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Effects of *Streptococcus thermophilus* Fermentation on the Flavors and Antioxidant Properties of Barley Juice

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Abstract: Lactic fermentation can improve the metabolic profile and functionality of juice, which is beneficial to human health. This study aimed to investigate the effect of *Streptococcus thermophilus* fermentation on the metabolic profiles and antioxidant activity of barley juice. The results demonstrated that *S. thermophilus* 7G10 dramatically increased the total titratable acidity and total phenolic and flavonoid contents in the barley juice after 24 h of fermentation. Only sixteen volatile compounds were detected in the fermented barley juice, including six acids, four ketones, three alcohols, and one aldehyde. In addition, based on non-targeted metabolomics, 30 important differential metabolites were screened among the 1460 non-volatile compounds. Notably, the barley juice fermented with *S. thermophilus* 7G10 had increased free radical (ABTS, DPPH, and O_2^-) scavenging activities. Furthermore, sensory evaluation showed that the barley juice fermented with *S. thermophilus* 7G10 was most attractive to consumers. These results show that LAB fermentation promotes the formation of volatile compounds and potentially enhances the antioxidant properties of barley juice.

Keywords: barley juice; *Streptococcus thermophilus*; metabolic profile; sensory evaluation; antioxidant activity

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1. Introduction

Barley belongs to the family Poaceae and is the fourth most widely produced cereal worldwide due to its ability to grow in many regions, including Europe, North America, Russia, and Australia. Tibet, Sichuan, Gansu, and Qinghai Provinces are the main sources of barley in China. Barley is largely rich in native phytochemicals, including phenolics, flavonoids, alkaloids, β-glucans, unsaturated fatty acids, and dietary fiber [1]. According to a previous report, barley has a certain regulatory effect on digestion. Moreover, emerging evidence has demonstrated that daily consumption of barley can reduce the severity of hyperlipidemia, regulate glucose metabolism, ameliorate oxidative stress, and relieve liver inflammatory responses [2]. For example, a barley intervention improved lipid metabolism and maintained liver function in mice fed a high-fat/cholesterol diet [3]. A recent study showed that barley treatment altered the intestinal microbiota structure by increasing the abundance of beneficial bacteria, such as *Bifidobacterium bifidum*, *Bifidobacterium fecale*, and *Akkermansia muciniphila* [4]. Traditionally, barley is mainly used for brewing or producing feed [5,6]. It is necessary to develop new methods of barley processing to expand the application field of barley.

Lactic acid bacteria (LAB) are regarded as important microbes in the modern pharmaceutical and food industries because they can improve flavor and reduce the content of non-digestible substances in raw materials [7]. Over the past decades, LAB, including

Streptococcus thermophilus, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lacticaseibacillus casei*, and *Lactiplantibacillus plantarum* subsp. *plantarum*, have been widely used to ferment milk to further increase its nutritive value and absorptivity. *S. thermophilus* is a crucial starter in dairy products, which is strongly associated with its rapid acidification capability [8]. Early research confirmed that *S. thermophilus* fermentation produced a series of functional molecules, including exopolysaccharides, short-chain fatty acids, polypeptides, and vitamins [9]. In addition, *S. thermophilus* fermentation alters the texture, improves the viscosity, and adjusts the mouthfeel of yogurts. Recently, fermented juice has attracted considerable interest due to its relatively high content of nutrients and unique flavors [10]. A previous study showed that *S. thermophilus* fermentation increased the contents of organic acids and phenolics as well as the antioxidant capacity of blackberry juices [11]. In addition, *S. thermophilus* exhibits a strong malolactic conversion ability [12]. However, there are few reports on the effects of *S. thermophilus* fermentation on the metabolic profiles of juices.

To expand the application field of barley juice, an *S. thermophilus* strain was selected to ferment barley juice. Fermented barley juice samples were collected at different times, and several physical and chemical indicators were determined, including pH, total titratable acidity, total phenols, and total flavonoids, as well as the volatile and non-volatile compounds. In addition, the antioxidant abilities of the unfermented and fermented barley juices were assessed by measuring their capacities to scavenge of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and superoxide anion (O_2^-). The acceptance of the barley juice to consumers was revealed based on the sensory analysis.

2. Materials and Methods

2.1. Bacterial Strain

S. thermophilus 7G10 was isolated from fermented bovine milk. The bacterial strain was routinely cultivated in MRS broth and incubated at 37 °C for 24 h. The bacterial cells were collected via centrifugation at 12,000× *g* for 5 min. Subsequently, the strain was washed three times with phosphate-buffered saline (PBS) and resuspended in pasteurized barley juice.

2.2. Fermented Barley Juice Preparation

Barley powder was provided by Xunweixuan Co., Ltd. (Danyang, China). Barley juice fermentation was carried out according to a previously described method with minor modifications [13]. In brief, the barley powder was dried at 65 °C for 1 h and mixed with distilled water at a ratio of 1:9 (*w*/*w*). Barley juice (100 mL), glucose (0.15%, *w*/*w*), and skim milk (0.075%, *w*/*w*) were mixed, pasteurized at 65 °C for 30 min, and then cooled in ice-cold water. Subsequently, *S. thermophilus* 7G10 was inoculated in 0.1 L of barley juice and fermented at 37 °C for 24 h. The initial number of viable *S. thermophilus* 7G10 cells was about 10^6 CFU/mL. The samples were obtained at 0, 2, 4, 6, 12, and 24 h of incubation and kept at −79 ± 1 °C for further analysis. There were three replicates for each time point, and a total of 18 samples were collected.

2.3. Microbiological and Physicochemical Analysis

2.3.1. Determination of Viable Bacterial Counts and pH

Viable counts of *S. thermophilus* 7G10 were measured using the standard plate count method [14]. Briefly, the sample (0.5 mL) was serially diluted to 10^{-6}, 10^{-7} and 10^{-8} with 4.5 mL of sterilized physiological saline water in test tubes. Then, 100 µL of diluted juice was dispensed in triplicate on MRS agar and incubated at 37 °C for 48 h. Plates containing 30–300 colonies were counted, and the results were recorded as log CFU/mL. The pH of the fermented barley juice was measured using a digital pH meter (Mettle-Toledo, Greifensee, Switzerland).

2.3.2. Determination of Total Sugars and Titratable Acidity

The total sugar content in the fermented barley juice is presented as glucose equivalents using the phenol–sulfuric acid method [13]. In brief, 1 mL of the diluted sample (1:5999), 1 mL of phenol (5%, *v*:*v*), and 5 mL of sulfuric acid were mixed and kept at 25 °C for 10 min. After vibration, all samples were placed in a 30 °C water bath for 10 min. The absorbance was detected at 490 nm using a UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). The titratable acid content was detected using titration with 0.1 M NaOH and computed using the lactic acid conversion coefficient.

2.3.3. Determination of Total Phenolic Content

The total phenolic content was determined according to the Folin–Ciocalteu method with slight modifications [14]. In brief, 2.0 mL of 10% Folin–Ciocalteu solution was added to 1.0 mL of diluted samples (1:79) and stored at 25 °C for 3 min. Na_2CO_3 (2.0 mL; 15% *w*/*v*) was added to the reaction solution and then stored in the dark at 25 °C for 30 min. The absorbance at 765 nm was detected using a UV–Vis spectrophotometer (Shimadzu, Japan). The results are presented as equivalents of gallic acid.

2.3.4. Determination Analysis of Total Flavonoid Content

The total flavonoid content of the fermented juice was measured using the aluminum chloride colorimetric method with slight modifications [14]. In brief, 5.0 mL of sodium nitrite (50 g/L) was transferred to 1.0 mL of diluted sample (1:19) and stored at 25 °C for 5 min. Then, 1.0 mL of $AlCl_3$ (10%, *v*:*v*) was added to the mixture, mixed, vibrated, and stored in a 25 °C incubator for 10 min. Immediately, 2.0 mL of sodium hydroxide (2 mol/L) was added to the mixture at 25 °C for 10 min, and the absorbance at 510 nm was detected using a UV–Vis spectrophotometer (Shimadzu, Japan). The results are presented as rutin equivalents.

2.4. Analysis of the Volatile Compounds

In brief, 2.0 g of NaCl, 5 mL of fermented juice, and 1.0 µL of 2-octanol (5 mg/L) were transferred to a headspace bottle. After incubation at 60 °C for 10 min, volatile compounds of the mixture were collected using 50/30 umcar/polydimethylsiloxane/divinylbenzene. After extraction for 30 min, an extractor was inserted into the GC-MS sample inlet and desorbed at 250 °C for 5 min. Volatile compounds of the fermented juice were measured using gas chromatography and mass spectrometry (GC-MS; Thermo Fisher Scientific, Waltham, MA, USA), and the volatiles were separated using a DB-5MS capillary column (30 m × 0.25 mm, 1.0 µm). The conditions of the column oven were as follows: The initial temperature was 50 °C, which was maintained for 3 min and then increased to 240 °C at the speed of 5 °C/min. The final temperature was 240 °C, which was maintained for 10 min. Electron ionization at 70 eV was applied to collect the mass spectra in the range of 40–450 amu. Volatile compounds were analyzed by matching the retention indices of the mass spectra and spectral components in the NIST11 and WILEY 07 libraries. The retention index (RI) was calculated using n-alkanes (C7-C30) as an external reference under the same operating conditions. For semi-quantitative analysis, the relative peak areas of samples were obtained and assumed to be linearly proportional to the compound, and the detector had the same molar response for all compounds. Semi-quantification of the volatile compounds was calculated according to the following formula:

$$C\ (mg/L) = A_c \times C_{is}/A_{is}\ (mg/L)$$

where C is the relative concentration of the sample; C_{is} is the final concentration of the internal standard in the sample; A_c is the peak area of the analyzed sample; A_{is} is the peak area of the internal standard. In this study, the concentration of 2-octanol (internal standard) was 0.5 mg/L. Principal component analysis (PCA) and construction of the PCA-based

loading plots were performed using Simca (v 14.1), based on the abundance of volatile compounds and hierarchical clustering analysis.

2.5. Analysis of the Non-Volatile Compounds

The preliminary treatment of barley juice referred to a previous study with minor modifications [15]. Briefly, the supernatant (400 µL) of the unfermented or fermented barley juice and 80% methanol (800 µL) were mixed, extracted, and then stored at 25 °C for 1 h. After centrifugation at 14,000 rpm for 10 min at 4 °C, the supernatant was collected and freeze-dried using a vacuum concentrator centrifuge. All samples were reconstituted in 80% methanol (400 µL) and vortexed. After centrifugation at 14,000 rpm for 10 min at 4 °C, the supernatant was collected and analyzed using UPLC-QTOF-MS (Thermo Fisher Scientific Inc.). Raw data were analyzed and filtered using CD software (v 3.3). The obtained data were analyzed using MetaboAnalyst 5.0 (McGill University, Montreal, QC, Canada) (https://www.metaboanalyst.ca, 22 April 2023).

2.6. Antioxidant Activity Analyses

2.6.1. ABTS Radical Scavenging Ability

ABTS radical scavenging ability was measured using a previously described method with slight modifications [16]. Briefly, ABTS solution (7 mmol/L) and K_2SO_4 solution (2.45 mmol/L) were mixed in equal volumes and transferred to a dark environment at 25 °C for 16 h. The absorbance of the mixture at 734 nm was adjusted to 0.70 ± 0.01 using PBS and then mixed with fermented juice in equal volumes and kept at 25 °C for 6 min. The absorbance of each sample was detected at 734 nm using an ultraviolet–visible (UV–Vis) spectrophotometer. The blank control consisted of distilled water and the mixture. The ABTS radical scavenging ability was calculated using Equation (1):

$$\text{ABTS radical scavenging ability (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100 \quad (1)$$

2.6.2. DPPH Radical Scavenging Ability

DPPH radical scavenging ability was determined based on a previous report with slight modifications [16]. Briefly, 3.9 mL of DPPH solution (0.02 mol/L) was added to 0.1 mL fermented juice and then stored in the dark at 25 °C for 30 min. The absorbance at 517 nm was detected using a UV–Vis spectrophotometer (Shimadzu, Japan). Blank controls consisted of absolute ethanol and DPPH solutions. The DPPH radical scavenging ability was calculated using Equation (2):

$$\text{DPPH radical scavenging ability (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100 \quad (2)$$

2.6.3. O_2^- Radical Scavenging Ability

O_2^- radical scavenging ability was determined according to a previously described method with slight modifications [13]. Briefly, nitroblue tetrazolium salt (NBT, 300 µmol/L), β-nicotinamide adenine dinucleotide (NADH, 936 µmol/L), and phenazine methosulfate (PMS, 120 µmol/L) were prepared in 100 mM PBS (pH 7.4). The reaction mixture consisted of an equal volume of samples or distilled water (control), NBT, NADH, and PMS. After incubation at 37 °C for 5 min, the absorbance at 560 nm was measured using a UV–Vis spectrophotometer. O_2^- radical scavenging ability was calculated using Equation (3):

$$O_2^- \text{ radical scavenging ability (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100 \quad (3)$$

2.7. Sensory Analysis

A total of 23 volunteers aged between 23 and 27 were recruited, including 12 males and 11 females. All the volunteers received 6 h of training according to a previous study [17] and met the requirements for the sensory evaluation panel. Sensory tests were performed in a quiet environment with white fluorescent lights. The unfermented and fermented barley

juices were distributed to the volunteers in a random sequence of coded plastic disposable cups. The samples were evaluated according to five sensory attributes, including overall acceptability, sourness, sweetness, odor, and color of the juice. The numbers ranged from 1 (poor) to 9 (excellent), indicating the extent to which the volunteer liked each attribute. The scores for each sample given by the volunteers were collected, and the average value was calculated.

2.8. Statistical Analysis

Data are shown as the means ± standard deviations of three experiments. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used for data analysis, and differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Dynamic Changes in Viable Cell Counts and pH of Barley Juice during Fermentation

Vegetables, fruits, and grains are suitable substrates for LAB growth [18]. A previous report showed that barley was rich in carbohydrates, amino acids, vitamins, minerals, and fatty acids, being very suitable for microbial growth [19]. As illustrated in Figure 1, the initial viable cell count of *S. thermophilus* 7G10 was about 6.11 log CFU/mL and increased to 8.68 log CFU/mL at the termination of fermentation, suggesting that *S. thermophilus* 7G10 could utilize the nutrients in barley juice for growth. Meanwhile, *S. thermophilus* 7G10 fermentation decreased the pH of the barley juice from 6.22 to 4.44.

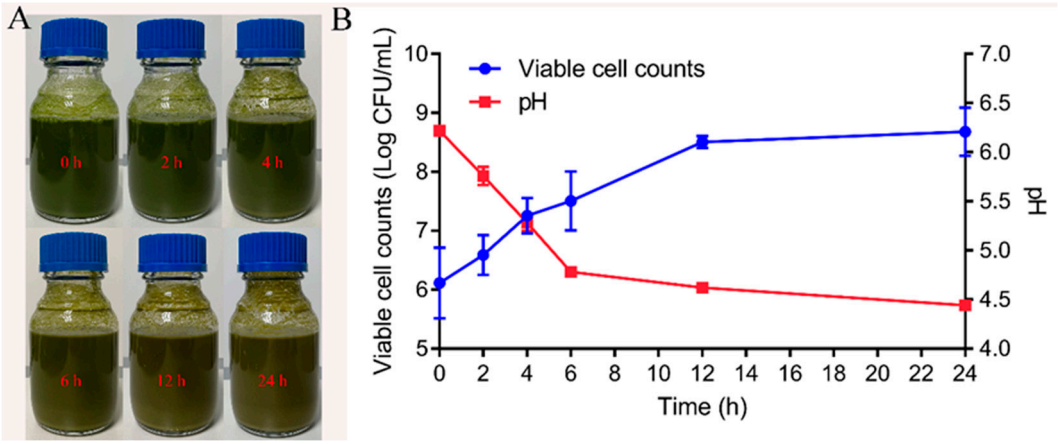

Figure 1. Photograph of the barley juice during the fermentation (**A**), and changes in viable cell counts and pH of the barley juice fermented with *S. thermophilus* 7G10 (**B**).

3.2. Dynamic Changes in Total Sugars, Titratable Acidity, Phenols, and Flavonoids in Barley Juice during Fermentation

Sugar serves as an important carbon source for bacterial growth. Changes in total sugar contents can directly reflect LAB growth [18]. As shown in Figure 2A, the total sugar content of the barley juice was significantly increased after 4 h of fermentation at 37 °C, which may be due to the degradation of polysaccharides in the barley juice by *S. thermophilus* 7G10. Subsequently, the total sugar content of the barley juice decreased dramatically at 6 h and consequently decreased to 0.38 ± 0.00 mg/mL at 24 h. In addition, LAB fermentation is confirmed to promote the formation of organic acids in vegetables, fruits, and grain juices [20]. In the present study, the total titratable acidity of the barley juice increased rapidly after 6 h and reached the highest concentration at 24 h (Figure 2B). However, high levels of total titratable acidity may suppress LAB growth, resulting in minor changes in the fermented juice quality [21].

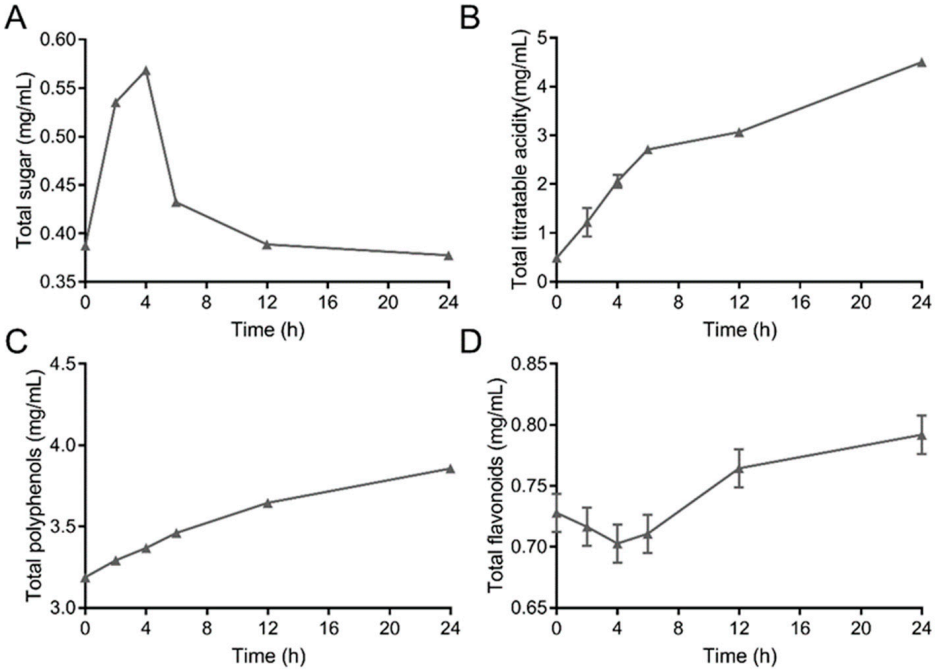

Figure 2. Effects of *S. thermophilus* 7G10 on total sugars (**A**), total titratable acidity (**B**), total polyphenols (**C**), and total flavonoids (**D**).

Phenols and flavonoids are widely present in vegetables and fruits in the form of bound or free states. Some reports have suggested that LAB fermentation can increase the content of active substances in food, including total phenols and total flavonoids [18,22]. As shown in Figure 2C, the content of total phenols showed an upward trend during fermentation and reached the highest point of 3.86 ± 0.02 mg/mL at 24 h. Complex polyphenols can be decomposed into simple polyphenols via enzymatic breakdown, and the phenolic transformation ability is strongly related to LAB growth [23]. A previous study indicated that LAB fermentation could transform polyphenolic compounds into others with lower molecular weights and release the conjugated phenolic compounds, which is beneficial for increasing the bioavailability of phenols [24]. In addition, the content of total flavonoids decreased from 0.73 ± 0.02 mg/mL to 0.70 ± 0.02 mg/mL (0–4 h) (Figure 2D) and then displayed a stepwise upward trend.

3.3. Volatile Compound Profiles of Unfermented and Fermented Barley Juices

Volatile compounds are regarded as an important index for assessing the quality of fermented juices. The profiles of the volatile compounds in the barley juice were measured using HS-SPME coupled with GC-MS during *S. thermophilus* 7G10 fermentation. As shown in Figure 3, 71 volatile compounds were detected and identified, including 22 acids, 15 ketones, 13 alcohols, and 9 aldehydes. Among them, 16 new volatile compounds formed during the barley juice fermentation, including 6 acids, 4 ketones, 3 alcohols, and 1 aldehyde.

PCA was performed to reveal the overall changes in the volatile compounds in the barley juice during the fermentation (Figure 4A). The samples of barley juice were spontaneously clustered according to different sampling times (R_2X = 0.71, Q_2 = 0.44). The first and second principal components of the PCA accounted for 39.4% and 17.6% of the total variability, respectively. The unfermented barley juice was located in the second and third quadrants of the PCA loading plot, whereas the fermented barley juice samples were located in the first and fourth quadrants after 24 h of fermentation, indicating that *S. thermophilus* 7G10 fermentation significantly altered the volatile compounds in the barley juice. Numerous studies have shown that fruit and vegetable juices fermented with LAB

have unique flavors [25]. In addition, the above appearance was further reflected in the HCA analysis (Figure 4B).

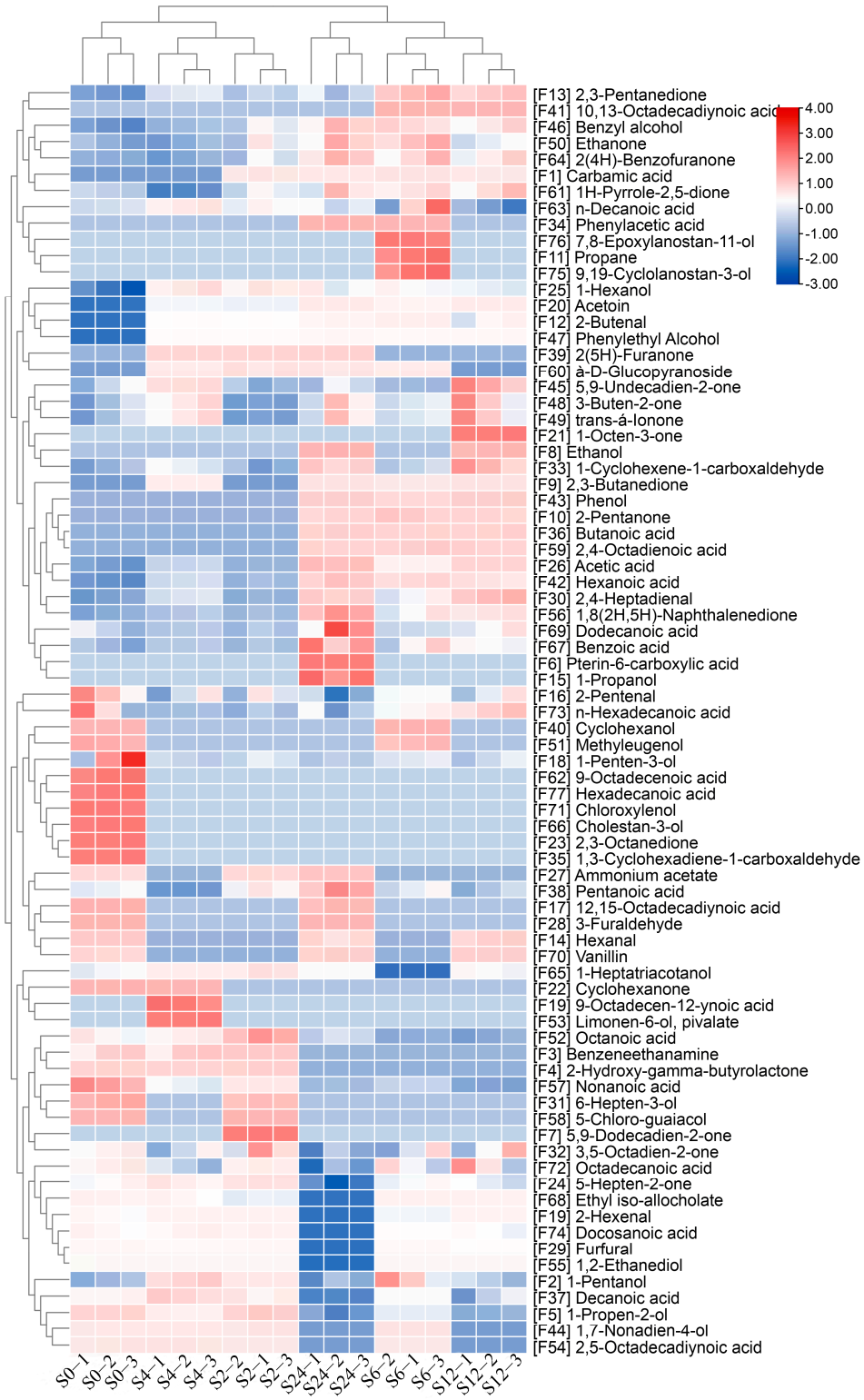

Figure 3. Heatmap of the volatile compounds in the barley juice during the fermentation. S0: unfermented barley juice; S2, S4, S6, S12, S24: fermented barley juice at 2 h, 4 h, 6 h, 12 h, and 24 h, respectively; "−1, −2, −3": three replicates for the corresponding samples.

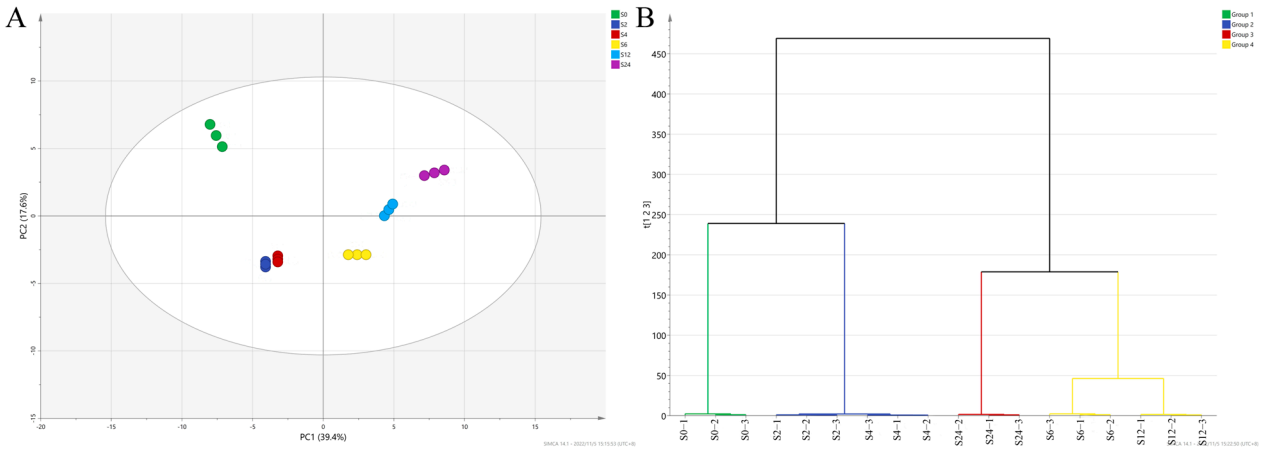

Figure 4. Score plot (**A**) and hierarchical cluster plot (**B**) based on PCA (n = 3) of the volatile compounds in the barley juice during the fermentation. S0: unfermented barley juice; S2, S4, S6, S12, S24: fermented barley juice at 2 h, 4 h, 6 h, 12 h, and 24 h, respectively; "−1, −2, −3": three replicates for the corresponding samples.

A PCA loading plot was constructed to identify the key volatile compounds between the unfermented and fermented barley juices (Figure 5). The main compounds identified in the unfermented barley juice were benzeneethanamine [F3], 1-penten-3-ol [F16], 12,15-octadecadiynoic acid [F15], 3,5-octadien-2-one [F29], 6-hepten-3-ol [F28], 9-octadecenoic acid [F58], octanoic acid [F49], 2-pentenal [F14], 2-hexenal [F17], methyleugenol [F48], and docosanoic acid [F70]. 1-Penten-3-ol is widely present in barley juice, and its contents gradually decrease during fermentation [26]. 9-Octadecenoic acid is usually present in dietary fats and oils, and long-term consumption can increase the risk of cardiovascular disease due to its effects on lipoproteins in plasma [27]. Methyleugenol, an alkenylbenzene compound, is present as a component of leaf and floral essential oils from many plant species, which is consistent with this study [28]. Fermented barley juice is mainly composed of pentanoic acid [F35], vanillin [F66], benzyl alcohol [F43], ethanone [F47], ethanol [F7], 2-pentanone [F9], 2,3-pentanedione [F11], and benzoic acid [F63]. Pentanoic acid is a carboxylic acid produced through microbial fermentation of carbohydrates [29]. Vanillin is a natural aromatic flavoring compound that is widely used to enhance food aromas and antimicrobial properties [30]. Benzyl alcohol is a member of the fragrance structural group of aryl alkyl alcohols and is used as a flavoring substance in foods [31]. In addition, enrichment of 2-pentanone and 2,3-pentanedione can be attributed to the oxidation of unsaturated fatty acids in LAB fermentation [32]. These results suggest that LAB fermentation significantly alters the volatile compounds present in barley juice.

3.4. Non-Volatile Compound Profiles of Unfermented and Fermented Barley Juices

The nature and concentration of non-volatile compounds directly affect the organoleptic quality of juice [33]. Therefore, the non-volatile compound profiles of the unfermented and fermented barley juices were analyzed using UPLC-QTOF-MS. A total of 1063 and 397 non-volatile compounds were identified in the unfermented and fermented barley juices, respectively, in positive (ESI+) and negative (ESI-) ion modes using untargeted metabolomics based on UPLC-QTOF-MS. As illustrated in Figure 6A,B, the principal components accounted for 69.0% (PC1 = 50.9% and PC2 = 18.1%) and 86.6% (PC1 = 73.9% and PC2 = 12.7%) of the total variation in the ESI+ and ESI- modes, respectively. The unfermented and fermented barley juices were obviously separated, suggesting that *S. thermophilus* 7G10 significantly altered the non-volatile compounds in the barley juice. In addition, the above appearance was also reflected in the OPLS-DA scores plots (Figure 6C,D). A previous study showed that *S. thermophilus* fermentation endowed the juice with more metabolic characteristics, such as phenolic acids, flavonoids, and alkaloids [12].

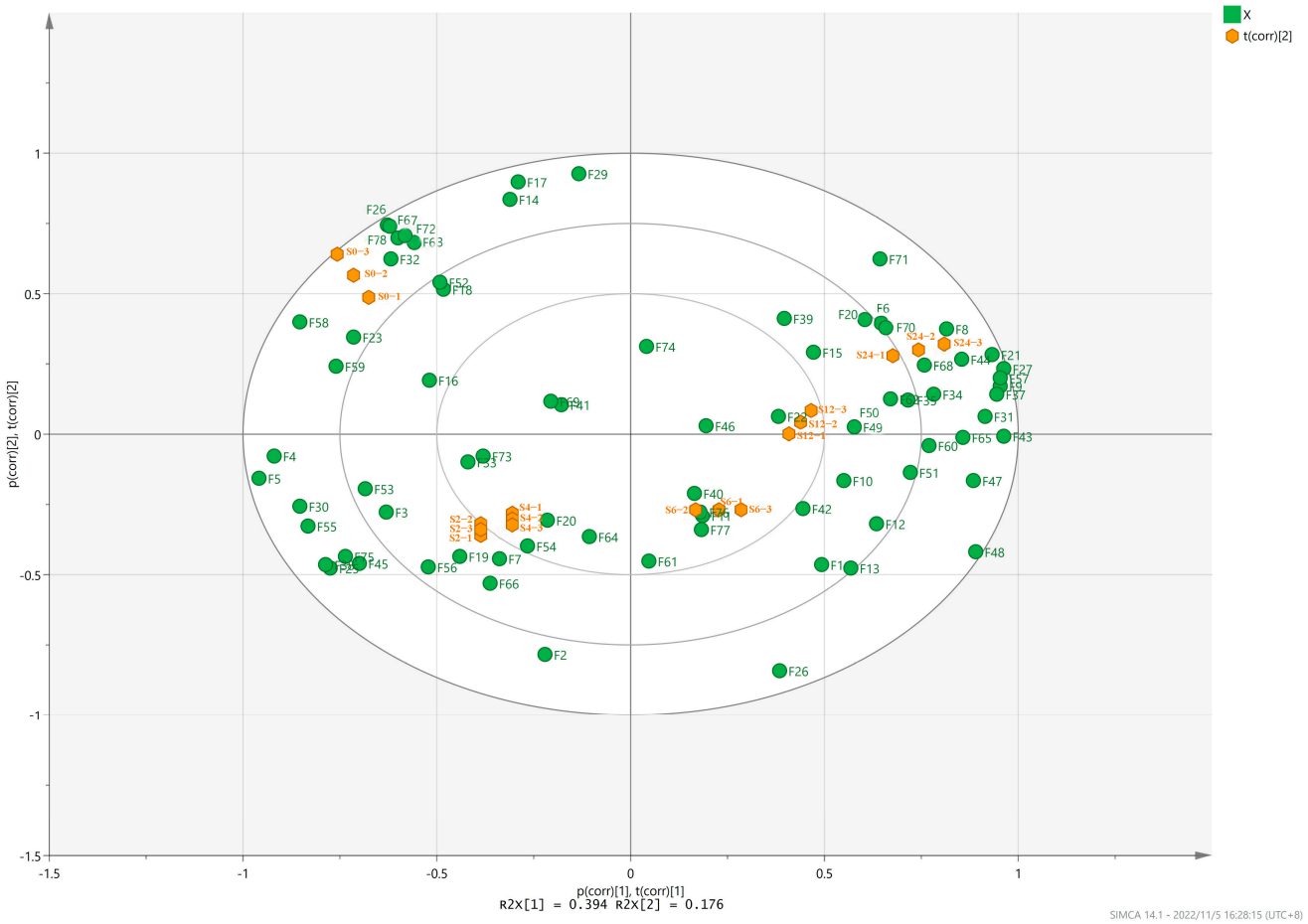

Figure 5. Loading plot of the two principal components based on PCA. S0: unfermented barley juice; S2, S4, S6, S12, S24: fermented barley juice at 2 h, 4 h, 6 h, 12 h, and 24 h, respectively; "−1, −2, −3": three replicates for the corresponding samples; F1–F71: the volatile compounds identified in the barley juice during the fermentation.

As illustrated in Table 1, 30 key non-volatile compounds were obtained according to their variable importance in projection (VIP). Compared with the unfermented barley juice, the contents of S-adenosylmethionine, iminoerythrose 4-phosphate, tolycaine, nonane-4,6-dione, 5-methyldeoxycytidine, N-ribosylnicotinamide, uracil, tetramethylene sulfoxide, anatabine, azobenzene, hypoxanthine, inosine, D(+)-phenyllactic acid, DL-lactic acid, pyruvic acid, (2R)-2,3-dihydroxypropanoic acid, hypoxanthine, guanine, 6-hydroxycaproic acid, cytarabine, deoxyguanosine, and pseudouridine in the fermented barley juice were significantly increased. S-Adenosylmethionine is an important substance that plays an important role in regulating glycolysis and alcoholic fermentation, and its content is strongly associated with the abundance of bacteria and fungi [34]. Uracil exists in relatively low amounts in foods, but its content is significantly increased in foods fermented with LAB, which is in accordance with our study [35]. Hypoxanthine, inosine, and guanine are reaction intermediates of purine metabolism and are regarded as potential markers for assessing food freshness [36]. In addition, an increase in the hypoxanthine content may result from an increase in hypoxanthine synthesis and a reduction in hypoxanthine degradation [37]. However, the contents of nicotinamide, L-lysine, L(-)-pipecolinic acid, glyoxylic acid, DL-malic acid, fumaric acid, and (3S)-3_6-diaminohexanoate deoxyguanosine in the barley juice were significantly reduced after *S. thermophilus* 7G10 fermentation. L-Lysine plays a key role in LAB growth, and its content was reduced in the barley juice after *S. thermophilus* 7G10 fermentation [38]. DL-Malic and fumaric acids are components of the tricarboxylic acid cycle, which significantly shorten the fermentation time [39].

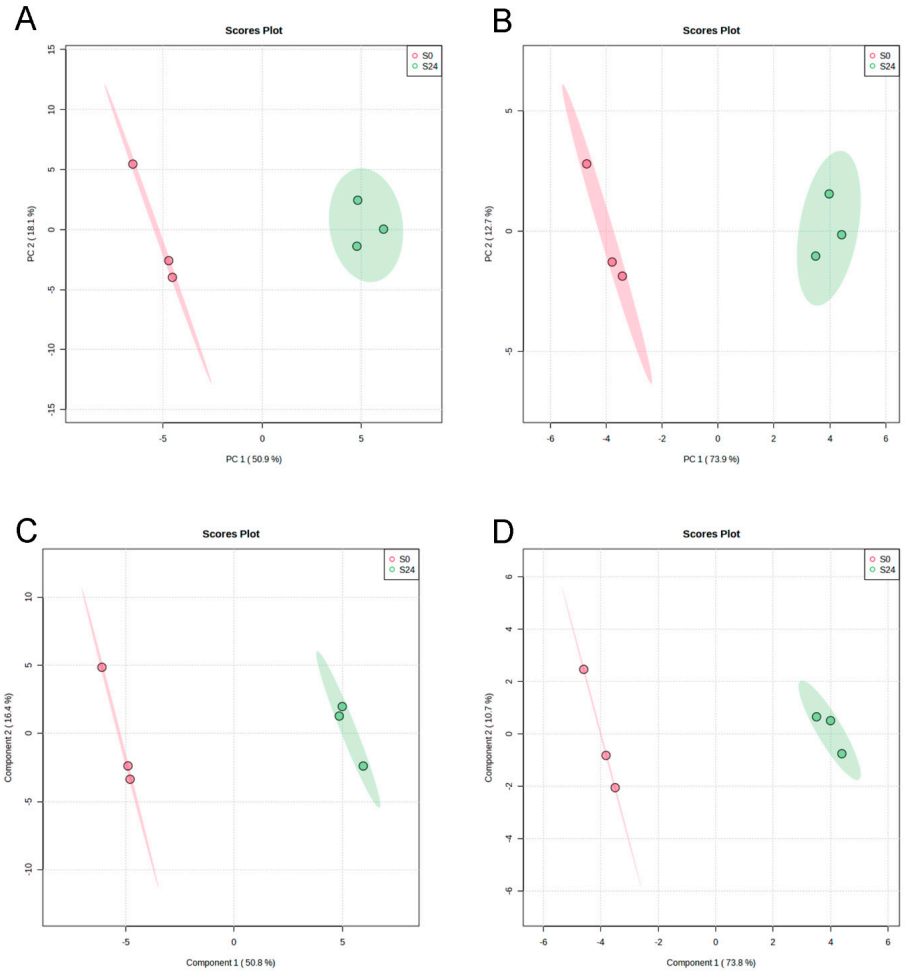

Figure 6. Non-volatile compounds of unfermented and fermented barley juices were detected using UPLC-QTOF-MS/MS. PCA score plots for ESI+ mode (**A**) and ESI− mode (**B**). OPLS-DA score plots for ESI+ mode (**C**) and ESI− mode (**D**).

Table 1. Identification of 30 total non-volatile compounds as biomarkers between the unfermented and fermented barley juices.

No.	*m/z*	Chemical Formula	Compound Name	S24 vs. S0
ESI+				
1	399.144	$C_{15}H_{22}N_6O_5S$	S-Adenosylmethionine	up
2	200.032	$C_4H_{10}NO_6P$	Iminoerythrose-4-phosphate	up
3	279.170	$C_{15}H_{22}N_2O_3$	Tolycaine	up
4	156.115	$C_9H_{16}O_2$	Nonane-4,6-dione	up
5	258.228	$C_{10}H_{15}N_3O_4$	5-Methyldeoxycytidine	up
6	255.247	$C_{11}H_{14}N_2O_5$	N-Ribosylnicotinamide	up
7	112.027	$C_4H_4N_2O_2$	Uracil	up
8	104.030	C_4H_8OS	Tetramethylene sulfoxide	up
9	122.048	$C_6H_6N_2O$	Nicotinamide	down
10	146.106	$C_6H_{14}N_2O_2$	L-Lysine	down
11	129.079	$C_6H_{11}NO_2$	L(-)-Pipecolinic acid	down
12	160.100	$C_{10}H_{12}N_2$	Anatabine	up
13	182.084	$C_{12}H_{10}N_2$	Azobenzene	up
14	136.039	$C_5H_4N_4O$	Hypoxanthine	up
15	268.081	$C_{10}H_{12}N_4O_5$	Inosine	up

Table 1. *Cont.*

No.	*m/z*	Chemical Formula	Compound Name	S24 vs. S0
ESI−				
1	165.055	$C_9H_{10}O_3$	D(+)-Phenyllactic acid	up
2	89.024	$C_3H_6O_3$	DL-Lactic acid	up
3	267.072	$C_{10}H_{12}N_4O_5$	Inosine	up
4	87.008	$C_3H_4O_3$	Pyruvic acid	up
5	135.031	$C_5H_4N_4O$	Hypoxanthine	up
6	150.041	$C_5H_5N_5O$	Guanine	up
7	131.071	$C_6H_{12}O_3$	6-Hydroxycaproic acid	up
8	242.077	$C_9H_{13}N_3O_5$	Cytarabine	up
9	313.091	$C_4H_5N_3O_2$	5-Amino-4-imidazolecarboxylate	up
10	266.088	$C_{10}H_{13}N_5O_4$	Deoxyguanosine	up
11	133.014	$C_4H_6O_5$	DL-Malic acid	down
12	115.003	$C_4H_4O_4$	Fumaric acid	down
13	243.061	$C_9H_{12}N_2O_6$	Pseudouridine	up
14	134.037	$C_6H_5N_3O$	HOBT	down
15	145.100	$C_6H_{14}N_2O_2$	(3S)-3,6-Diaminohexanoate	down

3.5. Antioxidant Activity of Unfermented and Fermented Barley Juices

LAB are widely used to ferment fruit juices and can enhance their physiological function, which may be due to the formation of bioactive ingredients that suppress the accumulation of free radicals. ABTS, DPPH, and O_2^- assays are extensively applied to measure the free radical scavenging ability of fermented juice in vitro. A single electron of free radicals can be paired with a radical scavenger, which degrades its violet color and decreases its absorbance at 517 nm [40]. As illustrated in Figure 7, the ABTS free radical scavenging activity of the barley juice was remarkably increased from 88.56 ± 1.13% to 98.69 ± 0.57% after 24 h of fermentation. A previous report showed that the antioxidant ability of pear juice fermented with *L. acidophilus* CH-2 was significantly increased compared to that of pear juice owing to the increase in the DL-malic acid and fumaric acid contents after fermentation [41]. A dramatic increase in the DPPH free radical scavenging ability in the fermented juice was observed, ranging from 79.96 ± 0.47% to 91.41 ± 0.81%. In addition, the fermented barley juice had a significantly high O_2^- radical scavenging activity (95.65 ± 1.75%) compared with the unfermented barley juice (85.37 ± 2.75%). These changes in the antioxidant activities of the fermented barley juice are strongly associated with the increases in the total phenolic and flavonoid contents. However, LAB fermentation can change the form and constituents of phenolics, which can increase the antioxidant activity of the juice [42,43].

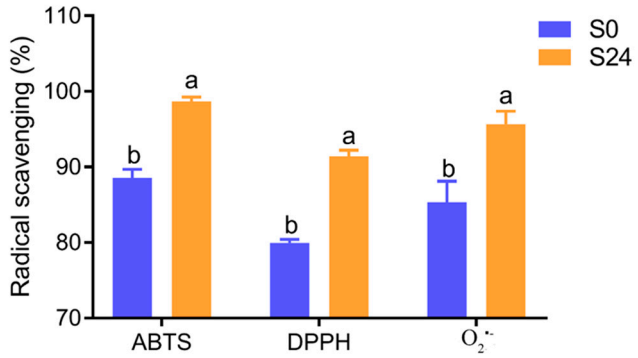

Figure 7. ABTS, DPPH, and O_2^- radical scavenging abilities (%) of unfermented and fermented barley juices. S0: unfermented barley juice; S24: fermented barley juice. Significant differences ($p < 0.05$) are indicated with different letters (a, b).

3.6. Sensory Evaluation

Whether barley juice fermented with *S. thermophilus* 7G10 has an unpleasant taste must be considered. The sensory evaluation of the barley juice fermented with *S. thermophilus* 7G10 is shown in Figure 8. The scores for color, odor, and sourness increased in the barley juice fermented with *S. thermophilus* 7G10, which agrees with a previous study [44]. In contrast, sweetness scores were reduced in the barley juice fermented with *S. thermophilus* 7G10, which may be due to the low sugar content after fermentation. In addition, *S. thermophilus* 7G10 fermentation increased the overall acceptability of the barley juice. Sun et al. also found that pumpkin juice fermented with *L. plantarum* was more popular among consumers, which was associated with its acceptable sugar-to-acid ratio [20]. In addition, benzyl alcohol and phenylethyl alcohol are the principal benzenic compounds that impart fruity and floral/rose-like scents, which is helpful in increasing the diversity of odors [45].

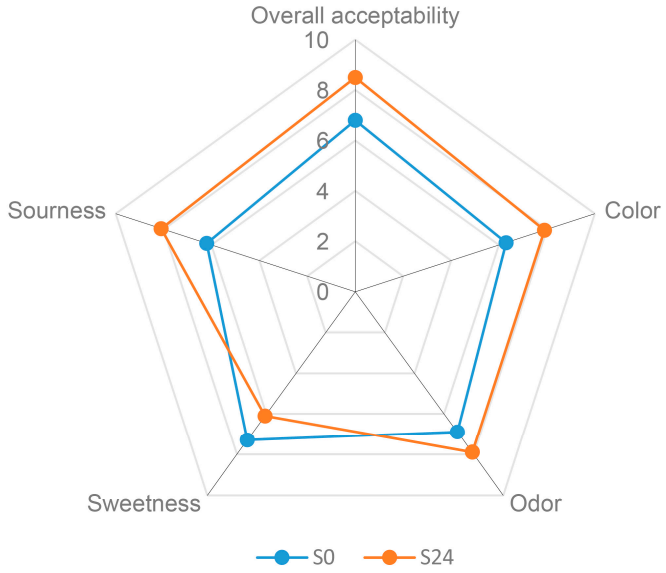

Figure 8. Sensory evaluation of unfermented and fermented barley juices. S0: unfermented barley juice; S24: fermented barley juice.

4. Conclusions

In conclusion, *S. thermophilus* 7G10 could grow well in barley juice, which decreased the pH and increased the contents of total polyphenols and flavonoids. The fermentation of barley juice with *S. thermophilus* 7G10 introduced a variety of volatile compounds (including acids, ketones, alcohols, and aldehydes) and non-volatile compounds (including S-adenosylmethionine, hypoxanthine, inosine, and guanine). In addition, barley juice fermented with *S. thermophilus* 7G10 displayed strong ABTS, DPPH, and O_2^- radical scavenging capacities. Taken together, the results of the present study offer a suitable strategy for the further development of fermented barley juice.

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