Evaluation of the effect of *Saccharomyces cerevisiae* on fermentation characteristics and volatile compounds of sourdough

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Revised: 2 March 2018 / Accepted: 12 March 2018 / Published online: 19 March 2018
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Abstract The objective of this study was to unveil insights into the effects of *Saccharomyces cerevisiae* on the development of volatile compounds and metabolites during the dough fermentation in making Chinese steamed bread. Changes in gluten structure under the influence of baker's yeast were studied using scanning electron micrographs (SEM). A unique aroma profile was found comprising some previously reported aromatic compounds and some unreported aromatic aldehydes ((E)-2-Decenal and 2-Undecenal) and ketones (2-Heptanone and 2-Nonanone) in the baker's yeast fermentation. Among metabolites, the most preferred sugar for this yeast (glucose) showed a significant decrease in contents during the initial few hours of the fermentation and at last an increase was observed. However, most of the amino acids increased either slightly or decreased by the fermentation time. SEM of fermented dough showed that the yeast had a very little effect on

starch stability. This study provided some fermentation features of the bakers' yeast which could be used for the tailored production of steamed bread.

Keywords *Saccharomyces cerevisiae* · Metabolites · Scanning electron micrograph · Volatile compounds

Introduction

Chinese steamed bread (CSB) is a wheat-based fermented product of Chinese origin representing around 40% of the total wheat consumption of China. It is now becoming popular in other Asian as well as American and some European countries (Zhu 2014). The microbial ecosystem of sourdough with a great microbial stability confers a plethora of benefits to steamed bread beyond simply endowing it with a unique flavor. CSB can be fermented by either a pure culture of yeast especially *Saccharomyces cerevisiae* or a combination of lactic acid bacteria (LAB) and mold/yeast as in traditional starter cultures, like sourdough, Jiaotou and Jiaozi (Luangsakul et al. 2009; Liu et al. 2012; Zhang and He 2013).

It is well established that, starter cultures with a mixed microbial population are more useful in terms of improving bread's texture, aroma, nutritional value and shelf life due to greater metabolic activities and microbial symbiotic relation (Liu et al. 2012; Zhu 2014). Therefore, it is of great importance to understand microbial interactions and their metabolic activities by designing mixed culture fermentations in order to select specific and suitable starter cultures for fermented products while retaining the traditional traits. However, relying on spontaneously fermented products renders their quality parameters either uncontrolled or unpredictable (Navarrete-Bolaños 2012). The use of

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-018-3122-1>) contains supplementary material, which is available to authorized users.

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selected strains is the need of controlled, optimized and standardized fermentation process.

The role of sourdough (Hansen and Schieberle 2005; Kim et al. 2009; Wu et al. 2012) and some individual strains from its microbiota like *L. plantarum* (Li et al. 2014), *L. sanfranciscensis* (Gobbetti and Corsetti 1997) in developing aromatic compounds in steamed bread has been well studied.

Saccharomyces cerevisiae (baker's yeast) is an important part of the microbial consortia of sourdough steamed bread and plays a key role as a leavening agent by producing carbon dioxide through the alcoholic fermentation of sugars and thus increased the volume of loaf (Paramithiotis et al. 2000). It is important to mention that, as a leavening agent, baker's yeast is still better than traditional starter cultures. For instance, it is about ten times more efficient (as a leavening agent) as compared to Jiaozi (Liu et al. 2012). Yeast fermentation is known to substantially reduce the proofing time of dough, which makes the process of steamed bread production simpler (Liu et al., 2012). Yeast also plays a role in the structure formation of gluten and the production of aromatic compounds like alcohols, aldehydes, carbonyls and esters (Montet and Ray 2016). Therefore, there is a lot of focus on the effects of selected yeast strains on aroma, flavor and texture of different types of dough like lean, sweet or frozen dough (Struyf et al. 2017).

Despite the great commercial importance of yeast and its prevalence in the sourdough environment, less attention has been paid in studying the role of *S. cerevisiae* in producing aromatic compounds and metabolites during the fermentation of Chinese steamed bread. Also, there is marked paucity of information on the role of *S. cerevisiae* towards impact on the gluten structure during the bread fermentation. Therefore, the objective of this study is to provide insights into all these properties of *S. cerevisiae* during the dough fermentation. We also combined solid-phase micro-extraction (SPME) and simultaneous distillation–extraction (SDE) to accurately determine aromatic compounds of steamed bread made by *S. cerevisiae*.

Materials and methods

Wheat flour

Flour with low gluten content was used for making CSB, which was purchased from a local supermarket. The protein, moisture, wet gluten and ash contents of the wheat flour were 7.56, 12.57, 18.5 and 0.05%, respectively. Instant dry yeast (Angel brand, made in China) was purchased from a local supermarket.

Fermentation of steamed bread

The steamed bread was made by the modification of the procedure described by Kim et al. (2009). Firstly, wheat flour and instant yeast (0.5% of wheat flour) were remixed and stirred for 10 min, the dough was fermented for 1 h at 28 ± 2 °C and 75% relative humidity in a controlled fermentation cabinet (LX-X24, Shandong, China). Secondly, the dough was rolled into round shape by hands and fermented at 28 ± 2 °C and 75% relative humidity for 30 min in a cabinet. Then, the proofed dough was steamed for 30 min in a steamer (Joyong, Hangzhou, China).

pH and total titratable acidity (TTA)

The pH and TTA values of each sample were determined by standard method (AACC 2000). All experiments were carried out in triplicate.

Metabolite analysis

Concentration of glucose in fermented dough samples was determined by Glucose Assay Kit (Sigma-Aldrich, China).

The compositions of amino acids during *Saccharomyces cerevisiae*-ZJU315 fermentation were determined using amino acid analyzer (L-8900 Hitachi-hitech, Japan), following the method described by Mariod et al. (2010). Samples containing 0.10 g of dough were acid hydrolyzed with 4.0 mL of 6 mol/L HCl in a vacuum-sealed hydrolysis vials at 110 °C for 22 h. Ninhydrin was added to the HCl as an internal standard. The tubes were cooled after hydrolysis, opened and placed in a desiccator under vacuum until dry. The residue was dissolved in a suitable volume of a sample dilution with 0.02 mol/L HCl, filtered through a Millipore membrane (0.45 µm pore size) and analyzed for amino acids.

Dough microstructures

The fermented dough samples at the initial fermentation stage and end of fermentation stage were fixed in 30 g/L glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 4 h at 25 °C. Samples were washed with phosphate buffer for 15 min three times. Then grains were post fixed in 10 g/L osmium tetroxide in phosphate buffer for 1 h at 25 °C. After washing with phosphate buffer, samples were dehydrated in ethanol: 15, 30, 50 and 70% ethanol for 10 min each, 85 and 95% for 15 min each, and 99.5% for 1 h. After dehydrating, samples were critical-point dried and coated with gold. The preparations were observed using a scanning electron microscope (XL30ESEM; Philips Amsterdam, Netherlands).

Volatile compounds analysis

SDE method

Volatile compounds in the steamed bread, fermented by *S. cerevisiae*, as well the control bread were determined by the SDE method using dichloromethane as the extraction solvent as previously described by Zhang et al. (2016). Anhydrous sodium sulphate (Na_2SO_4) was mixed with the solvent-phase extract which was then concentrated to 1 mL at 40 °C using a Vigreux column (30 cm × 2 cm). The samples were then stored in a freezer at − 80 °C for GC–O–MS (Gas Chromatography–Olfactometry–Mass Spectrometer) analysis.

SPME method

Volatile compounds in the samples were determined using the SPME method as previously described by Zhang et al. (2016). Steamed bread crumbs (1.5 g) were placed in 15 mL vials and spiked with 1.5 mg of internal standard d4-pyrazine and sealed with an aluminum crimp cap provided with a needle-pierceable polytetrafluoroethylene/silicone septum. SPME was performed with 75 µm CAR/PDMS (Carboxen/Polydimethylsiloxane) fiber mounted in a manual SPME holder assembly (Supelco, Bellefonte, USA). The sample vial was placed in a 75 °C water bath for 30 min to equilibrate and then the septum was pierced with the SPME needle. The fiber was exposed to the headspace of the sample for 30 min and after the extraction, the fiber was retracted into the needle and immediately transferred to the injection port of a gas chromatograph and desorbed in the GC injection port for 10 min at 250 °C.

Gas chromatograph-mass spectrometer (GC-MS) analysis

The analysis of volatiles was performed on a GC 7890A (Agilent, Palo Alto, CA) coupled to a Triple Quad 7000B (Agilent, Palo Alto, CA), and equipped with a Sniffer 9000 Olfactometer (Gerstel, KG, Germany). Separations in GC were performed on DB-WAX (30 m × 0.320 mm i.d., 0.25 µm film thickness; J & W Scientific, USA). The carrier gas used was ultra-high purity helium and the column had a flow rate of 1.0 mL/min.

The oven temperature was programmed from 40 °C for 3 min, then increases at 5 °C/min to 200 °C, and then increasing at 10 °C/min to 230 °C, whereby it was held for 5 min. The temperatures of the injector and the GC/MS interface were 250 and 280 °C.

Electron-impact mass spectra were generated at 70 eV, with m/z scan range from 35 to 550 amu, with the ion source temperature of 230 °C. Compounds were identified

according to NIST 2.0 mass spectra libraries installed in the GC–MS equipment.

Compounds identification

The identification of volatile compounds was based on the comparison of the mass spectrum and retention index with reference compounds. The RI values and odour descriptions on DB-Wax column, with those of linear retention indices (RIs), having the same/similar odor quality and RI, previously have been reported in www.odour.org.uk. n-Alkanes ($\text{C}_7\text{--C}_{22}$) were analyzed under the same conditions to calculate LRIs: $\text{LRI} = 100 \text{ N} + 100 \text{ n} (\text{t}_{\text{Ra}} - \text{t}_{\text{RN}}) / (\text{t}_{\text{R(N+n)}} - \text{t}_{\text{RN}})$, which was described by Dool and Kratz (1963).

Statistical analysis

Statistical analyses were performed by SPSS.17.0.

Results and discussion

Changes in pH and TTA values

The changes in pH and TTA values (g/100 g of the sample) during the fermentation are presented in Fig. 1. A gradual decrease in pH with a corresponding increase in TTA was noticed during the fermentation process. The initial pH and TTA values of the sourdough sample at the beginning of fermentation was 5.43 ± 0.05 and 7.46 ± 0.11 , respectively, which reached to the final values of 4.8 ± 0.03 and 8.11 ± 0.06 , respectively after 3.5 h of fermentation. It is known that low pH as a result of high acid contents during the fermentation enhances the capacity of dough to retain gases which are produced as a result of LAB or yeast fermentation (Rocha and Malcata 2012).

Fig. 1 Changes of pH and TTA during *S. cerevisiae* fermentation

Changes in metabolites during fermentation

Metabolite changes in terms of glucose and amino acid contents were studied during the dough fermentation of yeasted and non-yeasted samples. Changes in the glucose content during the fermentation process are shown in Fig. 2. It is known that the trace amounts (0.3–0.5%) of free sugars in dough (glucose, sucrose, maltose and fructose) are important sources of carbon for the metabolism of yeast fermentation. These sugars are utilized by yeast producing CO₂ and ethanol. In our trial, we only focused on glucose as it is considered as the most important and preferred sugar for *S. cerevisiae* during the fermentation (Hopkins and Roberts 1936). The results show that during the initial stage (30 min) of fermentation, the control dough (without yeast) contained 1.02 ± 0.08 mg/g glucose contents, which increased to 1.56 ± 0.06 mg/g after 220 min of fermentation. However, in the fermented dough, the glucose contents decreased rapidly during the initial few hours of the fermentation and at last an increase in glucose content was observed. A sharp decrease in glucose content just after the fermentation process shows a huge consumption of glucose as a result of yeast metabolic activity. It is well established that yeast preferentially utilizes glucose and assimilates other sugars like fructose or maltose (Hopkins and Roberts 1936; Verstrepen et al. 2004). Transcriptomic analysis of three different strains of *S. cerevisiae* in bread dough fermentation revealed that glucose genes are expressed just after the onset of fermentation (Aslankoohi et al. 2013). The increase in glucose content in control bread and in yeasted dough at the end of fermentation may be attributed to the hydrolysis of starch or sucrose by the activity of indigenous amylases (α -amylase) or inverses in wheat flour that act on damaged starch granules (Jayaram et al. 2013).

Amino acids are essential component of dough and serve as important flavor precursors. Variation in the concentrations of different amino acids, which include total amino acids, aromatic amino acids, essential amino acids,

Fig. 2 Changes of glucose contents during *S. cerevisiae* fermentation

branched-chain amino acids, sulphuric amino acids and glutamic acid in control and fermented dough at three different stages is shown in Fig. 3. A very slight increase in amino acid contents (essential, branched and aromatic) was observed in yeasted dough sample with a slight decrease in sulphuric and glutamic acid groups. It is known that only a little increase in amino acid contents could be observed during the yeast fermentation of dough as compared to a mixed culture of bacteria and yeast (Spicher and Nierle 1988). The amino acid metabolism of the yeast *S. cerevisiae* has recently been confirmed by transcriptomic studies of different strains of this species during the dough fermentation. It was revealed that different strains (regardless of their genetic background) expressed genes involved in amino acid metabolism in the middle fermentation phase (Aslankoohi et al. 2013).

Microstructural changes during *S. cerevisiae* fermentation

Gluten network plays an important role in retaining the gases produced by yeast and bacteria during the fermentation process. In dough, normally starch granules are embedded firmly in the protein matrix. However, starch granules adapt the reticular pattern or become swollen (Tester 1997) once gluten network is damaged.

SEMs (scale bar 100 and 50 μ m) of yeasted dough samples at the initial and end stages of fermentation are shown in Fig. 4. Before the fermentation, it can be seen that the smooth and uniform starch granules are entrapped in an extensible protein or gluten network. These observations are in line with the previously described observation for a dough microstructure (Kim et al. 2003).

After fermentation only a slight change in starch granules can be observed. However, some starch granules are deformed or ditches are formed over its surface. This may be attributed to the action of endogenous α -amylase in flour or the activity of microorganisms in sourdough on damaged starch granules, which resulted in the alteration of the dough consistency as it is well known that *S. cerevisiae* does not produce starch degrading enzymes, amylases (Ostergaard et al. 2000). It can be concluded that yeast may help in developing the dough structure by less affecting the starch granules and thus helps in improving the gas retention capacity of dough.

Volatile aromatic compounds

A sum of 27 volatile aromatic compounds was extracted using two different methods of volatile extraction (SDE and SPME) followed by their identification using the GC coupled with an Olfactometric detector. Results of the identification are shown in Table 1. A total of 11

Fig. 3 Changes of amino acids during *S. cerevisiae* fermentation (1: beginning of fermentation stage; 2: middle of fermentation stage; 3: end of fermentation stage)

aldehydes, 7 alcohols, 4 ketones, 1 acid, 1 ester and 3 others compounds were detected. In a previous study, over 40 flavor compounds (20 alcohols, 7 esters, 3 alkanes, 1 sulphur, 6 lactones, 6 aldehydes and 3 alkenes) were detected in French sourdoughs fermented with *S. cerevisiae* using the vacuum desorption method (Frasse et al. 1993). Similarly, in another study, a total of 7 different types of volatile compounds (acetaldehyde, ethyl acetate, acetone, ethanol, hexanal, isobutanol and propanol) were found in bread doughs fermented with *S. cerevisiae* using SPME (Torner et al. 1992). Compared to these two reports, more aldehydes were detected in the current study. Among, alcohols, propanol was not found in our study, however, 1-Pentanol, 1-Hexanol and 1-Octanol were recovered that remained undetected in the mentioned study (Torner et al.

1992). A variation in different result is probably due to the usage of different wheat flour varieties as well as different strains of leavening agent. In this context, it is also important to mention that the ability of forming volatile aromatic compounds significantly varies among the species as well among the strains of the same species (Hansen and Schieberle 2005).

More aldehydes were detected using SDE method, while more alcohols were detected using SPME method (Table 1). Among aldehydes, (E)-2-Octenal, Heptanal, (E)-2-Nonenal and (E,E)-2,4-Decadienal were detected in the control bread as well as in the bread fermented with the yeast *S. cerevisiae*. The presence of aromatic compounds in the control bread may be due to the endogenous odorants of wheat flour which are produced as a result of natural

Fig. 4 SEM observation of dough during *S. cerevisiae* fermentation. **A** Beginning of fermentation stage, **B** end of fermentation stage

enzymes and microflora of flour (Hansen and Schieberle 2005). Also, the variety and type of wheat flour affect the aroma generation in sourdough bread to a great extent (Starr et al. 2015).

Results of aldehydes, detected by both SDE and SPME, show that the bread fermented by *S. cerevisiae* produced only four aldehydes that could not be detected in the control bread, namely Nonanal, Furfural, (E)-2-Decenal, and 2-Undecenal. The rest of the seven aldehydes were found in both bread samples by either of the two methods. To the best of our knowledge, the two aldehydes, (E)-2-Decenal and 2-Undecenal and the two ketones (2-Heptanone and 2-Nonanone) have not previously been reported in Chinese steamed breads. The two aldehydes E,E)-2,4-Decadienal and (E)-2-Nonenal are key aroma components of bread crumb (Grosch and Schieberle 1997). It was noted that, in the absence of the yeast the control bread produced higher contents of (E,E)-2,4-Decadienal, (E)-2-Nonenal, (E)-2-Octenal and Heptanal, while in the presence of the yeast high contents of 3-methyl-1-Butanol, 2-methyl-1-Butanol, 2,3-Butanedione and Phenylethyl alcohol were obtained. These findings are in agreement with a previous report (Frasse et al. 1993). It could be explained from the

fact that 2,4-(E,E)-Decadienal, (E)-2-Nonenal, (E)-2-Octenal and Heptanal are lipid oxidation products (Birch et al. 2013), whilst 3-methyl-1-Butanol, 2-methyl-1-Butanol, 2,3-Butanedione and Phenylethyl alcohols are fermentation products from the Ehrlich pathway (Pico et al. 2015). In the presence of low yeast content (control bread) there was an abundant availability of oxygen and thus lipooxygenases efficiently utilized oxygen to convert lipids into aldehydes, while in the presence of yeast, there was a deficiency of oxygen as it was rapidly consumed by yeast to produce the products via Ehrlich pathway. Thus, lipooxygenase enzymes remained unable to transform lipids into aldehydes and ketones (Pauline et al. 2008).

Yeast also converts amino acids into alcohols through transaminase, decarboxylase and reductase reactions in the Ehrlich pathway. For instance, phenylalanine, leucine, isoleucine and valine are converted into aromatic compounds (mainly 2-Phenylethanol and 3-methyl-1-Butanol) by the metabolic activities of yeasts which impart highly desirable flavors to bread crumb (Dickinson et al. 2003). Among all alcohols, 3-methyl-1-Butanol showed the highest proportion, which was surprisingly higher than ethanol. It shows a high amino acid metabolism of the

Table 1 GC-MS identification of volatiles of *S. cerevisiae* steamed bread obtained with SDE and SPME methods

Compounds	RI	SDE		SPME		Odor
		Control	<i>S. cerevisiae</i>	Control	<i>S. cerevisiae</i>	
Aldehydes (11)						
Hexanal	1081	–	19.22	5.78	–	Green
Heptanal	1183	37.13	15.22	–	1.90	Planty green
(E)-2-Heptenal	1318	–	–	1.06	1.88	Fatty
Nonanal	1392	–	2.23	–	3.98	Fattt
(E)-2-Octenal	1425	30.50	9.02	–	–	Nutty
Furfural	1460	–	–	–	2.82	–
Benzaldehyde	1515	–	–	13.27	5.71	Almond
(E)-2-Nonenal	1532	11.53	6.88	–	–	Cucumber
(E)-2-Decenal	1642	–	2.08	–	–	Fatty
2-Undecenal	1750	–	1.41	–	–	Bitter
(E,E)-2,4-Decadienal	1807	19.84	6.24	–	–	Fried potato
Total		100.00	62.30	20.11	16.29	
Alcohols (7)						
Ethanol	925	–	–	–	12.78	Alcohol
3-Methyl-1-Butanol	1198	–	–	–	27.24	Sweet,green
2-Methyl-1-Butanol						
1-Pentanol	1252	–	15.29	7.01	2.60	Alcoholic
1-Hexanol	1361	–	–	19.71	7.37	–
1-Octanol	1551	–	–	1.42	1.02	–
Phenylethyl alcohol	1903	–	–	–	16.66	–
Total		0	15.29	28.14	67.67	
Ketones (4)						
2-Heptanone	1180	–	6.04	–	–	Cheese
2-Octanone	1283	–	8.34	–	–	Fruity
3-Hydroxy-2-Butanone	1292	–	–	–	1.21	
2-Nonanone	1387	–	2.16	–	–	–
Total		0	16.54	0	1.21	
Acids (1)						
Acetic acid	1449	–	–	–	9.46	Acid
Total			0	0	9.46	
Ester (1)						
Ethyl acetate	880	–	–	–	1.10	Fruity
Total			0	0	1.10	
Others (3)						
2-Pentylfuran	1231	–	5.87	43.95	1.60	Fruity
Naphthalene	1740	–	–	7.80	1.25	Camphoric
2,3-butanedione	945	–	–	–	1.42	Sweet
Total		0	5.87	51.75	4.27	

yeast during the fermentation period. It is well known that the production of free amino acids via proteolysis during ferment at ion greatly influence the aroma profile.

Conclusion

This study provided information about the role of *S. cerevisiae* in the production of volatile aromatic compounds and metabolites during the fermentation of steamed bread. A decrease in the concentration of glucose over the course of fermentation was probably related with the metabolism of the yeast during the fermentation. Most of

the amino acids increased either slightly or decreased during the fermentation time. A total of 27 aromatic compounds were identified. SEM of fermented dough showed that the yeast had a very little effect on starch stability. This study may be helpful in selecting desirable flavors and metabolites correlated with the specific *S. cerevisiae* strain used in this study which may further allow tailored formulation of CSB.

Acknowledgements This research was supported by National Natural Science Foundation of China (Grant No. 31601461).

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