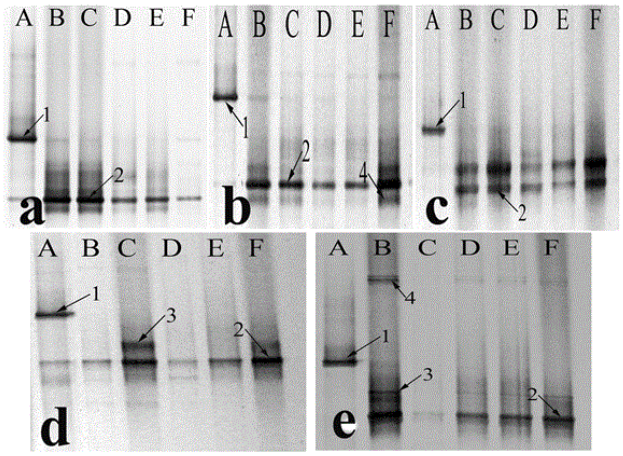

Figure 4: DGGE profiles obtained with fungus primers. a-e: sourdoughs wheat, wheat + rice, wheat + buckwheat, wheat + sweet potato, wheat + corn; A-F: fermented for 1d, 3d, 5d, 7d, 9d and 11d. The identification of the bands is reported in Table 1, bands indicated by numbers were excised, and, after re-amplification and cloning, they were subjected to sequencing.

Strain No.	Closest relatives	GenBank No.
Wheat		
1	Restionaceae environmental sample*	EF024896.1
2	*Saccharomyces cerevisiae*	AB536786.1
Wheat + Rice		
1	Restionaceae environmental sample*	EF024896.1
2	*Saccharomyces cerevisiae*	AB536786.1
Wheat + Buckwheat		
1	Restionaceae environmental sample*	EF024896.1
2	*Saccharomyces cerevisiae*	AB536786.1
Wheat + Sweet potato		
1	Restionaceae environmental sample*	EF024896.1
2	*Saccharomyces cerevisiae*	AB536786.1
3	Fallopia multiflora voucher	EF153715.1
Wheat + Corn		
1	Restionaceae environmental sample*	EF024896.1
2	*Saccharomyces cerevisiae*	AB536786.1
3	Fallopia multiflora voucher	EF153715.1
4	*Pichia pseudocactophila*	EF550380.1

Table 2: Identification of fungi species by 23S rRNA fragment sequencing results of the selected bands from the DGGE fingerprint.

Yeast population dynamics in sourdough

To detect the fluctuation of yeast communities during the sourdough fermentation process, the fungi primers FF390 and FR1 were used, whose feasibility had been verified in our previous studies [30]. DGGE analysis showed that in the sourdough fermentation process, *Saccharomyces cerevisiae* possessed the dominant position in 5 sourdoughs, and *Pichia pseudocactophila* was detected in sample W +C (Table 2 and Figure 4). Though the viable cell count results indicated that the microbial number on YPD plate were below the detection limit (102 CFU/mL) from samples of W+R, W+C since the 7th day of incubation, the detecting of yeast using DGGE method indicated that the combination of culture method and culture-independent method is powerful for the monitor of microbial diversity than the single one. However, some undesirable bands standing environmental samples were also obtained from DGGE gel for the low DNA concentrations of yeasts (Figure 4 and Table 2).

Sensory evolution of Mantou

Though Chinese government have developed the Ministry of Commerce of PR China standard (SB/T10139-93) to regulate the production of Mantou, the evaluation parameters, e. g. exterior, color, structure, elasticity, stickness and flavour, were greatly influenced by human dietary preferences. The typical Mantou should be made solely using wheat in Northern China, while the addition of rice, buckwheat, sweet potato and corn met the eating habits in other areas. Moreover, the high nutritional value and health care function is the main reason for the choice of the hybrid Mantou. For example, buckwheat is rich in amino acids, oleic acid, linoleic acid, vitamins and trace elements, which are benefit for the prevention and treatment of diabetes, cardiovascular disease, ulcers and cancers [31]; the elements in sweet potato are useful for the prevention of cancer and cardiovascular diseases [32] and the vitamins and trace elements containing in corn is claimed to possessed the anti-aging and anti-cancer effects [33].

So, though the scores of specific volume, exterior, color, structure, elasticity, stickness were not so good as wheat Mantou (Table 3), the hybrid Mantou is still liked by most people, especially for the elder in Southern China. In addition, the addition of rice, buckwheat, sweet potato and corn have changed the flavours of Mantou. Take sweet potato for example, the unique sweetness of sweet potato gain a high score of flavoue, though its shape is not so good as other Mantou. Moreover, we just made Mantou following the process of hand workshop, the score of the sensory is just for the Mantou we made and for the experts we invited. For the rates of starter, matrixes, the fermented time, temperature, even the kneading time, will greatly influence the physical and chemical properties and flavour of Mantou.

Mantou types	Vol (mL)	Wgt (g)	Specific volume (mL/g)	Specific volume (score)	Exterior	Color	Elasticity	stickness	Flavour	Total score of sensory
wheat	182.85	75	2.42	18.35	13.8	8	16.45	13.55	6.1	89.35
wheat: rice = 1:1	152.21	75	2.02	15.32	11.32	5.21	14.78	12.67	8.31	80.06
wheat: buckwheat = 1:1	171.32	75	2.28	18.53	12.49	7.87	15.33	14.55	7.23	90.31
wheat: sweet potato = 1:1	167.32	75	2.23	17.65	12.11	6.32	13.21	13.87	8.87	83.42
wheat: corn = 1:1	163.41	75	2.18	16.32	10.23	6.18	13.75	13.27	7.21	77.74

Table 3: Effects of different matrixs on the sensory quality of Mantou.

Conclusion

Though it was widely accepted that fermentation parameters and type of substrate determined the selection of the competitive biota in sourdoughs [34], it was still a challenge to discuss whether the flour and its autochthonous microorganisms drive the selection of the dominant species [35]. Previous studies on the characterization of the sourdough biota showed that the flour itself did not play a key role in driving the selection of the competitive species, and the bakery environment had been proven to define and select the dominant species in sourdoughs [36,11]. However, recent studies indicated that the nature of the substrate posed an important role on the establishment of stable consortia of LABs and yeasts when sourdoughs from gluten-free substrates, cereal and pseudocereal [28,11], which was confirmed in the present study.

In the present study, we found that the matrixes could conversely influence the microbial diversity and number, the microbial yields of metabolites (e.g. unsaturated fatty acids), and the sensory of Mantou. Currently, the steamed breads are widely consumed in the whole China as one of the important staple foods, while the shelf life of Mantou was only 1 week and easily to be contaminated or spoiled by spoilage organisms. Therefore, it is an essential work to investigate the interaction between the microbial community and matrixes during sourdough fermentation, which are beneficial for the shelf life of Mantou, as well as the enhancement of its flavour and nutrients.

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