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ORIGINAL PAPER

Flavor and antioxidant activity improvement of carrot juice by fermentation with *Lactobacillus plantarum* WZ-01

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Abstract

A strain WZ-01 that can improve the flavor of carrot juice was isolated from naturally fermented orange and identified as *Lactobacillus plantarum* by 16S rRNA sequence analysis. After inoculation into carrot juice, *L. plantarum* WZ-01 could proliferate rapidly, and the bacterial population increased from 5.5×10^4 to 5.3×10^8 CFU mL^{-1} in 48 h. Organic acids were produced, resulting in a decrease in the pH value. Fermentation simultaneously enhanced the antioxidant activity of carrot juice and slightly changed the color and carotenoids content of the carrot juice. Solid-phase microextraction-gas chromatography–mass spectrometry analysis showed that β-pinene and β-phellandrene disappeared, and the relative contents of α-pinene, sabinene and β-myrcene decreased, while some aldehydes and ketones were formed by fermentation. Fermentation by strain WZ-01 could significantly weaken the terpene flavor of carrot juice and improve its sensory qualities.

Keywords Carrot juice · *Lactobacillus plantarum* · Fermentation · Flavor · Antioxidant activity

Introduction

Carrot (*Daucus carota* L.), one of the most commonly consumed and well-known root vegetables in the world, is rich in carotenoids (mainly β-carotene), vitamins (A, D, B, E, C and K), polyphenols, calcium, phosphorus, iron, zinc, manganese, molybdenum and other trace elements and has various biological activities such as antioxidant, anti-aging, eyesight protection, immunity improvement and hypoglycemic activities [1–3]. According to the statistics of the Food and Agriculture Organization (FAO) of the United Nations, the world production of carrots and turnips in 2016 was approximately 42.71 million tons, and almost half were cultivated in China. Carrot is the major vegetable source of provitamin A carotenoids in the Chinese diet. However, carrot has a terpene flavor due to the abundant mono- and sesquiterpenes, such as pinene, caryophyllene, myrcene, limonene, sabinene, terpinene, cymene, terpinolene, humulene, and bisabolene [4, 5]. This unique flavor of carrots is poorly accepted by some consumers, especially children and young people in China [6].

Fermentation is a traditional preservation and processing technology used to extend the storage period and enhance the nutritional and organoleptic qualities of foods [7]. Some pickled vegetables, such as kimichi, suan-cai and wax gourd, have a long history and are very popular in China and Southeast Asia [8, 9]. During fermentation, many microbial and enzymatic reactions occur, leading to changes in the constituents and compounds in raw food materials, and strains of *Saccharomyces*, *Bacillus* and lactic acid bacteria are common microbes in fermented foods [10]. Recently, the application of lactic acid bacteria in the fermentation of vegetables and fruits has attracted considerable attention owing to the improved sensory properties, nutritional quality and health benefits of fermented foods. Di Cagno et al. [11] reported that lactic acid fermentation through selected *Lactobacillus plantarum* strains could enhance the flavor profile of pomegranate juice. The volatile compounds in horse gram sprouts could also be bio-transformed by *L. plantarum* [12]. Fermentation of carrot juice by selected lactic acid bacteria could improve its biological functions, such as hypoglycemic and cholesterol-lowering effects with

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L. plantarum NCU116 [13, 14], pathogen inhibition activity with *L. rhamnosus* LOCK900 [15], and probiotic function with *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and *L. rhamnosus* DSM 20711 [16]. Lactic acid bacteria fermentation has also increased the antioxidant activity of some vegetative materials, such as mixed berry juice [17], sweet lemon juice [18], acorn dough [19], and red ginseng extract [20]. The objective of this study was to screen strains of lactic acid bacteria suitable for improving the flavor of carrot juice and analyze the changes in the volatile compounds after fermentation. Moreover, the physicochemical properties, antioxidant activity and sensory index of the carrot juice throughout fermentation by the selected strain were determined. The results from this work will be helpful for the development of carrot beverages with good nutrition and flavor properties.

Materials and methods

Materials, chemicals and instruments

Carrots and other fruits and vegetables were purchased from a local supermarket in Wenzhou, China. Pickled fruits (orange, tomato, blueberry, pineapple, and apple) and vegetables (cabbage, carrot, leaf mustard, and cucumber) were prepared by adding 10% NaCl and naturally fermenting them for 30 days.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-l-picrylhydrazyl (DPPH) and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO, USA). An Ezupbacterial DNA extraction kit was purchased from Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). Glucose, lactose, NaCl, K_2HPO_4, $Na(CH_3COO)_2$, yeast extract, bromocresol green and all other chemicals and solvents were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

The main instruments used were a GCM-003 juice extractor (Crown electrical Co., China), a CR-5 Minolta chroma meter (Konica Minolta, Japan), a SevenEasy pH meter (Mettler Toledo (Shanghai) Instruments, China), a UV-1810 spectrophotometer (Beijing Puxi General Analysis Instrument, China), a 5424R centrifuge (Eppendorf, Germany), a T100^{TM} thermal cycler (Biorad, USA), and a 7980A-5975C gas chromatograph-mass spectrometer (Agilent Technologies, USA).

Isolation of flavor-improving *Lactobacillus* strain

The solutions of pickled fruits and vegetables were serially diluted with aseptic saline, and 100 µL samples from different dilutions were spread over de Man-Rogosa-Sharpe (MRS) agar supplemented with 0.06% bromocresol green. After incubation at 37 °C for 48 h, the bacterial colonies that could form a clear yellow zone on the plate were screened and further purified by streaking plates until pure colonies were obtained. The purified bacteria were inoculated into carrot juice and incubated at 37 °C for 72 h. The strains that could improve the terpene flavor of carrot juice were selected by sensory evaluation.

Identification of strain WZ-01 by 16S rDNA sequencing

Total DNA extraction and purification were carried out according to the instructions of the kit provided by the manufacturer. The isolated genomic DNA was used as a template to amplify the 16S rDNA gene by PCR with the following primers: forward primer (5′-CAGTTTTGATC CTGGCT-3′) and reverse primer (5′-AGGAGGTGATCC AGCCGCA-3′). PCR was carried out according to the following thermal cycling conditions: 94 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 45 s, primer annealing at 55 °C for 45 s, elongation at 72 °C for 1 min and thermal retardation at 72 °C for 10 min. The PCR products were determined, and a suitable sample was sent to Sangon Biotech (Shanghai) for sequencing. The resulting sequence was submitted to the GenBank database and compared with known sequences in the database by BLAST. The phylogenetic tree was constructed by MEGA 7.0 software.

Preparation and fermentation of carrot juice

Fresh carrots were washed thoroughly, peeled and ground into juice. The juice was pasteurized at 90 °C for 15 min, cooled and inoculated with strain WZ-01. The fermentation was carried out at 37 °C for 120 h.

Colony forming unit (CFU) determination of lactic acid bacteria

The carrot juice was serially diluted in saline to assay the CFUs. Samples (100 µL) from different dilutions were spread on MRS agar plates. The colonies on the media were counted after 48 h of incubation at 37 °C, and the number of lactic acid bacteria populations in each sample was calculated.

Physico-chemical characterization determination

The color attributes (L^*, a^* and b^*) were measured by a chroma meter using the CIE scale. The pH values were determined by a pH meter. The titratable acidity (TA) was determined by titration with 0.025 mol L^{-1} NaOH to pH 8.2, and the results are expressed as a percentage of lactic acid.

The total carotenoids were determined according to the method of Martínez-Flores et al. [21] with slight modifications. Two milliliters of carrot juice were thoroughly mixed with 10 mL of acetone/petroleum ether (1:1). The organic phase was collected, and the aqueous phase was repeatedly extracted with 5 mL of acetone/petroleum ether until it was colorless. The combined organic phases were mixed and suitably diluted. The absorbance was measured at 450 nm, and the concentration of carotenoids was calculated based on a calibration curve prepared using standard solutions of β-carotene.

Antioxidant activity determinations

Carrot juice (1 mL) was fully mixed with 10 mL of methanol and centrifuged for 10 min at 10,000×*g*. The supernatant was used to measure the antioxidant activity as described by Ekinci et al. [22]. The scavenging abilities toward ABTS radical (ABTS·) and DPPH radical (DPPH·) were analyzed using the methods of Chen et al. [23] with minor modifications. For DPPH· scavenging, 2 mL of sample was added to 2 mL of methanolic DPPH solution (0.03 mg mL^{-1}). The absorbance was measured at 517 nm after 30 min of reaction at 30 °C in the dark. The ABTS radicals were generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) for 12 h. The absorbance of the $ABTS^{+}$ solution was adjusted to 0.70 ± 0.02 at 734 nm. Samples (1 mL) were added to 3.0 mL of $ABTS^{\cdot+}$ solution, and the absorbance was measured at 734 nm after 6 min of reaction at 30 °C.

The scavenging rate was calculated according to $(\%) = [1 - A_i/A_0] \times 100$, where A_0 was the absorbance of the blank (methanol instead of sample) and A_i was the absorbance of the sample. The same formula was used for both antioxidant assays.

Determination of volatile compounds

The volatile compounds were extracted from the carrot juice by headspace solid-phase microextraction (HS-SPME) and determined by gas chromatography-mass spectroscopy (GC–MS) as described by Liu et al. [24] with some modifications. Briefly, the sample (5 mL) was transferred to a 15-mL SPME glass vial, tightly capped and then kept at 60 °C in a water bath for 15 min to equilibrate. The volatile compounds were extracted and adsorbed by a 75 μm CAR-PDMS Extractive Fiber (Supelco, Bellefonte, USA) for 30 min at the same temperature. After extraction, the fiber was retracted and immediately inserted into the injection port of an Agilent GC–MS system equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, USA) for 3 min to desorb the volatile compounds.

The GC–MS operating conditions were as follows: the injection port temperature, detector temperature and interface temperature were all 250 °C; the GC oven temperature was initially maintained at 40 °C for 5 min, increased to 250 °C at a rate of 8 °C min^{-1} and held at 250 °C for 5 min; the flow rate of helium was 1.0 mL min^{-1}; the mass spectrometer was operated in electron impact mode with the electron energy set at 70 eV and a scan range of 30–500 m/z; the ion source and quadrupole temperatures were set at 230 °C and 250 °C, respectively.

The extracted volatile compounds were identified by matching their mass spectra with those in the mass spectral database (NIST 1.6 and Wiley 6.0) and previous reports. The relative percentages of the detected peaks were obtained by peak-area normalization.

Sensory evaluation

The sensory evaluations of carrot juice were carried out by the scoring method described by Riganakos et al. [2] with some modifications. In brief, samples were evaluated by a trained sensory panel consisting of 10 members chosen among undergraduate students of Wenzhou University between 17 and 25 years of age. Panelists compared the fresh juice and fermented juices with different fermentation times at room temperature based on sensory evaluation criteria including aroma, taste and color on a ten-point scale (10: excellent, 1: very poor). All samples were kept refrigerated until the time of sensory evaluation.

Statistical analysis

All analyses were performed in triplicate, and error bars indicate the standard deviation. Statistical analyses were performed using STATISTICA6.0, and one-way analysis of variance by Duncan's multiple range test was performed to test the significance of the differences.

Results and discussion

Identification of flavor-improving strain WZ-01

Thirty-five lactic acid-producing bacterial strains were isolated from naturally fermented fruits and vegetables. Sensory evaluation confirmed that a bacterial strain (named WZ-01) isolated from fermented orange had the strongest ability to improve the terpene flavor of carrot juice. It was Gram-positive, and the colony on the MRS plate was white, round, wet and had regular edges. The 16S rDNA gene of strain WZ-01 was successfully amplified and gave a unique band on the electropherogram (Fig. 1a), and the gene sequence (1308 bp) was determined and deposited in the GenBank database under the accession number MH016560. StrainWZ-01 was identified as *L. plantarum*

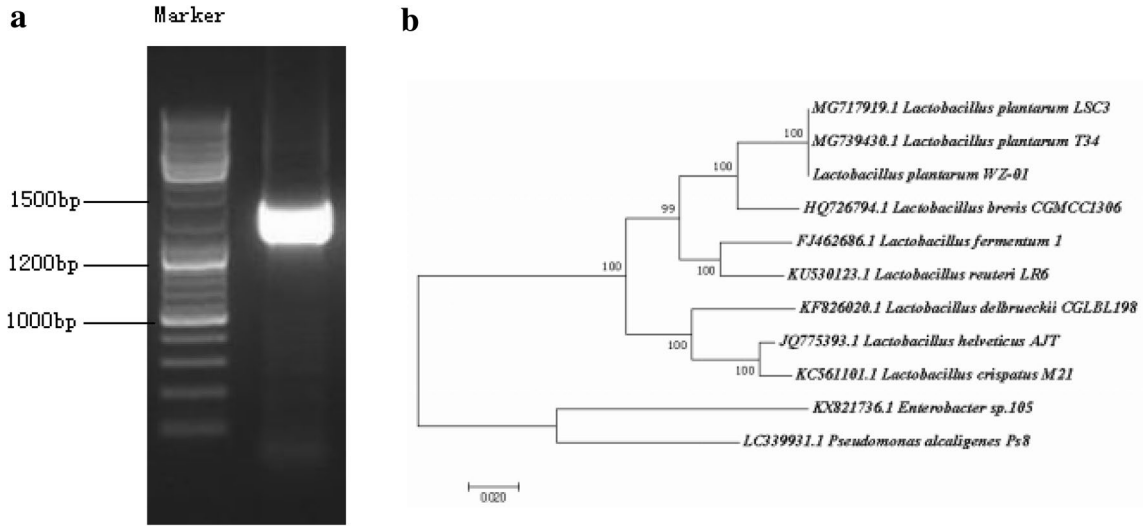

Fig. 1 Agarose gel electropherogram of PCR amplified 16S rDNA (**a**) and phylogenetic tree (**b**) of *L. plantarum* WZ-01

based on sequence similarity analysis (Fig. 1b), and it exhibited 100% homology to *L. plantarum* T34 (accession number MG739430.1). The strain WZ-01 was stored in the China General Microbiological Culture Collection Center (Beijing, China) under the No. CGMCC13338. *L. plantarum* is a safe strain for food processing and has been approved for use in the food industry by the State Administration for Market Regulation of China. Previous studies have also reported that *L. plantarum* strains could improve the flavor or sensory properties of carrot juice [6], pomegranate juice [11], horse gram sprouts [12] and tomato juice [25].

Growth of *L. plantarum* WZ-01 in carrot juice

During fermentation, the flavor of fruits and vegetables is improved by the growth and metabolism of bacteria or the catalytic reactions of their extracellular enzymes, and the number of bacteria is directly related to the flavor of the fermented product [26]. The differences in strains and nutritive factors could affect the growth rate of *L. plantarum*. For instance, *L. plantarum* strains (LP3, AF1 and LU5) grew quickly in sarshir, and the populations reached approximately 8.5 log CFU g^{-1} in 12 h of fermentation [27]. However, it took 72 h for *L. plantarum* LS5 to reach 8.5 log CFU mL^{-1} when grown in sweet lemon juice [18]. As shown in Fig. 2, the *L. plantarum* WZ-01 population increased logarithmically during the first 48 h in carrot juice and reached 5.3×10^8 CFU mL^{-1} from an initial population of 5.5×10^4 CFU mL^{-1}. Afterwards, the number of *L. plantarum* WZ-01 decreased gradually because nutrition reduction and metabolite accumulation restrict further growth of the bacteria and lead to cell autolysis [19].

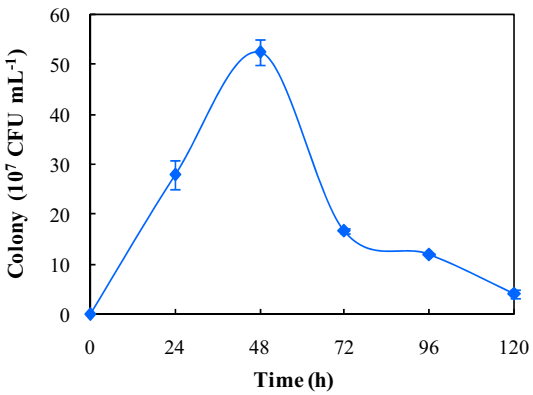

Fig. 2 Population change of *L. plantarum* WZ-01 in carrot juice during fermentation

Changes in the physico-chemical properties of carrot juice during fermentation

Color is one of the important indexes for consumers to evaluate the quality of food/beverage products. As shown in Fig. 3, the color parameters (L^*, lightness; a^*, redness; and b^*, yellowness) of carrot juice did not change significantly ($p > 0.05$) during fermentation by *L. plantarum* WZ-01. There was also no noticeable difference among the fermented samples based on visual sensory evaluation. The orange color in carrot juice is most influenced by its carotenoids content [2], which is stable during fermentation (Fig. 4c).

During fermentation with lactic acid bacteria, some organic acids (especially lactic acid) are synthesized and secreted into the media due to bacterial growth and metabolism, and this cause a decrease in pH until a critical limit.

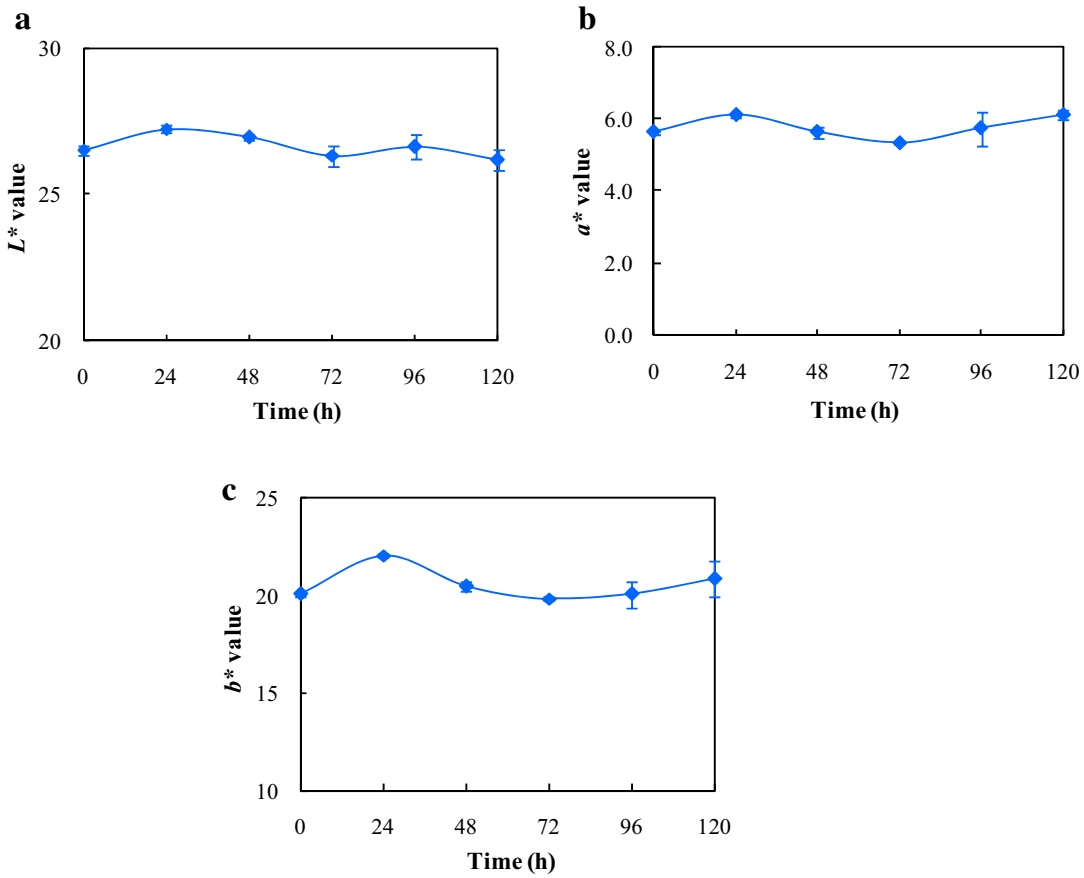

Fig. 3 Change of color in carrot juice during fermentation by *L. plantarum* WZ-01. **a** *L** value; **b** *a** value; **c** *b** value

L. plantarum strains LP3, AF1 and LU5 decreased the pH of sarshir from 6.0 to 3.6 after 10 h of fermentation [27]. *L. plantarum* PTCC 1745 reduced the pH of acorn dough from 6.4 to 3.5 in 24 h of fermentation [19]. As shown in Fig. 4a, b, the pH of carrot juice decreased from 5.23 to 3.85 in 48 h with the growth of *L. plantarum* WZ-01 and then remained stable. The titratable acidity increased from an initial content of 0.52 g 100 mL^{-1} to 0.81 g 100 mL^{-1} in 24 h and then remained relatively stable ($p > 0.05$).

Carotenoids are important natural pigments that not only give carrots their characteristic appearance but also have strong biological functions. It has been reported that 100 g of carrots contain 6–15 mg of carotenoids, which can enhance the synthesis of vitamin A and play an important role in radical scavenging and/or oxidation inhibition and therefore protect humans against cancer and cardiovascular diseases [1]. Nazzaro et al. [16] reported that the carotenoids in carrot juice were not degraded by *L. delbrueckii* subsp. *bulgaricus* or *L. rhamnosus*. Similar results were also observed in this work, in which no significant ($p > 0.05$) change in the carotenoids content was observed in the carrot juice during fermentation by *L. plantarum* WZ-01 (Fig. 4c).

Change in the sensory qualities of carrot juice during fermentation

As shown in Fig. 5, the sensory score increased during fermentation, indicating that fermentation by *L. plantarum* WZ-01 could improve the flavor of carrot juice. The scores significantly ($p < 0.05$) increased from 3.01 to 7.20 in the first 72 h. Although the scores continued to increase after 72 h, the changes were not significant. Therefore, 72 h is a suitable fermentation time for the production of fermented carrot juice.

Change in the antioxidant activity of carrot juice during fermentation

Free radicals are formed uncontrollably in biological processes and may cause oxidative damage to membrane lipids, proteins, enzymes, and DNA in cells and tissues, leading to degenerative diseases. Antioxidants present in food react directly with reactive oxygen or intermediate forms metabolites of the oxidoreduction reaction, resulting the free radicals and reactive oxygen to more stable and non-reactive forms [28–30]. Carrots are rich in

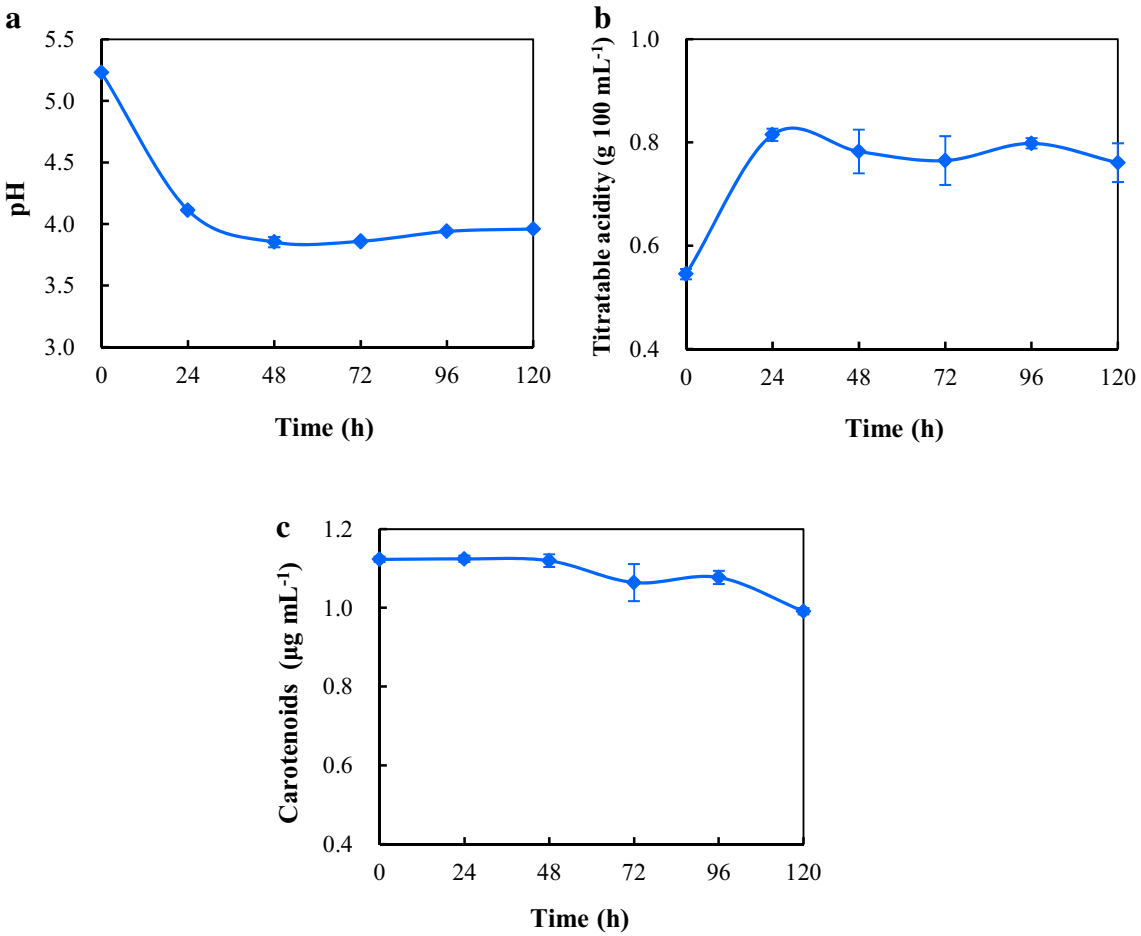

Fig. 4 Changes of pH (**a**), titratable acidity (**b**) and carotenoids content (**c**) in carrot juice during fermentation by *L. plantarum* WZ-01

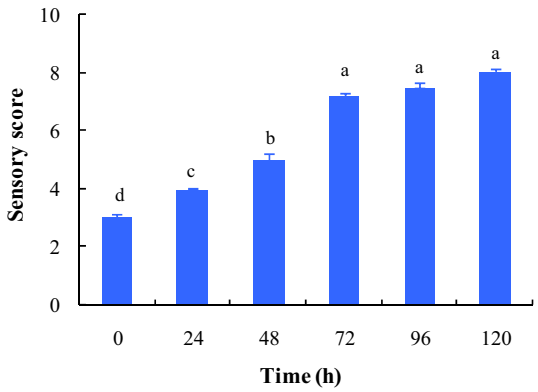

Fig. 5 Effect of *L. plantarum* WZ-01 fermentation on the sensory score of carrot juice Different letters indicate significant differences at $P < 0.05$

carotenoids, vitamin C and phenolic compounds, which are capable of scavenging free radicals [2, 21, 31, 32]. Therefore, it is important to preserve or enhance the antioxidant activity of carrot juice by emerging technologies. Fermentation by selected lactic acid bacteria can be considered a suitable strategy because these bacteria can synthesize antioxidant exo-polysaccharides [33], peptides [34, 35], glutathione [25], superoxide dismutase [36], and other antioxidant compounds such as benzoic acid and benzaldehyde [17], and they have been shown to increase the phenolic contents in acorn dough [19], pomegranate juice [37] and soy whey [38]. As shown in Fig. 6, the DPPH free radical scavenging rate of carrot juice increased from an initial value of 76.2% to 81.7% in 72 h of fermentation by *L. plantarum* WZ-01, and the ABTS free radical scavenging rate increased from an initial value of 63.2% to 78.3% in 48 h, indicating that fermentation by the strain WZ-01 could enhance the free radical scavenging activity of carrot juice to some extent. These results were supported by Nazzaro et al., who determined that *L. bulgaricus* fermentation could enhance the DPPH free radical scavenging activity of carrot juice [16]. *L. plantarum* strains were also reported to enhance the antioxidant activity of tomato juice [25], berry juice [17], sweet lemon juice [18] and pomegranate juice [37].

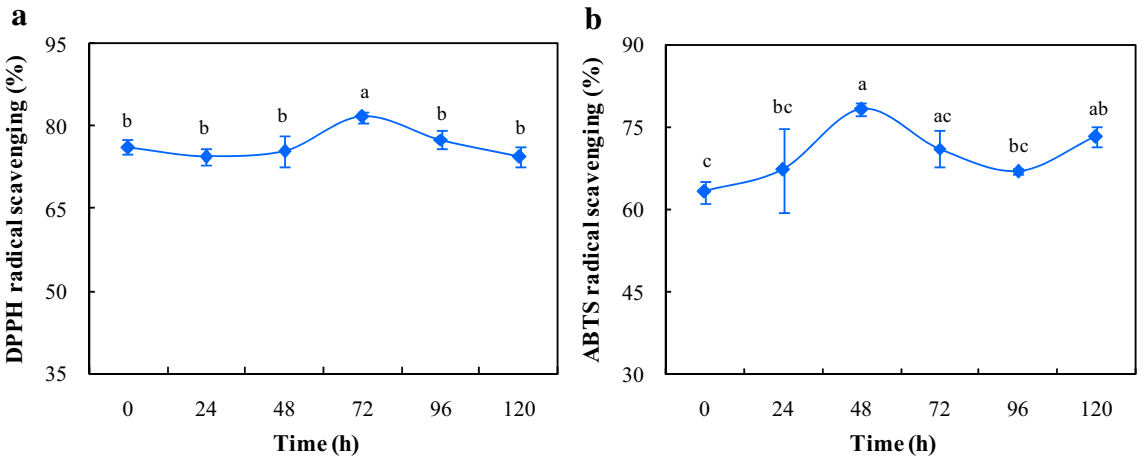

Fig. 6 Effect of *L. plantarum* WZ-01 fermentation on the DPPH radical (**a**) and ABTS radical (**b**) scavenging activity of carrot juice. Different letters indicate significant differences at $P < 0.05$

Identification of the volatile compounds in fresh carrot juice and fermented carrot juice by the strain WZ-01

Carrots have a number of important quality characteristics, including the contents of sugars, dry matter, nonvolatile bitter compounds and volatile compounds, which directly affect its sensory qualities. Monoterpenes and sesquiterpenes are the most abundant volatile components in carrots, and the major monoterpenes are pinene, sabinene, myrcene, limonene, terpinene, cymene and terpinolene. The major sesquiterpenes are caryophyllene, humulene, cuparene and bisabolene [39, 40]. In this work, the extraction and separation of volatile compounds from fresh and fermented carrot juice were performed by headspace SPME-GC. Full multicompound quantitation using SPME is impossible in complex organic matrices [4] and is not the primary aim of this research. Therefore, the data given in Table 1 are relative contents. By comparison of their MS data with those in the NIST database and references [4, 5, 39, 40], fifty-five volatile compounds were identified in fresh carrot juice, while fifty were identified in the fermented samples, and these compounds included terpenes, aldehydes, ketones, alcohols, esters and aromatics, and terpenes were the most abundant. These findings are in accordance with previous studies on volatiles in carrots [4, 5, 39, 40].

Gas chromatography–olfactometry showed that the characteristic "carrot top" odor is related to α-pinene, β-pinene, sabinene, phellandrene and β-myrcene, and the odor thresholds for β-pinene, sabinene and β-myrcene are low; 140 ppb, 75 ppb and 13 ppb, respectively [5]. As shown in Table 1, β-pinene and β-phellandrene disappeared upon fermentation by *L. plantarum* WZ-01, and the relative contents of α-pinene, sabinene and β-myrcene were reduced by 29.37%, 5.40% and 9.38%, respectively. In addition, the relative contents of some aldehydes and ketones, such as hexanal, heptanal, benzaldehyde, 2-nonenal, 3-hydroxy-2-butanone and octanone, increased during fermentation. Therefore, the terpene flavor of carrot juice was improved by the fermentation of *L. plantarum* WZ-01.

Conclusions

In recent years, vegetable fermentation using lactic acid bacteria has regained popularity because a variety of amino acids, vitamins, antibacterial substances, organic acids and aromatic compounds are produced, enhancing nutrition and improving flavor [41]. *L. plantarum* WZ-01 could enhance the DPPH and ABTS radical scavenging activities to some extent and improve the terpene flavor of carrot juice by degrading β-pinene and β-phellandrene and decreasing the relative contents of α-pinene, sabinene and β-myrcene, resulting in good sensory properties. It can be concluded that *L. plantarum* WZ-01 has practical application potential in the fermentation of carrot juice and that fermented carrot juice can be developed into functional drinks with enhanced health benefits and good flavor.

Table 1 Identified volatile compounds in fresh carrot juice and fermented carrot juice by *L. plantarum* WZ-01

No	Retention time	CAS#	Compound	Relative content (%)	
				Fresh juice	Fermented juice
Terpenes					
1	2.30	007785-26-4	α-pinene	3.03 ± 0.18	2.14 ± 0.15
2	2.88	000079-92-5	Camphene	0.56 ± 0.03	0.53 ± 0.03
3	3.52	018172-67-3	β-pinene	0.31 ± 0.02	ND[a]
4	5.46	002,009-00-9	Sabinene	2.03 ± 0.12	1.92 ± 0.11
5	5.52	000123-35-3	β-mycrene	0.64 ± 0.04	0.58 ± 0.04
6	6.25	005989-27-5	D-limonene	1.01 ± 0.07	0.87 ± 0.05
7	6.35	004221-98-1	β-phellandrene	0.16 ± 0.00	ND
8	7.41	013877-91-3	Ocimene	0.16 ± 0.01	ND
9	7.62	000099-85-4	γ-terpinene	3.42 ± 0.17	3.91 ± 0.18
10	8.46	000586-62-9	Terpinolene	2.45 ± 0.13	2.93 ± 0.17
11	14.22	000087-44-5	β-caryophyllene	14.58 ± 1.04	13.97 ± 1.12
12	15.29	006753-98-6	α-caryophyllene	1.28 ± 0.10	1.20 ± 0.07
13	15.49	018794-84-8	β-farnesene	0.81 ± 0.06	0.73 ± 0.05
14	15.76	004630-07-3	Valencene	0.46 ± 0.03	1.02 ± 0.07
15	15.90	023986-74-5	Germacrene D	1.08 ± 0.09	1.79 ± 0.07
16	16.19	26560-14-5	α-farnesene	0.37 ± 0.02	0.31 ± 0.01
17	16.29	000495-61-4	β-bisabolene	2.31 ± 0.14	2.35 ± 0.15
18	16.71	000495-62-0	γ-bisabolene	16.76 ± 0.97	16.95 ± 0.102
19	16.76	000560-32-7	α-patchoulene	0.43 ± 0.02	0.41 ± 0.02
20	16.95	000644-30-4	Curcumene	5.80 ± 0.42	5.54 ± 0.36
21	19.50	001139-30-6	Caryophyllene oxide	0.61 ± 0.04	ND
Aldehydes					
22	3.33	000066-25-1	Hexanal	ND	0.26 ± 0.01
23	6.18	000111-71-7	Heptanal	ND	0.36 ± 0.02
24	8.73	000124-13-0	Octanal	0.18 ± 0.01	ND
25	13.18	000100-52-7	Benzaldehyde	ND	0.11 ± 0.00
26	13.40	018829-56-6	2-Nonenal	ND	0.55 ± 0.03
27	14.63	000432-25-7	β-cyclocitral	0.31 ± 0.02	0.17 ± 0.01
Ketones					
28	8.77	000513-86-0	3-hydroxy-2-butanone	2.45 ± 0.16	4.42 ± 0.27
29	8.84	000111-13-7	Octanone	ND	0.76 ± 0.03
30	9.91	000110-93-0	6-methyl-5-hepten-2-one	0.25 ± 0.02	0.24 ± 0.01
31	10.89	000821-55-6	2-nonanone	0.73 ± 0.05	0.35 ± 0.01
32	12.21	000076-22-2	Camphor	1.26 ± 0.09	0.95 ± 0.05
33	17.53	023696-85-7	Damascenone	ND	0.49 ± 0.03
34	17.91	000127-41-3	α-ionone	0.42 ± 0.02	0.14 ± 0.00
35	18.05	003796-70-1	Geranylaceton	0.69 ± 0.03	0.41 ± 0.02
36	19.04	000079-77-6	β-ionone	1.72 ± 0.08	1.37 ± 0.07
37	21.42	000502-69-2	Perhydrofarnesyl acetone	0.15 ± 0.01	0.18 ± 0.00
Alcohols					
38	7.11	000111-70-6	1-heptanol	0.43 ± 0.02	0.34 ± 0.01
39	12.79	000104-76-7	2-ethylhexanol	0.36 ± 0.02	0.46 ± 0.03
40	13.60	000513-85-9	2,3-butanediol	0.22 ± 0.01	0.48 ± 0.02
41	13.75	000078-70-6	Linalool	0.16 ± 0.01	0.49 ± 0.03
42	13.88	000111-87-5	1-octanol	3.46 ± 0.19	1.27 ± 0.08
43	14.46	020126-76-5	4-terpineol	4.03 ± 0.31	4.13 ± 0.27
44	14.87	029803-82-5	cis-p-menth-2-en-1-ol	0.10 ± 0.00	ND
45	15.41	000143-08-8	1-nonanol	0.68 ± 0.04	0.25 ± 0.02

Table 1 (continued)

No	Retention time	CAS#	Compound	Relative content (%)	
				Fresh juice	Fermented juice
46	17.51	000106-25-2	Geraniol	0.30 ± 0.01	ND
47	22.42	000515-69-5	α-Bisabolol	0.13 ± 0.01	ND
Esters					
48	1.95	000108-05-4	vinyl acetate	0.59 ± 0.04	0.67 ± 0.04
49	14.05	005655-61-8	Bornyl acetate	3.16 ± 0.20	3.85 ± 0.31
50	18.63	000141-12-8	Neryl acetate	2.10 ± 0.13	1.88 ± 0.06
Aromatics					
51	8.21	000099-87-6	p-cymene	3.02 ± 0.17	2.49 ± 0.15
52	11.72	001195-32-0	2,4-dimethyl styrene	0.49 ± 0.02	1.0 ± 0.06
53	11.79	007399-49-7	o-isopropenyltoluene	0.14 ± 0.00	ND
54	15.07	000122-78-1	Benzeneacetaldehyde	ND	0.24 ± 0.01
55	16.88	00012200-9	p-Methylacetophenone	ND	0.48 ± 0.02
56	17.37	005973-71-7	3,4-Dimethylbenzaldehyde	ND	0.13 ± 0.01
57	18.14	000090-05-1	Guaiacol	0.27 ± 0.02	0.18 ± 0.01
58	18.77	000060-12-8	Phenethyl alcohol	0.21 ± 0.02	0.13 ± 0.01
59	22.21	007786-61-0	2-Methoxy-4-vinylphenol	0.43 ± 0.02	0.10 ± 0.00
60	22.60	000487-11-6	Elemicin	0.18 ± 0.01	0.10 ± 0.01
Others					
61	12.33	000098-01-1	Furfural	ND	1.08 ± 0.08
62	15.61	000097-61-0	2-Pentanecarboxylic acid	ND	1.28 ± 0.05
63	20.24	00048378-3	Cadalin	0.21 ± 0.01	ND
64	22.93	000607-91-0	Myristicin	0.46 ± 0.02	0.37 ± 0.02
65	23.51	000487-19-4	β-Nicotyrine	0.36 ± 0.02	ND
66	24.39	000496-16-2	Coumaran	0.12 ± 0.01	ND

[a]Not detected

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