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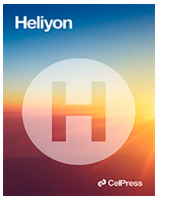

Research article

Production of γ–aminobutyric acid–enriched sourdough bread using an isolated *Pediococcus pentosaceus* strain JC30

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ABSTRACT

A γ–aminobutyric acid (GABA)–producing strain JC30 was isolated from traditional kimchi, which was identified as *Pediococcus pentosaceus* by 16S rDNA sequencing. *P. pentosaceus* JC30 was highly tolerant to acid, bile salt, and high temperatures. The survival rate of JC30 in MRS medium (pH 2.5) for 3 h was 60.96 %. Furthermore, the survival rate of JC30 in MRS medium with 3 mg/mL bile salt for 24 h was 86.62 %. The survival rate of JC30 in MRS medium at 56 °C and 58 °C for 10 min was 97.17 % and 78.20 %, respectively. When 2 % v/v JC30 (8.0 log10 CFU/mL) was added to prepare sourdough and the sourdough was then used to make bread, the bread had a higher specific volume (5.13 ± 0.12 mL/g) and GABA content (3.32 ± 0.04 mg/g DW) than the control.

1. Introduction

γ–Aminobutyric acid (GABA) is widely present in organisms and is well known as a neurotransmitter that plays an inhibitory role in the mammalian nervous system [1,2]. GABA has physiological functions such as lowering blood pressure, improving sleep, and antiaging [3–5]. When the body is deficient in GABA, symptoms such as anxiety, restlessness, fatigue, and insomnia appear. Hinton et al. [6] reported that oolong tea is rich in GABA, which significantly reduces stress, plays an anxiolytic role, and alters heart rate variability. A safety assessment of GABA was conducted by the United States Pharmacopeia, and the data were collected from clinical studies, adverse event information, and toxicology trials. The results showed that there were no serious adverse events in the human body with an intake of 18 g/day GABA for 4 days or 120 mg/day GABA for 12 weeks [7].

Currently, GABA used in food is mainly derived from microbial fermentation. The activity of glutamate decarboxylase (GAD) in microbial strains largely determines the production of GABA. GAD catalyzes the conversion of glutamic acid to GABA in their fermentation products [8]. Some lactic acid bacteria (LAB) strains have GAD activity and can catalyze the production of GABA from L–sodium hydrogen glutamate (L–MSG) [9]. In particular, LABs produce lactic acid to lower the pH value of the fermentation broth to 4.0–5.0, which is the optimal pH for GAD to produce GABA [10]. Traditional fermented foods are a relevant source of LAB strains [11].

Abbreviations: GABA, γ–aminobutyric acid; GAD, glutamate decarboxylase; LAB, lactic acid bacteria; L–MSG, sodium hydrogen glutamate; MRS, DeMan–Rogosa–Sharpe medium; .SPP, silkworm pupa protein; TTA, total titrable acidity; DFP, dough fermentation power; TPA, texture profile analysis.

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Screening GABA–producing strains from traditional fermented foods and using them to produce functional foods is an effective strategy. Diana et al. [12] obtained a GABA–producing strain *Lactobacillus shortum* CECT 8183 from traditional cheese products. Nakatani et al. [13] screened a GABA–producing strain, *Lactiplantibacillus plantarum* KB1253, from Japanese kimchi and used it to enhance the health benefits of tomato juice. In addition, LAB fermentation always improves the flavor of foods. Liang et al. [14] reported that a *Pediococcus pentosaceus* strain was used in the fermentation of suancai and increased the content of 14 volatile flavor compounds, including benzene acetaldehyde and phenylethyl alcohol, which brought a sweet scent of fruit and rose fragrance.

Bread is one of the most common staple foods, and sourdough bread has been increasingly focused on due to its high nutritional value [15]. *L. shortum* CECT 8183 was isolated by Diana et al. [12] and used to prepare sourdough, which was then added at 21 % to the bread recipe. The results revealed that the GABA content of the sourdough bread reached 24.2 mg/100 g. Venturi et al. [16] used a GABA–producing strain, *Lactobacillus farciminis* A11, to make bread with 20 % amaranth flour; further, the results indicated that the GABA concentration in the bread reached up to 39 mg/kg.

Screening for GABA–producing strains from different sources can provide resources for the production of GABA–enriched foods. The purpose of this study is to use the *P. pentosaceus* JC30 strain in the preparation of GABA rich sourdough bread as a case study to shed light on how microorganisms with specific functions improve the sensory quality and nutritional value of food.

2. Materials and methods

2.1. Materials

Traditional kimchi was from Changde City, Hunan Province (from vegetables including cabbage and purple kale). MRS broth and agar medium were purchased from Baisi Biotechnology (Hangzhou, China). γ-Aminobutyric acid (GABA) standard (≥99 %) was purchased from Sigma Aldrich (St Louis, MO, USA). Other chemical reagents were analytical grade reagents, which were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The MRS–MSG liquid medium consisted of 52.4 g MRS broth medium, 10.0 g sodium glutamate, and distilled water added to 1000 mL. Whole wheat flour was purchased from Xinliang (Xinxiang, China) and silkworm pupa protein (SPP) was purchased from Quanzhou Xinwang Biotechnology (Quanzhou, China).

2.2. Screening of γ-aminobutyric acid-producing LAB strains

LAB strains were isolated from traditional Chinese kimchi. Chopped kimchi was added to 0.85 % sodium chloride solution and ground to homogenate. The homogenate was diluted to different concentrations, and applied to $CaCO_3$–MRS plates. The plates were incubated anaerobically at 37 °C for 48 h and then calcium dissolution circles were picked out. The colonies were separated by streaking on MRS plates and purified repeatedly. The selected colonies were subjected to Gram staining, microscopic examination, and H_2O_2 contact enzyme test. The colonies with Gram–positive strains without spores and negative for contact enzymes were selected for further use.

LAB strains were activated in the MRS liquid medium at 30 °C for 24 h prior to use; 2 % (v/v) fermentation broth was then cultured in MRS–MSG liquid medium and fermented at 30 °C for 48 h. GABA–producing capacity was evaluated according to the GABA concentration in the fermentation broth, using the method of Zhuo et al. [17].

2.3. Sequence analyses of the 16 S rRNA gene

Genomic DNA of the strain was extracted using Bacterial Genomic DNA Extraction kit SK8255 (Tiangen Biotech Co., LTD., Beijing, China). Bacterial 16S rDNA universal primers were used to amplify the target fragment by PCR. The forward primer was 27F (5′-AGTTTGATCMTGGCTCAG-3′), and the reverse primer was 1492R (5′-GGTTACCTTGTTACGACTT-3′). Sequence was determined by Sangon Biotech (Shanghai, China), and the BLAST analysis was performed on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Determination of tolerance characteristics of the strain

The JC30 strain was cultured to the logarithmic growth stage, and the acid tolerance test and bile salt tolerance test were performed as follows [18]. The JC30 culture was centrifuged at 5000 rpm for 10 min at 4 °C, and the precipitate was washed twice with 0.01 mol/L PBS (pH = 7.2). The precipitate was resuspended in equal volumes in sterile MRS broth with pH 2.5 and 3.0, respectively, and then incubated at 30 °C for 3 and 6 h, respectively. The bile salt tolerance test was performed as follows. An equal volume of JC30 culture resuspension was added to sterile MRS broth containing 3 mg/mL bile salt, and then incubated at 30 °C for 24 h. The heat resistance was measured as follows. The bacterial solution reached the logarithmic phase and was then incubated at 54 °C, 56 °C, and 58 °C for 10 min each. The tubes were then placed on ice for 5 min. A diluted bacterial solution was prepared and applied to MRS solid culture medium, and then incubated at 37 °C for 48 h. The growing colonies were counted, and the survival rate of the strain was calculated according to equation (1).

$$\text{Survival rate} \Big/ \% = \frac{\log 10\ \text{CFUN}_1}{\log 10\ \text{CFUN}_2} \times 100 \quad (1)$$

where:

N_1 = the number of viable bacteria on the plate.

N_2 = the initial number of viable bacteria.

2.5. Sourdough preparation

Pediococcus pentosaceus JC30 was inoculated into MRS liquid medium and cultured at 37 °C for 18 h, and then the precipitate was obtained by centrifugation at 8000 rpm for 5 min. The precipitate was washed twice with 8.5 mg/mL of sterile saline and then resuspended in sterile water for use. The sourdough preparation method followed by Diana et al. [12] with slight modifications. The formula for the various sourdoughs is provided in Table 1. The sourdough used for further bread baking was obtained by shaking the culture at 120 rpm for 24 h at 37 °C and 85 % humidity.

2.6. Determination of pH, total titrable acidity (TTA), and lactic acid bacteria colony number of sourdough

10 g of sourdough with different fermentation time was accurately weighed. 90 mL of deionised water was added to prepare a solution to be tested. The solution was mixed and stired for 15 min, then left to stand for 10 min. The pH was measured using a pH meter (E−201F, Shanghai INESA Scientific Instrument CO., LTD, China). Referring to the method of Zhang et al. [19], TTA was determined by measuring the volume of NaOH consumed during titration with a 0.1 mol/L NaOH solution until the pH of the solution reached 8.5. The sourdough fermented for 24 h was diluted to an appropriate concentration and applied to MRS solid culture medium to culture at 37 °C for 48 h.

2.7. Bread preparation

The method of bread preparation was according to Jin et al. [20] with slight modifications. All ingredients were listed in Table 2. They were mixed in a bread maker (Donlim DLT06A, China) for 6 min. After fermentation (90 min, 32 °C, 80 % relative humidity), shaping and proofing (90 min, 32 °C, 80 % relative humidity), the dough was baked in an oven (Supor K42FK823, China) for 30 min (170 °C/200 °C). The bread was cooled for 2 h for further evaluation.

2.8. Determination of dough fermentation power

Refer to Balestra et al. [21] for calculating the dough fermentation power (DFP). 40 g of dough was placed inside a 250 mL graduated cylinder. The dough was placed in a 32 °C incubator and monitored every 20 min. The DFP was calculated according to equation (2).

$$DFP = \frac{v_1 - v_0}{v_0} \tag{2}$$

where:

v_0 = starting volume of the dough.

v_1 = volume after the leavening time.

2.9. Valuation of the sourdough bread

The specific volume of the bread was calculated using the rapeseed replacement method, expressed in mL/g. Texture profile analysis (TPA) (TMS–PRO, Gene–Science Scientific Instrments Inc., USA) and the degree of bread crumbs ageing were determined according to the method described by Jin et al. [20].

The determination of GABA content were according the method of Zhuo et al. [17]. 1.0 g of bread was soaked in 40 mL of boiling distilled water and placed in a 40 °C water bath for 2 h. The supernatant was filtered through 0.45 μm film and lyophilised for use.

Table 1
Formula of sourdoughs.

Sourdough samples	CK	S(JC30)	S(SPP)	S(JC30 + SPP)
Whole wheat flour/g	10	10	9	9
Silkworm pupa protein/g	/	/	1	1
Water/mL	40	39	40	39
JC30/mL	/	1	/	1

Sample notation corresponds to: CK (natural sourdough without JC30 and SPP), S(JC30) means (sourdough containing JC30), S(SPP) means (sourdough containing SPP), S(JC30 + SPP) means (sourdough containing JC30 and SPP).

Table 2
Formula of breads.

Ingredients	CK bread	S(JC30) bread	S(SPP) bread	S(JC30 + SPP) bread	S(JC30) + SPP bread
CK sourdough/g	50	/	/	/	/
S(JC30) sourdough/g	/	50	/	/	50
S(SPP) sourdough/g	/	/	50	/	/
S(JC30 + SPP) sourdough/g	/	/	/	50	/
SPP/g	/	/	/	/	1
Flour/g	80	80	80	80	79
Water/g	14	14	14	14	14
Sugar/g	2.7	2.7	2.7	2.7	2.7
Salt/g	0.5	0.5	0.5	0.5	0.5
Instant yeast/g	1.0	1.0	1.0	1.0	1.0
Butter/g	1.8	1.8	1.8	1.8	1.8
Milk powder/g	0.7	0.7	0.7	0.7	0.7

CK bread (bread without JC30 and SPP), S(JC30) bread means (bread containing JC30 sourdough), S(SPP) bread means (bread containing SPP sourdough), S(JC30 + SPP) bread means (bread containing JC30 and SPP sourdough), S(JC30) + SPP bread means (bread containing JC30 sourdough and SPP).

2.10. Statistical analysis

All measurements were repeated for three times, and the experimental data were expressed by $\bar{x} \pm s$. The data were analyzed and plotted using GraphPad Prism 8.0 (GraphPad Software, California, USA) and Excel. A one–way ANOVA was performed with SPSS 26.0 (SPSS, Inc., Chicago, IL, USA). Duncan's method was used to test the significance of differences amomg samples, and the results with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Screening of GABA–producing LAB

Sixty strains of potential LAB numbered JC1–JC60 were isolated from traditional Hunan kimchi (China). The 60 strains were individually inoculated into MRS–MSG liquid medium. After 48 h of fermentation at 30 °C, the fermentation liquid was centrifuged, and the supernatant was selected for GABA determination. The results revealed that there were six strains whose GABA production capacities were higher than 1.0 mg/mL (Fig. 1A). The highest GABA production was obtained from the JC30 strain, which reached 2.12 mg/mL. Therefore, the JC30 strain was selected for further investigation.

3.2. Identification of the JC30 strain

The results of the colony morphology and Gram staining of the JC30 strain are shown in Fig. 1. The colony of the JC30 strain was white in color with a smooth, rounded, slightly raised surface (Fig. 1B). After Gram staining, the bacteria were purple and positive (Fig. 1C). The result of the polymerase chain reaction of 16S rDNA of the strain is shown in Fig. 1D. The 16S rDNA sequence was compared by BLAST on NCBI, and the GenBank accession number was SUB12245622 JC30 OP764403. The JC30 strain was identified as *P. pentosaceus*. *P. pentosaceus* has been reported to have numerous beneficial effects, such as antioxidant, cholesterol-lowering, and

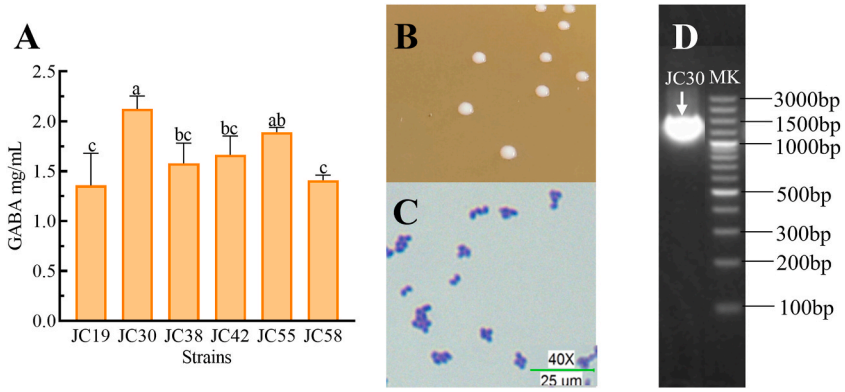

Fig. 1. Morphological, molecular biological identification and optimization of strains culture conditions. (A) GABA content in fermentation broth of different strains. (B) Colony morphology. (C) Observation of cell morphology after Gram staining (1000 ×). (D) Polymerase chain reaction of 16S rDNA. Different superscripted letters (a–d) indicate a significant difference ($P < 0.05$).

bacteriostatic capacities [11,22,23]. Recently, the application of *P. pentosaceus* in probiotic fermentation products has received increasing attention. It was reported that *P. pentosaceus* had been widely applied to fermented dairy products, meat products, and pasta [24].

3.3. Tolerance of P. pentosaceus JC30 to heat, acid, and bile salt

The pH of human gastric juice is between 2.5 and 3.0 [25]. LABs need a strong ability to neutralize gastric acid to survive in the intestine [26]. Therefore, it is necessary for selected strains to have strong acid resistance, and acid tolerance for the evaluation of probiotic strains. Therefore, the survival rate of the JC30 strain was measured at pH 3.0 and 2.5. As shown in Table 3, the survival rate of the JC30 strain reached 85.30 % after 3 h of incubation in a pH 3.0 medium. As the pH decreased and the incubation time increased, the survival rate of the JC30 strain decreased. After 6 h of incubation in a pH 2.5 medium, the survival rate of the JC30 strain was still 40.56 %. Ramadhanti et al. [27] screened *Lactobacillus fermentum* strain 1743 from palm sugar, which showed a 74.71 % survival rate in pH 3 medium for 3 h. And the strain showed its potential as a probiotic. That is to say, the JC30 strain had good tolerance to acid.

Bile salt tolerance is another capacity that is usually considered in the application of LABs. It is a relevant criterion to evaluate the probiotic performance of LAB because good tolerance to bile salts means the LABs can survive in the intestinal fluid for a certain time [28]. *P. pentosaceus* JC30 had a survival rate of 86.62 % after 24 h of incubation in a medium containing 3 mg/mL bile salts. Thao et al. [29] isolated a strain of *P. pentosaceus* HN10 from fermented loblolly, and its survival rate was 62.78 % at a bile salt concentration of 3 mg/mL for 24 h. Compared with *P. pentosaceus* HN10, the JC30 strain had good bile salt tolerance.

The ability of LABs to survive in high–temperature environments is conducive to their viability in a thermal environment, which can provide favorable conditions for the production and storage of active products by LABs [30]. As shown in Table 3, the survival rate of the JC30 strain was 100 % at 54 °C for 10 min, and the survival rate was 78.20 % at 58 °C for 10 min. The results indicated that the JC30 strain had good heat resistance, which is conducive to its stability during further application and preservation.

3.4. Changes in pH, TTA, and lactic acid bacteria colony number during the preparation of sourdough

Traditional sourdough is a mixture of flour and water, which is spontaneously fermented by a complex microbial ecosystem consisting of LABs and yeast from raw materials [15]. Recently, some specific cultures were used in the fermentation process of sourdough, such as LABs, which can produce metabolites having positive effects on the texture and staling of bread. In this study, JC30 strain was used to prepare sourdough. As shown in Fig. 2, the pH and TTA values of sourdough at different fermentation times were determined. The pH values of all sourdough samples decreased during 24 h, while the TTA value increased. At the 24th h, the pH values were 3.82–4.05, and the TTA values were 7.47–10.24 mL. The S(SPP) and S(JC30 + SPP) groups showed higher TTA values, which may be relate to the addition of silkworm pupae protein (SPP) because it improves the buffering capacity [31]. SPP is a protein obtained from edible insects that was encouraged to be used in human food for its good nutritional quality [32]. The pH and TTA values of the S(JC30) and S(JC30 + SPP) groups changed in a similar trend. Within 12 h of fermentation, the pH value of these two sourdough decreased rapidly, while the TTA value increased quickly, and then the changes slowed down during 12–24 h. In the S(SPP) and CK groups, the pH and TTA values changed slightly during 0–8 h, then the pH value decreased rapidly and the TTA value increased quickly in the following 8 h. As shown in Fig. 2C, after 24 h of fermentation, the number of LAB colonies in S(JC30 + SPP) was the largest, and the measured value was 8.68 ± 0.04 log10 CFU/g. At the same time, the count of LAB colonies in S(JC30) sourdough was 8.28 ± 0.17 log10 CFU/g. The addition of SPP to sourdough benefits the growth of lactic acid bacteria JC30.

3.5. Effects of different sourdoughs on the dough fermentation ability

The dough fermentation ability reflects the growth and metabolic activity of lactic acid bacteria and yeast during the fermentation process. The dough fermentation power is shown in Fig. 3. During the fermentation process, the expansion volumes of the five groups of dough were recorded, and which exhibited a pattern of initially rising and then stabilizing. During 0–100 min of fermentation, the

Table 3
Tolerance of *P. pentosaceus* JC30 to heat, acid, and bile salt.

Tolerance characteristic	Tolerance condition	JC30/log10 CFU/mL	SR/%
Acid resistance	0 h, pH 2.5	8.58 ± 0.18^{a}	100 %
	0 h, pH 3.0	8.58 ± 0.18^{a}	100 %
	3 h, pH 2.5	5.23 ± 0.09^{c}	60.96 %
	3 h, pH 3.0	7.32 ± 0.01^{b}	85.30 %
	6 h, pH 2.5	3.48 ± 0.11^{d}	40.56 %
	6 h, pH 3.0	5.77 ± 0.06^{c}	67.25 %
Bile salt tolerance	0 h, 3 mg/mL bile salt	7.55 ± 0.01^{a}	100 %
	24 h, 3 mg/mL bile salt	6.54 ± 0.15^{b}	86.62 %
Heat resistance	10 min, 30 °C	8.12 ± 0.16^{a}	100 %
	10 min, 54 °C	8.11 ± 0.02^{a}	100 %
	10 min, 56 °C	7.89 ± 0.14^{a}	97.17 %
	10 min, 58 °C	6.35 ± 0.02^{b}	78.20 %

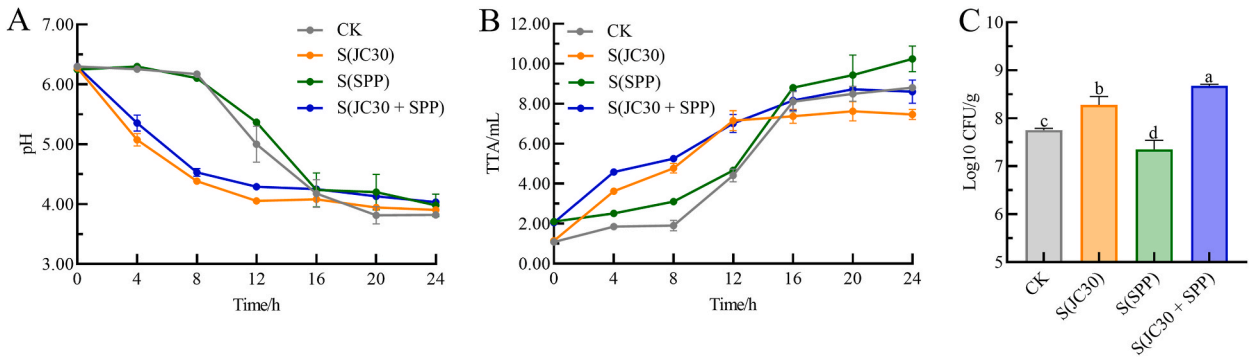

Fig. 2. The pH (A), TTA (B), and lactic acid bacteria colony number (C) of different sourdoughs. Different superscripted letters (a–d) indicate a significant difference ($P < 0.05$).

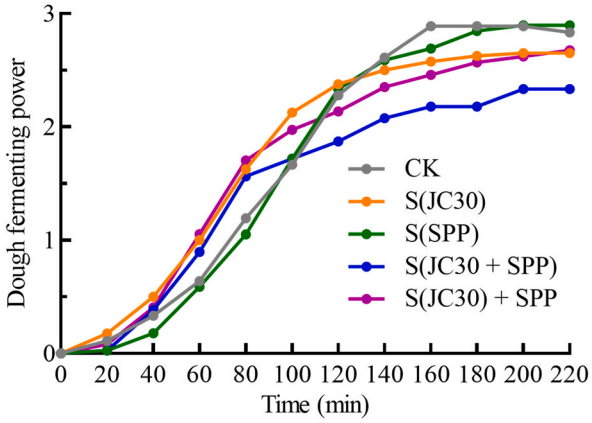

Fig. 3. The dough fermentation ability of different formulations of dough.

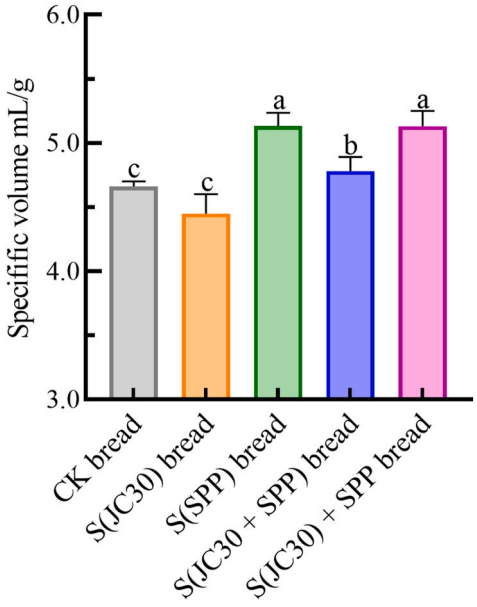

Fig. 4. The specific volume of different groups bread. Different superscripted letters (a–d) indicate a significant difference ($P < 0.05$).

expansion volumes of the dough containing S(JC30), S(JC30 + SPP), S(JC30) + SPP increased quickly. After 100 min, the expansion volumes of the dough containing CK and S(SPP) sourdough continued to rapidly increase, while that of the other three groups dough started to slow down. The reducing of the fermentability of dough may be due to the over-acidification by JC30.

3.6. Effects of different formulations on the quality of bread

3.6.1. Effects of different formulations on a specific volume of bread

The specific volume of bread reflects the volume expansion rate and gas holding capacity of bread. The specific volumes of the bread made from different formulations are shown in Fig. 4. Compared with CK group bread (4.66 ± 0.04 mL/g), the specific volumes of the bread in the S(SPP), S(JC30 + SPP), and S(JC30) + SPP groups were significantly increased by 10.15 %, 2.58 %, and 10.09 %, respectively, while that of the bread in the S(JC30) group decreased by 4.51 %. The above results demonstrated that the addition of SPP was helpful to improve the specific volume of bread. Whether the SPP was added during the production of sourdough or in the subsequent preparation of bread, it made the sourdough bread fluffier and softer. Compared with the bread in S(JC30) group, the specific volumes of the bread in S(JC30 + SPP) and S(JC30) + SPP groups were increased by 7.42 % and 15.28 %, respectively. In summary, *P. pentosaceus* JC30 promoted the quick acidification of the dough, which was not conducive to the formation of gluten structure in the later stage, and SPP effectively alleviated this negative impact and promoted the formation of gluten structure.

3.6.2. Effects of different formulations on the internal texture and structure of bread

Sourdough is a combination of flour and water that is fermented by LAB and the yeasts naturally present in raw materials [15]. In the present study, *P. pentosaceus* JC30 was added to prepare sourdough, and then the different sourdoughs were used to prepare bread. Meanwhile, high–quality insect protein SPP is also added in a certain amount. The different formulations of sourdough bread are listed in Table 1. The texture and structure of the bread were determined. The results are shown in Fig. 5. The bread from S(SPP), S(JC30 + SPP), and S(JC30) + SPP groups had more uniform pores and larger volumes, which is consistent with the specific volume results in Fig. 4. The pores of bread directly reflect the compactness of the bread. Compared with CK group, the bread from S(JC30) + SPP group formed a better grid structure with a uniform distribution of pores.

3.6.3. Effects of different formulations on the texture profile of bread

It was reported that the gumminess, springiness, and cohesiveness of bread are positively correlated with bread quality, while the values of hardness, adhesiveness, and chewiness are negatively correlated with bread quality [33]. The TPA of the bread with different formulations was measured after 2 h of cooling and 72 h of cold storage, and the results are listed in Table 4. The bread from S(SPP) and S(JC30) + SPP groups had the same hardness, which was 1.74 N after 2 h of cooling. The hardness was the smallest. The hardness of S

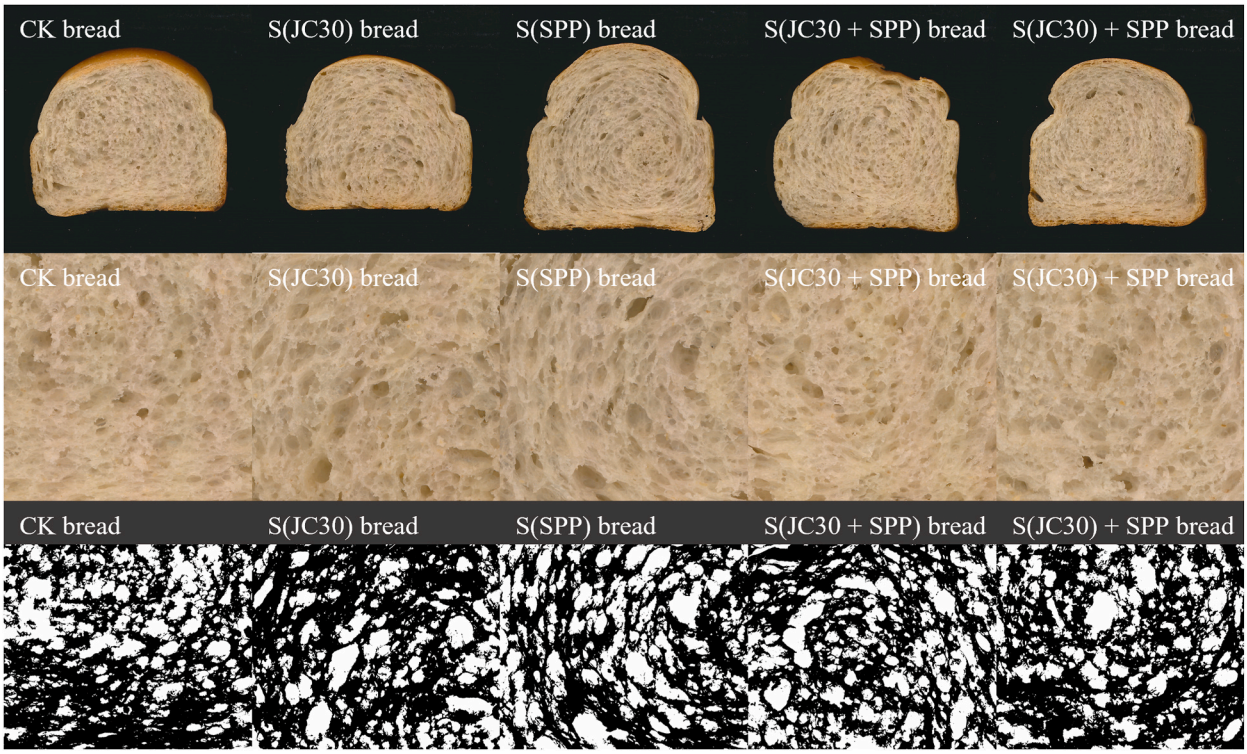

Fig. 5. The physical appearance of different groups bread.

Table 4
Texture analysis of bread.

Time	Sample	CK bread	S(JC30) bread	S(SPP) bread	S(JC30 + SPP) bread	S(JC30) + SPP bread
0 d	Hardness/N	2.13 ± 0.12^{bc}	2.69 ± 0.20^{b}	1.74 ± 0.40^{c}	4.12 ± 0.83^{a}	1.74 ± 0.25^{c}
	Cohesiveness/Ratio	0.74 ± 0.02^{a}	0.73 ± 0.02^{a}	0.71 ± 0.01^{a}	0.68 ± 0.04^{b}	0.72 ± 0.01^{a}
	Adhesiveness/ mj	0.04 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
	Springiness/mm	3.48 ± 0.58^{ab}	3.58 ± 0.32^{ab}	2.80 ± 0.25^{b}	3.65 ± 0.46^{a}	3.54 ± 0.36^{ab}
	Chewiness/mj	5.54 ± 1.42^{b}	7.03 ± 1.03^{bc}	3.56 ± 1.12^{d}	10.02 ± 0.92^{a}	4.73 ± 0.61^{cd}
	Gumminess/N	1.58 ± 0.13^{b}	1.96 ± 0.11^{bc}	1.26 ± 0.28^{c}	2.77 ± 0.44^{a}	1.34 ± 0.04^{c}
3 d	Hardness/N	10.15 ± 1.11^{b}	14.93 ± 0.49^{a}	8.50 ± 0.35^{cd}	9.28 ± 0.71^{bc}	7.33 ± 0.88^{d}
	Cohesiveness/Ratio	0.47 ± 0.02^{b}	0.47 ± 0.02^{ab}	0.52 ± 0.04^{a}	0.48 ± 0.04^{ab}	0.47 ± 0.02^{ab}
	Adhesiveness/ mj	0.06 ± 0.04^{ab}	0.04 ± 0.01^{b}	0.05 ± 0.00^{ab}	0.05 ± 0.00^{b}	0.12 ± 0.06^{a}
	Springiness/mm	3.48 ± 0.33^{a}	3.44 ± 0.09^{a}	3.29 ± 0.05^{a}	3.40 ± 0.44^{a}	3.53 ± 0.58^{a}
	Chewiness/mj	16.46 ± 2.28^{b}	24.12 ± 0.37^{a}	14.64 ± 1.11^{bc}	14.89 ± 1.87^{bc}	12.07 ± 1.01^{c}
	Gumminess/N	4.74 ± 0.62^{b}	7.07 ± 0.27^{a}	4.46 ± 0.39^{b}	4.65 ± 0.40^{b}	3.46 ± 0.44^{c}

The data are the means of three independent experiments ± standard deviations (n = 3).
a-d Means within a raw with different superscript letters are significantly different ($P < 0.05$).

(JC30 + SPP) group bread was the largest, followed by S(JC30) group bread. The addition of JC30 to the sourdough led to the excessive production of organic acids in the sourdough and affected the final hardness of the bread. After storage for 72 h, the overall hardness of the bread increased. Compared with CK group, the hardness of S(JC30) group bread increased by 47.92 %, while the hardness of S (SPP), S(JC30 + SPP), and S(JC30) + SPP group bread decreased by 16.26 %, 5.57 %, and 27.78 %, respectively. S(JC30) + SPP group bread was the softest after 3 days of storage. The results indicated that the addition of SPP during the sourdough bread making process slowed down the staling of the bread.

Chewability refers to the energy required to chew a solid sample into a swallowing state. As shown in Table 4, the changes in chewiness were consistent with hardness. Among the five groups of bread, the chewiness of S(SPP) group was the smallest, and that of S (JC30) + SPP group was in the middle level, similar to the CK group. The chewability of all groups of bread increased after 3 days of storage, and the bread of the S(JC30) + SPP group had the minimum chewiness. A comprehensive evaluation of the texture of the bread with different formulations showed that the bread in S(JC30) + SPP group had the optimal texture characteristics.

3.6.4. Effects of different formulations on the staling of bread

The staling degree of bread is shown in Fig. 6. The staling of bread refers to the decline in quality during storage, which leads to the loss of flavor, taste, and economic value [34]. The staling of bread is related to its hardness (Table 4). The degree of bread staling in the CK group was 354 %, while that in the S(JC30) + SPP group was 327 %. Although the staling degree of bread in the S(JC30 + SPP) group significantly decreased to 125 %, the initial hardness of the bread (0 day) in this group was far higher than that of S(JC30) + SPP. Thus, the S(JC30) + SPP group was a more reasonable choice.

3.6.5. Effects of different formulations on the GABA content in bread

The GABA content of the bread is shown in Fig. 7. The GABA contents of different groups of bread were significantly different ($P < 0.05$). The bread of S(JC30) + SPP group had the highest GABA content, which reached 3.32 mg/g DW. Compared with the CK bread, the GABA contents in S(JC30 + SPP) group and S(JC30) + SPP group increased by 53.89 % and 98.80 %, respectively. The GABA–producing capacity of JC30 was increased in the presence of SPP during the fermentation process after the addition of sourdough.

LABs and their metabolites are generally recognized as safe [35]. In recent years, more and more experiments have shown that the soluble factors secreted by LABs or released after bacterial lysis have additional biological activities, such as regulating immune function, improving allergic reactions, and preventing obesity [36]. *P. pentosaceus* JC30 was isolated from Chinese traditional kimchi and then used to prepare sourdough. SPP is a protein obtained from edible insects that was encouraged to be used in human food for its good nutritional quality [32]. SPP is increasingly being utilized in varieties of food products, such as bread, and yogurts [37]. Torres et al. [38] reported a preparation method for high–protein cookies enriched with SPP. Other recent studies have shown positive results from the inclusion of insect powders and LAB in cereal–based products [39]. Thus, it is a practical experience to determine the effects of LABs and SPP on the technological properties, sensory acceptance, and nutritional value of sourdough bread. The above results revealed that the JC30 strain as a starter culture and SPP as a supplement effectively improved the quality of the sourdough bread.

4. Conclusion

In the current study, a GABA–producing strain, JC30, was screened from Hunan traditional kimchi. Identified by morphological observation and 16S rDNA sequencing, the strain belongs to *P. pentosaceus*. The strain JC30 had good resistance to heat, low pH, and bile salts. JC30 was used to make sourdough, and then the sourdough was used to prepare bread. The bread in S(JC30) + SPP group had a higher specific volume (5.13 ± 0.12 mL/g) and GABA content (3.32 ± 0.04 mg/g DW) than the control. JC30 has the potential to further enhance the health value of sourdough bread.

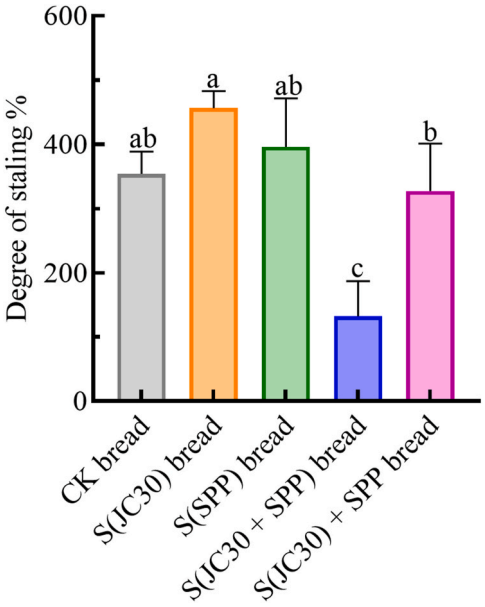

Fig. 6. The degree of bread staling in different groups. Different superscripted letters (a–d) indicate a significant difference ($P < 0.05$).

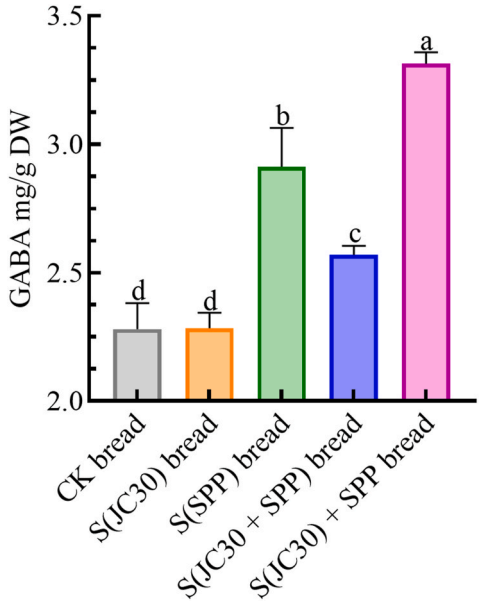

Fig. 7. The GABA content in different groups bread. Different superscripted letters (a–d) indicate a significant difference ($P < 0.05$).

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Data availability statement

Data is avaible on Mendeley data (https://doi.org/10.17632/668pff5byx.1).

CRediT authorship contribution statement

Jiajia Xuan: Writing – original draft, Validation, Software, Investigation, Formal analysis. **Xinyao Han:** Data curation. **Junjia Che:** Visualization. **Jun Zhuo:** Data curation. **Jingjie Xu:** Investigation. **Jianliang Lu:** Resources. **Huirong Mu:** Methodology. **Jun Wang:** Writing – review & editing. **Jie Tu:** Validation, Supervision, Software, Project administration, Methodology, Conceptualization. **Guanhui Liu:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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