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RESEARCH PAPER

Enhancement of nutritional characteristics of Tartary buckwheat (*Fagopyrum tataricum*) sprouts, passion and pineapple juice fermented by yeast (*Saccharomyces cerevisiae*) and *Lactobacillus plantarum*

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Abstract

The study examined the functional properties of lactic yeast-fermented Tartary buckwheat sprout with passion and pineapple juice (Tatary buckwheat sprout juice). Pineapple and passion fruit were included during fermentation to improve the sensory and nutritional quality of the juice. We initially created a juice with Tartary buckwheat sprouts, and then fermented it with *Lactobacillus plantarum* and yeast (*Saccharomyces cerevisiae*) to produce a novel fermented sprout juice. Our results indicated that the best organoleptic quality was achieved when the inoculum of *Lactobacillus plantarum* was 2%, the inoculum of yeast was 1%, and the fermentation time was 30 hours. The results showed that fermentation resulted in a 1.55-fold increase in the total amino acid content, with fresh sweet amino acids increasing by 1.75-fold and sour-bitter amino acids increasing by 1.33-fold. Additionally, the levels of acetic acid, lactic acid, citric acid, and tartaric acid in the buckwheat sprout juice increased by 12.31%, 11.45%, 4.22%, and 3.88%, respectively. GC-MS analysis revealed a significant increase of volatile flavoring substances, such as alcohols and esters, by more than 24.7% in the fermentation products. From the results, yeast-Lactobacillus fermentation process may effectively improve the nutritional values of Tartary buckwheat sprout juice.

Keywords

Tartary buckwheat sprout, Organic acid, Aroma compound, Amino acid, antioxidant ability

Introduction

Buckwheat, mainly divided into common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*), originated in the southwest of China and has gradually spread to other countries. It is rich in various active ingredients and is a dual-purpose crop that integrates health care with nutritional value (Li et al. 2022). A series of physiological and metabolic changes, mainly in the contents of various nutritional

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functional compounds, occurs in the germinated Tartary buckwheat. Lin showed that the bioavailability of Tartary buckwheat functional compounds and their nutritional value improve upon germination (Lin et al. 2008). In particular, they significantly increase in flavonoids, quercetin, rutin, and other active components (Liu et al. 2008). The flavonoid content in grains of different Tartary buckwheat varieties after germination showed a 1.76–2.33-fold increase during the sprouting period, and active substances such as rutin increased 4.1–6.5-fold (Ishii et al. 2014; Wiczkowski et al. 2014). The low digestibility of protein and starch in buckwheat grains consumed by humans might be related to the presence of tannin, phytic acid and protease inhibitors (Zhang et al. 2015). Germination improved the contents of amino acids, reduced sugars in the grains, and reduced their protein, thus improving the digestibility of proteins and starch. A better balance of amino acids in Tartary buckwheat sprouts compared to seeds, with significant improvement in nutritional quality and easier absorption by the human body (Singh et al. 2019). Germination can reduce or eliminate the content of toxic, harmful, or antinutritional substances in grains (Alvarez et al. 2008). However, the strong grassy smell of Tartary buckwheat sprouts results in poor taste and smell, which seriously affects consumer acceptability.

Fermentation is a traditional method of food processing and storage with a history spanning thousands of years. In recent decades, there has been a resurgence of interest in this technique on a global scale. This is attributable to the enhanced sensory characteristics and superior nutritional benefits of fermented foods (Domenico and Angelo 2024; Mukherjee et al. 2024). Fermentation converts the macromolecular substances in raw materials into active micromolecular substances, resulting in easier absorption; the changed composition of the food matrix significantly changes aromatic substances and improves sensory quality (Wei et al. 2018). Studies have showed that fermented juice can improve the intestinal environment (Reig et al. 2013), and enhance anti-inflammation (Tasdemir and Sanlier 2020) and anticancer activities (Villarreal-Soto et al. 2019). In addition, fermented products have the particular effects of alleviating hangovers and protecting the liver (Jung et al. 2016). Research has confirmed that fermentation by a combination of yeast and *Lactobacillus plantarum* strains can produce antibacterial substances that act on the intestinal mucosa and can bind to the binding sites of intestinal pathogens competing for host cells (Moshiri et al. 2017). This potential ability to adhere to the intestinal surface has a protective effect that improves the body's immunity. Nowadays, the raw materials available in markets are rich and varied, but the development of related fermented products with buckwheat sprouts has not yet been reported. Therefore, the development of fermented products for the enrichment of buckwheat-related products is of great practical significance.

Many countries worldwide have begun to use and process the Tartary buckwheat sprouts, such as South Korea (Giménez-Bastida et al. 2015), Japan (Nakamura et al. 2013) and China (Chen et al. 2020a). Because of its tender green color, crisp taste and rich nutrition, the Tartary buckwheat sprouts can be eaten raw and is deeply appreciated by consumers. At present, these sprouts are mainly used to produce Tartary buckwheat tea (Noda et al. 2021), noodles (Ma et al. 2013), biscuits (Ishiguro et al. 2016), bread (Giménez-Bastida et al. 2015), cakes and other products (Sturza et al. 2020). However, research on the development of fermented products from Tartary buckwheat sprouts are lacking. Thus, the current study was designed to produce a probiotic Tartary buckwheat sprout juice with tropical fruit and pineapple using yeast and *Lactobacillus plantarum* for fermentation (Fujita et al. 2017; Hashemi and Jafarpour 2021). The unpleasant grassy and bitter flavors in Tartary buckwheat sprouts reduce consumer acceptability compared to fruits and vegetables. Therefore, we hypothesis that the use of passion fruit and pineapple in fermentation would increase the organoleptic and nutritional properties of the fermentation product. To test this hypothesis, we prepared buckwheat sprout fermented juice using *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in a mixed fermentation with buckwheat sprouts, pineapple and passion fruit. The organoleptic properties, organic acids, amino acids, flavonoid content, volatile compounds and other nutritional properties were determined in the buckwheat sprout juice before and after fermentation. In addition, they also conducted storage experiments. These findings suggest a new method of processing Tartary buckwheat with potential health benefits.

Materials and methods

Sample processing and germination

Tartary buckwheat variety Xi-qiao no. 1 seeds from Chengdu University were germinated at 25 °C, and the sprouts were harvested 15 days after germination. Pineapple and purple passion fruit were bought at market. The average weight of each pineapple fruit was 500–600 g, and of the passion fruit, 80 g. Yeast was produced by Angel Yeast Co. Ltd. *Lactobacillus plantarum* was obtained from Shaanxi Agricultural Biological Collection Center, and Chengdu Cologne Chemicals Co. Ltd supplied all the chemicals.

Preparation of Tartary buckwheat sprout and fruit flavor enzymes

The technological process for fruit-flavored Tartary buckwheat sprout juice fermentation was as follows (Fig. 1), the raw materials were washed and cut into sections, consisting of 100 g Tartary buckwheat sprouts, 50 g passion fruit juice, and 100 g pineapple pieces, accurately weighed at a ratio of 2:1:2; 200 mL of water was added to make the fermentation substrate. The substrate was sterilized (pasteurization method, 60 °C for 30 minutes, cool and then inoculate) and fermented in two stages: at 35 °C for 16 h and then at 32 °C for 14 h. Pasteurization was conducted at 80 °C for 30 min.

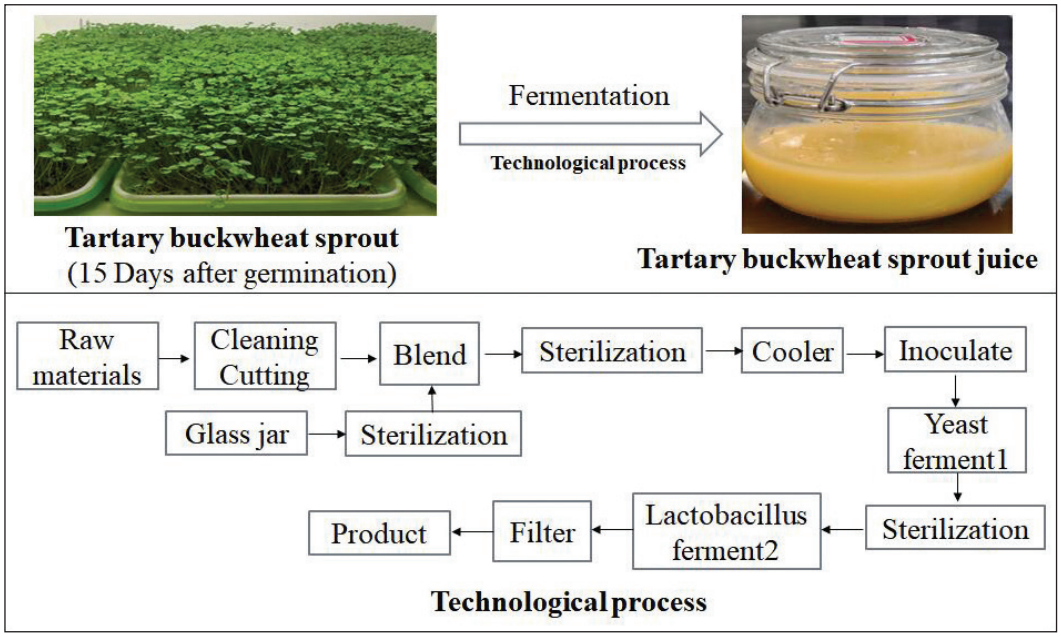

Figure 1. Fermentation process of fruity Tartary buckwheat sprout juice.

Nutrition composition analysis

Sensory evaluation

Sensory evaluation was performed according to the method of (Hashemi et al. 2016a), with some modifications. Sensory indexes of the Tartary buckwheat sprout juice before fermentation were evaluated for: color (20 points), flavor, taste and impurities, for a total maximum score of 100 points. Twelve testers performed the sensory evaluation. Finally, the evaluation scores of the 12 testers were analyzed by SPSS statistical software to determine the differences between pre- and post-fermentation samples. The scoring criteria are shown in Suppl. material 1: table S1.

An electronic eye (E-eye IRIS VA400; Alpha M.O.S., France) (Stefániková et al. 2020) was used for high-resolution imaging under controlled lighting and imaging conditions in a closed chamber with white light uniformly dispersed to avoid shadows. An electronic tongue was used according to the method of (Valente et al. 2018) with slight modifications. A taste sensing system (α-Astree; Alpha MOS Company, Toulouse, France) was used to evaluate the sour, bitter, aftertaste, fresh and sweet flavors. 50 ml of buckwheat bud juice before and after fermentation were measured separately at room temperature.

Determination of organic acid content

5 mL of sample in a 100 mL volumetric flask, add 2 mL of 10.6% (m/m) potassium ferrocyanide solution and 2 mL of 30% (m/m) zinc sulfate solution, shake well, fix the volume, let stand for 0.5 h, filter, and then processed by a Sep-Pak C18 solid-phase extraction cartridge, the sample solution was slowly flowed through the cartridge at a flow rate of 1 mL/min, and the resulting solution was used for HPLC analysis. The resulting solution was used for HPLC analysis, passed through a 0.22 um filter membrane, and put on the machine. The quantitative determination of organic acids (lactic acid and citric acid) was based on the method (Mousavi et al. 2013), using a U3000 HPLC (Thermo Fisher Scientific) at a detector wavelength of 214 nm.

Analysis of volatile aroma components

8 mL of sample was transferred into a 15 mL extraction bottle; 2.5 g NaCl was added with magnetic stirring, and the bottle was quickly sealed. The solid-phase microextraction (SPME) fiber head was preconditioned at 230 °C in the inlet of the GC-MS until there were no impurity peaks. The sample bottle was placed on the SPME device, and the temperature was set to 80 °C. The sample bottle was preheated in the extraction device for 15 min. The SPME fiber was inserted into the headspace of the sample through the bottle cap to about 1.0 cm above the upper surface of the sample, and headspace extraction was conducted for 60 min. The extraction fiber was then inserted into the sample inlet of the GC-MS and analyzed at 230 °C for 3 min. The sample was then taken for further analysis.

The volatile aroma compounds of fermented Tartary buckwheat sprout juice were determined by an Agilent 7890A-5975C GC-MS system (Qi et al. 2018). The area normalization method was used to quantify the percentage of the peak area of the identified components out of the total area of all identified components.

Determination of amino acids content

5 mL of 5% sulfosalicylic acid solution was added to 5 mL of sample, mixed well, and centrifuged at 6000 g for 10 min. The supernatant was evaporated to dryness

in a rotary evaporator, dissolved in 1 mL of sodium citrate buffer solution, and filtered through a 0.45 μm membrane. Amino acid content was determined according to the method at an ultraviolet detection wavelength of 440 nm–570 nm (Zhu et al. 2016).

Contents and antioxidant activity of various substances during storage

Determination of DPPH free radical clearance

The method described for determining DPPH free radical scavenging capacity was performed with slight modifications (Chen et al. 2020b). 400 μL of sample was taken, then 600 μL of methanol (14.26 mol/L) was added, and finally 600 μL of DPPH solution was added. The absorbance was measured at 527 nm. DPPH radical scavenging activity was expressed as the percentage inhibition of DPPH, and the expression was as follows: DPPH% = (1 − (As − Ac) ÷ A0) × 100%, where A0 is the initial absorbance and Ac is the control.

Determination of Superoxide dismutase (SOD)

The method described for determining superoxide dismutase was performed with slight modifications (SOD) (Fernando et al. 2021). 10 mL of sample was centrifuged at about 3500 rpm for 10 min, and the supernatant was taken and set aside. The supernatant was diluted 5 times with saline, 20 μL of sample was taken, 20 μL of enzyme dilution solution and 20 μL of enzyme working solution were added, and finally, 200 μL of substrate application solution was added and reacted for 20 min at 37 °C. The absorbance value was measured at 450 nm. Distilled water was used as a blank control group.

Determination of ABTS free radical clearance

The free radical scavenging capacity was also studied using the ABTS radical cation decolorization assay with slight modifications (Dudonné et al. 2009). The sample were diluted 5, 10, 15 and 20 times, respectively. 20 μL of the samples and 20 μL of VC were taken at different dilutions, and 20 μL of phosphate buffer were taken. 200 μL of ABTS working solution was added to the above solutions, and the mixture was allowed to stand at 37 °C for 6 min, and the absorbance was measured at 734 nm. The expression was as follows: ABTS%= (1 − (Ai − Aj) ÷ A0) × 100%, where A_i is the absorbance of the sample and ABTS working solution, A_j is the absorbance of phosphate buffer and sample, and A_0 is the absorbance of the mixture of ultrapure water and ABTS working solution.

Determination of protein content

The samples were centrifuged and diluted 5, 10, 15, and 20 times proportionally. Take 10 μL of the samples with different dilutions (Nakamura et al. 2009), 10 μL of the standards, and 250 μL of the protein working solution, respectively. Mix well and then react at 37 °C for 30 min before measuring the absorbance at 562 nm. Distilled water was used as a blank control group. Calculation formula for protein content: Pro = As − A_0 / Ac − A_0 × 524 μg/mL × dilution factor.

Determination of total flavonoid content

Total flavonoids were determined using the aluminum chloride colorimetric method (Stanojevic et al. 2009). 1 mL of sample was taken and methanol (2 mL, 0.3 g/mL) was mixed with 0.1 mL of 10% aluminum chloride hexahydrate, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction was fixed to 10 mL with methanol solution and reacted for 40 min at room temperature and the absorbance was measured at 450 nm. Based on the standard curve: y = 0.0582x − 0.0027, calculate the amount of flavonoids in the sample solution (mg). X = m / (W × d × 1000) × 100%, where X is the total flavonoid content (%), m is the amount of flavonoids in the sample calculated from the regression equation (mg), W is the mass or volume of the sample (g/mL), and d is the dilution ratio.

Determination of total polyphenol content

Total polyphenol content was determined following the method (Toro-Uribe et al. 2020), The sample was diluted 5, 10, 15, and 20 times, and then 1 mL of the dilution gradient was aspirated into a 10 mL cuvette. 2.5 mL of Folin-Phenol reagent was added and shaken well. 2.5 mL of 15% sodium bicarbonate solution was added, and the volume was adjusted to the scale by adding water and shaking well. After 60 min of a water bath at 40 °C and 20 min of cooling, the absorbance was measured at 778 nm. Based on the standard curve: y = 0.0137x + 0.0265. Calculation formula for total polyphenol content: X = C · 10 · N, where X is the total polyphenol content in the sample (mg/L), C is the total polyphenol content in the solution to be measured (mg/L) calculated from the table standard curve, 10 is the number of times the filtrate was diluted, and N is the number of times the sample was diluted.

Statistical analysis

Experimental data was analyzed statistically through Excel and SPSS software, and Origin 2020 software was used for plotting. Data were subjected to one-way ANOVA; statistical significance was set at $p < 0.05$.

Results and discussion

Processing of fermented Tartary buckwheat sprout juice

Selection of Tartary buckwheat sprouts at different growth stages

The morphology of Tartary buckwheat sprouts of different germination days (0, 5, 10, 15 d) was observed, and their flavonoid content was determined. As shown in the Table 1, the flavonoid content of buckwheat seeds was

18.46 mg/g; with the prolongation of germination time, the flavonoid content tended to be positively correlated. At 20 d germination, the flavonoid content in buckwheat sprouts was 43.42 mg/g. Although 20 d sprouts have the highest flavonoid content, 15 d buckwheat sprouts have the highest biomass and brittle leaves. Therefore, 15 d Tartary buckwheat sprouts were selected for subsequent product development of raw materials.

Table 1. Changes in the morphology of buckwheat seeds after germination and their flavonoid content.

Day	Leaf number and Morphology	Content of Total Flavonoids
0	seeds	18.46±0.37 mg/g
5	cotyledon stage: small blade area	40.78±0.68 mg/g
10	cotyledon stage: blade shaping	42.21±0.43 mg/g
15	cotyledon stage: bright green leaf colour and crisp texture	43.26±0.72 mg/g
20	cotyledon stage: dark green leaf colour and crisp texture	43.42±0.99 mg/g

Note: Each experiment was repeated 3 times.

Processing of fermented Tartary buckwheat sprout juice by *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

The quality of fermented juice products is primarily influenced by the type of bacteria and the duration of fermentation. Yeast and lactic acid bacteria are the most commonly utilized fermentation strains. Different types of yeast result in varied flavors, aromas, and nutritional profiles in the fermented products. *Saccharomyces cerevisiae* is a widely used strain in fermenting juices, with studies demonstrated that significant enhancements in sensory and nutritional attributes of apple juice fermented with Saccharomyces cerevisiae (Li et al. 2021). In most fermented foods, such as kimchi, fermented fruit and vegetable juices, and fermented fruit wine, *Lactobacillus plantarum* is commonly used as the main fermentation strain (Fonseca et al. 2021; Urbina et al. 2021). However, relying solely on lactic acid bacteria can result in an overly acidic and bitter product with reduced functionality. *Lactobacillus* also requires additional nutrients to thrive during fermentation. Introducing yeast as an initial inoculant can supply the necessary nutrients for Lactobacillus growth. Implementing a staged fermentation approach can help prevent debris formation and optimize yeast activity, ultimately minimizing the negative effects of lactic acid bacteria and laying a solid foundation for subsequent lactic acid bacteria fermentation (Li et al. 2021).

Fermentation temperature plays a crucial role in the growth and fermentation performance of strains, significantly influencing product fermentation. It also impacts the development of flavor compounds during the fermentation process. The ideal temperature for *Lactobacillus* growth is 35 °C, while yeast thrives at 30 °C. The specific fermentation temperatures for yeast and lactic acid bacteria will be determined in future experiments, without considering single-factor test screening. Following yeast fermentation at 30 °C, *Lactobacillus plantarum* fermentation at 35 °C is conducted to leverage the mutually beneficial symbiotic relationship between the two microorganisms for a complete fermentation process (Chen et al. 2017).

Optimization of the yeast amount

From Fig. 2B, it can be seen that when the inoculum quantity of yeast is 1.5%, the sensory score and SOD activity reach the highest value. When the inoculum quantity is lower than 1.5%, all the indexes positively correlate with the inoculum quantity. When the inoculum quantity is higher than 1.5%, all the indexes are negatively correlated with the inoculum quantity, and the overall tendency is firstly increasing and then decreasing. When the inoculum quantity reaches a certain amount, the appropriate carbon, nitrogen, and other energy substances fully promote the growth of microorganisms so the product reaches the best state. When the inoculum amount exceeds 1.5%, the nutrients in the fermentation broth may be consumed entirely due to the massive reproduction of microorganisms in the inoculum amount, and the depletion of carbon and nitrogen energy sources will lead to autolysis and premature aging of the strain, which will make it impossible for it to survive.

Optimization of the *Lactobacillus plantarum* Amount

The quantity of Lactobacillus plantarum used as an inoculum is crucial for the final taste and nutritional quality of the product. In the staged fermentation process, yeast is inoculated first followed by lactic acid bacteria. The optimal amount of yeast inoculation is determined, and the inoculation amount of lactic acid bacteria is also considered. As illustrated in Fig. 2A, when the inoculum amount of Lactobacillus plantarum is between 2% and 3%, both sensory score and SOD activity peak. Beyond 3% inoculation, these indicators exhibit a decreasing trend, possibly due to accelerated bacterial growth leading to premature decline, slight spoilage, and browning of the product, consequently impacting quality and sensory scores. Therefore, for superior quality in the later stages of fermentation, an inoculation amount between 1% and 3% is recommended.

Optimization of the fermentation time

The fermentation time significantly influences the strain. A short period of incomplete fermentation fails to meet the high nutrient requirements of fermented juice, while an extended fermentation period can lead to reduced nutrient utilization and adverse effects. As illustrated in Fig. 2C, an overall increasing trend was observed with longer fermentation times, with sensory score and SOD vigor gradually increasing. The sensory score and SOD vigor peaked at 30 h. However, organoleptic score and SOD viability

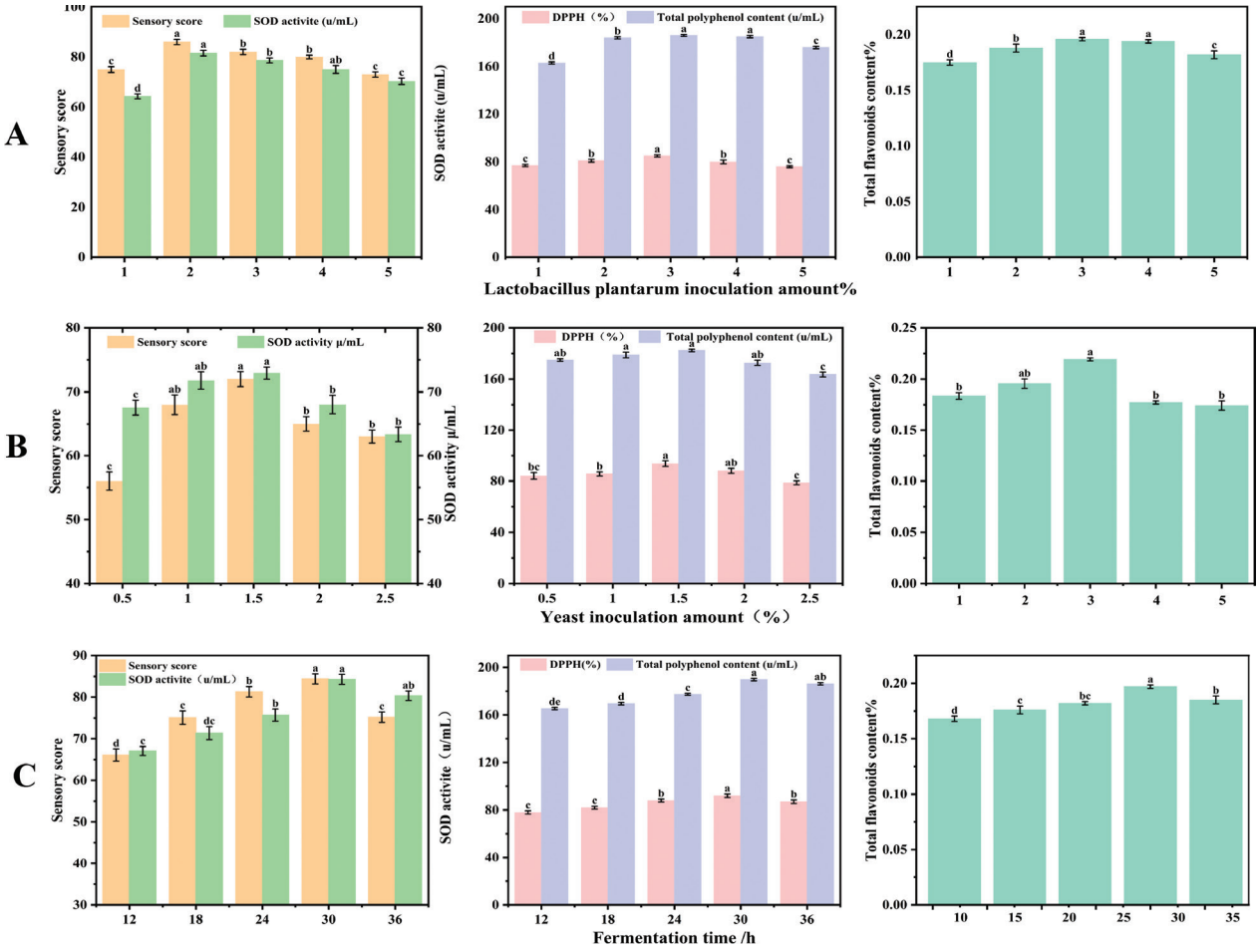

Figure 2. Effects of fermentation conditions on the quality of fermented Tartary buckwheat sprout juice; **A.** *Lactobacillus plantarum* inoculation amount; **B.** Yeast inoculation amount; **C.** Fermentation time. Each experiment was repeated 3 times.

tended to decrease after 30 hours of fermentation. The total polyphenol content of the fermented samples increased with longer fermentation times. Additionally, total polyphenols, total flavonoids, and DPPH radical scavenging rate showed a positive correlation during fermentation, reaching their maximum values at 30 h. Prolonged fermentation resulted in the decay of raw materials, leading to decreased organoleptic quality and product deterioration.

After orthogonal optimization of fermentation conditions based on sensory quality and nutrient composition, the optimal fermentation protocol for buckwheat sprout juice products was finally determined, the fermentation process involves inoculating yeast at 2% for 16 hours at 30 °C, followed by inoculating *Lactobacillus plantarum* at 3% for 14 hours at 35 °C.

Quality evaluation of fermented Tartary buckwheat sprout juice

Sensory evaluation

Fermentation improved the product's color and texture. The Tartary buckwheat sprout fruit juice was golden yellow, and the solution was transparent and glossy with no impurities. Suppl. material 1: table S2 shows the sensory evaluation before and after fermentation.

In order to objectively evaluate the flavor of buckwheat sprout juice, the test was carried out using an electronic tongue (Liu et al. 2023a). Suppl. material 1: fig. S1 presents the results of the electronic eye sensory analysis. The color area of the fermented product was relatively small, and its purity and luster were uniform. Principal component analysis (PCA) showed more than 97% of PC1's contribution, effectively improving the color of the product. In addition, the five fermented color varieties were aggregated into two varieties, 4048 and 4064, better to distinguish the degree of color between the two. Pre-fermentation and post-fermentation products were well distinguished and did not intersect, clustered in separate regions on the PCA graph (left and right, respectively). The significant difference between the two indicated that the electronic eye could better distinguish the liquid's color changes before and after fermentation (Stefániková et al. 2020).

Sensory analysis and detection results for the electronic tongue showed the changes in each flavor substance before and after fermentation (Fig. 3). The image shows that the acid and bitter taste and fresh and sweet taste thresholds were reduced after fermentation. The reduction of bitter and

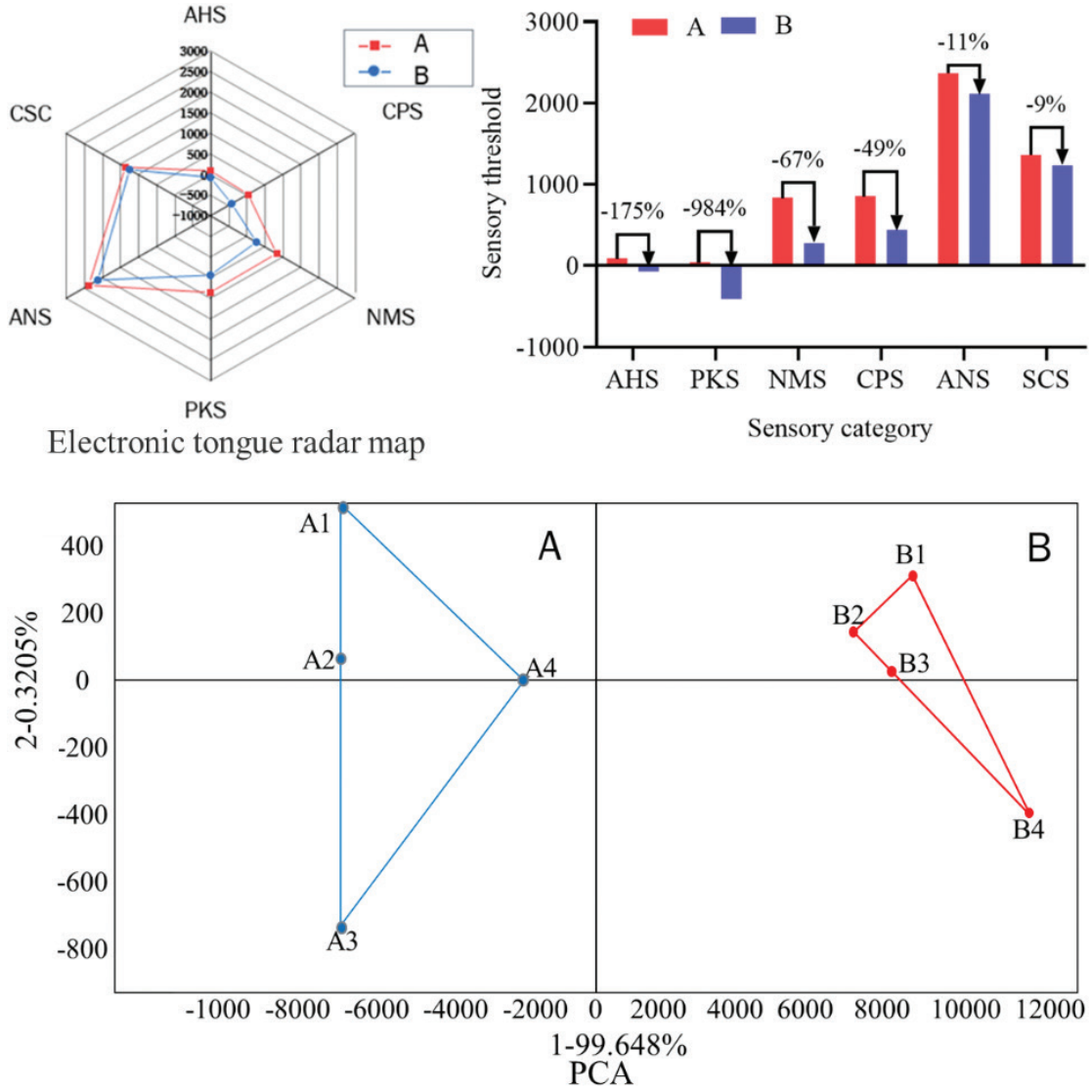

Figure 3. The electronic tongue sensory analysis of Tartary buckwheat sprout juice. **A** is the sample without fermentation; **B** is the sample filtered after fermentation, AHS is sour, CSC is bitter, ANS is sweet, NMS is umami, CPS and PKS are generic flavors.

astringent taste was related to the reduction in polyphenols, flavonoids, and other substances, and it might also be related to the increase in amino acid content. PCA showed that PC1 contributes 99% as the main component, and the taste of the product before and after fermentation could be better distinguished. There was no overlap between the product flavors before and after fermentation, clustering in the left and right regions of the PCA graph, respectively, indicating a significant difference between the two; this also indicated that the electronic tongue could effectively distinguish between the pre-and post-fermentation samples.

Quantitative HPLC analysis of organic acid content

Table 2 shows a significant difference in organic acid contents before and after fermentation. Among them, the contents of acetic acid, lactic acid, citric acid, and tartaric acid increased by 12.31%, 11.45%, 4.22%, and 3.88% post-fermentation ($p < 0.05$), respectively, which might be due to the two-stage fermentation with the two fermentation strains, making full use of a large number of substrates to produce a large number of organic acids, which was similar to previous reports (Hashemi et al. 2016b). In our study, the higher lactic acid content in fermented Tartary buckwheat sprout juice and the decrease in malic acid is due to malic acid being used as the leading carbon source for lactic acid production during fermentation, at the same time, higher levels of malic acid produce a pungent flavor (Liu et al. 2023b). The concentrations of acetic acid and lactic acid significantly increased post-fermentation, from 168.43±1.09 to 1399 mg/L, and 6012.2±3.2 to 7157.4±2.1 respectively, and were identified as the primary characteristic organic acids ($p < 0.05$). This finding aligns with previous studies shown that lactic acid and acetic acid are key organic acids generated through fermentation (Menzi et al. 2023). Organic acids produced by microbial

Table 2. Organic Acid Content of Tartary buckwheat sprout juice with fermentation and pre-fermentation.

Project	Pre-fermentation (mg/L)	Fermentation (mg/L)	Increase degree%
Lactic acid	6012.2±3.2^{b}	7157.4±2.1^{a}	11.45
Malonic acid	321.73±2.65^{b}	492.63±1.11^{a}	1.71
Oxalic acid	36.14±1.1^{b}	132.40±1.28^{a}	0.96
Tartaric acid	149.87±1.95^{b}	537.98±1.64^{a}	3.88
Formic acid	547.58±1.79^{b}	725.45±2.07^{a}	1.78
Malic acid	3205.40±2.8^{a}	2130.87±1.51^{b}	-10.75
Acetic acid	168.43±1.09^{b}	1399±1.5^{a}	12.31
Citric acid	286.43±1.78^{b}	708.21±1.22^{a}	4.22
Butanedioic acid	69.49±1.74^{b}	319.70±1.93^{a}	2.5
Propanoic acid	587.50±1.8^{b}	595.56±2.18^{a}	0.08

Different letters in the same row mean statistically significant differences ($p < 0.05$) (n = 3).

fermentation are the main flavor nutrients in fruit and can prevent or reduce food spoilage (Li and Liu 2015).

Furthermore, organic acids produced during microbial fermentation contribute to maintaining the body's acid-base balance, stimulating digestive juice secretion, aiding nutrient absorption, strengthening the stomach, enhancing appetite, reducing fatigue, softening blood vessels, and facilitating the absorption of calcium and iron. They not only inhibit the growth of harmful microorganisms but also support fat digestion by gastric juices, potentially preventing diseases and promoting metabolism (Kwon and Ricke 1998).

Aroma compound analysis

Aroma composition is an essential indicator for evaluating the flavor and quality of a product. Fig. 4 shows the changes in the flavor components of the fermented product compared to pre-fermentation. Volatile aroma compounds of Tartary buckwheat sprouts and the fruits' flavor products were highly enriched after fermentation ($p < 0.05$); among them, alcohols, esters, and aldehydes play a significant role in the aroma of fermented products. Before fermentation, 35 main volatile aroma compounds were determined, including aldehydes, alcohols, esters, and others, accounting for 34.37%, 29.76%, 19.18%, and 9.74%, respective-

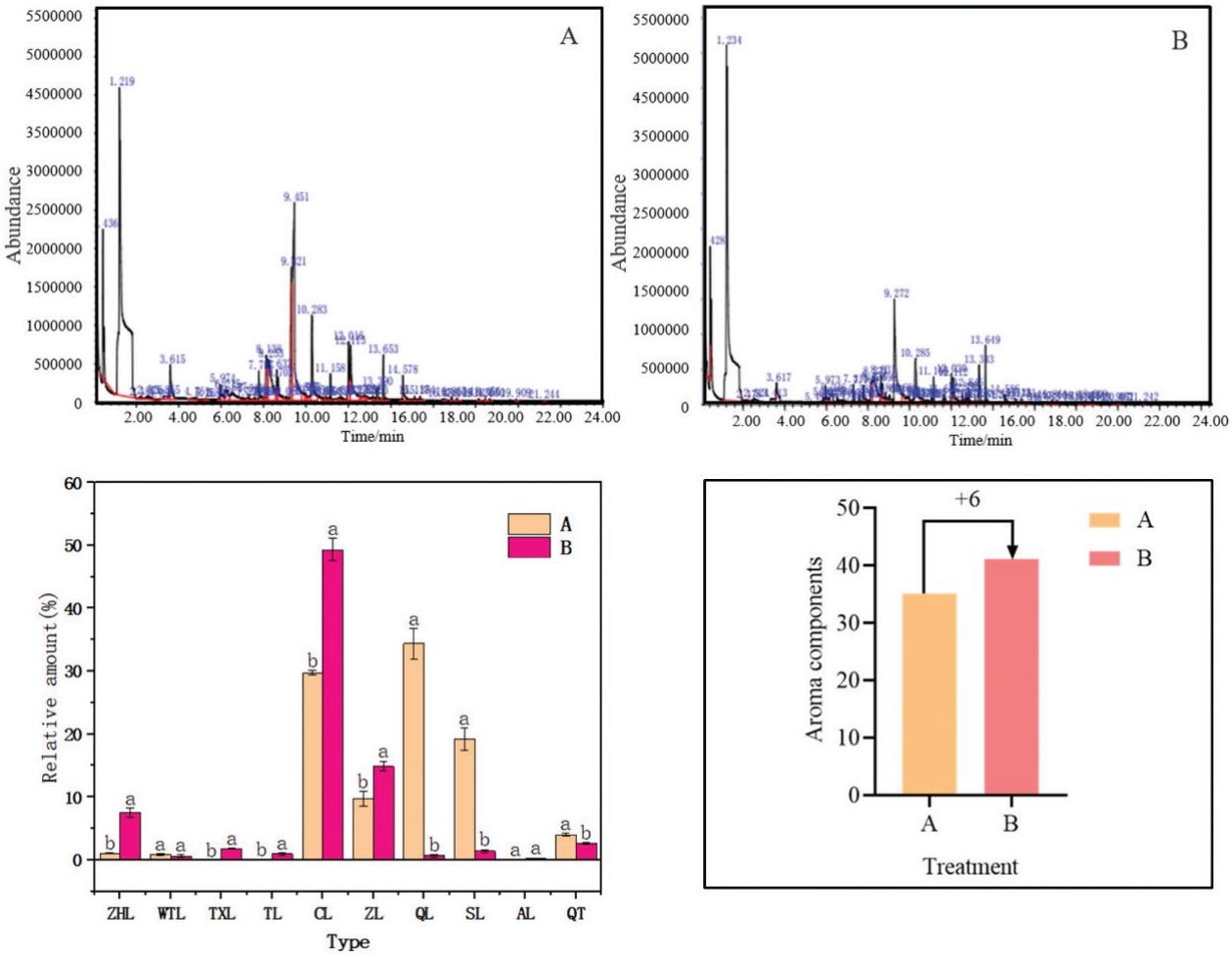

Figure 4. The changes in aroma components before and after fermentation of Tartary buckwheat sprout juice. **A.** The sample without fermentation; **B.** The sample filtered after fermentation. ZHL, WHT, TXL, TL, CL, ZL, QL, SL, AL and QT represent heterocyclic compounds, Alkane compounds, Terpene compounds, Ketone compounds, Alcohol compounds, Ester compounds, Aldehyde compounds, Acid compounds, Amine compounds and other compounds, respectively.)

ly ($p < 0.05$). After fermentation, 41 main volatile aroma compounds were identified, including alcohols, esters, heterocyclic compounds, and other aroma compounds, accounting for 49.29%, 14.91%, 7.55%, and 2.63%, respectively. Thus, alcohols and esters can be considered the characteristic aroma compounds in the product. One study showed that the proportion of aldehyde aroma compounds is highest before fermentation, and that of alcohol aroma compounds is highest after fermentation, which might be the main difference in volatile aroma compounds before and after fermentation (Wei et al. 2011; Zhang et al. 2022). It has been shown that pineapple aroma volatiles are mainly esters, followed by internal alcohols and terpenes (Montero-Calderón et al. 2010). In our study, no terpene aroma compounds were detected in the enzyme products before fermentation and the detection of three terpene aroma compounds after fermentation might be due to the new substances produced by the self-contained aroma compounds of pineapple and passion fruit during fermentation.

Amino acid content analysis

Table 3 shows the changes in amino acid contents of the product before and after fermentation, divided into fresh–sweet amino acids, acid–bitter amino acids and non-taste amino acids (Liu et al. 2015). Following fermentation, there was a significant increase in proline (Pro) from 218.864±1.76 to 404.56 μg/mL, and phenylalanine (Phe) from 0.731±0.162 to 15.578±2.495μg/mL compared to pre-fermentation juice($p < 0.05$). This observed rise in amino acids such as proline and phenylalanine post-fermentation is likely attributed to the metabolic activity of lactic acid bacteria converting bioactive molecules into amino acids during the fermentation process (Han et al. 2025). Glycine (3.041–2.453 μg/mL) and valine (21.348–17.05 μg/mL) decreased significantly after fermentation($p < 0.05$). This reduction in amino acids can be attributed to the utilization of these compounds as flavor precursors during the yeast fermentation process. They are metabolized to alcohols and aldehydes by microorganisms, ultimately contributing to the formation of characteristic flavor compounds (Tian et al. 2024).

Table 3. The effect of fermentation on amino acid and protein content of Tartary Buckwheat sprout juice.

Project	Type	Pre-fermentation (μg/mL)	Fermentation (μg/mL)	Increase degree%
Fresh sweet amino acid	Asn	5.472±0.8405^{b}	9.408±0.8095^{a}	3.93
	Thr	5.809±0.282^{b}	10.166±0.209^{a}	4.36
	Ser	2.645±0.478^{b}	4.482±1.101^{a}	1.84
	Glu	1.96±0.14^{b}	7.6±0.7^{a}	5.64
	Gly	3.041±0.226^{a}	2.453±0.4735^{ba}	-0.59
	Ala	4.424±1.448^{b}	5.031±1.694^{a}	0.61
	Pro	218.864±1.767^{b}	404.564±2.156^{a}	185.7
	Val	21.348±1.135^{a}	17.053±1.214ba	-4.3
	Total	263.563±6.3165^{b}	460.757±8.357a	
Acid bitter amino acid	Arg	8.026±0.241^{b}	12.048±0.678^{a}	4.02
	Ile	59.488±2.269^{b}	67.757±2.066^{a}	8.27
	Leu	22.132±1.901^{b}	23.555±2.263^{a}	1.43
	Tyr	2.155±0.25^{b}	4.279±0.422^{a}	2.12
	Phe	0.731±0.162^{b}	15.578±2.495^{a}	14.84
	His	2.772±0.023^{b}	3.37±0.417^{a}	0.6
	Total	95.304±4.864^{b}	126.587±8.341^{a}	
Non-taste amino acid	Lys	0.562±0.094^{b}	1.407±0.228^{a}	0.85
	Met	45.406±1.89^{a}	44.957±0.974^{a}	-0.45
	Cys	14.151±1.802^{b}	16.008±0.616^{a}	1.85
	Total	60.119±3.786^{b}	62.372±1.818^{a}	
Total amino acids		418.986±14.9665^{b}	649.716±18.516^{a}	
Protein (mg/mL)		3.24±0.42^{b}	9.82±0.58^{a}	

Different letters in the same row mean statistically significant differences ($p < 0.05$) (n = 3).

Among them, the changes in bitter amino acids and fresh–sweet and sour taste were significant before and after fermentation, increasing from 263% and 95% to 461% and 127%, respectively, a 1.75-fold and 1.33-fold increase in content ($p < 0.05$). The total amino acid content increased 1.55-fold. The increase in the content of each component after fermentation was significant ($p < 0.05$). The effect of fermentation on nutritional value and taste, as well as amino acids, played an essential role in the growth and development of the fermenting organisms, thereby increasing the taste and mouthfeel of the food (Sun et al. 2016). In addition, protein content was also significantly increased by fermentation, and the decomposition of protein into amino acids and polypeptides might be the main reason for the increase in amino acid content.

Basic indicators of products' antioxidant activity before and after fermentation

Antioxidant activity has been recognized as a key mechanism contributing to the beneficial effects of fermented juice. Active antioxidant substances work by inhibiting the reaction of free radicals through the transfer of hydrogen ions, ultimately reducing the risk of diseases such as aging and coronary heart disease (Wang et al. 2024). DPPH, SOD, ABTS, total polyphenol and total flavonoid contents of the samples before and after fermentation were determined (Table 4). The antioxidant index of the 5-fold diluted samples after fermentation was significantly improved compared to pre-fermentation. In addition, the significant increase ($p < 0.05$) in total polyphenol content after fermentation was positively correlated with the antioxidant capacity, while the total flavonoid content did not change significantly during fermentation.

Evaluation of nutrition quality and antioxidant property during storage

As shown in Fig. 5A, the antioxidant activity of the Tartary buckwheat sprout and fruit flavor enzyme product changed during storage, with the overall curve showing an increasing and then decreasing trend, followed by another increase and decrease. The free-radical-scavenging

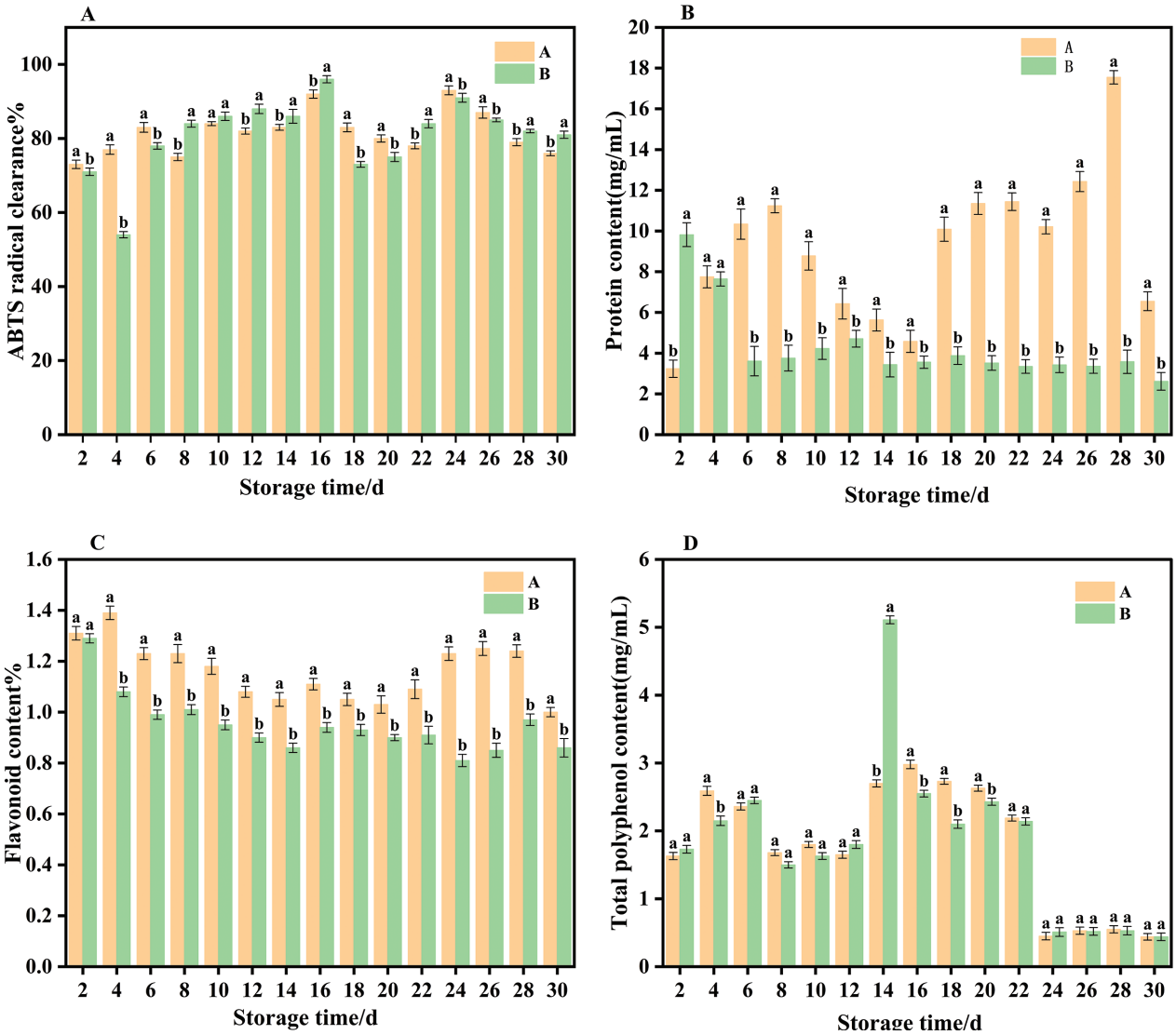

Figure 5. The changes in flavonoid, total polyphenol and protein content, ABTS radical clearance of Tartary buckwheat sprout juice during storage. **A.** The sample without fermentation; **B.** The sample filtered after fermentation.

Table 4. The antioxidant activity of Tartary Buckwheat sprout juice before and after fermentation.

Treatment	DPPH (%)	ABTS (%)	SOD(U/mL)	TPC (mg/mL)	Flavone (%)
Pre-fermentation	56.13±3.25^{b}	67.65±2.56^{a}	45.48±2.02^{b}	1.63±0.052^{b}	1.31±0.026^{a}
Fermentation	86.47±2.13^{a}	66.24±2.47^{b}	85.15±1.58^{a}	1.73±0.057^{a}	1.29±0.017^{b}

Different letters in the same row mean statistically significant differences ($p < 0.05$) (n = 3).

capacity of ABTS gradually increased during the first 6 days of storage, and its antioxidant capacity was higher at 6 days after storage, and 8 to 16 days after fermentation than before fermentation, reaching the highest level on day 16, then decreasing and increasing again thereafter. This is the same trend as previous studies with results showing that the antioxidant capacity of fermented fruit juices during storage is mainly related to their phenolic content (Kim et al. 2011; Wang et al. 2021).

Fig. 5B presents the change in protein content during storage before and after fermentation of the Tartary buckwheat sprout-fruit juice product; protein content first increased then decreased, and then increased again before fermentation, and then gradually decreased, and maintained a stable state after fermentation. The protein content after fermentation was significantly higher ($p < 0.05$) at 0–2 days of storage than before fermentation. The change in proteins with increasing storage time was greater for the non-fermented vs. fermented product, and the protein content of the fermented sample tended to stabilize on day 6 of storage. Protein content directly affects the final foaming ability of their product, and the protein was mainly decomposed into amino acids and peptides during the fermentation, which proves that the increase in amino acid content plays a leading role in protein content (Shokribousjein et al. 2011).

Fig. 5C, D shows the changes in total flavonoids and total polyphenols, during storage. The content of total flavonoids before fermentation was higher than that after fermentation ($p < 0.05$), and this difference became less obvious during storage. The change in total polyphenol content was positively correlated with antioxidant capacity, which also confirms the validity of the previous antioxidant data. The decrease in the total amount of phenolic compounds is due to their breakdown and utilization by micro-organisms and enzymes, etc. during the fermentation process through chemical reactions, as well as the effect of storage time and temperature (Klimczak et al. 2007; Ben et al. 2009).

Conclusion

All research findings indicated that the sensory attributes and nutritional composition of Tartary buckwheat sprout juice can be enhanced through a dual fermentation process involving yeast and lactobacilli. When compared to non-fermented Tartary buckwheat sprout juice, the fermented counterpart exhibits elevated levels of organic acids, proteins, amino acids, total polyphenols, and aromatic compounds. Moreover, co-fermentation contributes to the preservation of sensory and nutritional characteristics over time. Consequently, the combined yeast-Lactobacillus fermentation presents a promising approach for the production of functional fermented beverages using Tartary buckwheat sprouts.

Author contributions

Xiao Han: data processing and writing. Gen Ma: resources and software processing. Xiaoqin Fu: experiments conduction. Xin Zou, Jieyu Zhang, Jie Wen – visualization. Yu Fan, Yan Wan, Liangzhen Jiang, Chao Song, Dabing Xiang – writing-reviewing and editing. All authors read and approved the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary material

Supplementary material 1

fig S1. The electronic eye sensory analysis of Tartary buckwheat sprout juice; table S1. Sensory evaluation standard of Tartary buckwheat sprout juice; table S2. The score of Tartary buckwheat sprout juice with fermentation and pre-fermentation based on sensory evaluation standard (docx. file).

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