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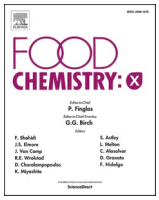

Biological characteristics of the gluten-free sourdough system fermented by Lactobacillus plantarum ST-III and its effect on dough quality and nutritional value during freezing

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ABSTRACT

In this study, the biological characteristics of wheat, tartary buckwheat, and wheat-tartary buckwheat sourdough systems fermented by *Lactobacillus plantarum ST-III* at different times were explored, and the quality and nutritional characteristics of sourdough frozen dough bread products with different fermentation substrates were investigated. Comparing the metabolic properties of sourdough during fermentation, it was found that the density of colony growth in TBS was the best, when fermented for 12 h, the protein degradation effect was the best, and the content of EPS (3.109 g/kg) and free amino acid was the highest. Although the quality of fresh TBS bread was worse than that of WS, after 13 weeks of frozen storage, the quality deterioration of TBS frozen dough bread was the smallest, the water migration rate was slower, and the protein digestibility was higher. Therefore, tartary buckwheat sourdough system can delay the degradation of bread quality and nutrition.

Introduction

In recent years, high-nutrition baked goods have become increasingly popular among consumers due to the accelerating pace of people's modern lives. By using freezing technology to process dough, the baking industry can provide consumers with quickly prepared fresh bread. Considering that this technology eliminates the need for extensive manpower and reduces repetitive investment wastage (Coda et al 2017; Angioloni and Collar, 2012), it can be used to produce baked goods on a standardized large scale. However, previous studies have shown that the bread prepared from wheat flour alone can only be frozen for a short time, has poor specific volume and baking quality (Arte et al 2015), and contains protein and amino acids will be lost, among other nutrients. When the freezing storage time of this bread is extended, its functional properties are lost due to the migration and ice crystal growth of the near-freezing moisture in the dough (He et al 2020). Moreover, long-term exposure to a low temperature environment destroys the yeast cells in the bread, weakens the network structure of the dough gluten, reduces the gas quality of the dough, speeds up moisture migration and fission, and reduces protein digestibility (Moroni et al 2011). Considering that the afore-mentioned limitations of freezing technology directly affect the manufacturing of baked goods on a standardized scale (Bigne et al 2019; Cui et al 2019), it is of great practical significance to design new products that can withstand these limitations in practical applications.

Tartary buckwheat is normally grown in low-temperature environments, and it has a higher content of antifreeze protein than regular wheat. As such, it can inhibit the growth or recrystallization of ice crystals and reduce cell damage (Jia et al 2017), thereby enhancing resistance to severe cold and stress. In general, tartary buckwheat is characterized by strong frost resistance and ecological adaptability (Benković and Kreft, 2015; Yang et al 2019; Pirzadah et al 2020). However, due to its high protein content (10–15%), tartary buckwheat is considered an indigestible grain that cannot be directly utilized by the body. Moreover, the stability of the gluten network structure in this grain is weak, which restricts its deep processing as a food product. According to previous studies, the air-holding capacity of frozen dough and the quality of the final bread product can be improved by using lactic acid bacteria to ferment the dough. In addition, this simple and cost-effective biotechnology can efficiently maintain the nutritional content and functional properties of the bread, reduce the content of anti-nutritional factors, and provide the product with a unique flavor. Previously, Chen et al. (2017), Coda et al. (2015) had shown that the formation of ice crystals can be effectively reduced by replacing wheat

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gluten with the EPS in gluten-free sourdough bread. Such replacement also delays system variations during freezing and thawing processes, protects the network structure of gluten, and improves the specific volume, texture, and flavor of tartary buckwheat and quinoa gluten-free bread, resulting in higher quality baked goods. Based on the available studies, the freezing resistance and nutritional quality of baked products can be enhanced by adding tartary buckwheat powder during the process of dough fermentation with lactic acid bacteria.

Lactobacillus plantarum ST-III has good acid, bile salt, and cold resistance, as well as moderate acid production ability. Therefore, it can promote the degradation of proteins and regulate intestinal flora, in addition to other advantageous effects (Wang et al 2011; Gobbetti et al., 2019). In this study, we explore the freezing resistance, protein distribution, amino acid composition, product quality, and nutritional function of the bread, added wheat flour and tartary buckwheat powder fermented by *Lactobacillus plantarum ST-III* into the dough with the proportion of 30%. The fermentation physicochemical characteristics of EPS-producing Tatary Buckwheat Sourdough (TBS), Wheat Sourdough (WS), and Wheat-Tartary Buckwheat Sourdough (WTBS) are compared, and the influence of *Lactobacillus plantarum ST-III* fermentation on the quality and nutritional value of frozen bread is analyzed. The obtained results show that the combination of biological fermentation and frozen dough technologies has great potential for the future development of high-nutrition frozen dough.

Materials and methods

Materials

Lactobacillus plantarum ST-III (CGMCC NO.0847) was provided by the State Key Laboratory of Dairy Biotechnology. Buckwheat flour with dry protein and ash contents of 11.09 and 1.42 g/100 g, respectively, was purchased from Yanmen Qinggao Co., Ltd. (Shanxi, China). Jinxiang bread special wheat flour (Jinxiang, China), active dry yeast (Anqi, China), sugar (Huiyi, China), and edible salt (Taigu, China) were purchased from local supermarkets. The protein content in wheat flour was 12.67 g/100 g, and the flour was kept in a dry cupboard at room temperature.

Sourdough fermentation

Before fermentation, *Lactobacillus plantarum ST-III* was cultured at 37 °C to the late exponential growth period (about 8 h). Collect the bacterial paste (4500 RPM, 15 min, 4 °C), washed twice with sterile saline, and then resuspended in sterile water for dough preparation (The initial inoculation amount of lactic acid bacteria is about 8.0 log CFU/g sourdough). Then, according to the ratio of m_1 (raw material powder): m_2 (bacterial liquid) = 1: 1, respectively weigh wheat flour, tartary buckwheat flour, and mixed flour (m_3 (wheat flour): m_4 (tartary buckwheat flour) = 1: 1), add 5% sucrose and sterile water in ratio 1:1 and mix well, then the dough is fermented at 30 °C for 24 h to obtain wheat fermented sourdough (WC), tartary buckwheat fermented sourdough (TBS) and mixed fermented sourdough (WTBS), all of which were used as one of the raw materials for making dough mentioned in 2.7, and the pH value of the dough is measured with an FE20 pH meter. Use the Yin et al (2015) method to determine the total titer acidity (TTA) of 10 g of dough. The lactic acid bacteria in the dough were counted with MRS AGAR medium (Qingdao Haibo, China), and cultured upside down at 37 ℃ for 48 h (Houngbédji et al. 2021).

Determination of organic acid content in sourdough

The contents of organic acids (lactic acid and acetic acid) in the three types of sourdough investigated herein (WS, TBS and WTBS) were determined using high performance liquid chromatography (HPLC; Shimadzu Management Co., Ltd., China), as per the method of Kola et al (2015).

Extraction and determination of extracellular polysaccharides in sourdough

The sugar metabolism in sourdough was determined according to the method published by Vrancken et al (2008). The extracellular polysaccharides were extracted based on the method reported by Ketabi et al (2008).

Changes in protein content during sourdough fermentation

Determination of protein content

During the 24 h fermentation period, 1 g of TBS, WS, and WTBS was collected every 6 h. The protein content in the collected samples was analyzed according to the method of Bradford (Ferreyra et al. 2021).

SDS-PAGE analysis of protein components during sourdough fermentation

The SDS-PAGE kit was used to determine the molecular weight distribution of total protein, albumin, and prolamin in TBS, WS, and WTBS at 0, 6, 12, 18, and 24 h. After gel staining and decolorization, images of the samples were recorded and analyzed using the Quantity One software v4.6.6(The discovery Serises, America) (Lu et al., 2019; Lynch et al. 2018).

Determination of amino acid content in sourdough

The free amino acids in the sourdough samples were analyzed by HPLC, as described by Demirkesen et al (2016) and Ua-Arak et al (2017).

Preparation of frozen dough

To prepare the frozen dough, high-gluten wheat flour, yeast, sugar, and salt were evenly mixed and then added to 12-h-fermented sourdough at the ratio of 70:30. Subsequently, water was added, and the mixture was slowly kneaded for 3 min to form a dough (Zhu et al. 2019). After standing for 10 min, the dough was divided into 70 g pieces, rounded, and then placed at − 33 °C for 3 h to attain a temperature of − 18 °C at the center of the dough. Thereafter, the dough was placed in a refrigerator at − 18 °C for 0, 3, 5, 7, 9, or 13 weeks. Before testing, the frozen dough was thawed at room temperature for 1 h.

To prepare the bread, thaw the dough at room temperature for 1 h, then put it in the wake box (38 ℃, relative humidity 85%) for 90 min. The upper/lower heat temperature was 170/180 ℃, and baked for 20 min.

Determination of the quality of frozen dough bread

Determination of the specific volume and texture of frozen dough bread

The specific volume of bread was determined according to AACC 10–05.01 (Wang et al. 2017; Yin et al. 2021):

$P = V/m$.

Where, P is the specific volume of bread, mL/g; V is the volume of bread, mL; M is the quality of the bread, g.

Using P/36R cylindrical probe, the parameters were set as 3 mm/s before test, 1.5 mm/s during test, 5 mm/s after test and 5 mm compression depth. Interval time 10 s; Compress twice. Measure the hardness, ductility, viscosity and chewability of the product. Each experiment is carried out in 3 parallel experiments, and the average value is taken (Xu et al., 2019).

Determination of moisture migration in frozen dough bread

The water mass fraction was determined by direct drying method, according to AACC 44–01. The experiment was repeated three times for each group (Kowalczewski et al. 2019).

In vitro determination of protein digestibility

Protein digestibility in common wheat bread (control group), fresh dough, and frozen dough was analyzed according to the method reported by Desai et al. (2018) and by Wu et al (2017).

Statistical analyses

SPSS 20.0 was used to process the data. Each group of data was repeated for three times. The mean value of the data was taken and the significance analysis was conducted. Meanwhile, prism 8 is used for drawing.

Results and discussion

Physicochemical properties of the sourdough fermented by Lactobacillus plantarum ST-III

Lactobacillus plantarum ST-III is a representative strain of lactic acid bacteria isolated during the yeast fermentation process. These bacteria speed fermentation and improve its quality, resulting in enhanced antifreeze performance of the product (Zhao et al. 2016; Jeon et al. 2016). As shown in Fig. 1(a), *Lactobacillus plantarum ST-III* experiences a 2 h lag phase after fermentation, then it enters the logarithmic growth phase. Fig. 1(b) shows that after 16 h of fermentation, colony growth tends to be stable, with the colony density of *Lactobacillus plantarum ST-III* in TBS being slightly higher than those in WS and WTBS (1.04 and 1.01 times, respectively). Compared to WS, *Lactobacillus plantarum ST-III*

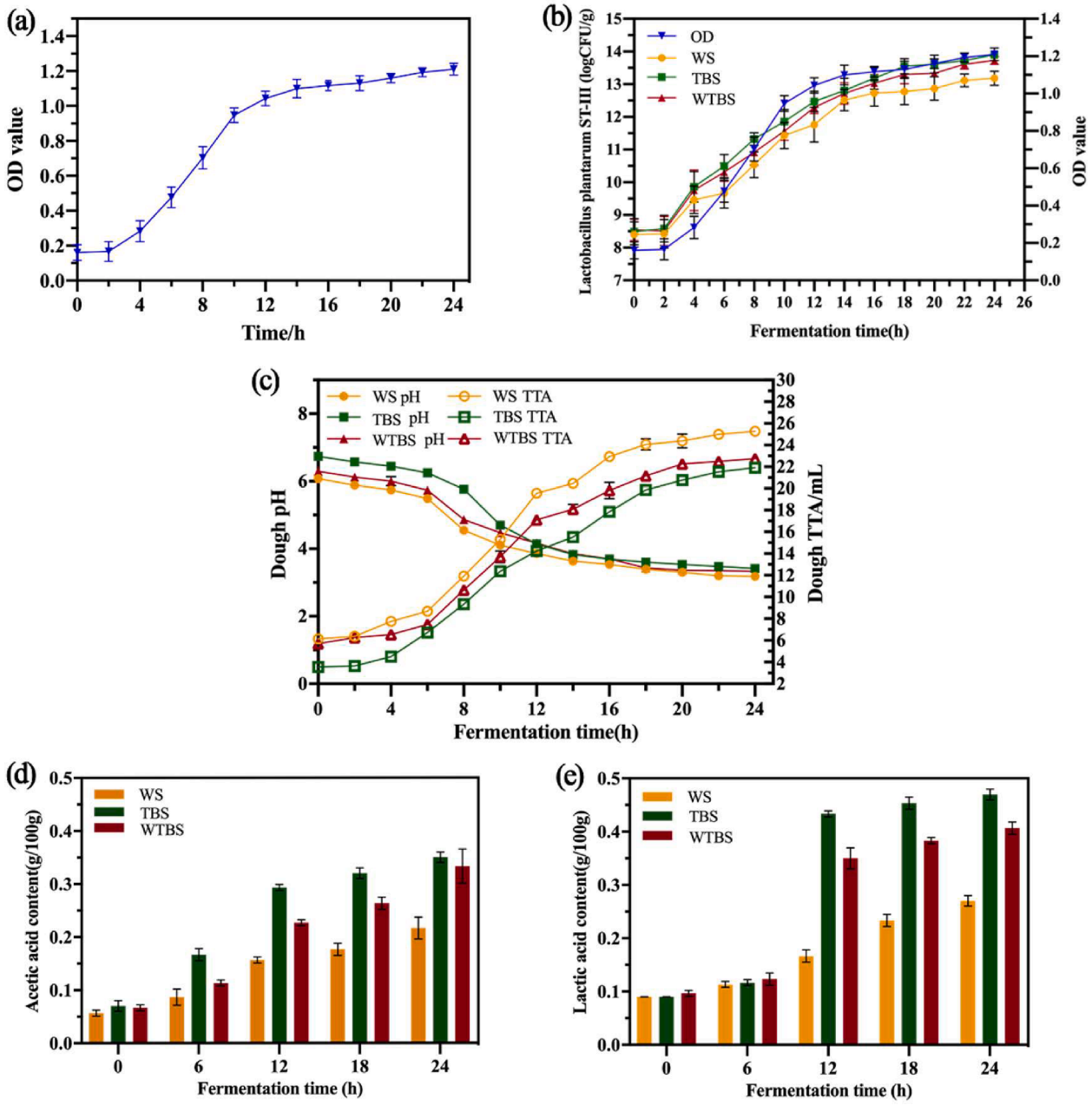


Fig. 1. The physical and chemical properties of sourdough fermentation system (a) Growth curve of *Lactobacillus plantarum ST-III* (b) Changes of Lactobacillus colony density during *Lactobacillus plantarum ST-III* fermentation of sourdough (c) Changes of pH value and TTA during *Lactobacillus plantarum ST-III* fermentation of sourdough (d) The change of acetic acid content in sourdough (e) Changes in lactic acid content in sourdough.

grows more vigorously in TBS and WTBS during the logarithmic growth phase due to the acidic environment of this sourdough (optimal pH value for the growth of *Lactobacillus plantarum ST-III* is 3.2–3.8).

The growth of lactic acid bacteria during sourdough fermentation increases the total acidity of the dough (reduces pH) (Debonne et al. 2020). As shown in Fig. 1(c), the pH values of WS, TBS, and WTBS before fermentation are 6.08, 6.74, and 6.29, respectively, and the TTA values are 6.14, 3.55, and 5.7 mL, respectively. The latter values (TTA) increase sharply with increasing fermentation time, whereas the former values (pH) decrease greatly (between 2 and 12 h). After 16 h of fermentation, the acidification rate becomes slow and almost stable, probably due to the fact that substrate consumption in the system at this stage increases acidity. This increase inhibits the growth of lactic acid bacteria, which agrees well with the trend of the total number of colonies. Finally, after 24 h of fermentation, the degree of acidification of TBS is less than those of WS and WTBS, possibly due to the buffering effect of tartary buckwheat on the pH of the fermenting sourdough system.

Organic acid content in sourdough

As shown in Fig. 1(d) and 1(e), the contents of acetic acid and lactic acid in the three groups of sourdough are very low before fermentation. After fermentation, the content of lactic acid greatly increases; however, the content of acetic acid does not change significantly. Due to the variation of fermentation substrates, the carbon sources and contents in the HMP pathway of fermented TBS, WS, and WTBS are different. As shown in Fig. 1(c), lactic acid bacteria grow and metabolize faster in tartary buckwheat substrate than in wheat and wheat-tartary buckwheat substrates. Therefore, the contents of lactic acid and acetic acid in fermented TBS are higher than those detected in fermented WS and WTBS samples. After 12 h of fermentation, the acetic acid content in TBS is 1.89 and 1.28 times higher than those in WS and WTBS, respectively, whereas the lactic acid content is 2.62 and 1.24 times greater than those in WS and WTBS, respectively. The amount of acetic acid detected in 100 g TBS at the end of fermentation (24 h) is 0.351, which is 1.61 and 1.06 times higher than the acetic acid contents in WS and WTBS, respectively. Meanwhile, the lactic acid content in TBS at 24 h is 0.469 g/100 g, which is 1.75 and 1.15 times greater than the corresponding contents in WS and WTBS, respectively. Considering that the acidification of organic acids may lead to more extensive protein hydrolysis in the dough, it is expected that the content of free amino acids in TBS is greater than those in WS and WTBS.

Extraction and determination of extracellular polysaccharides in sourdough samples

The contents of monosaccharides, disaccharides, and extracellular polysaccharides determined at different stages of sourdough fermentation are summarized in Table 1. Among these components, the extracellular polysaccharides produced by *Lactobacillus plantarum ST-III* can replace hydrocolloids, thereby improving the stability and quality of the frozen dough (Tieking et al. 2003). In TBS, the fructose content increases significantly during fermentation, reaching 9.03 mmol/kg at 12 h. Meanwhile, the EPS output in this group reaches 3.109 mmol/kg, which is appreciably higher than the outputs detected in other groups. The lowest fructose and EPS contents are detected in WS. As for sucrose, its content varies significantly depending on the type of sourdough, as well as on the fermentation time. In TBS, the variation may be attributed to the consumption of large amounts of sucrose at 30 °C. This is consistent with the production of high quantities of EPS and fructose in this group. In addition, the rate of growth and acid production in TBS is low, which means that this sourdough is more inclined to synthesize EPS under the action of a protective mechanism, thereby increasing stress resistance and cell survival. The synthesis of EPS and the accumulation of fermentable sugars maintain the fermentation temperature of TBS at 30 °C. Overall, the results show that tartary buckwheat can promote the production of EPS in sourdough.

Variation in sourdough protein during fermentation

Determination of protein content

Depending on the nature of the cereal substrate, the fermentation of sourdough by lactic acid leads to different types and contents of organic acids, resulting in varied degrees of protein degradation. Based on the values listed in Table 2, the albumin and prolamin contents in WS, TBS, and WTBS vary significantly during the fermentation process. The albumin in the dough is continuously degraded, and after 24 h of

Table 2
Changes in the content of protein components during the fermentation of two types of sourdough by *Lactobacillus plantarum ST-III*.

Fermentation time/h		0	6	12	18	24
Albumin content (g/100 g)	WS	0.859 ± 0.012^{a}	0.551 ± 0.017^{b}	0.290 ± 0.011^{c}	0.169 ± 0.007^{d}	0.084 ± 0.012^{e}
	TBS	6.296 ± 0.046^{b}	6.328 ± 0.018^{a}	5.931 ± 0.031^{c}	4.907 ± 0.011^{d}	4.425 ± 0.007^{e}
	WTBS	4.286 ± 0.011^{a}	4.218 ± 0.013^{a}	3.919 ± 0.011^{b}	3.705 ± 0.018^{c}	3.608 ± 0.018^{d}
Prolamin content(g/100 g)	WS	4.736 ± 0.015^{d}	4.887 ± 0.010^{c}	5.016 ± 0.060^{a}	4.955 ± 0.011^{b}	4.807 ± 0.016^{c}
	TBS	1.261 ± 0.018^{d}	1.448 ± 0.013^{c}	1.815 ± 0.006^{a}	1.781 ± 0.009^{a}	1.505 ± 0.007^{b}
	WTBS	3.056 ± 0.004^{e}	3.165 ± 0.012^{d}	3.545 ± 0.023^{a}	3.328 ± 0.011^{b}	3.247 ± 0.013^{c}

The data are the means of three independent experiments ± standard deviations.
^{ae} Values in the same row with different superscript letters differ significantly ($P < 0.05$).

Table 1
Sugar metabolism changes during sourdough fermentation.

Sample	Fermentation time	Fermentable sugar(mmol/kg Dough)				EPS(g/kg Dough)
		Glucose	Fructose	Sucrose	Maltose	
WS	6 h	1.79 ± 0.08^{a}	3.02 ± 0.01^{a}	34.11 ± 0.01^{a}	10.18 ± 0.04^{a}	0.944 ± 0.04^{a}
	12 h	1.76 ± 0.04^{b}	5.93 ± 0.03^{b}	20.59 ± 0.03^{b}	11.33 ± 0.07^{b}	1.334 ± 0.04^{b}
	18 h	1.79 ± 0.06^{c}	4.09 ± 0.05^{c}	13.61 ± 0.07^{c}	11.79 ± 0.03^{c}	1.165 ± 0.01^{c}
TBS	6 h	0.18 ± 0.01^{a}	7.09 ± 0.06^{a}	27.83 ± 0.02^{a}	10.54 ± 0.03^{a}	2.052 ± 0.06^{a}
	12 h	0.09 ± 0.01^{b}	9.03 ± 0.05^{b}	16.53 ± 0.02^{b}	11.99 ± 0.05^{b}	3.109 ± 0.06^{b}
	18 h	0.12 ± 0.03^{b}	8.84 ± 0.01^{c}	9.03 ± 0.07^{c}	12.36 ± 0.05^{c}	2.945 ± 0.04^{b}
WTBS	6 h	1.33 ± 0.02^{a}	5.83 ± 0.06^{a}	31.87 ± 0.03^{a}	9.03 ± 0.02^{a}	1.164 ± 0.04^{a}
	12 h	1.54 ± 0.02^{b}	6.53 ± 0.08^{b}	19.34 ± 0.02^{b}	9.73 ± 0.01^{b}	1.863 ± 0.03^{b}
	18 h	1.55 ± 0.06^{b}	6.28 ± 0.08^{c}	10.87 ± 0.05^{c}	10.38 ± 0.09^{c}	1.632 ± 0.02^{c}

The data are the means of three independent experiments ± standard deviations.
^{ae} Significant difference of different superscript letter values in the same column *($P < 0.05$)*.

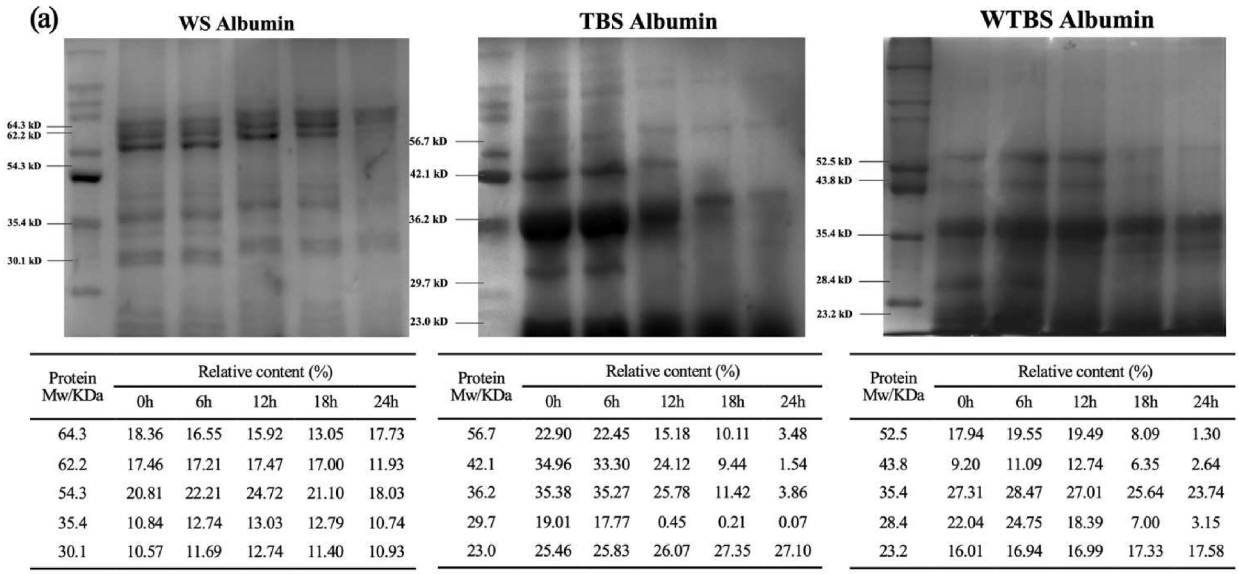
fermentation, the amounts of degraded albumin in WS, TBS, and WTBS are 90%, 3%, and 15.8%, respectively. Further analysis shows that *Lactobacillus plantarum ST-III* can effectively degrade the soluble proteins in wheat dough during fermentation and that the degradation of albumin in sourdough mainly occurs between 4 and 16 h. In TBS and WTBS, the rate of albumin degradation decreases with the extension of fermentation time.

Unlike albumin, the content of prolamin in WS, TBS, and WTBS increases first and then decreases during fermentation. The rates of increase vary depending on the nature of the grain, and maximum contents are detected at 12 h for all three samples. At the end of fermentation (24 h), an overall increase of 3.19%, 19.34%, and 6.25% is observed in the amounts of prolamin in WS, TBS, and WTBS, respectively. This increase may be attributed to the effects of *Lactobacillus plantarum ST-III* reproduction and metabolic acid production in promoting the dissolution of insoluble proteins such as globulin in the sourdough (Turksoy et al., 2020). Based on the trend of prolamin content variation, it is speculated that the fermentation of tartary buckwheat sourdough by *Lactobacillus plantarum ST-III* can improve the quality of tartary buckwheat processing.

SDS-PAGE analysis of fermented sourdough

As shown in Fig. 2(a) and 2(b), *Lactobacillus plantarum ST-III* degrades the albumin of the main molecular segment in all three sourdough samples investigated herein. In TBS albumin, the 36.2 k_D protein drops from 35.38% to 3.86% after 24 h of fermentation, and the 29.7 k_D protein drops from 19.01% to 0.07%. However, molecules accumulate in the 23.0 k_D segment of TBS albumin.

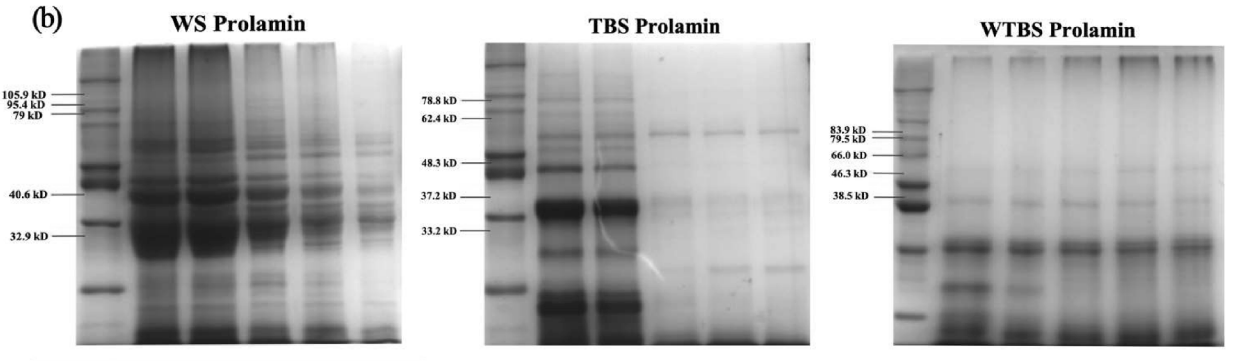
The influence of lactic acid bacteria fermentation on prolamin content in wheat and tartary buckwheat was investigated using SDS-PAGE analysis. The results shown in Fig. 2(b) and the relative content analysis of gliadin reveal that the 37.2 k_D protein in TBS accumulates after 12 h of fermentation, while the 78.8, 62.4, 48.3, and 33.2 k_D proteins degrade continuously after brief accumulation. In WS, the protein of the 32.9 k_D segment increases from 3.22% to 19.82% after 6 h of fermentation, while other large-molecule proteins are slowly degraded. Compared to the total protein and albumin contents, the relative content of gliadin in WS, TBS, and WTBS changes slightly during fermentation.



Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
64.3	18.36	16.55	15.92	13.05	17.73
62.2	17.46	17.21	17.47	17.00	11.93
54.3	20.81	22.21	24.72	21.10	18.03
35.4	10.84	12.74	13.03	12.79	10.74
30.1	10.57	11.69	12.74	11.40	10.93

Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
56.7	22.90	22.45	15.18	10.11	3.48
42.1	34.96	33.30	24.12	9.44	1.54
36.2	35.38	35.27	25.78	11.42	3.86
29.7	19.01	17.77	0.45	0.21	0.07
23.0	25.46	25.83	26.07	27.35	27.10

Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
52.5	17.94	19.55	19.49	8.09	1.30
43.8	9.20	11.09	12.74	6.35	2.64
35.4	27.31	28.47	27.01	25.64	23.74
28.4	22.04	24.75	18.39	7.00	3.15
23.2	16.01	16.94	16.99	17.33	17.58



Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
105.9	3.23	3.84	4.48	2.32	1.73
95.4	3.06	2.22	1.81	1.94	1.93
79.0	5.17	4.45	3.95	3.25	2.03
40.6	10.26	10.26	9.72	8.62	7.74
32.9	3.22	19.82	19.38	18.47	10.93

Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
78.8	6.44	7.26	3.36	2.03	0.83
62.4	4.01	4.04	2.16	1.09	0.32
48.3	4.36	4.53	2.95	1.03	0.54
37.2	3.77	3.92	4.58	3.72	2.17
33.2	26.22	20.71	18.72	14.33	5.22

Protein Mw/KDa	Relative content (%)				
	0h	6h	12h	18h	24h
85.9	2.44	1.25	3.73	2.65	1.07
79.5	1.32	2.27	1.24	2.12	1.26
66.0	1.76	2.15	1.97	2.85	1.03
46.3	2.47	3.28	2.54	1.93	1.92
38.5	2.22	1.59	2.00	1.08	1.42

Fig. 2. Changes in SDS-PAGE profile of *Lactobacillus plantarum ST-III* during sourdough fermentation (a) WS, TBS, WTBS albumin SDS-PAGE profile and changes in relative content (b) WS, TBS, WTBS albumin SDS-PAGE spectrum and changes in relative content.

Determination of amino acid content in sourdough

The values listed in Table 3 indicate that TBS contains more total free amino acids (6.77–8.03 g/100 g) than WS. The essential amino acids account for 31.91–32.88% of the total, with the highest percentage detected after 18 h of fermentation. In TBS, the content of free amino acids gradually increases during fermentation. Specifically, the contents of essential and non-essential amino acids in this group increase by 22.22 and 16.92%, respectively, between 6 and 18 h of fermentation. This increase may be attributed to the degradation of proteins in TBS into small peptide chains first and then into free amino acids after 12 h. The activation of endogenous protease and amylase during the fermentation of tartary buckwheat powder also promotes the metabolism of free amino acids. In WS and WTBS, the content of free amino acids increases and then decreases. After 18 h, this content is lower than that detected initially, which indicates that the fermentation of tartary buckwheat by *Lactobacillus plantarum ST-III* is more conducive to protein hydrolysis than wheat fermentation.

Among the different amino acids investigated in this study (17 in total), the essential amino acids, particularly leucine, phenylalanine, and lysine, exhibit significantly increasing contents in TBS. These amino acids can coordinate and promote the absorption of nutrients, thereby enhancing their beneficial effects in the human body (Payne et al. 2016; Rios et al. 2020). Overall, the results show that the addition of fermented (by *Lactobacillus plantarum*) TBS to baked products effectively improves their nutritional value.

Determination of the specific volume and texture of frozen dough bread

As shown in Fig. 3(a), the specific volume of frozen dough in all of the groups decreases gradually with the extension of freezing time. This is mainly due to the production of EPS during the sourdough fermentation process and the freezing resistance of the tartary buckwheat powder itself. EPS improves the freeze–thaw stability of frozen dough, thereby reducing the specific volume of frozen dough bread. After 13 weeks of freezing, TBS bread has the highest specific volume.

The results illustrated in Fig. 3(b) demonstrate that as the storage time increases, the hardness of WS, TBS, and WTBS bread also increases. At 0 weeks of storage, the TBS bread shows lower gluten content and higher hardness than the WS and WTBS breads due to the higher content of tartary buckwheat in the TBS. With time, the hardness of the bread increases; however, the rate of increase in the hardness of TBS bread is lower than those detected for WS and WTBS breads, due to higher EPS content. Studies conducted on the effect of buckwheat yeast exopolysaccharide on the aging characteristics of bread yield similar results (Yang et al. 2019). Compared to the corresponding fresh doughs, the hardness of WS, TBS, and WTBS breads after 13 weeks of freezing are 26.76, 12.94, and 17.09 times greater, respectively. Therefore, the addition of tartary buckwheat can effectively delay the destruction of the gluten protein network and the dissolution of starch granules in the frozen dough.

Moisture migration in frozen sourdough bread

Fig. 3(c) shows that the moisture content in the bread crust and bread core is large and that with time, the moisture migrates from the bread core to the outer skin, along the moisture gradient. The speed of migration increases with the extension of storage time, but moisture migration in TBS bread is generally slower than that in WS and WTBS breads. Moreover, the aging and regeneration of starch accelerate during the freezing process, and the Aw change of TBS bread is slower than that of WS and WTBS breads (Fig. 3(e)). This is mainly due to the production of a large amount of organic acids in TBS bread during freeze storage, which reduces the starch/protein migration of water and effectively prevents the spreading of moisture from the bread core to the bread surface. As a result, the water is retained in the bread, and a stable quality of the frozen dough is ensured.

In vitro digestibility of the protein in the bread

In the fermentation process of sourdough, lactic acid bacteria can degrade large protein into small peptide chains and free amino acids, thus further improving the utilization rate of protein in the product. The protein in tartary buckwheat flour cake was digested by pepsin-trypsin two-step digestion method (Bredariol et al. 2020).

It can be seen from Table 4 that for fresh dough bread, the protein digestibility of TBS fermented bread was 48.47% after gastric digestion for 90 min, which was 1.30 times and 1.63 times that of WTBS fermented bread and control bread, respectively. After entering the trypsin digestion stage, the digestibility of the three kinds of breads increased gradually. After 180 min of digestion, the protein digestibility of TBS fermented bread reached 64.39%, which was significantly higher than the other two breads. And for the control bread, the protein digestibility of the sourdough bread fermented with *Lactobacillus plantarum ST-III*

Table 3
Changes of essential amino acid content during the fermentation of sourdough by *Lactobacillus plantarum ST-III.*

Amino acid content (g/100 g)		WS 6 h	WS 12 h	WS 18 h	TBS 6 h	TBS 12 h	TBS 18 h	WTBS 6 h	WTBS 12 h	WTBS 18 h
Essential amino acid	Threonine	0.34	0.36	0.31	0.30	0.30	0.34	0.31	0.32	0.31
	Valine	0.51	0.53	0.47	0.37	0.39	0.43	0.43	0.43	0.42
	Methionine	0.09	0.13	0.09	0.06	0.07	0.10	0.09	0.09	0.07
	Isoleucine	0.37	0.40	0.36	0.22	0.22	0.28	0.29	0.30	0.30
	Leucine	0.92	0.96	0.86	0.48	0.50	0.60	0.68	0.68	0.66
	Phenylalanine	0.63	0.71	0.59	0.32	0.35	0.40	0.45	0.48	0.43
	Lysine	0.23	0.23	0.21	0.41	0.44	0.49	0.34	0.33	0.32
Total content		3.09	3.32	2.89	2.16	2.27	2.64	2.59	2.63	2.51
Nonessential amino acid	Aspartic acid	0.48	0.50	0.43	0.72	0.74	0.82	0.61	0.61	0.59
	Serine	0.62	0.64	0.56	0.39	0.39	0.44	0.48	0.47	0.45
	Glutamate	4.84	5.04	4.45	1.36	1.39	1.58	2.79	2.80	2.68
	Glycine	0.46	0.48	0.42	0.45	0.48	0.54	0.46	0.46	0.45
	Alanine	0.39	0.42	0.37	0.36	0.38	0.43	0.38	0.39	0.38
	Cystine	0.18	0.20	0.16	0.12	0.11	0.13	0.14	0.15	0.14
	Tyrosine	0.18	0.26	0.16	0.11	0.15	0.13	0.15	0.15	0.13
	Histidine	0.25	0.28	0.24	0.18	0.19	0.21	0.22	0.22	0.21
	Arginin	0.39	0.43	0.35	0.65	0.66	0.72	0.52	0.52	0.49
	Proline	1.65	1.71	1.55	0.27	0.28	0.39	0.88	0.88	0.85
Total content		9.44	9.96	8.69	4.61	4.77	5.39	6.63	6.65	6.37
Total content (Essential amino acid and nonessential amino acid)		12.53	13.28	11.58	6.77	7.04	8.03	9.22	9.28	8.88
Essential amino acid: Nonessential amino acid		1: 3.06	1:3	1: 3.01	1: 2.13	1: 2.1	1: 2.04	1: 2.56	1: 2.53	1: 2.54

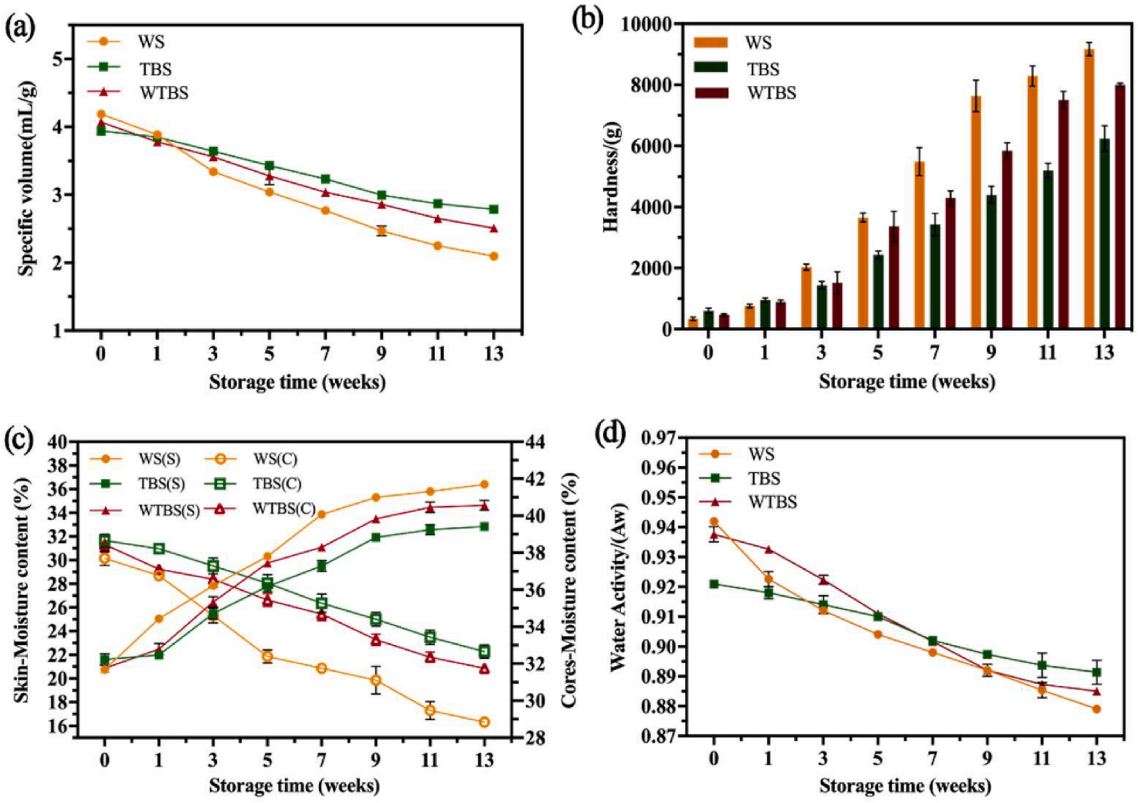


Fig. 3. Quality index of sourdough bread (a) Specific volume of frozen dough bread (b) Firmness of frozen dough bread (c) Changes in moisture content of frozen dough bread skin and cores (d) Changes in water activity of frozen dough bread.

Table 4
In vitro digestibility of bread protein in frozen dough fermented by *Lactobacillus plantarum ST-III.*

Group	0 min		30 min		60 min		90 min		120 min		150 min		180 min	
	0 weeks	13 weeks	0 weeks	13 weeks	0 weeks	13 weeks	0 weeks	13 weeks	0 weeks	13 weeks	0 weeks	13 weeks	0 weeks	13 weeks
Control	0	0	7.58 ± 0.57 ^{Da}	5.28 ± 0.31^{Db}	12.34 ± 0.42 ^{Da}	10.16 ± 1.10^{Db}	27.34 ± 0.31 ^{Da}	25.02 ± 0.17^{Db}	35.99 ± 0.56 ^{Da}	33.60 ± 0.53 ^{Db}	42.51 ± 0.37 ^{Da}	40.87 ± 0.33 ^{Db}	46.73 ± 0.56 ^{Da}	44.48 ± 0.16 ^{Db}
WS	0	0	8.24 ± 0.52^{Ca}	6.44 ± 0.57^{Cb}	14.13 ± 0.63^{Ca}	12.37 ± 0.66^{Cb}	30.29 ± 0.53^{Ca}	27.86 ± 0.58^{Cb}	37.57 ± 0.28 ^{Ca}	34.99 ± 0.62 ^{Cb}	44.50 ± 0.52 ^{Ca}	42.91 ± 0.87 ^{Cb}	47.77 ± 0.45 ^{Ca}	45.87 ± 0.42 ^{Cb}
TBS	0	0	14.58 ± 0.82^{Aa}	13.19 ± 0.64^{Ab}	24.87 ± 0.43^{Aa}	23.65 ± 0.26^{Ab}	48.54 ± 0.34^{Aa}	47.83 ± 0.52^{Ab}	52.84 ± 0.67 ^{Aa}	51.68 ± 0.34 ^{Ab}	58.40 ± 0.63 ^{Aa}	57.77 ± 0.31 ^{Ab}	65.50 ± 0.34 ^{Aa}	64.97 ± 0.28 ^{Ab}
WTBS	0	0	12.47 ± 0.55^{Ba}	10.57 ± 0.38^{Bb}	16.36 ± 0.38^{Ba}	15.07 ± 0.45^{Bb}	37.48 ± 0.43^{Ba}	36.31 ± 0.34^{Bb}	44.48 ± 0.61 ^{Ba}	42.67 ± 0.26 ^{Bb}	48.31 ± 0.81 ^{Ba}	47.18 ± 0.21 ^{Bb}	53.11 ± 0.56 ^{Ba}	51.95 ± 0.76 ^{Bb}

Values in the same row with different superscript letters differ significantly *($P < 0.05$)*.

was higher in vitro, which could be attributed to the activation of protease during the fermentation process leading to protein hydrolysis, thus improving the protein digestion rate during the digestion process. It can be seen that the degradation of the protein in the sourdough by lactic acid bacteria can improve the utilization rate of the protein, thereby further improving the nutritional value of the product.

In addition, whether fresh or frozen for 13 weeks, the protein digestibility of TBS bread was always higher than that of WTBS bread and control bread, but after frozen for 13 weeks, the protein digestibility of each group showed a downward trend, and TBS decreased more slowly than other groups. This may be because the protein content and amino acid content of TBS are higher than those of WS and WTBS, and the low temperature environment has little influence on the protein content of frozen dough bread with TBS added, so the frozen dough bread with TBS added has higher nutritional value.

Conclusion

This study fully clarified the biological characteristics of the gluten-free sourdough system fermented by *Lactobacillus plantarum ST-III* and the positive effect of adding 12 h TBS on the quality and nutritional value of fresh bread and frozen dough bread. Analyses of the contents and distribution of proteins and amino acids in the sourdough show that compared to WS (fermented with the same strain of bacteria), TBS is more capable of degrading proteins into small molecule protein and amino acids. Therefore, in the sourdough fermented by Lactobacillus *plantarum ST-III*, the formed EPS enhances the water binding capacity in the dough. Among them, the EPS content in TBS is the highest, so it has an impact on the freezing and thawing process. The fission caused by the recrystallization of medium ice is more tolerant, and the bread and frozen dough bread obtained by its processing have better quality and

nutritional content than ordinary wheat bread. Overall, our results demonstrate that the use of *Lactobacillus plantarum ST-III* to ferment tartary buckwheat sourdough improves the processing quality of low-gluten tartary buckwheat flour and minimizes nutrient loss in the frozen dough. This provides a basis for the development of high-nutrition bread and frozen dough. Subsequent studies on the water holding capacity and hydration properties of the extracted and purified EPS on frozen tartary buckwheat wet gluten and frozen starch gel will provide a more complete explanation of the antifreeze mechanism of frozen dough.

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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