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Microbial characterization of five Chinese traditional sourdoughs by high-throughput sequencing and their impact on the quality of potato steamed bread

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ABSTRACT

To improve the quality of potato steamed bread, the microbial diversity of 5 different sourdoughs and their effects on potato steamed bread were studied. The proximate composition analysis differentiated the Chinese traditional sourdough (CTS) with different levels of nutrients and pH (3.82–6.22). High-throughput sequencing revealed the predominant microbes in the five CTSs were *Lactobacillus*, *Pediococcus*, and *Wickerhamomyces*. Similarities of bacterial and fungal compositions were observed within them. The fermentation rheological results suggested CTS1 and CTS3 had higher total gas production value (2355.5 mL and 2249.5 mL respectively) than baker's yeast. CTSs resulted in various effects on the appearance and texture properties of potato steamed bread with CTS1 showed the highest specific volume (2.38 mL/g) and sensory score (7.43). *Wickerhamomyces* significantly correlated with the total gas production, hardness, resilience, and lightness of potato steamed bread. The study suggested a potential application of CTSs in fermented potato staple foods.

1. Introduction

Potato is the fourth largest crop from the perennial nightshade *Solanum tuberosum* L. Every 0.25 kg fresh potato can generate 100 kcal energy (Kolasa, 1993) and result in a good feeling of satiety which makes it a good option for resisting hungry. Plus it has balanced nutrition and plain flavor and therefore becomes an ideal alternative staple food. Nowadays, many parts of the world have made potato as their staple foods due to its composite nutritional value and plentiful resources. China is the leading country for potato production and has 96 million tonnes potato produced in 2016 (FAO, 2018). In order to improve the diet structure of its population and enhance their physical health, the Chinese government has initiated the national potato staple food products and industrial development strategy in 2015. New staple foods made out of potato like steamed bread are getting popular in the market and citizens' dining room.

Until now, most of the potato staple foods are still fermented by the usual baker's yeast (fresh or dry) because of its convenience and efficiency. While this kind of dominant starter has an obvious drawback – the plain flavor. Besides its high fermentation efficiency can be a defect when processing potato staple food. Normally, the potato doughs are of higher viscosity than their wheat counterparts as the potato flour used was gelatinized and their viscosity increase along with the fermentation (Mackey, Ofoli, Morgan, & Steffe, 2010). When using baker's yeast to ferment potato dough, the high fermentation rate resulted in faster viscosity increment which will make the dough too sticky to the machine before steaming. So, a temperate control of the fermentation rate can relieve this technical issue somehow.

Sourdough is a traditional leaven agent that has been used for thousands of years in many parts of the world. Because of the lacking of the standard processing method, sourdough is still a niche product in the food industry. It is prepared either by spontaneous or starter-cultured fermentation (De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016). The formula and processing procedure can be varied from region to region thus result in different microbe grown in it (Vuyst, Kerrebroeck, & Leroy, 2017). While generally, the dominant microorganisms are lactic acid bacteria (LAB) and yeasts (Chavan & Chavan, 2011). According to the previous study, sourdough can have various benefits on fermented foods including but not limited to an improved nutritional value, delayed staling and prolonged storage periods (Siepmann,

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Ripari, Waszczynskyj, & Spier, 2018). Recently, certain amounts of studies have investigated the effect of sourdough on the quality of cereals or pseudo-cereals foods (Rinaldi, Paciulli, Caligiani, Scazzina, & Chiavaro, 2017; Schober, Bean, & Boyle, 2007; Wu et al., 2012). However, the impact of sourdough on potato-based foods has not been explored.

High-throughput sequencing (HTS) is an emerging technology in the food industry as it allows the accurate identification of complex microbes in different food matrix, therefore becomes a useful tool for further food study (Ercolini, 2013; Ercolini, De, La, & Iacono, 2012). Compared to the traditional sequencing techniques, HTS is easier to conduct and cheaper to operate.

In this study, HTS technique is employed to identify the microorganisms of 5 different sourdough matrices in order to get an informed understanding of them. The effect of sourdough on the ferment rheology properties and technological quality of potato steamed bread are also evaluated. The correlation analysis between the predominant microbe of sourdough and various characteristics of the potato steamed bread can offer a new prospect in the production of potato staple foods.

2. Materials and methods

2.1. Materials

Wheat flour (protein: 11.8 g/100 g, fat: 1.8 g/100 g, ash: 0.6 g/100 g, dietary fiber: 1.9 g/100 g, moisture 9.9 g/100 g, and carbohydrate: 74 g/100 g) was purchased from Guchuan Food Industry Co., Ltd (Beijing, China). Potato flour (protein: 9.9 g/100 g, fat: 0.3 g/100 g, ash: 2.0 g/100 g, dietary fiber: 6.3 g/100 g, moisture: 12.7 g/100 g, and carbohydrate: 68.8 g/100 g) was provided by Inner Mongolia Linkage Potato Co., Ltd (Inner Mongolia, China). Gluten (protein: 77.5 g/100 g, fat: 0.7 g/100 g, ash: 2.0 g/100 g, moisture: 7.4 g/100 g, and carbohydrate: 12.4 g/100 g) was obtained from Henan Zhong Xing Chemical Co., Ltd. (Zhengzhou, Henan, China). Instant dry yeast, sugar, and dietary sodium carbonate were purchased from the local supermarket.

Five representative sourdough samples are collected from private households and factories in different places of China where they all take sourdough steamed bread as staple food. Samples are freshly prepared by spontaneous fermentation and transported to the laboratory under cooling conditions and stored at −20 °C until analysis. The detailed recipe and processing information of the 5 sourdough samples is shown in Table 1.

2.2. Proximate composition analysis

Sourdough samples are freeze-dried and grounded then subjected to chemical analysis. Moisture, protein, fat, ash and total dietary fiber content were determined according to their corresponding AOAC protocol (Association of Analytical Chemists, 2000). Specifically, moisture content determination was conducted under AOAC method 935.29. Protein content was determined by Kjeldahl method (AOAC method 955.04) with nitrogen conversion factor at 6.25. Crude fat, ash, and total dietary fiber content were analyzed by AOAC method 960.39, 942.05 and 991.43 respectively.

2.3. pH and total titratable acidity determination

The pH and total titratable acidity (TTA) determination were carried out according to Tilman (Schober et al., 2007) with little modifications. 10 g sample was mixed with 90 mL sterile distilled water and homogenized for 2 min. The pH was recorded by a pH meter and TTA was expressed as the volume of 0.1 N NaOH used to neutralize the solution to pH 8.3.

2.4. HTS analysis

All the fresh samples are divided in laminar flow cabinet under aseptic conditions and sealed with sterilized tubes before sending to Biomarker Technologies (Beijing, China) for HTS analysis.

2.4.1. Total DNA extraction and assessment

Total DNA was extracted using OMEGA DNA isolation kit (Omega, D5625-01, USA) according to manufacturer protocol. The quantity and concentration of the extracted DNA are evaluated by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

2.4.2. Amplification of 16S rDNA and ITS region

The V3 + V4 region of the 16S rDNA and ITS region were amplified respectively for the identification of bacteria and fungi. The polymerase chain reaction (PCR) was carried out firstly with 2 min denaturation at 98 °C and then 25 cycles at 98 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min for 16S rDNA and 30 cycles for ITS region. The amplification ended with 7 min extension at 72 °C.

Table 1
Source information, proximate composition, pH, and TTA of Chinese traditional sourdough samples.

Sample ID	Sources	Main Ingredients	Basic manufacture process	Moisture [a] (g/100g)	Protein [b] (g/100g)	Fat [b] (g/100g)	Ash [b] (g/100g)	Total dietary fiber [b] (g/100g)	pH	TTA (mL)
CTS1	Beijing Haileda Food Co., Ltd (Henan branch)	Wheat flour	Flour + Sourdough flour → Appropriate **amount** of water → Spontaneous fermentation for 3 to 5 days → Dry in the shade	10.77±0.00b	8.59±0.03c	0.15±0.03c	0.70±0.01b	2.02±0.07bc	6.22±0.14a	6.38±0.18c
CTS2	Inner Mongolia Wajie Food Co., Ltd	Wheat flour		10.52±0.00b	13.98±0.16b	0.50±0.03b	0.47±0.00d	2.25±0.22b	3.82±0.03d	15.38±0.53a
CTS3	Private household from Shandong Province	Maize flour		11.87±0.00a	8.55±0.11c	3.03±0.01a	1.36±0.01a	3.62±0.15a	5.68±0.07b	5.38±0.18d
CTS4	Private household from Shandong Province	Rice flour		12.73±0.00a	8.15±0.09d	0.18±0.04c	0.21±0.01e	0.87±0.01d	4.67±0.03c	9.25±0.35b
CTS5	Beijing Haileda Food Co., Ltd	Wheat flour		12.24±0.00a	14.38±0.13a	0.49±0.03b	0.57±0.01c	1.70±0.24c	3.92±0.04d	14.5±0.35a

CTS, Chinese traditional sourdough. Results were expressed as means ± standard deviation (n ≥ 2). Data with different letters in the same column were significantly different ($p < 0.05$).
[a]Moisture content was measured on flour basis. [b]The other components were measured on dry basis.

2.4.3. Illumina paired-end sequencing and bioinformatic analyses

The qualified PCR products which were tested by Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA) were sequenced paired-end with Illumina Highseq platform (Illumina Inc, San Diego, CA, USA) at Biomarker Technologies (Beijing, China) according to the company's own protocol. The original raw reads were merged, filtered and then clustered based on the similarity of 97% with UCLUST of QIIME software (version 1.8.0) to have different operational taxonomic units (OTUs). The various OTUs were aligned with the Silva (http://www.arb-silva.de/) and Unite (http://unite.ut.ee/index.php) reference gene database separately for the classification of bacteria and fungi.

The microbial diversity within one single sample was analyzed by alpha diversity (Grice et al., 2009). Indices like Chao1 and ACE richness estimators, Shannon and Simpson diversity indices are adopted to evaluate it. For the discrepancy between individual samples, beta diversity was assessed based on binary Jaccard similarity coefficient (Fligner, Verducci, & Blower, 2002).

2.5. Rheofermentometer measurements

The ferment rheology properties of all the sourdough samples were measured by Rheofermentometer F4 (Chopin Technologies, France). Mixed flour (30% potato flour plus 70% wheat flour) was fermented by different sourdoughs without the addition of baker's yeast. Besides, 2% gluten and 8% sourdough (flour basis) was employed. The dry matters were mixed at a low speed (80 rpm) in a Hobart mixer A-120 (The Hobart Manufacturing Company, Tory, OH, USA), lukewarm water was added properly until dough forms. The final 315 g dough was placed in the vat at the temperature of 35 °C for 6 h.

2.6. Potato steamed bread making

Based on the preliminary experiment, 30% potato steamed bread was prepared as follows. Batches of 200 g mixed flour (20 g sourdough, 60 g potato flour, and 120 g wheat flour), 4 g gluten, 1 g sugar and 140 g lukewarm water were mixed at low speed (80 rpm) in a Hobart mixer A-120 (The Hobart Manufacturing Company, Tory, OH, USA) for 15 min. The dough was leavened for first fermentation in a fermenting box (35 °C, 85% RH) until a polyporous structure was formed. After that, 1 g dietary sodium carbonate was added and the dough was kneaded for 5 min, divided and molded into 100g round-shaped patches and left for 15 min second fermentation. Finally, the well-fermented dough pieces were placed in a steam cooker with a lid covered (Supor Co., Ltd., Hangzhou, Zhejiang, China) and steamed over cold water for 30 min under atmospheric pressure. Instant dry yeast fermented 30% potato steamed bread was made as a control. The ingredients were: 60g potato flour, 140g wheat flour, 1.5g yeast, 1g sugar, 4g gluten, and 130g lukewarm water. The first fermentation time for control bread was 1 h and second was 15 min.

2.7. Specific volume, height/diameter ratio, color measurements, sensory evaluation, and texture profile analysis of potato steamed bread

Specific volume was evaluated by rapeseed displacement method. Height/diameter ratio was measured using a vernier caliper. A Color i5 spectrometer (XRite, USA) was used to test the crust color of the steamed bread in CIE $L^*a^*b^*$ system performed by reflectance method. The setting parameters were as follows: measuring geometry d/8, illuminant D65, observer 10°, slit width 25 mm.

Sensory evaluation was carried out based on a 9-scale hedonic test (1-extremely dislike; 9-extremely like) according to a previous study (Meilgaard, Civille, & Carr, 2016). Questionnaires were handed out to 14 people (6 males and 8 females, age 21–55) who had steamed bread as their staple food to evaluate the general acceptance.

The texture profile analysis (TPA) was carried out using a TA-XT2i Texture Analyser (Stable Microsystems Ltd., Godalming, London, UK). Four slices (20 mm thickness) taken from the central part of each potato steamed bread were evaluated with a 50 mm diameter cylinder probe. The pre-test speed was 2.0 mm/s, test speed 1.0 mm/s, post-test speed 2.0 mm/s, distance 50% and the test gap was 5 s. Hardness, resilience, chewiness, and adhesiveness were calculated from the graphic.

2.8. Statistical analysis

All the experiments were conducted at least in triplicate, the average and standard deviation values were expressed. Statistical analysis and Pearson correlation coefficients (two-tailed) were performed using the Statistical Analysis System (SAS) version 9.2 software (SAS Institute Inc., Cary, NC, USA), $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Proximate composition

As is shown in Table 1, all the sourdough samples had a moisture content of 10.52–12.73 g/100 g. The protein content varied significantly between all the samples where CTS5 possessed the highest amount and CTS4 had the lowest protein content. This might be attributed to the differences of flour used in them (Table 1) because rice flour and corn flour contain lower protein than wheat flour. A former study on *injera* (Baye, Mouquet-Rivier, Icard-Vernière, Rochette, & Guyot, 2013) had indicated that flour composition would influence the fermentation types therefore lead to composition discrepancy of sourdough. For CTS3, the fat, ash and dietary fiber content are the highest which were 3.03, 1.36, and 3.62 g/100 g DW, respectively. The higher ash and total dietary fiber levels could be explained by the higher fiber and mineral elements content in corn flour. The pH of the examined sourdoughs ranged from 3.82 to 6.22 with significant differences ($p < 0.05$). And TTA varied in accordance with pH change. According to previous studies, the pH of sourdoughs was normally between 4.0 and 4.5 (Hayta & Hendek Ertop, 2017) and they were generally classified into three types (Types I, II, and III) (Chavan & Chavan, 2011): Type I is an end-product of previous fermentation that usually has been called traditional sourdough. Type II and III are more industrial and both need baker's yeast (*S. cerevisiae*) for leavening (Böcker, Stolz, & Hammes, 1995), while Type II is usually liquid and Type III is dry. Although some of the examined sourdoughs in this article are not classic sourdoughs regarding the pH value, they are still much more like Type I because of the manufacture style. Acidity is a crucial indicator for sourdough maturity which associated with the production of organic acids by LAB metabolism. Different degrees of acidification will influence the structure-forming components like starch, gluten, and arabinoxylans (Arendt, Ryan, & Dal, 2007). The higher pH value, in this case, might be because of the variation of the processing technology such as starter, flour type, and fermentation conditions (Kati, Kaisa, & Karin, 2007).

3.2. Characterization of sequencing data

A total of 870,831 effective tags were obtained from bacterial analysis and 1,066,983 from the fungal analysis. These effective tags were further assigned to different operational taxonomic units (OTUs) based on 97% similarity. The amount of bacterial OTUs of each sample were: 88 for CTS1; 43 for CTS2; 58 for CTS3; 93 for CTS4 and 35 for CTS5. While the amount of fungal OTUs of each sample were: 54 for CTS1; 110 for CTS2; 58 for CTS3; 90 for CTS4 and 102 for CTS5 (Table 2).

Microbial richness estimators (ACE and Chao1) and diversity indices (Simpson and Shannon) are used to evaluate the alpha diversity within a certain sourdough sample (Table 2). ACE and Chao1 are both indices that estimate the OTU numbers in a microbial community but

Table 2
Numbers of effective tags, OTUs observed, coverage, and alpha diversity indices of Chinese traditional sourdough.

Sample ID	Bacteria						
	Effective Tags	OTUs Observed	ACE	Chao1	Simpson	Shannon	Coverage
CTS1	178,508	88	102.16	101.6	0.34	1.39	1.00
CTS2	174,988	43	81.36	65.75	0.7	0.71	1.00
CTS3	180,288	58	74.89	66.27	0.83	0.45	1.00
CTS4	175,917	93	98.95	93.75	0.54	1.19	1.00
CTS5	161,130	35	53.98	47	0.53	1	1.00
Sample ID	Fungi						
	Effective Tags	OTUs Observed	ACE	Chao1	Simpson	Shannon	Coverage
CTS1	219,275	54	62.23	58.67	0.51	0.98	1.00
CTS2	213,559	110	121.84	116.6	0.37	1.37	1.00
CTS3	218,579	58	62.54	62.67	0.71	0.65	1.00
CTS4	202,223	90	95.89	95.25	0.8	0.68	1.00
CTS5	213,347	102	102.76	102.2	0.31	1.47	1.00

CTS, Chinese traditional sourdough. OTUs, operational taxonomic units. Chao1 and ACE are both indices of the estimated OTU number. Simpson & Shannon indices represent the microbial diversity. Coverage value measures the representativeness of the sequenced library.

calculated according to different algorithms. Simpson and Shannon indices are used in ecology study to quantitatively decide the biodiversity in a region. Simpson index is negatively correlated with biodiversity while Shannon index is positively related to that.

From the viewpoint of bacteria, CTS1 had the highest richness as well as diversity (ACE: 102.16, Chao1: 101.6; Simpson: 0.34, Shannon: 1.39). While CTS5 had the lowest bacterial richness (ACE: 53.98, Chao1: 47) and CTS3 had the lowest diversity (Simpson: 0.83, Shannon: 0.45). From the fungal aspect, CTS2 had the highest richness (ACE: 121.84, Chao1:116.60) and CTS5 possess the biggest diversity indices (Simpson: 0.31, Shannon: 1.47). Both CTS1 and CTS3 had the lowest fungal richness. CTS3 and CTS4 had the lowest diversity indices. All the coverage rates are about 1.00 means the sequence depth is enough to represent the studied microbe.

Unweighted pair-group method with arithmetic mean (UPGMA) cluster tree based on binary jaccard algorithm was employed to evaluate the beta diversity of different samples (Fig. 1). The differences between different samples can be visually observed based on the length of the tree branch. Fig. 1A indicated that CTS1 and CTS4, CTS2 and CTS5 had close bacterial composition and Fig. 1B showed that CTS1 and CTS3, CTS2 and CTS5 had similar fungal composition. This result is consistent with the following Fig. 2.

3.3. Microbial composition at genus and species levels

In order to identify the specific microorganisms of CTS, the relative abundance distribution of the bacterial and fungal composition at genus and species levels were shown in Fig. 2.

Except for CTS3 whose dominant genus was *Pediococcus* (91.17%), all the sourdough samples were mainly composed of *Lactobacillus* (Fig. 2A). Both *Pediococcus* and *Lactobacillus* are representative genera of lactic acid bacteria (LAB). LAB is considered a crucial microbe in sourdough due to its proteolytic activity, volatile, antibacterial and antifungal compounds formation capacity (Dertli & Yilmaz, 2016). These characteristics give sourdough the ability to ameliorate the quality and shelf life of fermented foods. Besides LAB, CTS1 also had 28.84% of *Acetobacter* and 4.33% of *Pediococcus*; CTS2 possessed 6.17% of *Weissella*; CTS3 had about 3.67% *Acetobacter* and CTS4 identified *Klebsiella* at 2.85%. These results are in consistence with previous researches (Li, Li, & Bian, 2016; Zhang & He, 2013) that the above-mentioned genera are commonly found in Chinese traditional sourdough.

A further identification of the bacterial composition at the species level (Fig. 2B) demonstrated that the dominant species of all the five studied samples remained unspecific due to the knowledge limitation. But in CTS1, CTS3, and CTS4, some *Lactobacillus_brevis* were identified and the relative abundance was 12.77%, 2.23%, and 16.13% respectively. And there was 5.87% *Weissella_confusa* existed in CTS2. *Lactobacillus_brevis* can have a positive effect on the sensory perception of bread by producing exopolysaccharides (EPS) (Di Monaco, Torrieri, Pepe, Masi, & Cavella, 2015). Research about *W. confusa* found that it can efficiently produce EPS especially dextran from the added sucrose in wheat sourdough without strong acid production (Katina et al., 2009).

Fig. 2C showed that the main fungal genus in CTS1 and CTS3 was *Wickerhamomyces* while CTS2 and CTS5 had no detail information about the dominant genera. CTS4 is mainly composed of *Saccharomyces* (91.43%). Also, CTS1 and CTS3 had a *Saccharomyces* proportion about

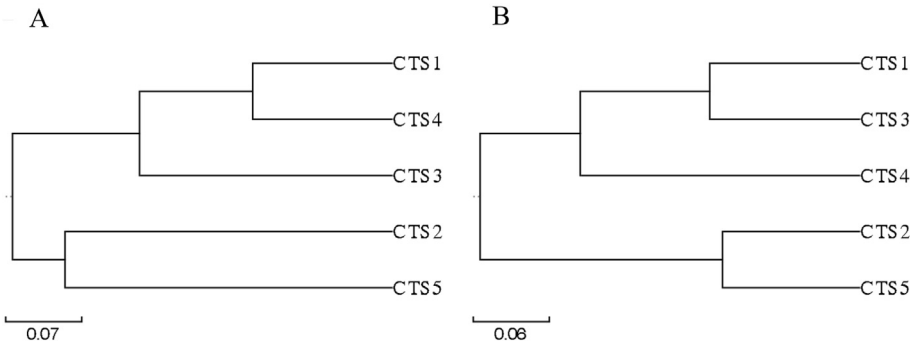

Fig. 1. Bacterial (A) and fungal (B) communities cluster tree of different CTS samples. CTS, Chinese traditional sourdough. CTS1, 2, and 5 are wheat sourdoughs collected from Henan, Beijing, and Inner Mongolia, CTS3 and 4 are maize and rice sourdoughs collected from Shandong province.

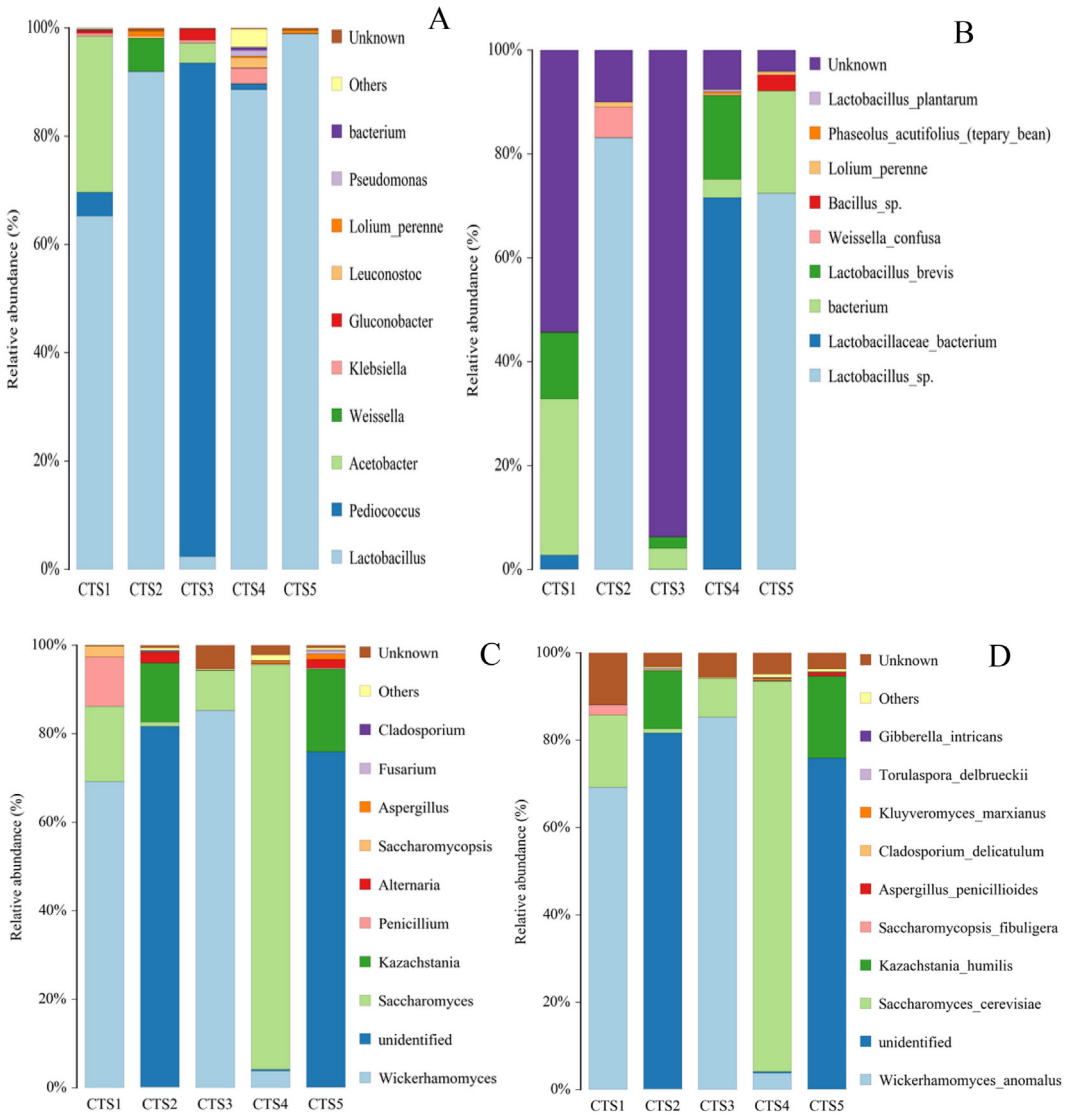

Fig. 2. Relative abundance of the microorganisms in different CTS samples: (A) Bacterial composition at genus level. (B) Bacterial composition at species level. (C) Fungal composition at genus level. (D) Fungal composition at species level. CTS, Chinese traditional sourdough. CTS1, 2, and 5 are wheat sourdoughs collected from Henan, Beijing, and Inner Mongolia, CTS3 and 4 are maize and rice sourdoughs collected from Shandong province.

16.9% and 9% respectively. The *Kazachstania* content in CTS2 was 13.32% and in CTS5 was 18.74%. Besides, an 11.22% of *Penicillium* and 2.37% of *Saccharomycopsis* was identified in CTS1.

A further identification at the species level (Fig. 2D) showed that the *Wickerhamomyces* in CTS1 and CTS3 was *Wickerhamomyces_anomalus*. The *Saccharomyces* in CTS1, CTS3, and CTS4 was found out to be *Saccharomyces_cerevisiae* which is also the species employed in baker's yeast. The *Kazachstania* in CTS2 and CTS5 turned out to be *Kazachstania_humilis* which also refers to *Candida_humilis* and *Kazachstania_exigua* due to the close phylogenetic relationship (Jacques et al., 2016). These abovementioned species are the most common yeast species found in stable sourdoughs (De Vuyst et al., 2016). It is known that *S. cerevisiae* and *W. anomalus* are both maltose-positive yeasts and they can survive low pH and high osmotic pressure conditions. *W. anomalus* is well adapted to the sourdough microbial ecosystems as the only yeast or in an inter-yeast species community (Daniel, Moons, Huret, Vrancken, & De, 2011). Besides, it can assimilate LAB to produce lactate or even phytase (Kurtzman, 2011). *C. humilis* and *K. exigua* are maltose negative and they got a capacity for dough leavening and the formation of small amounts of acetic acid (Kurtzman, 2011). However, they can form a nutritional mutualism relationship with the strictly heterofermentative *L. sanfranciscensis* so that they can hydrolyze sucrose and glucofructans (Gobbetti, Corsetti, & Rossi, 1994).

3.4. Fermentation rheological properties

In order to evaluate the effect of CTS on dough development and gas-production capacity, the fermentation rheological properties of 30% potato dough were demonstrated in Table 3, with baker's yeast as a control. Compared to other sourdoughs, CTS2 and CTS5 showed the lowest dough height and gas volume at maximum development time. CTS1 and CTS3 can produce more gas than baker's yeast as significant differences existed in their V_T values which implies a potential use of these sourdoughs in the amelioration of fermented foods. The discrepancy between sourdoughs' rheology behaviors could result from the different processing methods and diversified formulas. Previously study had shown that processing parameters like dough mixing temperature can have a positive effect on the dough height at maximum development time (Hm) and the addition of salt can negatively affect the fermentation rheological properties (Huang, Kim, Li, & Rayas-Duarte, 2008). It is known that the dominant LAB species in sourdough varied according to its raw material. In laboratory quinoa sourdoughs,

Table 3
Effect of Chinese traditional sourdough on the fermentation rheological properties and technological parameters of potato steamed bread.

Sample ID	Fermentation rheological properties		Technological parameters of potato steamed bread									
	Hm (mm)	V_T (mL)	Specific volume (mL/g)	Height/Diameter ratio	Sensory score	Color			Texture			
						L^*	a^*	b^*	Hardness (g)	Chewiness (g)	Resilience (%)	Adhesiveness
CTS1	16.85 ± 2.33b	2355.50 ± 51.62a	2.38 ± 0.06a	0.71 ± 0.01c	7.43 ± 1.23a	75.95 ± 0.98cd	0.23 ± 0.06c	20.09 ± 1.05ab	3379.82 ± 16.92c	2495.97 ± 39.78d	0.42 ± 0.03ab	0.77 ± -0.06a-b
CTS2	0.15 ± 0.06d	110.67 ± 3.06e	1.69 ± 0.12c	0.86 ± 0.04a	6.33 ± 1.88ab	77.21 ± 0.35bc	0.97 ± 0.16a	19.08 ± 0.69b	6100.53 ± 6.41a	3906.75 ± 8.44b	0.36 ± 0.01bc	0.71 ± -0.02c
CTS3	14.50 ± 0.99bc	2249.00 ± 0.11b	2.10 ± 0.10b	0.64 ± 0.02e	6.88 ± 1.28ab	74.92 ± 0.33d	0.06 ± 0.01e	20.05 ± 0.61ab	2507.83 ± 1.66d	1749.18 ± 8.13e	0.41 ± 0.01b	0.75 ± -0.02a-bc
CTS4	14.00 ± 0.00c	1051.00 ± 0.04d	2.34 ± 0.01a	0.69 ± 0.03cd	6.65 ± 1.57ab	77.28 ± 0.86bc	0.14 ± 0.02d	19.53 ± 0.93ab	2409.02 ± 2.44d	4763.89 ± 9.23a	0.43 ± 0.00a	0.72 ± -0.02b-c
CTS5	0.08 ± 0.05d	103.00 ± 0.00e	1.75 ± 0.17c	0.66 ± 0.01de	6.27 ± 1.37b	77.77 ± 1.59b	0.35 ± 0.05b	17.45 ± 0.16c	5126.92 ± 9.8b	3388.23 ± 7.39c	0.37 ± 0.01bc	0.73 ± -0abc
Control	20.00 ± 0.99a	2086.50 ± 4.95c	2.04 ± 0.18b	0.80 ± 0.00b	7.43 ± 1.32a	81.56 ± 0.71a	−0.14 ± 0.03d	20.38 ± 0.29a	3414.15 ± 5.03c	2644.17 ± 9.14d	0.42 ± 0.05ab	0.80 ± -0.08a

CTS, Chinese traditional sourdough. Control was fermented by commercial yeast. Results were expressed as means ± standard deviation (n ≥ 2). Data with different letters in the same column were significantly different ($p < 0.05$).
Hm, dough height at maximum development time (mm); V_T, the total volume of CO_2 (mL); R_C, the CO_2 retention coefficient (%).

Table 4
Pearson correlation coefficients of microorganisms and technological properties of potato steamed bread.

Microorganisms	TTA	V_T	Hardness	Resilience	L^*	Sensory score
Lactobacillus					0.933*	
Acetobacter						0.899*
Wickerhamomyces		0.934*	−0.892*	0.924*	−0.967**	
Kazachstania	0.913*					

Only significantly correlated variables and coefficients are shown. *$p < 0.05$; **$p < 0.01$.

species *L. plantarum* and *L. brevis* are the dominant ones (Ruiz Rodríguez et al., 2016). But in wheat and rye/maize sourdough ecosystems, *L. brevis* was found to be predominant (Minervini et al., 2012; Rocha & Malcata, 2012). Because of the various formulas of sourdough, different metabolisms of microbes are expected so that may result in diverse rheological behaviors.

3.5. Specific volume, height/diameter ratio, color, sensory, and texture evaluation of potato steamed bread

Specific volume, height/diameter ratio, color, sensory, and texture characters constitute the important quality attributes of potato steamed bread (Table 3). The higher specific volume and height/diameter ratio of steamed bread represent a better product appearance and customer satisfaction. In comparison with the yeast fermented potato steamed bread, only CTS2 and CTS5 demonstrated a significantly lower specific volume. It is known that specific volume is the combined result of the gas productivity of yeast and lactic acid bacteria fermentation and gluten strength of dough (Hoseney, 1994). Levels of acidification can have different effects on bread specific volume (Clarke, Schober, & Arendt, 2002). Therefore, it is reasonable to hypothesize that the acidification degree of different sourdough samples resulted in the various specific volume. This assumption is confirmed by the result in Table 1 that CTS2 and CTS5 showed the lowest pH value (3.82 and 3.92 respectively). In respect of height/diameter ratio, CTS2 had the highest value which was 0.86, while the others were significantly lower than the yeast one. The sensory evaluation showed that the acceptance of sourdough-fermented potato steamed bread had no significant difference ($p > 0.05$) with the baker's yeast fermented sample except for CTS5. All the sourdough-fermented potato steamed bread had a lower L^* and b^* values but higher a^* values. This indicated that the sourdough fermented potato steamed bread had a darker color than the baker's yeast fermented sample.

TPA parameters include hardness, chewiness, resilience, and adhesiveness. Generally speaking, the addition of sourdoughs had various impacts on the texture attributes of potato steamed bread. Among them, CTS2 exhibited the highest hardness value (6100.53 g) and CTS4 the lowest (2409.02 g). Besides, no significant differences ($p > 0.05$) existed between CTS1 and control potato steamed bread. Interestingly, there was a significant negative correlation ($R^2 = -0.857$, $p < 0.05$) between the hardness and specific volume of all the potato steamed bread which was also confirmed by a previous study (Liu et al., 2018). As for the chewiness value, only CTS1 had no significant differences with control potato steamed bread, others were higher. For the adhesiveness behavior, all the sourdough fermented potato steamed bread had lower values than the control ones which meant better mouthfeel. Resilience represents the ability of the bread to recover from deformation and it is normally a contributor to good bread quality. In this study, CTS1 and CTS4 had equivalent resilience value with the control potato steamed bread and others were significantly lower than that.

3.6. Correlations between microbial composition and technological properties

The Pearson correlation coefficient between the abundance of core microorganisms of sourdough and the technological properties of potato steamed bread was calculated with SAS 9.2 software to tentatively analyze the relationship between them. As shown in Table 4, *Wickerhamomyces* was positively correlated with the total gas production (V_T) and resilience of potato steamed bread while negatively correlated with the hardness and L^* value. This result is in accordance with previous research that breads that fermented with composite starter that contained *Wickerhamomyces_anomalus* showed the lower hardness and lightness value compared to those of baker's yeast (Coda et al., 2013). It is already confirmed that bread fermented with sourdough (inoculated with *L. plantarum* 1A7 and *W. anomalus* LCF1695) showed the most volume and softness than baker's yeast bread and appreciated elasticity, color, and overall taste (Coda et al., 2011). Therefore, *Wickerhamomyces* may facilitate the technological attributes of potato steamed bread thus offer a potential development of the non-saccharomyces yeasts in fermentation.

Also, the result showed that *Lactobacillus*, *Kazachstania*, *and Acetobacter* were positively correlated to the L^*, TTA, and sensory score of potato steamed bread respectively. However, other microbial genera like *Pediococcus* and *Saccharomyces* didn't show significant relations to the potato steamed bread's technological qualities. The contrary effect of *Lactobacillus* and *Wickerhamomyces* on the lightness of potato steamed bread suggested that LAB might be efficacious for the improvement of color. A former study using LAB to develop gummy-supplements that showed higher lightness may support this assumption (Lele et al., 2018). The positive correlation between *Kazachstania* and TTA might because of the acid-tolerant character (Hui, Evranuz, & Arroyo-López, 2012) of it. Several studies have validated the improvement of *Acetobacter* on the flavor in liquor and vinegar (Sun et al., 2016; Wang et al., 2015). Therefore, the result of this study may also suggest applications of these microbes in the enhancement of potato steamed bread.

4. Conclusion

The composition of CTS is significantly different form one to another. Microbial composition of the five studied sourdoughs is various but dominated by *Lactobacillus*, *Pediococcus*, and *Wickerhamomyces*. The application of CTS on potato steamed bread showed different influences on the dough fermentation rheological and steamed bread's technological characteristics, with CTS1 and CTS3 performed better. The correlation analysis suggested that *Wickerhamomyces* had a better impact on the quality of potato steamed bread. Other species like *Lactobacillus*, *Kazachstania*, *and Acetobacter* may also help to improve the quality of potato steamed bread. The study suggested a potential employment of CTSs especially some microbial species in the amelioration of potato staple foods.

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Conflict of interest

The authors have no conflicts of interest to declare.

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