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Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread

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ABSTRACT

A total of 105 yeast isolates was obtained from 15 sourdough samples collected from different regions in China and subjected to random amplified polymorphic DNA (RAPD) analysis. Six species were identified including *Pichia membranifaciens*, which has not previously been reported in Chinese sourdoughs. Different species of yeast were used in single-culture fermentation to make Chinese steamed bread (CSB). The volatiles of the CSB were captured by solid-phase microextraction method, separated and identified by gas chromatography-mass spectrometry. In total, 41 volatile compounds were found in all the steamed breads. All CSBs showed a similar volatile profile; however, significant differences in the quantity of some volatile compounds were seen among the CSB fermented by different yeast species. A partial least squares discriminant analysis showed that the CSBs could be separated by their characteristic volatile profiles. The study suggested that the aromatic properties of CSB are determined by the yeast used.

1. Introduction

Sourdough has been traditionally used in making Chinese steamed bread (CSB), a staple food in China, for about 2000 years. CSB is generally formulated with wheat flour, water and sourdough/yeast (Kim, Huang, Zhu, & Rayas-Duarte, 2009), and is steamed after fermentation. Sourdough fermentation confers a remarkable influence on the overall quality of the resulting products because of an abundant metabolic repertoire of the complex microbial communities of sourdough (Gobbetti & Gänzle, 2012). Extensive research reports have revealed that the sourdough microbiota primarily consists of lactic acid bacteria (LAB) and yeasts (Gobbetti & Gänzle, 2012) and the metabolic activities of these microbes in dough fermentation mainly include leavening (yeasts and heterofermentative LAB species), flavor formation (yeasts and LAB) and acidification (LAB) (De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016). In addition, the LAB in sourdough has been considered biotechnologically significant for its various metabolites (Capozzi et al., 2012; Russo et al., 2014). However, a collaboration with yeast is always essential in making cereal-based products because of its remarkable leavening ability and contribution to aroma formation (De Vuyst et al., 2016).

Bread aroma is one of the most important attributes determining its acceptance by consumers (Ruiz, Quilez, Mestres, & Guasch, 2003). In bread making, the metabolic acitivites of yeasts and LAB, especially the yeasts, largely determine the volatile compound profiles of bread crumb (De Vuyst et al., 2016). Despite a tremendous number of studies focusing on volatile profiles of sourdough, sourdough bread or sourdough CSB, the relation between sourdough microbiota and its volatile profile is not fully understood (Ripari, Cecchi, & Berardi, 2016). Some attention has been paid towards revealing the influence of sourdough strains on flavor production by using different starters (Ravyts & De Vuyst, 2011; Ripari et al., 2016). By investigating the single-strain sourdoughs, desirable flavor could be selected and correlated with specific strains. These studies, however, mostly focused on specific strains of LAB. Less attention has been paid to unraveling the influence of different species of yeast on the flavor of bread/steamed bread, despite their prevalence in the sourdough environment and potential commercial importance.

In recent years, some efforts have been exerted to investigate the diversity of yeast in Chinese traditional sourdoughs. Zhang et al. (2011) investigated the yeast microbiota in sourdough samples collected from the western part of Inner Mongolia. Zhang et al. (2015) surveyed 5 sourdough samples from 5 provinces of China to provide some insights into their yeast composition. But the research on this complex microbial niche is far from enough, considering its wide spread prevalence and

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consumption in China. Until now, the microbial composition of traditional sourdough in most regions of China remains unknown, and the roles played by specific species in CSB making are not clear. In this study, we collected samples from more regions than in previous studies, including some areas that have never been covered before, like Shandong and Hebei province, to explore the main species of yeast in Chinese traditional sourdough, and further to illuminate the roles of the different yeast species towards conferring aromatic properties to CSB. This study could provide useful information, which may allow tailored formulation of CSB using specific yeasts for desirable aromas.

2. Materials and methods

2.1. Sourdough sampling and isolation of yeasts

Fifteen sourdough samples were collected from nine provinces of China (Liu et al., 2016). Prior to yeast enumeration and isolation, a refreshment of the sourdoughs was performed to active the microbiota following the procedures described in Fig. S1. Five grams of each sourdough were suspended in 45 mL sterile physiological saline and decimally diluted, and then aliquots of 100 μL of the highest three dilutions (10^{-4} to 10^{-6} or 10^{-3} to 10^{-5}) were plated onto yeast-peptone-dextrose (YPD) agar (yeast extract 10 g, peptone 20 g, dextrose 20 g, agar 18 g, per liter of water) containing 0.1 g of chloramphenicol (Sigma-Aldrich St. Louis, MO) per liter and incubated aerobically at 28 °C for 48 h. Enumeration was repeated twice with appropriate dilutions. For each sample, five to ten isolates were picked from the highest three dilutions based on colony morphology, and purified by successive streaking on YPD agar plate for at least three times. After that, the isolates were cultured in YPD broth for 24 h at 28 °C, and then stored at 4 °C for further analysis.

2.2. Random amplified polymorphic DNA (RAPD) analysis and yeast identification

Genomic DNA of the yeast isolates was extracted using a DNA extraction kit (Sangon Biotech, Shanghai, China). The primer M13 was used for RAPD analysis (Andrighetto, Psomas, Tzanetakis, Suzzi, & Lombardi, 2000). PCR mixture (25 μL) and conditions are listed below: 10× buffer (20 mM Mg^{2+}plus) 2.5 μL, dNTP 2 μL (2.5 mM), primer 1.5 μL (10 μM), DNA template 2 μL, ExTaq 0.25 μL (5 U/μL), ddH_2O 16.75 μL; preliminary denaturation for 5 min at 94 °C, followed by 34 cycles of 94 °C for 1 min, 45 °C for 2 min and 72 °C for 1.5 min, and terminated with an elongation step at 72 °C for 10 min. Electrophoresis of the PCR products and analysis of the obtained RAPD profiles were performed according to the previously described method (Sadiq et al., 2016). For identification of the yeast, one or more representatives of each RAPD fingerprint group were sequenced using the universal primer pairs NL1 and NL4 (Cocolin, Aggio, Manzano, Cantoni, & Comi, 2002). The obtained sequences were deposited in the GenBank database.

2.3. Fermentation properties of the yeasts

The abilities of the yeasts to ferment flour sugars were investigated according to the method described by Nasr et al. (2014). Glucose, fructose, sucrose and maltose, were separately used as the single carbohydrate source. Each test was performed in triplicates and tubes without inoculation were set as control.

The leavening ability of the yeast strains was determined according to the methods reported by Rad and Kasaie (2017), and Yeh, Charles, Ho, and Huang (2009) with some modifications. Outline of the testing device and detailed procedures are shown in Fig. S2. The amount of gas produced by the yeasts during dough fermentation was determined by measuring the volume of the liquid displaced. The dough inoculated with yeast isolated from commercial baker's yeast was used as a positive control. The experiments were repeated twice.

2.4. Chinese steamed bread making

The CSB was made according to the method given by Li, Deng, Li, Liu, and Bian (2015), with some modifications. Wheat flour, water and yeast cells (inoculum size of 10^8 cfu/g dough) were mixed in a dough maker (HMJ-D3826, Guangdong bear electric Co., Ltd. China) to make a dough with a dough yield (dough weight × 100/flour weight) of 150. After mixing for 10 min at a medium speed, the dough was kneaded and shaped manually for 5 min, followed by incubation at 30 °C and 80% RH for 2 h in a hygrothermostat (CTHI-250B; STIK (Shanghai) Co., Ltd, China). After that, the dough was put in a steamer (ASD, Zhejiang, China) when the water was boiling, and was steamed for 20 min. A 2-h fermentation was selected on the basis of production of CSB with baker's yeast.

2.5. Headspace-solid phase microextraction (HS-SPME)

To find out the most appropriate fiber for extracting the volatile compounds, four different fibers (Supelco Inc., Bellefonte, PA) were tested, and the 75 μm Carboxen/polydimethylsiloxane fiber (CAR/PDMS) showed the best extraction of the volatile compounds from steamed bread with more large-area and useful peaks (Fig. S3) agreeing with a previous report (Ruiz et al., 2003), and thus it was selected for subsequent aroma analysis in this work. To extract the volatile compounds, 3 g of steamed bread cut into pieces of about 0.2 × 0.2 × 0.2 cm, were placed into 20-mL headspace vials sealed with screw caps. After 15 min of equilibration at 60 °C, the volatile compounds were absorbed by the fiber during a 30 min extraction (Pacyński, Wojtasiak, & Mildner-Szkudlarz, 2015). The analysis was carried out in duplicate and was repeated twice.

2.6. Gas chromatography-mass spectrometry (GC-MS)

The absorbed volatiles were desorbed for 4 min into the injection port of the GC (7890B; Agilent Technologies) at 250 °C in splitless mode. The GC was equipped with a DB-WAX capillary column (J & W Scientific, 30 m long × 0.25 mm internal diameter, 0.25 μm film thickness). The column temperature program was 40 °C for 2 min, then heating at 5 °C/min to 230 °C. Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The GC was coupled to an MS detector (5977A; Agilent Technologies) used in scan mode (m/z 35–500) with an electronic impact of 70 eV. The temperatures of the quadrupole, ion source and transfer line were 150, 230 and 230 °C, respectively.

2.7. Compound identification

Volatile compounds were identified by (a) comparing their retention times and mass spectra with those of authentic standards, (b) matching their mass spectra with those of a commercial database (NIST 11), and (c) determining retention index (RI) using a C_7–C_{30} n-alkanes series (Sigma-Aldrich, St. Louis, MO). Semi-quantification of the compounds was conducted according to the method employed by Di Cagno et al. (2014).

2.8. Standards

For the identification of volatile compounds, the following standards were purchased. 2-Pentylfuran and trans-3-octen-2-one were obtained from Sigma Aldrich (St. Louis, MO). Ethanol, 3-methyl-1-butanol, 1-octen-3-ol, propanol, 2-methylpropanol, hexanol, pentanol, phenethyl alcohol, 3-methylbutanal, hexanal, heptanal, nonanal, octanal, benzaldehyde, 6-methyl-5-hepten-2-one, acetic acid, hexanoic acid, octanoic acid, pentanoic acid and ethyl acetate were purchased