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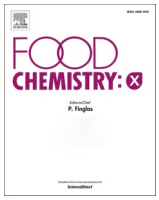

Mixed fermentation of lactic acid bacteria and sourdough on quality and storage characteristics of steamed bun

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ABSTRACT

The effect of mixed fermentation with sourdough and lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus thermophilus*), the physicochemical indexes, storage characteristics of dough and bun were investigated. Compared with sourdough-only dough and bun, the mixed fermentation significantly increase the total phenol, flavonoid and hydrolyzed amino acid content of the dough, the specific volume and height-diameter ratio of mixed fermentation bun increased significantly by 18.3 % and 7.9 %, respectively ($P < 0.05$), along with a significant improvement in sensory quality ($P < 0.05$), and exhibited enhanced skin whiteness (by 2.0 %), with an increase in stomatal density and porosity by 2.6 % and 16.5 %, respectively. During a nine-day storage period, the moisture content near the skin and bun core of steamed bun decreased by 3.9 %, and 1.6 %, respectively, and the aging enthalpy values of mixed fermentation bun were significantly lower than sourdough-only bun ($P < 0.05$). Mixed fermentation providing a theoretical basis for the development of novel steamed bun starters.

1. Introduction

Steamed bun, a fermented flour product produced from wheat flour, stands as a traditional staple food in China (Z. Li et al., 2016) with a rich cultural history, distinctive characteristics, and an enduring legacy spanning over 1500 years (Tang et al., 2023). In contrast to Western staple bun, steamed bun has unique features such as soft texture, low oil and sodium content, and superior nutrient retention (Deng et al., 2022), granting it wide consumer acceptance. Owing to its nutritional value and economic advantages, steamed bun occupies a significant portion of the market demand in China. Sourdough serves as the major starter in steamed bun production (S. Li et al., 2024), and comprises a blend of flour and water containing a variety of lactic acid bacteria (LAB), sourdough and other fungi, among which LAB play an important role in shaping the flavor and texture of dough mixed honey suckle (Y. Liu et al., 2023; Ran et al., 2023) LAB are Gram-positive bacteria with a long history of application in fermented flour products (Akamine et al., 2023). Several studies have reported the contribution of LAB fermentation to the sourdough microbiome and the distinctive characteristics of sourdough-based foods (Suo et al., 2021).

LAB are the most abundant microorganisms in traditional sourdough, and studies have shown that the synergistic fermentation of sourdough and LAB can improve the quality of steamed bun, by delaying aging, improving nutritional value, and prolonging shelf life (Sha et al., 2023);(Liu, Han and Zhou, 2011);(Shen et al., 2022); (Liu TongJie et al., 2016). More recently, the application of LAB in fermented flour products has emerged as a major research focus in the food industry. Ma (Ma et al., 2021) suggested that LAB metabolites can effectively enhance the rheological properties, nutritional components, and flavor substances of dough-derived products, thus significantly improving the quality of whole wheat bun. In contrast to dough fermented by only a single sourdough strain, dough fermented by multiple strains undergoes saccharification and esterification, resulting in the production of various flavor compounds such as esters, aldehydes, alcohols, and organic acids which contribute to imparting a unique flavor and taste to steamed bun (Warburton et al., 2022).

In general, the research investigated the effect of employing a lyophilized LAB starter on the quality and structural characteristics of

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steamed bun. Changes in pH, total titratable acidity, total phenol, flavonoid and hydrolyzed amino acid conten, specific volume, height-diameter ratio, color, sensory quality, core structure, texture characteristics, moisture content, and aging characteristics of steamed bun produced by single fermentation and mixed fermentation during a nine-day storage period were also evaluated. The findings discussed herein provide a theoretical basis for the utilization of a Lyophilized LAB starter in steamed bun fermentation and its potential application in the industrial production.

2. Materials and methods

2.1. Materials and reagents

Lactobacillus plantarum zrx03 (independent intellectual property rights, GenBank No.MN784485) and *Streptococcus thermophilus* 6063 were maintained at the Food Biotechnology Laboratory of the Food College at the Henan Institute of Science and Technology, China. Wheat flour (milling degree SB/T 10139–93) was sourced from COFCO Flour Industry Co., Ltd., Luohe, China. Sourdough powder was obtained from Angel Yeast Co., Ltd., Wuhan, China. White sugar was purchased from Yonghui Supermarket, Xinxiang, China. Skim milk powder, trehalose, and lactose were obtained from Henan Huarui Biotechnology Co., Ltd., Zhengzhou, China. MRS medium was obtained from Beijing Oberstar Biotechnology Co., Ltd., Beijing, China. All the above reagents were of analytical purity.

2.2. Activation and cultivation of bacterial strains

The two LAB strains were retrieved from a − 80 °C refrigerator and streaked onto MRS agar plates. After an incubation period, single colonies were selected and transferred into MRS liquid medium. Bacterial cells were activated after two to three sequential incubation cycles at 37 °C under shaking (100 ×g) for 24 h. The activated bacterial suspension was inoculated into MRS liquid medium and cultured at 37 °C under shaking (100 ×g) to the end of the logarithmic phase of bacterial growth, pH value reached 6.5.

2.3. Preparation of LAB powder

A 20 mL of the LAB suspension cultured until the end of the logarithmic phase was transferred to a 50-mL sterile centrifuge tube under sterile conditions, then submitted to centrifugation at 4 °C and 980 ×*g* for 15 min. The supernatant was discarded in order to collect LAB cells to which a protective agent (trehalose 9.19 %, lactose 14.61 %, and powdered skimmed milk powder 13.16 %) was added in a 1:10 ratio, followed by thoroughly mixing. Then, the mixture was pre-frozen at-80 °C for 4 h, followed by freeze-drying in a cold trap at −70 °C under vacuum in a 1 Pa atmosphere (ALPHA 1–4 LSC, MARTIN CHRIST, Germany) for 24 h.

2.4. Preparation of dough and steamed bun

In a mixing machine, 100 mL of sterile water, 200 g of flour, 2 g of dry sourdough, 2 g (3.0×10^{11} cfu/g) of LAB powder consisting of *L. plantarum* and *S. thermophilus* as lyophilized powder mixed at a ratio of 1:1 (*w/w*) (Preliminary test result), and 2 g of white granulated sugar were combined to prepared mixed fermentation sourdough-lactic acid bacteria steamed bun (L-SSB). The resulting dough was neutralized for 10 min until its surface was smooth. Steamed bun was prepared using the secondary fermentation method (S. Li et al., 2024). The kneaded dough was placed at constant temperature (35 °C) and humidity (85 %) conditions for 60 min, and then divided into 80-g pieces. After CO_2 removal, the second fermentation was carried out at 35 °C for 30 min, followed by steaming for 20 min in boiling water. After a ten-minute interval, the steamed dough was removed, covered with gauze, and allowed to cool down to room temperature for further testing. Dough obtained without LAB powder, i.e., fermented by sourdough only, was designated sourdough-only steamed bun (SSB) and served as the control sample.

2.5. Effect of mixed fermentation on physicochemical properties of dough

The sourdough-only dough (SD) and sourdough-lactic acid bacteria dough (L-SD) were frozen at −80 °C for 12 h. Freeze-dried at a cold trap temperature of −70 °C and a vacuum of 1 Pa for 48 h. The milled powder was sifted through a 1000-mesh sieve, and the freeze-dried powder was placed in a dry dish for storage.

2.5.1. Determination of pH value and total titratable acidity

The pH and total titratable acidity (TTA) of the dough were determined following the method described by Müller et al. (Müller et al., 2021). Accurately weighed the 10 g of dough, added 90 mL of sterile water, and stir evenly by using a magnetic stirrer (SN-MS-6D, Shanghai Shangpu Instrument & Equipment Co., Ltd., China). The dough pH was determined by pH meter (FE28, METTLER TOLEDO Instrument Co., Ltd., China). The suspension was titrated with 0.1 M NaOH, and the total volume of NaOH consumed at pH 8.5 was recorded, which represented the TTA of sample.

2.5.2. Determination of total phenols and flavonoids content

A 3 g of freeze-dried power were mixed with 97 mL of distilled water and heated in a water bath at 65 °C for 2 h. The extracting solution was centrifuged at 4 °C and 7100 ×*g* for 20 min, and the supernatant of each sample was collected.

The total polyphenol content was determined using the Folin-Ciocalteu colorimetric method (Imeneo et al., 2021) and with appropriate modifications. The gallic acid was configured into a standard solution of 0.1 mol/L, and a certain amount of standard solution was taken, and the volume of 1 mL was supplemented with deionized water. 1 mL of 0.25 mol/L Folin-Ciocalteu was added and mixed for 8 min. A 2 mL of 15 % $NaCO_3$ solution was added and reacted for 10 min, the deionized water was used as blank control. Then the sample was incubated in dark at room temperature for 2 h. The absorbance was determined at 760 nm. The standard curve of gallic acid was drawn with absorbance as ordinate and sample concentration as abscissa.

The determination of flavonoid content was carried out with reference to the method described by Papoutsis et al, (Papoutsis et al., 2018) and some modifications were made. The 0.5 mL of extract, 1 mL of deionized water and 0.15 mL of $NaNO_2$ (5 %, w / v) were placed in 5 mL centrifuge tubes and incubated at room temperature for 6 min. The 2 mL of NaOH (4 %, *w/v*) was added and 1.2 mL of deionized water was added to replenish the volume. The sample was incubated in the dark place for 15 min, with deionized water serving as the blank control. The absorbance was measured at 510 nm, and the standard curve of rutin was drawn.

2.5.3. Determination of hydrolyzed amino acid content

A 2.0 g of the sample was weighed in an ampoule bottle and added 4 mL of 6 M hydrochloric acid. The sample was hydrolyzed in a vacuum drying oven at 110 °C for 24 h after sealed with nitrogen. After cooling, the volume was diluted to 100 mL with double distilled water, and 2 mL was taken out and dried on a nitrogen blowing instrument. The sample was redissolved with 2 mL 0.02 M hydrochloric acid, filtered through 0.45 um filter membrane, and determined by amino acid automatic analyzer (S433D, Sykam, Germany).

2.5.4. Determination of flavor substances

The volatile flavor compounds in samples were determined by head space solid phase micro extraction and gas chromatography–mass spectrometry (TRACE1300 + TSQ9000, Thermo Fisher Scientific, US).

Solid-phase micro extraction: a 5 g of freeze-dried powder were

placed into the extraction vial, and the extraction head was conditioned at the gas chromatography inlet for 30 min. The conditioned extraction head was inserted into the membrane and extracted in a constant-temperature water bath at 60 °C for 40 min. Subsequently, the extraction head was removed. The needle was carefully withdrawn and swiftly inserted into the gas chromatography inlet. The volatile components were desorbed for 5 min at 250 °C in splitless mode.

The chromatographic conditions and mass spectrometry conditions refered to the method of Wu (Wu et al., 2012), and the components with similarity greater than or equal to 80 % were compared in the NIST library.

2.6. Effect of mixed fermentation on the quality of steamed bun

2.6.1. Determination of specific volume and height-diameter ratio of prepared steamed bun

Prepared steamed bun were weighed after cooling (m), and the volume (v) of steamed bun was determined using the rapeseed replacement method (S. Li et al., 2024). The specific volume of steamed bun was determined by v/m and the average value was obtained from three replicates.

Additionally, using a vernier caliper, the maximum height (H) and maximum diameter (D) of prepared steamed bun were measured, and height-diameter ratio was calculated by H/D and the average value was obtained from three replicates.

$$\text{Height} - \text{diameter ratio} = H/D \quad (1)$$

2.6.2. Sensory evaluation of prepared steamed bun

A sensory evaluation group consisting of ten individuals was enlisted to assess the sensory attributes of prepared steamed bun. The chosen panelists (five males and five females, aged between 25 and 45 years old) frequently carry out formal sensory evaluation along with regular informal consumption of baked goods, and did not report having food allergies or intolerances. Test samples were of food-grade quality. The sensory evaluation of this study did not require ethical permission. The research group and the participants of the sensory evaluation issued a consent statement, and all participants voluntarily participated in the sensory evaluation and protected their privacy. Sensory evaluation was carried out to assess six attributes in steamed bun samples, namely, skin color, skin structure, internal structure, springiness, taste, and flavor. Quantitative descriptive analysis (QDA) was employed to evaluate sensory analysis data, employing a score system ranging from 0 to 10 (in which 0 = the worst, 10 = the best) for skin color; from 0 to 15 (in which 0 = the worst, 15 = the best) for skin structure, internal structure, springiness and flavor; and from 0 to 30 (in which 0 = the worst, 30 = the best) for taste. Samples were randomly distributed to the panelists in triplicates. The maximal total score for each sample was 100. Specific scoring criteria are detailed in Table 4.

2.6.3. Determination of texture of prepared steamed bun

Prepared steamed bun were placed at room temperature for 1 h, sliced into 2.5 ± 0.5 cm pieces, and texture was assessed at room temperature using a texture analyzer (TA-XT2i, Stable Micro Systems, UK). Each sample was measured three times, and the average value was recorded. The test was conducted using a P/36R flat cylindrical probe under the following conditions: interval time, 5 s; pre-test speed, 2.00 mm/s; centering speed, 2.00 mm/s; measured speed, 2.00 mm/s; test distance, 50 % of sample height. Hardness, springiness, cohesiveness, chewiness, resilience, and gumminess of steamed bun samples were recorded.

2.6.4. Determination of color difference of prepared steamed bun

Steamed bun samples were sliced after cooling, and brightness (L), red-green (a), and yellow-blue (b) values of bun skin and core were recorded using a colorimeter (CR-400, Konica Minolta, Japan). Whiteness (WH) of the sample was calculated according to the Formula (2), and the average value was obtained from ten replicates of each measurement.

$$W_H = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (2)$$

2.6.5. Evaluation of steamed bun core structure

The core structure of steamed bun was analyzed according to the Coda method (Coda et al., 2017). Image J software (National Institute of Mental Health, Maryland, US) was used for analysis using the Ostu's method for image thresholding to calculate stomatal density and porosity of samples.

2.6.6. Determination of steamed bun storage conditions

Steamed bun, prepared as described in Section 2.4., were left to cool, sealed in a fresh-keeping bag, and then stored in a refrigerator at 4 °C. The hardness, gumminess, and chewiness of stored steamed bun was evaluated on day 1, 3, 5, 7, and 9 of storage.

2.6.7. Texture analysis of steamed bun during storage

Changes in hardness, gumminess, chewiness, springiness, cohesiveness, and resilience of stored steamed bun were recorded on day 1, 3, 5, 7, and 9 of storage according to the methods described in Section 2.7.

2.6.8. Determination of moisture content of steamed bun during storage

The moisture content of steamed bun was recorded near the bun skin and the bun core on day 1, 3, 5, 7, and 9 of storage using the 'National Food Safety Standard-Determination of Moisture in Food' (GB 5009.3–2016) employing the 105 °C constant weight method.

2.6.9. Determination of aging enthalpy of steamed bun during storage

Changes in the enthalpy of steamed bun samples stored in sealed bags at 4 °C and of steamed bun core samples freeze-dried after 1, 3, 5, 7 and 9 days of storage were evaluated in a differential scanning calorimeter (DSC) (STA449C, Naichi, Germany). The change of enthalpy can reflect the heat absorbed or released by steamed bread during storage due to physical and chemical changes such as water loss and starch retrogradation. The steamed bun core was freeze-dried and ground to powder, and 2.5 mg of the resulting powder was weighed and placed in an aluminum crucible, to which 7.5 μL of distilled water was added. A small piece of sample was cut and placed flat on the bottom of the sample plate after the mold was gently pressed. Then the sample was placed at 4 °C in a refrigerator for equilibrium overnight, and an aluminum crucible was used as a reference. DSC conditions included a heating rate of 10 °C/min and a scanning range of 20–120 °C. Recorded parameters included onset temperature (T_0), peak temperature (T_p), termination temperature (T_c), and enthalpy value (ΔH).

2.7. Statistical analysis

IBM SPSS Statistics 24 software (Armonk, New York United States, US) was used for one-way analysis of variance (ANOVA), and *P* values <0.05 were considered statistically significant. Graphs were generated using Origin 2022 software (Northampton, Massachusetts, US). Each set of tests was repeated three times, and results were expressed as mean ± standard deviation.

3. Results

3.1. The effects of mixed fermentation on pH and TTA of dough

In the process of dough fermentation, pH value was used to characterize the concentration of hydrogen ions in the dough, and TTA was used to characterize the sourness of the dough. The acidification degree of dough is an important index to evaluate the fermentation

performance, which has an important influence on the dough structure and product quality. It can be seen from Fig. 1 that the addition of lactic acid bacteria reduces the initial pH value of the dough. During the fermentation process, the pH of the dough in the control group decreased slowly from 5.9 to 5.4. The pH value of the mixed fermentation dough of sourdough and lactic acid bacteria decreased rapidly at 0–4 h, slowed down at 4–6 h, and tended to be flat at 6–8 h. The TTA of dough increased rapidly and then tended to be gentle, which corresponded to the change trend of pH.

3.2. The effect of mixed fermentation on the content of total phenols and total flavonoids in dough

Phenolic substances and flavonoids are present in wheat, which have certain antioxidant properties and affect the nutritional value of dough. It can be seen from Table 1 that the content of total phenols and flavonoids in L-SD increased significantly compared with SD ($P < 0.05$). The contents of total phenols and flavonoids in L-SD group were 213.5 μg GAE/g and 114.8 μg RE/g higher than those in SD group, respectively. Based on the analysis of the results, it was concluded that the mixed fermentation of sourdough and lactic acid bacteria could improve the antioxidant properties of the dough.

3.3. The effect of mixed fermentation on the content of hydrolyzed amino acids in dough

The amino acids play an important role in the final quality of dough as the precursor of flavor substances. As shown in Table 2, the content of hydrolyzed amino acids in dough fermented by mixed fermentation was generally higher than that of dough fermented by sourdough-only. The branched-chain amino acids, sulfur-containing amino acids and aromatic amino acids of L-SD were significantly higher than those of SD ($P < 0.05$). Among them, cystine in L-SD increased by 16.3 % compared with SD, phenylalanine, valine and leucine increased by 14.9 %, 11.3 % and 10.1 %, respectively.

3.4. The effect of mixed fermentation on volatile flavor compounds of dough

Flavor had been one of the important indexes to evaluate the quality of steamed bread. As shown in Table 3, there were some differences in the types and contents of volatile flavor substances in different dough. A total of 48 volatile flavor substances such as alcohols, aldehydes, acids and esters were detected in the different dough. The alcohols accounted for the largest proportion in SD and L-SD, followed by aldehydes, acids and esters.

3.5. Specific volume and height-diameter ratio of prepared steamed bun

The specific volume and height-diameter ratio of different steamed bun showed that L-SSB exhibited a significantly higher specific volume compared to the SSB group, respectively ($P < 0.05$), and a slightly higher height-diameter ratio.

Table 1
Contents of total phenols and flavonoids in dough.

Index	SD (μg GAE/g)	L-SD (μg RE/g)
TPC	369.6 ± 3.4[a]	583.1 ± 5.6[b]
FC	256.9 ± 5.3[a]	371.7 ± 2.3[b]

SD: sourdough-only dough. L-SD: sourdough-lactic acid bacteria dough. Significant statistical differences among values within the same line are indicated by different superscript letters ($P < 0.05$). The same below.

Table 2
Hydrolyzed amino acid content of dough.

Types of amino acids	SD g/100 g	L-SD g/100 g	Amino acids increase ratio (L-SD vs SD)
Asp	0.497	0.527	6.0 %
Thr	0.320	0.332	3.8 %
Ser	0.609	0.584	−4.1 %
Glu	3.712	4.021	8.3 %
Gly	0.434	0.464	6.9 %
Ala	0.381	0.413	8.4 %
Cys	0.086	0.100	16.3 %
Val	0.432	0.481	11.3 %
Met	0.149	0.153	2.7 %
Ile	0.406	0.430	5.9 %
Leu	0.784	0.863	10.1 %
Tyr	0.248	0.265	6.9 %
Phe	0.523	0.601	14.9 %
His	0.351	0.346	−1.4 %
Lys	0.269	0.283	5.2 %
Arg	0.408	0.424	3.9 %
Pro	1.239	1.334	7.7 %

SD: sourdough-only dough. L-SD: sourdough-lactic acid bacteria dough.
Note: Aspartic acid (Asp); Threonine (Thr); Serine (Ser); Glutamic acid (Glu); Glycine (Gly); Alanine (Ala); Cystine (Cys); Valine (Val); Methionine (Met); Isoleucine (Ile); Leucine (Leu); Tyrosine (Tyr); Phenylalanine (Phe); Histidine (His); Lysine (Lys); Arginine (Arg); Proline (Pro).

3.6. Sensory quality of prepared steamed bun

Compared to SSB, L-SSB showed varying degrees of improvement in skin color, skin structure, internal structure, springiness, taste, and flavor (Fig. 2). Notably, the taste of the L-SSB steamed bun significantly improved. Thus, the use of mixed fermentation with sourdough and LAB had a positive impact on the overall quality of steamed bun.

3.7. Texture characteristics of prepared steamed bun

Texture characteristics are indicators of steamed bun quality. Hardness, gumminess, and chewiness of SSB and L-SSB steamed bun

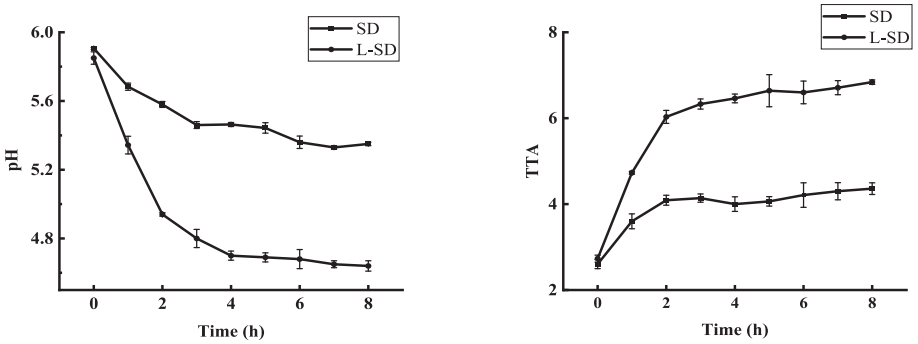

Fig. 1. Changes of pH and TTA in different dough during fermentation.

Table 3
Analysis of volatile flavor compounds in different dough.

description of sample		Proportion of various substances (%)					
		alcohols	aldehydes	acid	ester	others	grand total
SD	Relative content (%)	53.1	19.9	10.7	5.6	10.7	100
	class	6	4	3	2	2	17
L-SD	Relative content (%)	40.5	26.3	18.1	9.2	5.9	100
	class	10	8	4	4	5	31

SD: sourdough-only dough. L-SD: sourdough-lactic acid bacteria dough.

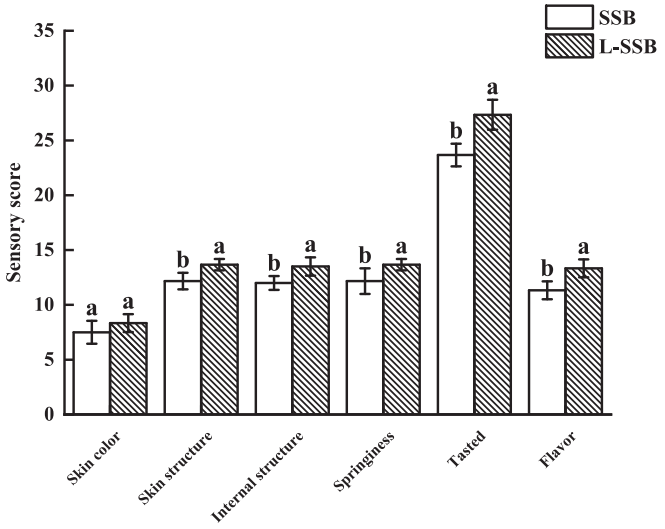

Fig. 2. Changes in steamed bun sensory score.

exhibited a negative correlation with quality, while springiness, cohesion, and resilience showed a positive correlation with quality. Hardness, gumminess, and chewiness of L-SSB were significantly lower than in SSB ($P < 0.05$; Table 5), being 35.8 %, 36.9 %, and 37.9 % lower, respectively, compared to SSB. Springiness, cohesion, and resilience were higher in L-SSB compared to SSB, but there was no significant ($P > 0.05$).

3.8. Color changes in prepared steamed bun

Compared to SSB, the Brightness (L) value of L-SSB skin increased significantly by 2.1 % ($P < 0.05$), although Red-green (a) and Yellow-blue (b) values did not change significantly ($P > 0.05$). Interestingly, whiteness in L-SSB also increased significantly by 2.0 % ($P < 0.05$; Table 6). The steamed bun core of L-SSB samples was significantly increased by 34.5 % ($P < 0.05$) for value a, although no significant changes in L, b, and whiteness (WH) values were observed ($P > 0.05$). In summary, these results showed that mixed fermentation with sourdough and LAB increased brightness and whiteness of steamed bun surface, and reduced greenness of the steamed bun core.

Table 4
Sensory scoring criteria of steamed bun prepared in the present study.

Attribute	Evaluation criteria	Score
Bun skin color	Bun skin color is bright and normal	8–10
	Bun skin color is normal, the brightness is poor	4–7
	Bun skin color is gray	1–3
Bun skin structure	Bun skin is smooth	11–15
	Bun skin is slightly blistered, and wrinkled	6–10
	Bun skin is severely spotted, blistered, and wrinkled	1–5
Bun internal structure	Bun pores are small and uniform	11–15
	Bun pores are uniform and coarse	6–10
	Bun pores are rough and uneven	1–5
Bun springiness	Fast rebound, able to recover	11–15
	Slow rebound, able to recover	6–10
	Cannot be restored	1–5
Bun taste	Refreshing, non-sticky to teeth, bite force	26–35
	Bite general, non-sticky to teeth	16–25
	No bite, non-sticky to teeth	6–15
	No bite, sticky to teeth	0–5
Bun flavor	Rich wheat flavor	11–15
	Low wheat aroma or no peculiar smell	6–10
	Odorous	0–5

Table 5
Texture characteristics of prepared steamed bun.

Attribute	SSB	L-SSB
Hardness	474.1 ± 8.3^{a}	304.5 ± 10.4^{b}
Springiness	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
Cohesiveness	0.9 ± 0.0^{a}	0.9 ± 0.0^{a}
Gumminess	410.4 ± 12.8^{a}	258.9 ± 15.8^{b}
Chewiness	396.3 ± 29.3^{a}	246.1 ± 19.4^{b}
Resilience	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}

SSB: sourdough-only steamed bun. L-SSB: sourdough-lactic acid bacteria steamed bun. Significant statistical differences among values within the same line are indicated by different superscript letters ($P < 0.05$). The same below.

3.9. Core structure of prepared steamed bun

The core structure is one of the major factors determining the quality of steamed bun. Specifically, stomatal density and porosity reflect pore distribution within steamed bun, which is an intuitive expression of dough gas production and gas holding capacity. Fig. 3 depicts changes in the core structure of different steamed bun prepared in the present study. SSB exhibited fewer and unevenly distributed pores, with large gas chambers. In contrast, L-SSB displayed denser stomata, hence stomatal distribution was more uniform and denser compared to SSB. The stomatal density and porosity were not statistically different between SSB and L-SSB (199.7 ± 2.1 vs. 205.0 ± 3.0), but the porosity was significantly increased from 29.8 ± 2.6 for SSB to 34.8 ± 1.5 for L-SSB ($P < 0.05$). Thus, mixed fermentation with sourdough and LAB was shown to improve dough gas production capacity and gas holding capacity, generating steamed bun with a more uniform and finer internal structure.

Table 6
Color differences in steamed bun skin and core prepared in the present study.

Sample type	Brightness (L)	Red-green (a)	Yellow-blue (b)	Whiteness (WH)
SSB bun skin	77.8 ± 0.3^{b}	-0.4 ± 0.0^{a}	14.6 ± 0.2^{a}	73.4 ± 0.3^{b}
L-SSB bun skin	79.4 ± 0.3^{a}	-0.4 ± 0.0^{a}	14.4 ± 0.1^{a}	74.9 ± 0.3^{a}
SSB bun core	72.2 ± 0.9^{a}	-0.3 ± 0.0^{b}	14.0 ± 0.3^{a}	68.9 ± 0.9^{a}
L-SSB bun core	73.2 ± 0.1^{a}	-0.2 ± 0.0^{a}	14.0 ± 0.0^{a}	69.7 ± 0.1^{a}

SSB: sourdough-only steamed bun. L-SSB: sourdough-lactic acid bacteria steamed bun. Significant statistical differences among values within the same line are indicated by different superscript letters ($P < 0.05$). The same below.

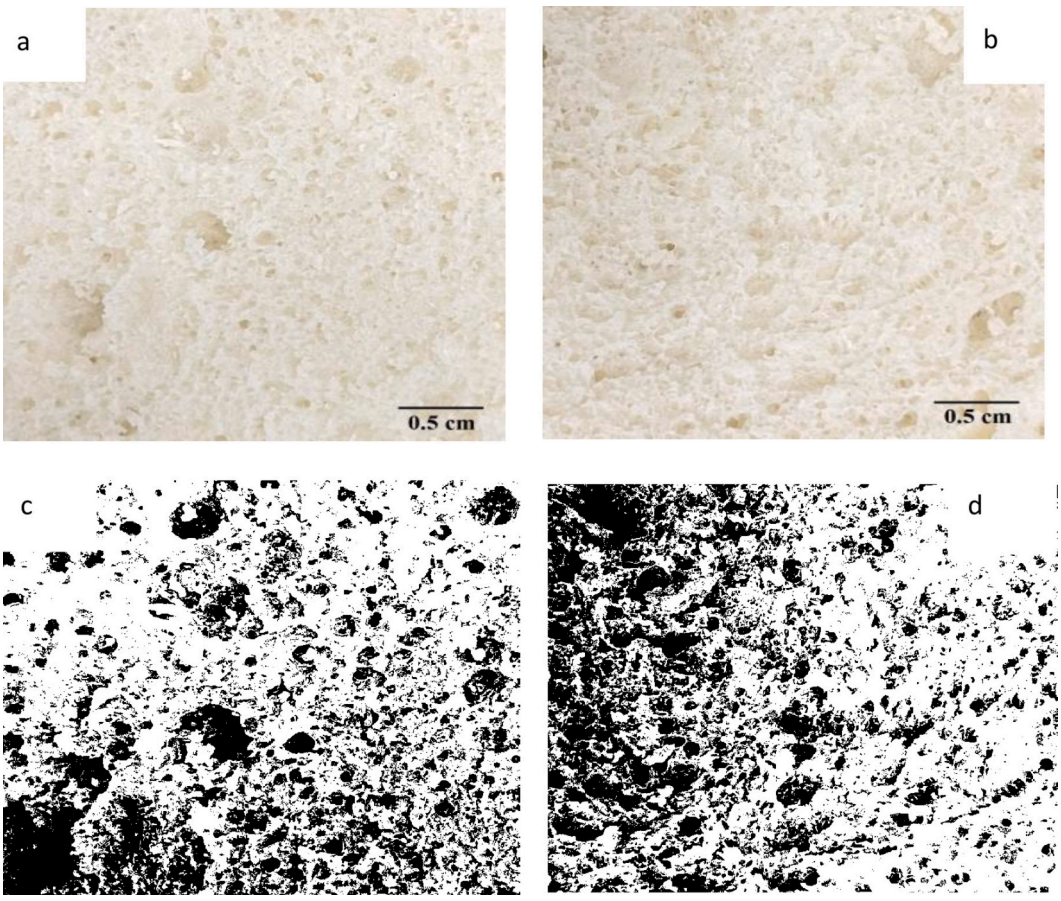

Fig. 3. Micrographs of the internal structure of sourdough-only steamed bun (SSB) and sourdough-lactic acid bacteria steamed bun (L-SSB). (a) SSB core structure diagram. (b) L-SSB core structure diagram. (c) SSB core structure diagram treated using Image J software. (d) L-SSB core structure diagram treated with Image J software.

3.10. Texture characteristics of steamed bun during storage

Texture traits such as hardness, gumminess, and chewiness, are negatively correlated with steamed bun quality, tending to increase when the quality of steamed bun deteriorates, which is the main aspect that negatively influences consumer acceptance. Springiness and cohesiveness belong to compressive relaxation, which are positively correlated with steamed bun quality, and an increase in these values indicates a strong and resilient steamed bun.

During storage hardness, gumminess, and chewiness of steamed bun showed an initial upward trend up to day 7 and then decreased (Fig. 4). This may be related to drying and loosening intitial structure due to water loss, which greatly reduced the internal force of steamed bun. Up to 7 days of storage, L-SSB had significantly lower hardness, chewiness and gumminess compared to SSB ($P < 0.05$), which could be attributed to an improve in the rheological properties of the dough and an increase in specific volume of steamed bun. On day 9 of storage, L-SSB showed a similar level compared to SSB (Fig. 4).

These findings showed that mixed fermentation with sourdough and LAB had a positive impact on hardness, chewiness and gumminess, also during storage up to 7 days. Springiness, cohesiveness, and resilience in steamed bun showed a decrease as storage time prolonged, which could be attributed to starch aging and water loss. In particular, cohesiveness and resilience of steamed bun decreased significantly ($P < 0.05$) in both sample group during the first three days of storage, and decreased at a slower rate during the subsequent days of storage, then gradually stabilized. No significant differences were found in springiness, cohesiveness, and resilience among the different types of steamed bun.

3.11. Changes in moisture content of steamed bun during storage

In steamed bun, water migrates during storage, which results in quality loss, and its texture change and starch aging are closely related to the water loss of steamed bun. Changes in moisture content near the skin of different steamed bun during a nine-day storage period are shown in Table 7. The moisture content near the skin of steamed bun decreased as storage time increased. During storage, the moisture content of L-SSB was higher than that of SSB. Specifically, after storage for nine days, the moisture content near the skin in SSB decreased by 4.6 % compared to the first day of storage, while in L-SSB decreased by 3.9 %. This indicates that the mixed fermentation with sourdough and LAB is beneficial in enhancing both the degree and rate of water loss during steamed bun storage. Different steamed bun decreased at a slower rate as storage time increased.

3.12. Changes in aging enthalpy of steamed bun during storage

It is known that changes in the quality of steamed bun during storage are closely related to changes in gel structure formed by protein, polysaccharide, and starch. During the steaming process, the dough's gluten skeleton becomes fixed, and starch molecules rapidly expand due to heating. During the gelatinization process, hydrogen bonds between starch molecules break, transforming starch from a crystal structure to an amorphous structure. During storage, the gelatinized starch molecules were reordered due to water loss. The crystal structure of insoluble starch molecules also re-forms, which destroys the stability of the gel network formed during the steaming process. This leads to the increase of steamed bread hardness and the deterioration of taste, which becomes

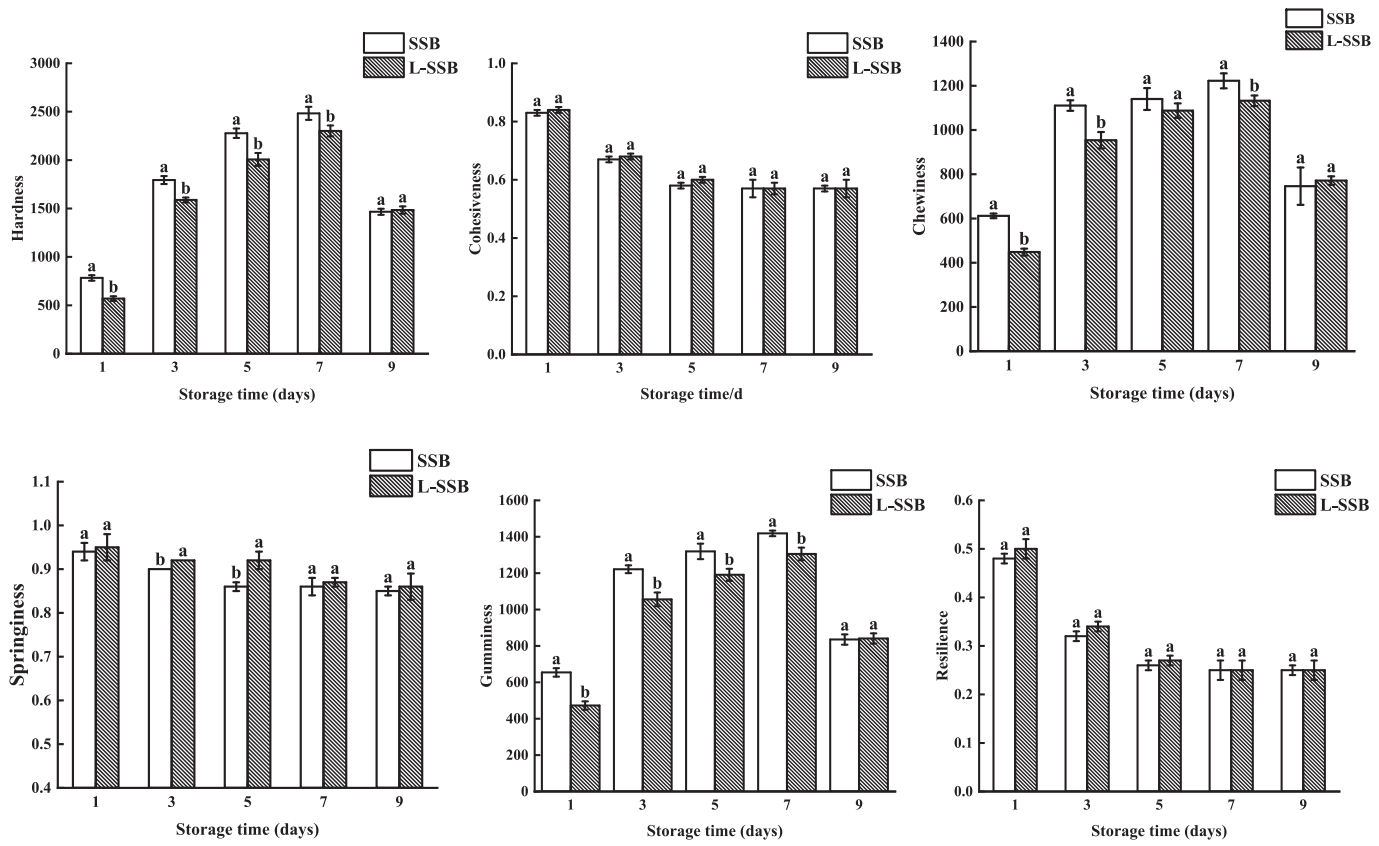

Fig. 4. Changes in hardness, gumminess, chewiness, springiness, cohesiveness and resilience of prepared steamed bun during 1 to 9 days of storage.

Table 7
Changes in moisture content in prepared steamed bun during 1 to 9 days of storage.

Day of storage	Steamed bun skin		Steamed bun core	
	SSB (%)	L-SSB (%)	SSB (%)	L-SSB (%)
1	42.8 ± 0.12^{ab}	44.4 ± 0.7^{a}	40.6 ± 0.6^{b}	40.2 ± 0.4^{b}
3	42.1 ± 0.3^{b}	43.7 ± 0.9^{a}	40.1 ± 0.2^{b}	40.2 ± 0.2^{b}
5	42.4 ± 2.0^{a}	43.4 ± 2.5^{a}	40.2 ± 0.2^{a}	40.1 ± 0.3^{a}
7	41.8 ± 1.4^{ab}	43.0 ± 2.0^{a}	40.0 ± 0.2^{b}	39.9 ± 0.2^{b}
9	40.9 ± 1.6^{a}	42.6 ± 1.1^{a}	40.0 ± 0.2^{a}	39.5 ± 0.1^{a}

SSB: sourdough-only steamed bun. L-SSB: sourdough-lactic acid bacteria steamed bun. Significant statistical differences among values within the same line are indicated by different superscript letters ($P < 0.05$). The same below.

the main factor of steamed bread quality deterioration (Siriamornpun et al., 2016; Wang ShuJun et al., 2015).

Changes in starch aging enthalpy during steamed bun storage are shown in Fig. 5. During day 1 to 7 of storage, ΔH values of SSB and L-SSB exhibited a gradual increase, and then stabilized during day 7 to 9 of storage. Furthermore, ΔH values of L-SSB were significantly lower than those of SSB throughout storage ($P < 0.05$). Taken together, these results indicate that mixed fermentation with sourdough and LAB can be considered an effective method for slowing down the deterioration rate of steamed bun and extending its shelf life.

4. Discussion

Steamed bun obtained by mixed fermentation with sourdough and LAB exhibited significantly improved quality. The effect of mixed fermentation on the physicochemical properties of dough was discussed. In the early stage of dough fermentation, lactic acid bacteria used carbohydrates in the dough to produce organic acids by rapid fermentation,

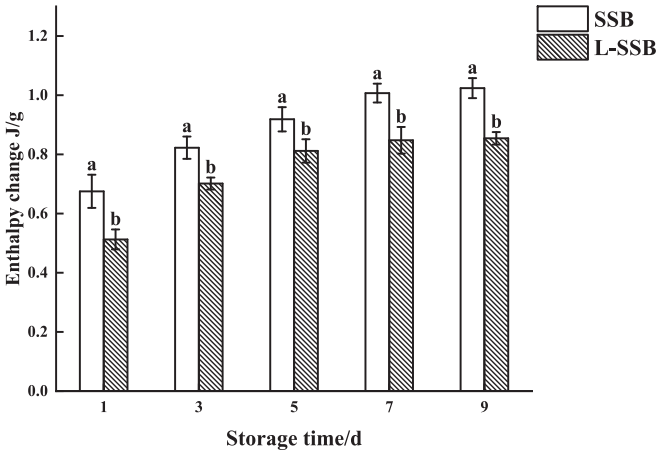

Fig. 5. Changes in aging enthalpy of steamed bun during a nine-day storage period.

which reduced the environmental pH value. At the same time, the titratable acid in the dough continued to accumulate, TTA increased, and sourdough fermentation produced alcohol and CO_2. The reason for the slight decrease in pH of the SD may have been due to the influence of lactic acid bacteria in the environment. Due to the consumption of a large amount of nutrients in the early stage of microbial fermentation, the acid production capacity in the later stage decreased, so the dough pH and TTA no longer changed significantly. The results were consistent with those of Cardinali (Cardinali et al., 2022), indicating that the mixed fermentation of sourdough and lactic acid bacteria was beneficial to the production and accumulation of organic acids. Phenolic acids and flavonoids had certain free radical scavenging ability(Kim et al., 2006).

The antioxidant activity was related to the antioxidant components in the dough and was also affected by pH. Acidic conditions were conducive to the scavenging of free radicals in the dough, and excessive acid in the dough led to a decrease in free radical scavenging. Appropriate acidification is beneficial to improve the antioxidant activity of the dough. Branched-chain amino acids (valine, isoleucine, leucine), sulfur-containing amino acids (cystine) and aromatic amino acids (tyrosine, phenylalanine) are the main amino acids involved in the formation of flavor compounds in whole wheat dough. They can form flavor precursors through the Ehrlich pathway(Hazelwood et al., 2008). As the main product of fermentation, the alcohols in L-SD gave steamed bread a special aroma and a lower aromatic domain.It could not only contribute to the flavor of steamed bread itself, but also could be used as a precursor to synthesize more flavor, such as the reaction of organic acids to esters, which had an indirect effect on the production of flavor(Paterson & Piggott, 2006).

This type of fermentation has been shown to positively influence specific volume and height-diameter ratio of steamed bun (Bartkiene et al., 2017). Improved dough rheological properties and gluten protein network structure, along with enhanced dough softness and air holding capacity, likely contributed to the improvement in steamed bun quality. Specifically, L-SSB exhibited significantly enhanced sensory quality compared to SSB, likely due to the acidic environment resulting from LAB fermentation activity, which reduces gluten protein mechanical strength, thereby improving rheological properties, hardness, color, and structure of steamed bun (Hu et al., 2022). Moreover, the interaction between organic acids produced by LAB fermentation and free amino acids improves steamed bun flavor (Demirkesen-Bicak et al., 2021). In addition, the improvement in texture characteristics of steamed bun may be attributed to the activation of endogenous proteases and amylases in an acidic environment, which likely facilitated protein and starch hydrolysis, improve the continuity among starch molecules, resulting in softer, easier-to-chew steamed bun, consistent with the findings by Wolter (Wolter et al., 2014). Zhu (Zhu et al., 2020) found a negative correlation between gluten protein and brightness of steamed bun skin, suggesting that improved whiteness might relate to internal structure uniformity. Thus, mixed fermentation with sourdough and LAB likely improved gluten network structure, thus enhancing internal structure uniformity of steamed bun, which resulted in better color. This is consistent with the findings reported by Liu (R. Liu et al., 2019), hence mixed fermentation with sourdough and LAB positively affects steamed bun color. Moreover, improvements in steamed bun core structure likely stemmed from enhanced gluten network structure. In addition, moderate acidification likely improved the crosslinking among small molecular proteins, promoting the formation of a more uniform, finer, and more stable gluten network structure within the dough, leading to a more delicate and porous internal structure in steamed bun (Chinma et al., 2023).

During storage, mixed fermentation with sourdough and LAB significantly reduced hardness, gumminess, and chewiness of steamed bun, as well as improved springiness, with no significant effect on cohesiveness and resilience. Fu (Fu et al., 2022) reported similar findings when employing LAB in bun fermentation, reporting a notable reduction in bun hardness, chewiness, and gumminess, while springiness and texture characteristics increased. These findings are in line with our findings and indicate that co-fermentation with sourdough and LAB can slow steamed bun deterioration. Water migration during storage contributes to steamed bun quality deterioration, with texture changes and starch aging closely linked to water loss. Other studies suggest that delaying moisture migration effectively delays starch aging, prolong the shelf life of steamed bun (Gray & Bemiller, 2003). Starch recrystallization causes aging during early storage, while water transfer from gluten protein to the crystalline region of starch induces aging during late storage (Cho et al., 1988). During storage, water loss and starch retrogradation increased the degree of starch recrystallization within steamed bun, whereas higher aging enthalpy indicated that steamed bun deterioration accelerated. Galle (Galle & Arendt, 2014) found that LAB fermentation could improve the aging rate of fermented flour products thus prolonging their shelf life. Similarly, Torrieri (Torrieri et al., 2014) found that the addition of 30 % sourdough could slow the water loss rate during bun storage and decrease bun aging enthalpy. Thus, mixed fermentation with sourdough and LAB appears to delay hardening, aging, and water loss of steamed bun.

A limitation of the present study is that only the effects of L. *plantarum* and *S. thermophilus* as starter cultures on fermented dough were explored. Thus, further research should explore the combined fermentation of multiple LAB and sourdough species found in traditional sourdough.

5. Conclusion

The use of freeze-dried LAB starters was shown to be highly feasible and applicable in steamed bun production. The mixed fermentation of sourdough and lactic acid bacteria was beneficial to the acidification of the dough, and could significantly increase the total phenol, flavonoid content and hydrolyzed amino acid content of the dough. The variety of flavor substances in the dough of mixed fermentation had increased, showing better flavor characteristics. Alcohols and aldehydes contributed greatly to the flavor of the mixed fermented dough.

Mixed fermentation with sourdough and LAB showed a significantly improved texture and sensory quality as well as storage characteristics of steamed bun. Mixed fermentation showed a more uniform and denser core structure, with increased stomatal density and porosity, and enhanced stomatal stability. During a nine-day storage period, hardness, gumminess, chewiness, and aging enthalpy of steamed bun were increased, while springiness, cohesiveness, resilience, and moisture content were unchanged. Our findings revealed that freeze-dried lyophilized LAB starter and sourdough could successfully used in steamed bun preparation. This provides a theoretical basis for LAB starter application in steamed bun and the development of novel steamed bun dough starters.

CRediT authorship contribution statement

Junjian Ran: Writing – original draft, Data curation, Conceptualization. **Yuhan Tang:** Writing – original draft, Visualization, Investigation. **Yue Zhang:** Writing – review & editing, Methodology. **Lingxia Jiao:** Formal analysis. **Chao Zhang:** Methodology. **Yongchao Li:** Writing – review & editing, Visualization, Funding acquisition. **Ruixiang Zhao:** Writing – review & editing, Resources.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

The data that has been used is confidential.

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