



Improved properties of dough fermented with rice wine prepared by mixed *Saccharomycopsis fibuligera* SF7 and *Saccharomyces cerevisiae* SC1

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Abstract

In view of the special aptitudes of the yeast population in both rice wine production and dough fermentation, in this study, the characteristics of dough fermented with rice wine prepared from mixed *Saccharomycopsis fibuligera* SF7 and *Saccharomyces cerevisiae* SC1 (JFC) were evaluated and compared with those of dough fermented directly with the two yeast co-cultures (FC). Dough inoculated with JFC showed higher acidity, reducing sugar content and leavening activity than dough fermented with FC. The water distribution pattern and pasting properties of the JFC-fermented dough changed dramatically after fermentation, and the dough microstructure and extensibility were improved. The steamed bread made with JFC-fermented dough had improved specific volume and showed similar volatile compound profiles to that made with commercial Jiuqu. These results indicated that mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1 could be used as defined starter cultures in rice wine preparation for steamed bread production.

Keywords Dough fermentation · Rice wine · Jiuqu · Yeast · Steamed bread

Introduction

Steamed bread is a type of fermented wheat-based staple food in China and has a history spanning over two millennia. Traditionally, the steamed bread production process involves mixing the dough, fermentation, re-mixing, shaping, leavening and steaming (Luangsakul et al., 2009). Of

these, fermentation is the crucial step in the process and is largely influenced by the type of starter used (Chang et al., 2020; Luangsakul et al., 2009). In addition to Jiaozi and sourdough, rice wine is also widely used in China as a starter for the preparation of steamed bread, as it is rich in yeast and natural enzymes (Huang et al., 2017). Rice wine is a traditional glutinous rice product fermented with the starter Jiuqu (koji) (Sun et al., 2020). Many studies have been conducted to improve the quality of steamed bread such as specific volume, texture, shelf life, nutritional value and sensory properties using this traditional rice wine fermentation biotechnology (Ai-Lati et al., 2018; Sun et al., 2020). However, most of these efforts focused on the optimization of processing parameters and the environmental factors affecting dough fermentation and product quality. There are few studies on the influence of microorganisms in Jiuqu in steamed bread production.

The microbiota in Jiuqu was mainly dominated by the amyolytic strains (*Rhizopus* genus, *Mucor* sp. and *S. fibuligera*) and alcohol-producing strains (*S. cerevisiae*, *Candida glabrata* and *Pichia burtonii*) (Cai et al., 2018; Jeyaram et al., 2008). Although the various microorganisms occurring in Jiuqu endow products with diverse quality characteristics (Son et al., 2018), it is unfortunately difficult to

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maintain a stable microbial community in the starter (Cai et al., 2018), which affected the final properties of rice wine and the quality of steamed bread (Kim et al., 2015). Recently, it has been found that defined starter cultures can be used as one of the approaches to improve the consistency of traditional fermented foods (Aryashad et al., 2023; Luangsakul et al., 2009; Zhao et al., 2023). However, to date, no study has investigated the technological performance of microbial cultures from Jiuqu in the dough fermentation, which may be useful in improving the quality of steamed bread.

Among the various types of microorganisms, yeasts play an important role in determining the CO₂ production, acidification and rheological properties of the dough, thus strongly influencing the quality attributes of the final product, such as texture and flavor (Condessa et al., 2022; Li et al., 2019b). Considering the ecological importance of yeasts in both rice wine and dough fermentation, suitable yeast strains could be selected to design mixed starters for rice wine production, which could further positively contribute to the quality of steamed bread. Traditional rice wine fermentation is a typical process of simultaneous saccharification and fermentation. Two types of yeast, namely *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae* are involved in these stages (Lv et al., 2015). *S. fibuligera* is a representative producer of amylolytic enzymes among yeasts (Cai et al., 2018). However, it showed poor gas production capacity. On the contrary, the efficient utilization of the carbon sources and production of CO₂ is a crucial attribute of *S. cerevisiae* (Li et al., 2019a). Therefore, a synergistic combination of *S. fibuligera* with *S. cerevisiae*, could be used to produce rice wine for dough fermentation. However, until now little information is available on this crucial aspect.

In this study, the use of mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1 isolated from Jiuqu in the production of steamed bread was investigated. The characteristics of dough fermented with rice wine prepared from mixed yeasts (JFC) and the quality of steamed bread were studied and compared with those fermented directly with mixed yeasts (FC). This study is the first to extensively investigate the use of mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1 isolated from Jiuqu in steamed bread production and is relevant for the future preparation and development of effective defined starter cultures.

Materials and methods

Materials

Wheat flour (10.73% protein, 0.37% ash, 0.75% fat and 13.9% moisture) was obtained from Jinyuan Flour Co., Ltd. (Zhengzhou, Henan, China). Glutinous rice (6.58% protein,

0.35% ash, 0.74% fat and 14.02% moisture) was supplied by Xingwang Rice Co., Ltd. (Wuchang, Heilongjiang, China). Two Jiuqu starters were obtained from Shaoguan, Guangdong, China (GS) and Yanbian, Jilin, China (JY), respectively, which was mainly composed of *Rhizopus oryzae*, *S. fibuligera* and *S. cerevisiae*. Instant dry yeast (*S. cerevisiae*) was Angel brand (Yichang, Hubei, China). *S. fibuligera* SF7 and *S. cerevisiae* SC1 were isolated from Jiuqu GS samples using the classical spread plating method (Luangsakul et al., 2009) on WL agar plate. Isolates were identified by partial sequencing of the internal transcribed spacer (ITS) regions. The GeneBank accession numbers for the ITS sequences of SF7 and SC1 are PP510614 and PP510615, respectively. Yeasts were grown in YPD medium at 30 °C and harvested at early stationary phase by centrifugation at 3000 r/min.

Preparation of rice wine

Rice wine was prepared as previously described (Sun et al., 2020). For the mixed yeast-inoculated sample, glutinous rice (500 g) was washed and then soaked in sterile distilled water (500 mL) at 30 °C for 12 h. After steaming for 45 min and cooling to room temperature, yeast cell suspension (cell number of *S. cerevisiae* SC1 and *S. fibuligera* SF7 was 1:1) in 500 mL sterile distilled water was added to the steamed glutinous rice (about 2×10^7 CFU/g rice) and immediately mixed. The mixture was then fermented in Bilon cabinet (HWS180, Shanghai, China) at 30 °C and 85% relative humidity (RH) for 24 h. For the rice wine prepared with Jiuqu starter, Jiuqu (1.5% (w/w) of the glutinous rice) was added as starter instead of mixed yeasts.

Full dough fermentation

To prepare the rice wine fermented dough, wheat flour (500 g) and rice wine (20% on flour basis) were first mixed with water (the total water content was limited to 45% on flour basis) using a mixer (Hauswirt HM790, Qingdao, China) and stirred for 10 min. The dough was then fermented at 30 °C and 85% RH for 8 h to produce rice wine dough. For the steamed bread fermented with mixed yeasts, yeast cells (2×10^7 CFU/g flour) were inoculated, and the water was 45% of the flour. A dough without yeast was prepared as control.

Cell population and chemical determination of dough

The sample for the determination of the microbial count, pH, total titratable acid (TTA), reducing sugars and ethanol content of the dough was prepared as previously described (Cai et al., 2018; Zhou and Li, 2021). The colony forming unit (CFU) count was assessed on WL agar containing

chloramphenicol (0.1 g/L) for yeast and on MRS agar supplemented with natamycin (0.1 g/L) for lactic acid bacteria (LAB) (Palacios et al., 2008). *S. cerevisiae* SC1 and *S. fibuligera* SF7 was identified by its distinct colony color (Supplementary Fig. S1). The pH was measured using a Leici REX pH meter (Shanghai, China). The TTA was defined as the amount (mL) of 0.1 N NaOH to titrate the solution to a final pH of 8.5 (Aryashad et al., 2023). The 3,5-dinitrosalicylic acid method was used to measure reducing sugar content (Miller, 1959). The alcohol content was determined by the potassium dichromate oxidation method (Crowell and Ough, 1979).

Fermentation behavior of dough

The fermentation behavior of dough was determined in a rheofermentometer (Li et al., 2019b). After 8 h of fermentation, the dough was remixed with 10% wheat flour and 4.5% water (on the fully fermented dough basis) for 8 min. Then, 315 g of the dough was placed in the F3 rheofermentometer (Chopin, Villeneuve-La-Garenne, Cedex, France) and fermented at 35 °C for 3 h.

Microstructure analysis

Scanning electron microscopy (SEM) (Quanta 200, FEI, Hillsboro, USA) was used to evaluate the morphology of the dough, and the microstructure of the protein network was visualized by confocal laser scanning microscopy (CLSM) (FV3000, Olympus, Japan) (Zhou and Li, 2021). Dough samples (10 µm) for CLSM were stained with rhodamine B 0.01% (w/v) and were observed at an excitation wavelength of 561 nm.

Water distribution and migration

The T_2 relaxation time of the proton distribution in the dough was measured using a low-field NMR (LF-NMR) spectrometer (MicroMR-CL-I, Shanghai Niumag Electronics Technology Co. Ltd., Shanghai, China) with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Li et al., 2019a).

Pasting property and X-ray diffraction (XRD) pattern

The pasting property of the doughs was determined by using a Rapid Visco Analyzer (RVA) (RVA-TM, Perten, Huddinge, Sweden) (Chang et al., 2020). The sample (3.5 g) and deionised water (25 g) were placed in an aluminium can and mixed for 30 s to form homogenised suspension. The canister was then placed in the RVA and subjected to the test.

The wide-angle XRD patterns of the doughs were obtained on a D8-Advance XRD instrument (Bruker AXS Inc., Karlsruhe, Germany) at 40 kV.

Steamed bread making and evaluation

The steamed bread was prepared as described previously (Sha et al., 2023). Wheat flour (40 g), water (18 g) and fully fermented dough (400 g) were mixed for 8 min. After rolling 20 times on the surface pressure machine, the dough was divided into pieces (100 g). The chunks were then molded and shaped into round dough balls. After proofing at 35 °C for 40 min at 85% relative humidity, the doughs were steamed for 30 min.

After cooling for 1 h, the qualities, including texture, specific volume and volatile components of the steamed bread were evaluated. Texture profile analysis (TPA) was performed using TA.XT2i (Stable Micro Systems, Ltd., Godalming, UK) as reported (Ai-Lati et al., 2018). The rapeseed displacement method was used to determine the specific volume of steamed bread (Yeh et al., 2009). The volatile components of steamed bread fermented with yeast and rice wine were determined by solid-phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC/MS), following the methods of a previous report (Li et al., 2019a). The volatile compounds were subjected to principal component analysis (PCA) using Origin 2018 (Origin Lab Corp., Northampton, USA).

Data analysis

Statistical analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, USA) and the data are presented as mean \pm SD based on triplicate analyses. One-way analysis of variance (ANOVA) and Duncan's multiple range comparison test were used to determine the significant differences between two samples at the 0.05 level.

Results and discussion

Microbiological and chemical properties of fermented dough

The microbial population, pH, TTA, reducing sugars and ethanol content of dough fermented with rice wine prepared from the mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1 (JFC) were compared with those of dough fermented directly with the mixed yeasts (FC) and spontaneously fermented dough (SF) (Table 1). In both JFC and FC doughs, the number of yeasts is much higher than that of LAB, indicating that the yeast is the dominant microbial group in the dough. The pH of FC dough was found to be around 5.04, which was

Table 1 pH, TTA, reducing sugar content, ethanol content and microbial counts of FC, JFC and SF doughs fermented for 8 h

	FC	JFC	SF
pH	5.04 ± 0.06 ^b	4.41 ± 0.12 ^c	5.36 ± 0.03 ^a
TTA (mL)	2.22 ± 0.08 ^b	5.01 ± 0.11 ^a	1.67 ± 0.02 ^c
Reducing sugar (mg/g)	0.15 ± 0.03 ^c	29.92 ± 2.05 ^a	20.4 ± 2.38 ^b
Ethanol (mg/g)	2.15 ± 0.16 ^a	1.43 ± 0.06 ^b	0.54 ± 0.03 ^c
LAB (log CFU)	3.92 ± 0.16 ^b	4.45 ± 0.12 ^a	4.01 ± 0.09 ^b
Yeast counts (log CFU)			
Sc	8.11 ± 0.07 ^a	7.45 ± 0.17 ^b	4.01 ± 0.11 ^{c#}
Sf	6.17 ± 0.19 ^a	4.95 ± 0.12 ^b	–*

FC: dough fermented directly with the *S. fibuligera* SF7 and *S. cerevisiae* SC1

JFC: dough fermented with rice wine prepared from the mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1

SF: spontaneously fermented dough

Sc: *S. cerevisiae*; Sf: *S. fibuligera*

Different superscript letters within the same raw indicate significant difference ($p < 0.05$)

#Indigenous yeast in flour identified as *S. cerevisiae*

*Not detected

higher than that of JFC. In contrast, a significantly higher TTA value was observed in the JFC dough ($p < 0.05$). The results suggested that more acidifying metabolites such as lactic acid were produced during the fermentation of JFC dough and confirmed its high number of LAB (Table 1).

Compared with the SF dough, the reducing sugar content of the FC dough was lower, whereas a higher content was observed in the JFC dough. The changes in reducing sugar content in the dough are determined by the imbalance between starch hydrolysis by the action of amylase and carbohydrate consumption by the yeast species. *S. cerevisiae* can consume carbohydrates remarkably (Li et al., 2019a), so the higher yeast count in FC dough (Table 1) implied that most of the hydrolysis sugar was used to support microbial growth and gas production during wheat dough fermentation. Conversely, the lower *S. cerevisiae* population in the dough and higher amylase activity in the rice wine led to an increase in reducing sugar content (Huang et al., 2017). The ethanol content of the FC and JFC samples was 2.15 mg/g and 1.43 mg/g dough, respectively, higher than that of the SF dough. This may be due to the predominance of the *S. cerevisiae* population in the dough, which is known to be the most efficient ethanol producer (Cai et al., 2018).

Fermentation behavior of dough

The fermentation behavior of the fermented doughs was investigated using a rheofermentometer (Table 2). The results show that the JFC dough had a higher CO₂ production (617 mL) and a comparable gas retention coefficient

Table 2 Fermentation parameters of FC and JFC dough

	FC	JFC
Dough development		
<i>Hm</i> (mm)	23.1 ± 0.2 ^b	35.4 ± 0.7 ^a
<i>h</i> (mm)	22.5 ± 0.05 ^b	35.4 ± 0.7 ^a
<i>T</i> ₁ (min)	127.5 ± 6 ^b	180 ± 0.0 ^a
Gaseous release		
<i>H'</i> m (mm)	37.0 ± 0.6 ^a	33.4 ± 0.3 ^b
<i>T'</i> ₁ (min)	21 ± 1.7 ^b	180 ± 0.0 ^a
Total volume (mL)	397 ± 13 ^b	617 ± 15 ^a
Retention coefficient (%)	97.8 ± 2.1 ^a	98.1 ± 1.3 ^a

Rheofermentometer parameters: *Hm* (mm), height under constraint of dough at maximum development time (mm); *h* (mm), height of dough at the end of the test (mm); *T*₁ (min), time needed to reach the maximum development; *H'*m (mm), maximum height of CO₂ production (mm); *T'*₁, time of the maximum gas formation (min)

FC: Dough fermented directly with the *S. fibuligera* SF7 and *S. cerevisiae* SC1

JFC: Dough fermented with rice wine prepared from the mixed *S. fibuligera* SF7 and *S. cerevisiae* SC1

Different superscript letters within the same raw indicate significant difference ($p < 0.05$)

(RC) compared with the FC dough. Additionally, the maximum gas formation time (min) (*T*₁) occurred later in the JFC dough. This indicated that the gas production rate of the JFC dough continued to increase throughout the whole fermentation process. The final dough height (*h*) and the time to maximum development (*T*₁) confirmed the increased dough development of the JFC dough. Therefore, although the maximum height of CO₂ production (*H'*m) of the JFC dough was lower, the dough resulted in a higher dough development (*Hm*) than the FC dough.

The fermentation behavior of dough is fundamentally determined by the CO₂ production and gas retention capacity of the dough, which was accordingly controlled by several different factors, e.g., yeast count and activity, available carbon source, gluten network structure and acidification level of the dough (Li et al., 2019a; Moroni et al., 2012). Although the *S. cerevisiae* count in the JFC dough was slightly lower, an adequate carbon source (reducing sugar content), and an appropriate acidification level from the LAB microflora could be favorable for its CO₂ generating capacity (Table 1). It was reported that the appropriate amount of organic acids obtained from LAB fermentation can improve the rheological properties and gas retention of the dough (Palacios et al., 2008). In addition, dough acidification could increase the CO₂ production activity of the yeast, allowing for better dough development (Moroni et al., 2012). Therefore, the use of rice wine made from mixed yeasts resulted in a better dough development

behavior than the dough fermented directly with mixed yeasts of *S. fibuligera* SF7 and *S. cerevisiae* SC1.

Water distribution of dough

Water distribution and migration in the fermented dough was obtained by LF-NMR (Fig. 1). In our system, the T_2 was divided into three populations: T_{21} (0.02–0.5 ms), T_{22} (0.5–30 ms) and T_{23} (50–200 ms), representing water molecules tightly bound, less tightly bound and weakly bound to the solids, respectively (Huang et al., 2017). The dough directly fermented with mixed yeasts (FC) showed minimal changes in the T_2 relaxation time profile after fermentation (Fig. 1A). However, when rice wine was used as a starter, both their distribution patterns and peak times shifted dramatically (Fig. 1B). It can be observed that after fermentation, T_{22} of JFC dough became a broader peak and there is a tendency to form a new peak. Meanwhile, the peak time of the proton population decreased dramatically. These changes suggest that dough fermentation with rice wine decreased water mobility and increased water–solid interactions, which may make them more compatible (Li et al., 2018). This redistribution of water may be due to the hydrolysis of starch molecules and acidification during the dough fermentation (Huang et al., 2017; Lu et al., 2022). The maltooligosaccharides degraded from the amorphous regions of starch have the ability to disrupt water interactions with starch, which is important for the distribution of water molecules (Zhang et al., 2019). Acidification during dough fermentation could also affect the glycosidic bonds in the starch granules, increasing their water-binding capacity (Falade et al., 2014).

Microstructure and elongation properties of dough

SEM and CLSM were used to observe the microstructure of the dough (Fig. 2). The FC and JFC dough groups at 0 h both showed a typical dense structure with fewer voids. Starch granules were found to be embedded in the gluten network and tightly bound to the gluten. After fermentation,

JFC dough showed larger gas holes compared to FC samples, and some embedded starch granules detached from the gluten matrix. The gas cells observed internally in the JFC dough indicated a better ability of the structure to trap CO_2 produced during the fermentation. The weakened adhesion between starch granules and gluten could be beneficial for the formation of gluten network in the dough.

The CLSM results showed that a similar gluten structure was found in FC and JFC doughs prior to fermentation, which contained distinct protein aggregation domains. After fermentation, irregular gluten networks and a number of sites of protein aggregation were observed in the FC dough. In contrast, the protein network in the JFC dough had a more homogeneous distribution, with few large protein aggregation sites. Therefore, rice wine fermentation results in a more evenly distributed and more continuous gluten structure, which could be fully stretched. The results were in line with the dough rheological behaviors of the JFC dough, which was characterized by higher dough strength and extensibility ($p < 0.05$) (Fig. 2E, F). The acidification and hydration of the gluten proteins in the dough may modify the hydrophobic and/or electrostatic interactions that are important for the aggregation and development of the three-dimensional gluten network (Sha et al., 2023; Sun et al., 2020; Wieser, 2007).

Pasting properties of dough

The pasting properties of the doughs were measured by RVA (Fig. 3). For the FC dough, the RVA profile after fermentation was slightly higher than that at 0 h, indicating that the viscosity parameters increased after fermentation. On the contrary, the fermented JFC dough showed significantly decreased viscosity parameters as compared with the unfermented dough. Research has shown that many factors can influence the gelatinisation properties of starch, such as starch structure, available moisture, starch-protein interactions and other components (Chang et al., 2020; De Bondt et al., 2020). It was found that the diffraction doublet of the XRD patterns at 2θ 17.1° and

Fig. 1 Relaxation T_2 distribution of (A) the dough fermented directly with mixed yeasts (FC) and (B) dough fermented with rice wine prepared with mixed yeasts (JFC) before and after fermentation

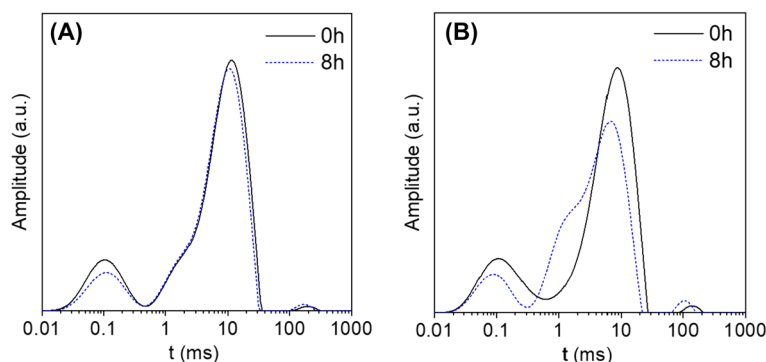


Fig. 2 SEM and CLSM micrographs of (A, B) the dough fermented with mixed yeast (FC) and (C, D) the dough fermented with rice wine made with mixed yeasts (JFC) (A, C) before and (B, D) after fermentation (8 h) (insets in SEM micrographs are the image of representative CLSM micrographs). (E, F) Elongation properties of FC and JFC dough. Different letters above bars in figure show significant difference ($p < 0.05$)

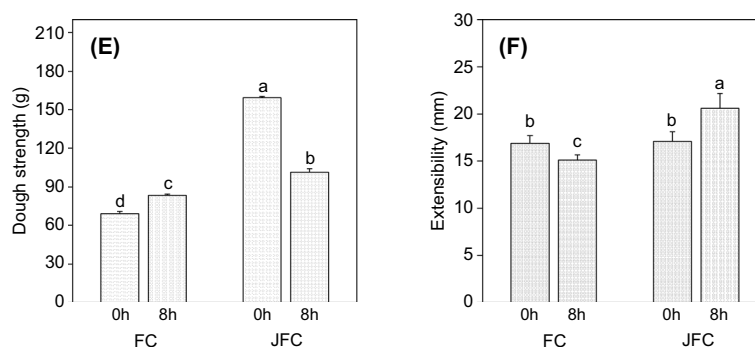
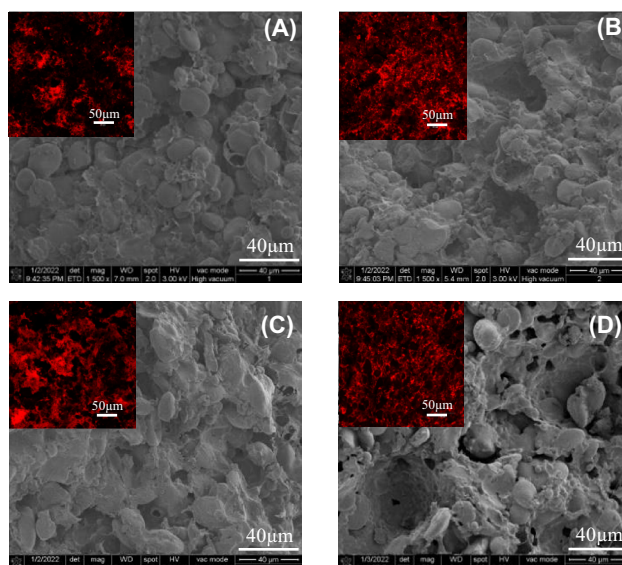
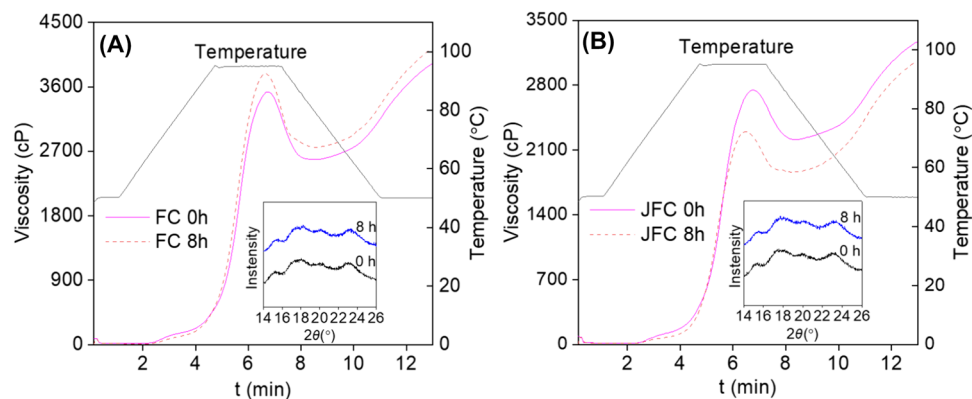


Fig. 3 Rapid visco analyzer (RVA) profile of (A) the dough directly fermented by mixed yeasts (FC) and (B) the dough fermented by rice wine prepared from mixed yeasts (JFC) before and after fermentation (8 h). Inset in the figure of RVA is the X-ray diffraction (XRD) pattern of the dough



18.1° of the JFC dough appeared to be weaker and became less distinguishable after fermentation compared with the FC dough (Fig. 3). Thus, the reduced pasting parameters of the fermented JFC dough could be due to the altered starch structure. On the other hand, the changes could be caused by the reduced connectivity between gluten and starch granules, changes in the water distribution pattern

and partial hydrolysis of starch during the fermentation stage (Ai-Lati et al., 2018; Huang et al., 2017; Li et al., 2018; Zhou and Li, 2021). In addition, sugars could also affect the gelatinization of the starch granules (De Bondt et al., 2020). As starch is the most abundant component in wheat flour, differences in the pasting properties of starch after fermentation could affect dough and product quality.

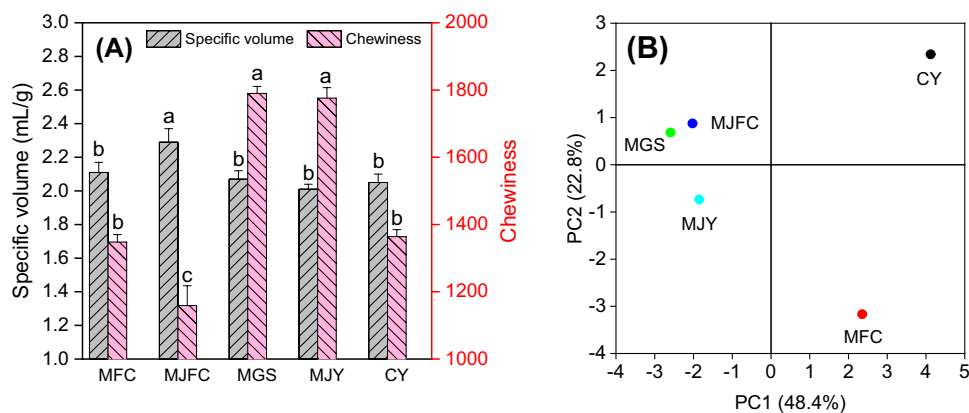


Fig. 4 (A) Comparison of the quality of steamed bread prepared with commercial yeast (CY), mixed yeast (MFC), rice wine fermented with mixed yeasts (MJFC), and rice wine fermented with Jiuqu GS and JY (MGS and MJY). (B) Score plot of PCA showing the aroma

compounds formed in the steamed bread samples fermented with CY, MFC, MJFC, MGS and MJY. Different letters above the bars indicate differences in the same quality parameter between the samples ($p < 0.05$)

Steamed bread quality

The qualities of steamed bread made from JFC dough (MJFC) were evaluated and compared with those made from FC dough (MFC), commercial yeast (CY) and Jiuqu GS and JY (MGS and MJY) (Fig. 4). The MJFC had the highest specific volume and the lowest chewiness value compared with the other samples (Fig. 4A). Specific volume is considered the most important parameter of bread as higher values are correlated with better quality (Na et al., 2024). It reflects the amount of gas produced by the yeast, the characteristics of the gluten network and the gas retention capacity of the dough (Huang et al., 2017). The higher specific volume and lower crumb chewiness of MJFC could be attributed to the higher CO₂ production capacity and acidification level of the JFC dough, which is conducive to the gas cell expansion and improvement of the gluten network strength. High specific volume and softer texture are preferred characteristics of steamed bread by consumers in China, which resulted in a slightly higher sensory score for MJFC (Supplementary Table S1).

PCA was used to get an overview of the differences in the volatile compounds of the steamed bread samples fermented with different starters. As shown in Fig. 4B, the accumulated contribution of the first two principal components was 71.2% and the steamed breads fermented with different starters could be well separated on the score plot, demonstrating the differences in their volatile profiles. The samples MJFC and MGS were grouped together, and closer to MJY, indicating the analogous volatile flavors of the three types of steamed bread compared to those of CY and MFC. The difference in volatile profiles between MGS and MJY may be due to the difference in other microbial populations such as LAB in the two Jiuqu in addition to yeast (Zhao et al., 2023). These

results suggest that satisfactory quality can be obtained when rice wine fermented with mixed yeast is used as a dough starter to make steamed bread.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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