


Comparison of physicochemical indexes, amino acids, phenolic compounds and volatile compounds in bog bilberry juice fermented by *Lactobacillus plantarum* under different pH conditions

Ming Wei¹ · Shaoyang Wang^{1,2} · Pan Gu¹ · Xiaoyu Ouyang¹ · Shuxun Liu¹ · Yiqing Li¹ · Bolin Zhang¹ · Baoqing Zhu¹ 

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Abstract This study aimed to investigate the effect of *Lactobacillus plantarum* strains on quality improvement of bog bilberry juice. Bog bilberry juice with different pH conditions was fermented by *Lactobacillus* B7 or C8-1 strain. Physicochemical index, amino acids, phenolic compounds, and volatiles of these fermented juices were compared. Results indicated that *Lactobacillus plantarum* strains preferred to metabolize malic acid and reducing sugar in non-pH-adjusted juice (NJ, pH 2.65), whereas quinic and citric acids were largely consumed in pH-adjusted juice (AJ, pH 3.50). Shikimic acid and aromatic amino acids were significantly accumulated in pH-adjusted juice, and phenolic compounds in both juices were significantly reduced. These strains enhanced the composition and concentration of volatiles compounds in non-pH-adjusted juice and improved the floral and fruity flavors. However, concentration and complexity of volatiles were reduced in pH-adjusted juices.

Keywords Bog bilberry juice · Lactic acid fermentation · *Lactobacillus plantarum* · Phenolic compounds · Volatile compounds

Introduction

Vaccinium uliginosum L, also known as bog bilberry, is a low-bush wild shrub distributed in cool regions of the northern hemisphere. Bog bilberry fruit contains numerous antioxidant compounds, such as phenolic compounds (Wang et al. 2014). It has been confirmed that consumption of bog bilberry fruits can decrease the risks of cardiovascular diseases, cancer, tumor, mutagenic activity, and diabetes (Pascual-Teresa et al. 2013). Compared to other berry fruits, high acids-to-sugar ratio in bog bilberry negatively affects their consumption as fresh fruits (Liu et al. 2015). Lactic acid fermented fruits and vegetables have gained much attention in the food industry since fermented fruits and vegetables could improve their sensory features. It has been reported that lactic acid fermentation has been applied to tomatoes, capers, pineapple, peppers, cabbages, carrots, and French beans with different lactic acid bacteria, such as *Lactobacillus*, *Leuconostoc*, *Weissella*, *Enterococcus*, and *Pediococcus* spp. (Di Cagno et al. 2013). However, no study has been carried out in bog bilberry juice.

Lactic acid fermentation of fruits can convert glucose and other hexoses into lactate. Meanwhile, the energy released during the conversion could help the growth of bacteria in fruits (Fugelsang and Edwards 2006). More importantly, the nutrition alterations during lactic acid fermentation can result in a significant improvement on the sensory attributes of fermented food products. For instance, organic acids play an important role in the sour taste of fruits (Mikulic-Petkovsek et al. 2012), while malic acid possesses a harsh mouthfeel. Lactic acid fermentation can convert malic acid into lactic acid, significantly reducing the harshness of fruits (Boulton et al. 1999). Moreover, some organic acids can be further metabolized into

✉ Baoqing Zhu
zhubaoqing@bjfu.edu.cn

¹ Beijing Key Laboratory of Forestry Food Processing and Safety, Department of Food Science, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China

² Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, P.O. Box 156, Archerfield BC, QLD 4108, Australia

aromatic compounds during fermentation (Gonzalez-Bello 2016). These aromatic compounds can play important roles in enhancing the fermented aroma of fermented food products (Helinck et al. 2004).

Lactobacillus plantarum is an acid-tolerant bacterium that widely exists in fermented plant foods. It has been widely applied in the fermentation industry due to its beneficial properties to fermented food products (Di Cagno et al. 2015). For example, lactic acid fermented food products exhibit a low level of lactose, which can benefit the consumption of consumers with the lactose intolerance (Di Cagno et al. 2013). *L. plantarum* has also been confirmed to be a good candidate for the development of probiotics. It can effectively reduce the allergenicity of food products and further improve the intestinal permeability compared to other microbes (Gobbetti et al. 2010).

Most of the previous studies have indicated that pH condition and lactic acid bacterial were the key factors that affect the performance of fruit lactic acid fermentation (Filannino et al. 2015, 2014; Mousavi et al. 2013). Compared to other fruits, bog bilberry fruits have high levels of organic acids with a low pH value (Colak et al. 2016). This could inhibit the growth of lactic acid bacteria during fermentation (Fugelsang and Edwards 2006). This could weaken the fermentation efficacy, which limits the sensory improvement of bog bilberry juice after fermentation. Different lactic acid bacterial strains have different adaptability, which could significantly alter the metabolic pathways of nutrients during lactic acid fermentation process (Di Cagno et al. 2015). This study aimed to investigate the effect of pH conditions and bacterial strains on the composition alteration of bog bilberry juice. The findings from this study could provide technical supports on the improvement of bog bilberry juice production.

Materials and methods

Juice

Wild bog bilberry fruits under a ripen stage were provided by Xinganlieshen Original Products Ltd. (Hulunbeir City, Inner Mongolia, China) in 2013. After cold-chain transportation to our lab, the berries were immediately crushed into juices and then filtered through 0.45 µm membranes. The juice had a 2.65 pH and was stored at −20 °C before lactic acid fermentation. Before inoculation, the juice was divided into two portions. One portion of the juice was kept at its original pH, whereas the other portion was adjusted to pH 3.50 using NaOH solid power. Both juices were pasteurized at 85 °C for 15 min.

Bacterial revitalization, inoculation, and fermentation

Lactobacillus plantarum B7 and C8-1 were used for bog bilberry juice fermentation. *L. plantarum* B7 and C8-1 were isolated from sourdough and pickle in our lab (Beijing Key Laboratory of Forestry Food Processing and Safety, Beijing Forestry University, Beijing, China), respectively. The bacteria were plated onto the MRS agar plates and incubated at 37 °C for 48–72 h for enumeration according to a reference method (De Man et al. 1960). When the cell number in each strain medium reached ca. 10⁹ CFU/mL, the strain (10 mL) was inoculated into 90-mL bog bilberry juice and transferred into a 100-mL capacity bottle. The lactic acid fermentation was carried out at 23 °C for 14 days. The juices before and after the fermentation process were sampled. Each fermentation was performed in duplicate.

Physicochemical indexes

Reducing sugar content and juice color intensity were determined using WineScan™ auto analyzer (FOSS Co., Ltd., Denmark). A PHS-3C pH meter (INESA Instrument Ltd., Shanghai, China) was used to measure the pH value of the juice. Organic acids in the juice were analyzed according to our published HPLC method (Wei et al. 2014). The quantitation of organic acids was carried out using external standards.

Amino acids

The derivatization of amino acids in the juice was carried out by mixing 500 µL of the juice with 10 µL of 1.00 g/L 2-amino adipic acid (internal standard), 15 µL of diethyl ethoxymethylenemalonate, 375 µL of methanol, and 875 µL of 1 mol/L borate buffer (pH 9.0). The mixture was sonicated for 30 min and then incubated at 70 °C in a water bath for 2 h (Gómez Alonso et al. 2007). After the derivatization, the derivatized amino acids were separated on a Venusil XSB-C18 column (4.6 × 250 mm, 5 µm, Bonna-Agela Technologies, Tianjin, China). The mobile phase consisted of (A) acetonitrile: methanol, (4:1, v/v) and (B) 25 mM acetate buffer with 0.02% sodium azide (pH 5.8). The flow rate was set at 0.9 mL/min and the injection volume was 20 µL. The elution program was as follows: 90% B for 20 min; 90–83% B for 10.5 min; 83% B for 3 min; 83–73% B for 31.5 min; 73–28% B for 8 min; 28–18% B for 5 min; 28–0% B for 4 min; 0% B for 3 min; 0–90% B for 5 min; and 90% B for 3 min. The detection wavelength was 280 nm. External amino acid standards were derivatized using the same protocol and used for the quantitation.

Phenolic compounds

Anthocyanins and non-anthocyanin phenolic compounds were analyzed on an Agilent 1200 HPLC system coupled with a MSD Trap VL mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) according to a published method (Gao et al. 2015). Before the injection, the juice was filtered through 0.45 μm microfiltration membranes. Anthocyanins were separated on a reverse C18 column (250 \times 4.6 mm, 5 μm , Kromasil, Sweden) under a flow rate of 1.0 mL/min. A 50 μL sample was injected into HPLC system, and the mobile phase consisted of (A) water: formic acid: acetonitrile (92:2:6, v/v/v) and (B) water: formic: acetonitrile (44:2:54, v/v/v). The elution was programmed as follows: 0–10% B for 1 min; 10–25% B for 17 min; 25% B for 2 min; 25–40% B for 10 min; 40–70% B for 5 min; and 70–100% B for 5 min. The detection wavelength was 525 nm and a positive electrospray ionization was used. Non-anthocyanin phenolic compounds were separated on a reverse Zorbax SB-C18 column (50 \times 3 mm, 1.8 μL , Agilent Technologies, Santa Clara, CA, USA) under a flow rate of 1.0 mL/min. A 2 μL sample was injected and the mobile phase consisted of (A) acetic acid: acetonitrile (1:99, v/v) and (B) acetic acid: water (1:99, v/v). The elution gradient was as follows: 0–5% B for 5 min; 5–8% B for 5 min; 8–12% B for 5 min; 12–18% B for 5 min; 18–22% B for 2 min; 22–35% B for 2 min; and 35–100% B for 4 min. The detection wavelength was 280 nm and a negative electrospray ionization mode was used. The nebulizer pressure, dry gas flow rate, and capillary voltage were set at 35 psi, 10 mL/min, and 325 $^{\circ}\text{C}$, respectively. The full scan mode from m/z 100 to 1500 was used to record mass spectrum of anthocyanins and non-anthocyanin phenolic compounds. Malvidin-3-*O*-glucoside, catechin, quercetin, gallic acid, caffeic acid, and chlorogenic acid were used as the external standard to quantify anthocyanins, flavanols, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, and chlorogenic acid, respectively.

Volatile compounds and sensory contribution

Volatile compounds in the juice were extracted using solid-phase micro-extraction according to a published method with minor modifications (Zhang et al. 2007). Briefly, 5 mL of the juice was mixed with 15 mL of NaCl in a vial tightly capped with a PTFE-silicon septum, and then heated at 40 $^{\circ}\text{C}$ for 30 min under an agitation speed of 400 rpm. Afterwards, a 50/30 μm DVB/Carboxen/PDMS fiber (Supelco, USA) was inserted into the headspace of the vial and adsorbed the volatiles for 30 min at 40 $^{\circ}\text{C}$ under the same agitation. Afterwards, the fiber was desorbed in the GC injector for 25 min. The volatile compounds were

separated using a HP-INNOWAX column (0.25 μm film thickness, 60 m \times 0.25 mm, J & W Scientific, USA) on an Agilent 6890 GC coupled with an Agilent 5975 mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). The flow rate of carrier gas (helium) was 1 mL/min, and the oven temperature was programmed as follows: 50 $^{\circ}\text{C}$ for 1 min; raising at 3 $^{\circ}\text{C}/\text{min}$ to 220 $^{\circ}\text{C}$; and 220 $^{\circ}\text{C}$ for 5 min. Electron impact mode was set at 70 eV with a mass scan range of m/z 20 to 450 under a selective ion mode. The volatiles were identified by comparing their mass spectrum with the available reference standard and further confirmed by the retention index on NIST and Wiley Mass Spectrometry. Volatiles were quantified using their corresponding external standard and calibrated by 4-methyl-2-pentanol as the internal standard. If the standard was not available, the quantitation of the volatiles was carried out using the external standard that possessed the similar structure or carbon atoms. Sensory contribution of the volatile in the juice was estimated using their odor activity value (OAV). OAV was calculated by the concentrations of volatiles in the juice over their odor threshold.

Statistical analyses

Data were expressed as the mean \pm standard deviation of duplicate tests. One-way analysis of variance (ANOVA) was used to evaluate the significant differences among the mean value of each compound at $p \leq 0.05$ on SPSS Statistics 23 (IBM, NY, USA).

Results and discussion

Physicochemical properties

Before the fermentation, the reducing sugar content was lower in the AJ than the NJ (Table 1). The fermentation resulted in a dramatic decrease on the reducing sugar content in the NJ, whereas the decrease on the reducing sugar content in the AJ was relatively slight.

The AJ and the NJ exhibited the similar content of organic acids before the fermentation. However, the composition of organic acids appeared to be significantly different in these juices after the fermentation. An increase on the total organic acids content was observed in the AJ fermented by both strains, whereas a content decrease took place in the NJ. Quinic acid was the predominant organic acid in the juice. It naturally exists in gooseberries, pears, cherries, blackberries, and strawberries (Whiting 1958). It can be produced through shikimate pathway (Dewick 1998), and it is a key precursor to form aromatic rings in fruits (Hulme 1958). A significant decrease on the quinic

Table 1 Physicochemical indexes of bog bilberry juice with different pH conditions fermented by *Lactobacillus plantarum* B7 and C8-1

| Compound | Before inoculation | | After fermentation | | | |
|-----------------------------|---------------------------|---------------------------|---------------------------|----------------------------|---------------------------|---------------------------|
| | pH 2.65 | pH 3.50 | pH 2.65 | | pH 3.50 | |
| | | | B7 | C8-1 | B7 | C8-1 |
| <i>Reducing sugar (g/L)</i> | | | | | | |
| Fructose (g/L) | 19.60 ± 0.00 ^a | 16.35 ± 0.07 ^b | 8.70 ± 0.00 ^d | 8.75 ± 0.07 ^d | 12.90 ± 0.99 ^c | 12.90 ± 0.28 ^c |
| Glucose (g/L) | 12.60 ± 0.00 ^a | 11.00 ± 0.00 ^b | 3.00 ± 0.00 ^d | 3.00 ± 0.14 ^d | 9.00 ± 0.85 ^c | 9.35 ± 0.35 ^c |
| Total | 32.20 ± 0.00 ^a | 27.35 ± 0.07 ^b | 11.70 ± 0.00 ^d | 11.75 ± 0.07 ^d | 21.90 ± 1.84 ^c | 22.25 ± 0.63 ^c |
| <i>Organic acids (g/L)</i> | | | | | | |
| Quinic acid | 6.84 ± 0.36 ^a | 6.49 ± 0.02 ^a | 6.41 ± 0.08 ^a | 6.77 ± 0.45 ^a | 1.83 ± 0.01 ^b | 1.39 ± 0.01 ^b |
| Malic acid | 3.45 ± 0.14 ^a | 3.70 ± 0.03 ^a | 0.29 ± 0.03 ^c | 0.56 ± 0.11 ^c | 1.38 ± 0.25 ^b | 1.33 ± 0.02 ^b |
| Citric acid | 3.41 ± 0.07 ^a | 3.50 ± 0.00 ^a | 2.82 ± 0.05 ^b | 2.78 ± 0.11 ^b | tr | nd |
| Shikimic acid | 0.02 ± 0.01 ^b | 0.03 ± 0.00 ^b | 0.03 ± 0.00 ^b | 0.02 ± 0.01 ^b | 0.88 ± 0.01 ^a | 0.87 ± 0.02 ^a |
| Lactic acid | nd | nd | 2.49 ± 0.14 ^c | 2.44 ± 0.35 ^c | 13.93 ± 0.30 ^a | 13.29 ± 0.31 ^b |
| Acetic acid | nd | nd | 0.25 ± 0.08 ^b | 0.29 ± 0.02 ^b | 2.24 ± 0.15 ^a | 2.30 ± 0.02 ^a |
| Total | 13.73 ± 0.28 ^b | 13.72 ± 0.01 ^b | 12.29 ± 0.13 ^c | 12.86 ± 0.81 ^{bc} | 20.27 ± 0.68 ^a | 19.16 ± 0.28 ^a |
| <i>pH</i> | | | | | | |
| pH | 2.65 ± 0.00 ^b | 3.50 ± 0.00 ^a | 2.58 ± 0.01 ^c | 2.63 ± 0.01 ^b | 3.47 ± 0.00 ^a | 3.50 ± 0.01 ^a |
| <i>Color attributes</i> | | | | | | |
| Color intensity | 9.80 ± 0.00 ^a | 9.55 ± 0.07 ^b | 9.60 ± 0.00 ^b | 9.60 ± 0.00 ^b | 9.20 ± 0.00 ^c | 9.25 ± 0.07 ^c |

Data are the mean ± standard deviation ($n = 2$). “nd”, not detected; “tr”, trace. Means in the same row with different superscript letters (a–d) are significantly different ($p < 0.05$)

acid level was observed in the AJ fermented by these strains. Meanwhile, shikimic acid was dramatically accumulated in the juice after the fermentation. However, the NJ did not show the significant differences on the quinic or shikimic acid level throughout the fermentation (Table 1). It has been reported that *L. plantarum* had the capability to degrade quinic acid as an electron acceptor, instead of sugar, to provide energy during fermentation (Filannino et al. 2015). Quinic acid could also be converted into shikimic acid (Ghosh et al. 2012). In this study, only the strains fermented juice (pH 3.50) showed the degradation of quinic acid and the accumulation of shikimic acid. Extremely low pH condition in the juice might inhibit this conversion. Regarding malic acid, a dramatic concentration decrease was observed in the juice after the fermentation by both strains. Similar results were also reported in other lactic acid bacteria fermented fruit juice (Filannino et al. 2014). Meanwhile, the concentration of lactic acid was enhanced in the juices after the fermentation. It was proposed that malic acid, during the lactic acid fermentation, was converted into lactic acid through the decarboxylation reaction under the activity of *L. plantarum*. This reaction could soften the mouthfeel of juice (Fugelsang and Edwards 2006). It should be noted that pH condition also affected the conversion ability of strains since the lactic acid level in the AJ after the fermentation was much higher

than in the NJ. This was consistent with the previous study (Filannino et al. 2014). Similarly, acetic acid was produced more in the AJ compared with the NJ, whereas citric acid concentration decreased significantly. These were because that citric acid was metabolized into acetic acid by *L. plantarum* (Nilchian et al. 2016), and this metabolism depended on the pH condition of the juices.

No significant changes were observed in the pH of the juice before and after the fermentation by these strains (Table 1). Nevertheless, the AJ and the NJ showed the decreases in their color intensities after the fermentation. This might be caused by the alteration of the anthocyanins composition (Liu et al. 2015).

Amino acids

The bog bilberry juice contained 22 free amino acids (including 7 essential amino acids) and ammonium amine (Table 2). Regarding the essential amino acids, most of the aromatic amino acids and branched-chain amino acids showed a significant concentration increase after the fermentation. Meanwhile, the levels of valine, isoleucine, leucine, and lysine were much higher in the AJ. It has been reported that lactic acid bacteria could metabolize aromatic and branched-chain amino acids to produce energy, which could result in a concentration decrease of these amino

Table 2 Concentration of amino acids in bog bilberry juice with different pH conditions fermented by *Lactobacillus plantarum* B7 and C8-1

| Compound (mg/L) | Before inoculation | | After fermentation | | | |
|-----------------------------------|---------------------------|---------------------------|----------------------------|---------------------------|----------------------------|----------------------------|
| | pH 2.65 | pH 3.50 | pH 2.65 | | pH 3.50 | |
| | | | B7 | C8-1 | B7 | C8-1 |
| Essential Amino Acids | | | | | | |
| <i>Aromatic amino acids</i> | | | | | | |
| Phenylalanine | 0.79 ± 0.20 ^b | 0.81 ± 0.22 ^b | 4.77 ± 0.48 ^a | 4.26 ± 0.06 ^a | 5.66 ± 1.24 ^a | 3.97 ± 1.29 ^a |
| <i>Branched-chain amino acids</i> | | | | | | |
| Valine | 1.03 ± 0.29 ^b | 1.03 ± 0.30 ^b | 1.49 ± 0.01 ^b | 1.17 ± 0.27 ^b | 5.13 ± 0.11 ^a | 4.76 ± 0.95 ^a |
| Leucine | 0.74 ± 0.18 ^b | 0.75 ± 0.16 ^b | 3.98 ± 1.20 ^b | 3.11 ± 0.42 ^b | 8.40 ± 1.32 ^a | 9.23 ± 0.99 ^a |
| Isoleucine | 0.33 ± 0.08 ^b | 0.71 ± 0.44 ^b | 1.02 ± 0.19 ^b | 0.57 ± 0.33 ^b | 5.31 ± 0.25 ^a | 5.61 ± 0.48 ^a |
| <i>Others</i> | | | | | | |
| Lysine | 1.53 ± 0.04 ^c | 1.61 ± 0.09 ^{bc} | 1.51 ± 0.28 ^c | 1.47 ± 0.21 ^c | 8.52 ± 4.21 ^{ab} | 9.34 ± 0.70 ^a |
| Threonine | 1.08 ± 0.25 ^a | 1.20 ± 0.25 ^a | 2.45 ± 0.18 ^{ab} | 1.62 ± 1.02 ^{ab} | 4.62 ± 2.64 ^a | 3.05 ± 0.92 ^{ab} |
| Methionine | 0.62 ± 0.13 ^a | 0.69 ± 0.17 ^a | 0.70 ± 0.02 ^a | 0.60 ± 0.01 ^a | 0.76 ± 0.11 ^a | 0.71 ± 0.02 ^a |
| Nonessential amino acids | | | | | | |
| γ-Aminobutyric acid | 5.68 ± 0.72 ^a | 5.61 ± 0.71 ^a | 8.45 ± 0.82 ^a | 8.03 ± 1.11 ^a | 6.00 ± 0.65 ^a | 6.38 ± 2.51 ^a |
| Arginine | 2.56 ± 0.13 ^c | 2.70 ± 0.26 ^c | 7.95 ± 0.12 ^b | 5.68 ± 0.68 ^{bc} | 13.91 ± 2.22 ^a | 13.23 ± 1.33 ^a |
| Asparaginate | 1.59 ± 0.29 ^a | 1.75 ± 0.25 ^a | 0.34 ± 0.07 ^{bc} | 0.30 ± 0.12 ^c | 0.99 ± 0.33 ^{abc} | 1.30 ± 0.30 ^{ab} |
| Cysteine | 1.48 ± 0.16 ^a | 1.51 ± 0.27 ^a | 0.37 ± 0.52 ^b | 0.53 ± 0.75 ^{ab} | nd | nd |
| Glycine | 1.36 ± 0.21 ^c | 1.33 ± 0.02 ^c | 12.33 ± 0.43 ^{ab} | 8.50 ± 2.88 ^b | 17.42 ± 1.66 ^a | 17.20 ± 0.91 ^a |
| α-Alanine | 1.35 ± 0.29 ^b | 1.36 ± 0.29 ^b | 6.12 ± 0.06 ^a | 5.00 ± 1.18 ^a | 5.27 ± 0.91 ^a | 4.70 ± 1.19 ^a |
| Ornithine | 0.86 ± 0.31 ^a | 0.89 ± 0.30 ^a | 1.98 ± 0.10 ^a | 1.82 ± 0.04 ^a | 1.57 ± 0.25 ^a | 2.08 ± 1.34 ^a |
| Tyrosine | 0.69 ± 0.02 ^b | 0.61 ± 0.05 ^b | 1.26 ± 0.00 ^a | 1.27 ± 0.11 ^a | 0.88 ± 0.15 ^{ab} | 1.13 ± 0.27 ^{ab} |
| Glutamic acid | 0.44 ± 0.62 ^a | 0.95 ± 0.04 ^a | nd | 1.98 ± 2.81 ^a | 0.45 ± 0.64 ^a | 1.59 ± 2.25 ^a |
| Serine | 0.42 ± 0.07 ^b | 1.12 ± 0.08 ^{ab} | 0.88 ± 0.04 ^{ab} | 1.05 ± 0.03 ^{ab} | 1.69 ± 0.26 ^a | 0.59 ± 0.83 ^b |
| β-Alanine | 0.30 ± 0.07 ^c | 0.32 ± 0.10 ^{bc} | 0.24 ± 0.01 ^c | 0.29 ± 0.05 ^c | 1.39 ± 0.66 ^a | 0.26 ± 0.02 ^c |
| Proline | 0.13 ± 0.18 ^b | 0.84 ± 0.80 ^{ab} | 2.13 ± 0.11 ^{ab} | 1.49 ± 0.59 ^{ab} | 3.26 ± 0.90 ^a | 2.09 ± 0.83 ^{ab} |
| Glutamine | nd | 1.72 ± 0.27 ^a | 0.95 ± 0.15 ^c | 1.10 ± 0.04 ^{bc} | 1.57 ± 0.11 ^{ab} | 1.61 ± 0.37 ^a |
| Histidine | nd | 1.55 ± 0.23 ^{ab} | nd | nd | 2.38 ± 0.07 ^b | 1.24 ± 1.75 ^{ab} |
| Aspartic acid | nd | tr | 0.44 ± 0.62 ^a | 0.65 ± 0.34 ^a | nd | 0.33 ± 0.02 ^a |
| <i>Total</i> | 22.98 ± 1.65 ^c | 29.05 ± 2.58 ^c | 59.34 ± 0.26 ^b | 50.48 ± 4.50 ^b | 95.18 ± 10.15 ^a | 90.39 ± 16.08 ^a |
| Ammonium amine | | | | | | |
| NH ₄ ⁺ | 0.63 ± 0.19 ^b | 1.24 ± 0.17 ^b | 1.54 ± 0.37 ^b | 1.19 ± 0.26 ^b | 22.51 ± 1.74 ^a | 21.38 ± 3.33 ^a |

Data are the mean ± standard deviation ($n = 2$). “nd”, not detected; “tr”, trace. Means in the same row with different superscript letters (a–c) are significantly different ($p < 0.05$)

acids during fermentation (Filannino et al. 2014). This was not consistent with our result. In this study, the consumption of quinic acid by these *L. plantarum* strains produced the energy for the bacterial growth. As a result, the biosynthesis of aromatic amino acids was promoted in the juice (Dewick 1998). In terms of the branched-chain amino acids, lysine and threonine were found to be higher in the AJ. This indicated that these strains might possess a high peptidase ability in a high pH condition during the lactic acid fermentation, which significantly enhanced the biosynthesis of the branched-chain amino acids through the peptide metabolisms (Pritchard and Coolbear 1993).

Additionally, most nonessential amino acids also increased on their concentration in the juice after the fermentation.

The *L. plantarum* strains preferred to metabolize quinic acid, citric acid, and malic acid in the AJ during the fermentation. Meanwhile, the consumption of the reducing sugar in the AJ was inhibited. However, the major energy source in the NJ resulted from malic acid and the reducing sugar. The consumption of the reducing sugar in this juice was much higher than that in the AJ, and the rapid consumption of malic acid during the fermentation could inhibit the activity of the *L. plantarum* strains to metabolize other nutrients. Besides, the lower malic acid level in the

NJ might be due to the greater energy requirement in acidic environment, since the decarboxylation of organic acids can supply extra ATP to sustain a proton balance inside and outside the cell membrane (Guchte et al. 2002).

Phenolic compounds

Anthocyanins are the major colorants in fruits that play an important role in the appearance of fruit juice (Pascual-Teresa et al. 2013). Before the fermentation, the major anthocyanins in the juice included malvidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside. Our result was consistent with the previous report (Wang et al. 2014). The fermentation decreased the anthocyanin levels in all the juices (Table 3). It has been reported that β -glucosidase released by *L. plantarum* can easily cleave anthocyanins into anthocyanidins and sugar moieties. Sugar moieties can be further metabolized by *L. plantarum* as the carbohydrate source (Mousavi et al. 2013). Moreover, anthocyanidins could interact with other phenolic compounds to form large polymers (Baranowski and Nagel 1983). As a result, the concentration of anthocyanins after the lactic acid fermentation was reduced, causing a decrease on the juice color intensity.

Protocatechuic and chlorogenic acids were the dominant phenolic acids in the juice. After the fermentation, protocatechuic acid showed a dramatic concentration decrease in all the juices. Similar evolution of protocatechuic acid was also observed in lactic acid fermentation of cherry juice (Filannino et al. 2015). Chlorogenic acid also exhibited a concentration decrease. It has been reported that *L. plantarum* could release decarboxylase and esterase, and these enzymes can degrade a wide range of phenolic acids (Di Cagno et al. 2015). However, the other phenolic acids remained at a low level in the juices during the fermentation process.

The bog bilberry juices contained the abundant amount of myricetin-3-*O*-galactoside and quercetin-3-*O*-galactoside before the fermentation. This was consistent with the previous study in bog bilberry wine (Liu et al. 2015). These two flavonols showed a dramatic concentration decrease in all the juices. Quercetin-3-*O*-galactoside remained the similar concentration in the AJ after the fermentation. However, the alteration on the concentration of quercetin was differentiated by the pH adjustment. A significant concentration increase of quercetin was observed in the NJ, whereas the AJ showed a reduced level. Compared with a previous study (Filannino et al. 2015), lactic acid fermentation of cherry juice did not cause the concentration alteration of quercetin. It has been reported that β -glucosidase produced by *L. plantarum* strains can hydrolyze glycosylated flavonols into their aglycones (Di Cagno et al.

2015), and low pH condition in the NJ might enhance the activity of β -glucosidase.

Epicatechin and epigallocatechin were the major flavanols in the bog bilberry juice. Both compounds showed a concentration decrease after the fermentation. Their concentration decrease might result from their oxidation, interactions with anthocyanins, or the metabolism by *L. plantarum* (Tabasco et al. 2011). However, the other flavanols did not significantly change on their level in the juice after the fermentation.

Volatile compounds

A total of 29 volatile compounds were detected in these bog bilberry juices before the fermentation. It was observed that the concentration of α -terpineol and ethyl octanoate in the juice decreased after adjusting the juice pH from 2.65 to 3.50 (Table 4). After the fermentation by these two strains, 32 volatile compounds were detected in the NJ, whereas the AJ juice contained 28 volatiles. The juice pH appeared to play an important role in affecting the evolution of the volatile compounds during the fermentation. For example, almost all the volatile compounds exhibited a significant concentration increase in the NJ by these two strains, whereas the concentrations of terpenoids, esters and acids decreased in the AJ.

Odor active value (OAV) is basically used to evaluate the contribution of the individual volatile compound to the overall aroma in foods. A volatile with its OAV value above 1 is normally considered to significantly contribute its flavor notes to the overall aroma (Zhang et al. 2007). A total of 9 volatile compounds in the juices exhibited its OAV above 1, including 4 alcohols, 1 acid, 3 esters, and 1 aldehyde (Table 5). These volatile compounds were thus suggested to have a significant impact on the overall aroma in the juices. It was the pH condition of the juice, instead of the strains, that significantly affected the evolution of these volatiles in the juice during the fermentation. For example, phenylethyl alcohol exhibits the floral note, whereas 1-heptanol can provide the oily flavor to juice (Lee et al. 2008). Both alcohols significantly increased the concentration in the NJ. However, the OAV of these two alcohols were reduced in the AJ after the fermentation. This indicated that these volatile compounds did not exert the significant contribution to the overall aroma of the AJ. Octanoic acid provides juice with the chemical flavor (Coelho et al. 2015), and its concentration was detected below its threshold in the AJ and NJ before the fermentation. However, the fermentation in the NJ resulted in its OVA above 1, indicating that octanoic acid significantly contributed its flavor note to the overall aroma in the juice. The AJ after the fermentation contained octanoic acid with its concentration below its threshold. Ethyl butanoate,

Table 3 Concentration of phenolic compounds in bog bilberry juice with different pH conditions fermented by *Lactobacillus plantarum* B7 and C8-1

| Compound (mg/L) | Before Inoculation | | After Fermentation | | | |
|---------------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | pH 2.65 | pH 3.50 | pH 2.65 | | pH 3.50 | |
| | | | B7 | C8-1 | B7 | C8-1 |
| <i>Anthocyanins</i> | | | | | | |
| Malvidin-3- <i>O</i> -glucoside | 110.64 ± 0.16 ^a | 108.28 ± 16.40 ^a | 37.24 ± 5.72 ^b | 30.98 ± 5.96 ^b | 40.24 ± 2.94 ^b | 36.68 ± 3.80 ^b |
| Petunidin-3- <i>O</i> -glucoside | 96.34 ± 0.64 ^a | 100.58 ± 3.44 ^a | 31.26 ± 4.46 ^b | 25.28 ± 3.96 ^b | 26.60 ± 0.72 ^b | 24.32 ± 2.52 ^b |
| Delphinidin-3- <i>O</i> -glucoside | 64.38 ± 0.92 ^a | 64.98 ± 8.86 ^a | 19.32 ± 2.68 ^b | 16.64 ± 2.90 ^b | 13.38 ± 0.64 ^b | 11.36 ± 0.96 ^b |
| Cyaniding-3- <i>O</i> -glucoside | 13.58 ± 0.40 ^a | 13.24 ± 1.62 ^a | 3.74 ± 0.56 ^b | 2.92 ± 0.44 ^b | 4.60 ± 0.02 ^b | 4.14 ± 0.30 ^b |
| Peonidin-3- <i>O</i> -glucoside | 9.20 ± 0.08 ^a | 9.18 ± 0.98 ^a | 2.70 ± 0.38 ^b | 2.26 ± 0.38 ^b | 3.46 ± 0.22 ^b | 3.12 ± 0.20 ^b |
| <i>Total</i> | 294.12 ± 2.14 ^a | 296.24 ± 31.30 ^a | 94.28 ± 13.78 ^b | 78.08 ± 13.64 ^b | 88.28 ± 4.56 ^b | 79.62 ± 7.76 ^b |
| <i>Phenolic acids</i> | | | | | | |
| Protocatechuic acid | 40.38 ± 0.64 ^a | 42.04 ± 0.24 ^a | 2.24 ± 0.40 ^b | 1.70 ± 0.08 ^b | 2.76 ± 0.68 ^b | 2.12 ± 0.30 ^b |
| Chlorogenic acid | 30.26 ± 0.20 ^a | 29.88 ± 0.04 ^{ab} | 20.14 ± 1.50 ^{abc} | 19.12 ± 2.92 ^{bc} | 18.12 ± 1.34 ^c | 22.32 ± 5.76 ^{abc} |
| Gallic acid | 2.44 ± 0.00 ^b | 3.04 ± 0.24 ^a | tr | tr | tr | tr |
| Caffeic acid | 2.30 ± 0.08 ^a | 2.44 ± 0.18 ^a | 1.30 ± 0.40 ^b | 0.96 ± 0.76 ^b | tr | tr |
| 4-Hydroxycinnamic acid | 1.12 ± 0.04 ^a | 0.64 ± 0.90 ^a | tr | tr | tr | tr |
| 2-Hydroxybenzoic acid | tr | tr | 0.16 ± 0.02 | 0.16 ± 0.10 | 0.14 ± 0.06 | 0.14 ± 0.08 |
| <i>Total</i> | 76.50 ± 0.56 ^a | 78.04 ± 1.62 ^a | 23.88 ± 2.32 ^b | 21.92 ± 3.88 ^b | 21.02 ± 2.08 ^b | 24.58 ± 5.40 ^b |
| <i>Flavonols</i> | | | | | | |
| Myricetin-3- <i>O</i> -galactoside | 124.20 ± 0.04 ^a | 121.58 ± 1.30 ^a | 71.12 ± 5.10 ^b | 65.72 ± 10.72 ^b | 79.16 ± 6.28 ^b | 76.72 ± 11.52 ^b |
| Quercetin-3- <i>O</i> -galactoside | 101.86 ± 0.18 ^a | 103.18 ± 0.16 ^a | 52.68 ± 3.42 ^b | 48.22 ± 7.94 ^b | 80.72 ± 5.06 ^a | 80.18 ± 10.10 ^a |
| Quercetin-3- <i>O</i> -glucuronide | 29.66 ± 0.04 ^a | 29.50 ± 0.10 ^a | 14.68 ± 1.24 ^b | 13.08 ± 2.74 ^b | 17.46 ± 1.62 ^b | 16.80 ± 2.68 ^b |
| Syringetin-3- <i>O</i> -galactoside | 12.54 ± 0.12 ^a | 13.12 ± 0.58 ^a | 8.02 ± 0.40 ^b | 7.52 ± 1.26 ^b | 7.56 ± 0.56 ^b | 7.28 ± 1.06 ^b |
| Quercetin | 5.10 ± 0.90 ^b | 1.72 ± 0.22 ^c | 20.92 ± 2.52 ^a | 19.90 ± 0.01 ^a | tr | tr |
| Myricetin | 4.90 ± 0.26 ^a | 4.20 ± 0.06 ^a | 4.52 ± 0.36 ^a | 4.06 ± 1.02 ^a | 0.84 ± 0.26 ^b | 0.72 ± 0.02 ^b |
| Isorhamnetin-3- <i>O</i> -galactoside | 3.52 ± 0.08 ^a | 3.90 ± 0.04 ^a | 1.42 ± 0.14 ^b | 1.22 ± 0.34 ^b | 2.16 ± 0.30 ^b | 2.12 ± 0.34 ^b |
| Syringetin-3- <i>O</i> -glucoside | 3.12 ± 0.10 ^a | 2.82 ± 0.46 ^{ab} | 1.94 ± 0.12 ^b | 1.80 ± 0.36 ^b | 1.84 ± 0.14 ^b | 1.76 ± 0.24 ^b |
| Myricetin-3- <i>O</i> -glucoside | 1.96 ± 0.26 ^a | 1.76 ± 0.42 ^a | 0.52 ± 0.22 ^b | 0.40 ± 0.26 ^b | 1.40 ± 0.18 ^{ab} | 1.38 ± 0.32 ^{ab} |
| <i>Total</i> | 286.88 ± 1.96 ^a | 281.70 ± 0.32 ^a | 177.88 ± 13.60 ^b | 161.92 ± 24.62 ^b | 191.14 ± 14.40 ^b | 186.94 ± 26.28 ^b |
| <i>Flavanols</i> | | | | | | |
| Epicatechin | 26.30 ± 0.96 ^a | 28.70 ± 1.36 ^a | 2.18 ± 0.94 ^b | 0.86 ± 1.00 ^b | 3.96 ± 0.02 ^b | 2.80 ± 0.14 ^b |
| Epigallocatechin | 23.38 ± 0.80 ^a | 23.78 ± 0.90 ^a | 6.50 ± 0.84 ^b | 5.40 ± 0.46 ^b | 6.00 ± 0.02 ^b | 4.68 ± 0.16 ^b |
| Gallocatechin | 2.22 ± 0.12 ^a | 2.32 ± 0.26 ^a | 0.48 ± 0.20 ^{bc} | 0.28 ± 0.02 ^c | 0.88 ± 0.08 ^b | 0.64 ± 0.02 ^{bc} |
| Catechin | 1.12 ± 0.16 ^a | 1.44 ± 0.10 ^a | 0.10 ± 0.06 ^b | 0.06 ± 0.02 ^b | 0.26 ± 0.36 ^b | 0.40 ± 0.02 ^b |
| <i>Total</i> | 53.00 ± 1.78 ^a | 56.22 ± 2.46 ^a | 9.26 ± 2.06 ^b | 6.58 ± 1.46 ^b | 11.08 ± 0.48 ^b | 8.52 ± 0.08 ^b |

Data are the mean ± standard deviation ($n = 2$). “nd”, not detected; “tr”, trace. Means in the same row with different superscript letters (a–c) are significantly different ($p < 0.05$)

isopentyl acetate, and ethyl hexanoate were the major esters in the juices with their OAV above 1, and these esters contributed the fruity aroma to the juice regarding their aromatic feature (Wu et al. 2016). A dramatic increase on the OAV of these esters occurred in the NJ after the fermentation, whereas their aroma contributions were neglectable in the AJ. Nonanal is an aldehyde with the fruity note (Wen et al. 2014). Its OAV value remained

similar after the fermentation by these strains. These results indicated that the NJ after the fermentation significantly improved the floral and fruity notes. However, the overall aroma of the AJ after the fermentation was significantly weakened.

It has been confirmed that amino acids and phenolic compounds can be converted into aromatic compounds by lactic acid bacteria (Helinck et al. 2004). As a result, the

Table 4 Concentration of volatile compounds in bog bilberry juice with different pH conditions fermented by *Lactobacillus plantarum* B7 and C8-1

| Compound (µg/L) | Before inoculation | | | After fermentation | | |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|-----------------------------|
| | pH 2.65 | | pH 3.50 | pH 2.65 | | pH 3.50 |
| | B7 | C8-1 | B7 | C8-1 | B7 | C8-1 |
| <i>Terpenoids</i> | | | | | | |
| α-Terpineol | 4.28 ± 0.66 ^b | 2.98 ± 0.16 ^c | 5.93 ± 0.18 ^a | 5.22 ± 0.03 ^{ab} | 2.53 ± 0.09 ^c | 2.76 ± 0.01 ^c |
| Linalool | 2.69 ± 0.91 ^{ab} | 3.37 ± 0.33 ^a | 2.94 ± 0.36 ^{ab} | 2.28 ± 0.04 ^{ab} | 1.66 ± 0.10 ^b | 1.58 ± 0.06 ^b |
| 6-Methyl-5-hepten-2-one | 1.52 ± 0.32 ^a | 1.59 ± 0.06 ^a | 1.76 ± 0.21 ^a | 1.63 ± 0.07 ^a | 1.35 ± 0.01 ^a | 1.45 ± 0.08 ^a |
| Citronellyl formate | nd | nd | 1.18 ± 0.04 | 0.57 ± 0.81 | 1.18 ± 0.02 | 1.16 ± 0.01 |
| <i>Total</i> | 8.49 ± 0.57 ^{bc} | 7.94 ± 0.56 ^{cd} | 11.81 ± 0.36 ^a | 9.70 ± 0.82 ^b | 6.72 ± 0.21 ^d | 6.95 ± 0.16 ^d |
| <i>Alcohols</i> | | | | | | |
| Phenylethyl alcohol | 663.48 ± 46.43 ^b | 617.49 ± 15.47 ^b | 1731.21 ± 93.00 ^a | 1775.80 ± 8.84 ^a | 459.67 ± 9.07 ^c | 463.40 ± 6.83 ^c |
| (Z)-3-Hexen-1-ol | 75.51 ± 8.84 ^{bc} | 63.76 ± 0.75 ^c | 258.14 ± 18.03 ^a | 265.64 ± 37.99 ^a | 127.70 ± 13.37 ^{bc} | 142.05 ± 0.94 ^b |
| 1-Hexanol | 42.79 ± 2.13 ^b | 36.13 ± 1.76 ^b | 68.98 ± 3.10 ^a | 66.86 ± 2.96 ^a | 25.07 ± 1.93 ^c | 26.02 ± 0.02 ^c |
| 1-Heptanol | 10.98 ± 0.06 ^b | 9.78 ± 0.52 ^b | 16.14 ± 0.37 ^a | 15.21 ± 0.92 ^a | 4.56 ± 0.04 ^c | 4.60 ± 0.08 ^c |
| 1-Octen-3-ol | 1.01 ± 0.05 ^a | 0.93 ± 0.05 ^{ab} | 0.85 ± 0.02 ^{bc} | 0.76 ± 0.03 ^c | 0.50 ± 0.01 ^d | 0.49 ± 0.01 ^d |
| 1-Octanol | 0.31 ± 0.00 ^b | 0.29 ± 0.00 ^{bc} | 0.68 ± 0.03 ^a | 0.62 ± 0.02 ^a | 0.26 ± 0.01 ^{bc} | 0.24 ± 0.00 ^c |
| 3-Octanol | 0.10 ± 0.02 ^a | 0.08 ± 0.00 ^a | 0.03 ± 0.04 ^a | nd | nd | nd |
| 2-Ethyl-1-hexanol | tr | tr | 1.58 ± 0.83 ^a | 1.14 ± 0.33 ^a | 0.97 ± 0.10 ^a | 0.84 ± 0.10 ^a |
| Benzyl alcohol | nd | nd | 135.42 ± 10.01 ^a | 134.47 ± 6.14 ^a | 99.19 ± 6.27 ^a | 97.46 ± 2.51 ^a |
| 1-Dodecanol | nd | nd | 0.64 ± 0.01 ^a | 0.64 ± 0.00 ^a | nd | nd |
| 2-Heptanol | nd | nd | nd | nd | 2.16 ± 0.00 ^a | 2.26 ± 0.11 ^a |
| <i>Total</i> | 794.17 ± 57.28 ^b | 728.45 ± 12.39 ^b | 2213.67 ± 83.17 ^a | 2261.13 ± 24.67 ^a | 720.07 ± 30.79 ^b | 737.35 ± 10.60 ^b |
| <i>Acids</i> | | | | | | |
| Octanoic acid | 1538.15 ± 280.98 ^b | 1156.53 ± 13.95 ^{bc} | 3921.58 ± 449.01 ^a | 4013.15 ± 454.80 ^a | 655.34 ± 108.56 ^c | 722.93 ± 30.91 ^c |
| Hexanoic acid | 147.01 ± 7.05 ^b | 132.57 ± 0.36 ^b | 272.10 ± 8.54 ^a | 266.00 ± 12.95 ^a | 136.89 ± 16.86 ^b | 123.18 ± 1.06 ^b |
| Pentanoic acid | nd | nd | nd | nd | 17.39 ± 1.35 ^a | 16.24 ± 0.05 ^a |
| <i>Total</i> | 1685.16 ± 288.03 ^b | 1289.09 ± 14.30 ^{bc} | 4193.69 ± 457.55 ^a | 4279.15 ± 467.74 ^a | 809.63 ± 90.35 ^c | 862.35 ± 32.02 ^c |
| <i>Esters</i> | | | | | | |
| Ethyl nonanoate | 19.50 ± 0.99 ^b | 18.42 ± 0.82 ^b | 24.37 ± 4.17 ^{ab} | 28.46 ± 6.90 ^a | nd | nd |
| Ethyl octanoate | 18.05 ± 1.13 ^b | 8.69 ± 1.32 ^c | 26.24 ± 1.29 ^{ab} | 25.03 ± 0.40 ^a | 4.66 ± 0.06 ^d | 4.60 ± 0.01 ^d |
| Ethyl laurate | 10.00 ± 1.06 ^a | 9.61 ± 0.70 ^a | 6.07 ± 8.59 ^a | 13.39 ± 3.07 ^a | nd | 8.98 ± 0.09 ^a |
| Isopentyl acetate | 7.65 ± 1.80 ^b | 7.27 ± 0.32 ^b | 30.34 ± 2.54 ^a | 27.51 ± 4.15 ^a | nd | nd |
| Ethyl hexanoate | 3.86 ± 0.87 ^b | 3.26 ± 0.19 ^b | 7.79 ± 0.24 ^a | 7.62 ± 1.51 ^a | nd | nd |
| Ethyl butanoate | 1.82 ± 0.25 ^b | 1.63 ± 0.06 ^b | 2.43 ± 0.25 ^a | 2.58 ± 0.36 ^a | nd | nd |
| Methyl salicylate | 1.67 ± 0.05 ^b | 1.54 ± 0.04 ^b | 2.35 ± 0.07 ^a | 2.13 ± 0.10 ^a | 2.09 ± 0.22 ^a | 1.76 ± 0.07 ^b |
| Isobutyl acetate | 1.60 ± 2.26 ^b | 3.23 ± 0.03 ^b | 5.98 ± 0.35 ^{ab} | 2.99 ± 4.23 ^{ab} | 9.85 ± 0.60 ^a | nd |

Table 4 continued

| Compound ($\mu\text{g/L}$) | Before inoculation | | After fermentation | | | | | | |
|------------------------------|-----------------------|------|-----------------------|------|-----------------------|------|-----------------------|------|------------------------|
| | pH 2.65 | | pH 3.50 | | pH 2.65 | | pH 3.50 | | |
| | B7 | C8-1 | B7 | C8-1 | B7 | C8-1 | B7 | C8-1 | |
| Ethyl phenylacetate | 0.60 ± 0.02^b | | 0.54 ± 0.02^b | | 1.00 ± 0.06^a | | nd | | nd |
| <i>Total</i> | 64.74 ± 4.18^b | | 54.20 ± 3.39^b | | 106.57 ± 9.06^a | | 110.66 ± 0.65^a | | 15.35 ± 0.02^c |
| <i>Aldehyde/Ketones</i> | | | | | | | | | |
| Benzaldehyde | 41.78 ± 4.50^{cd} | | 39.20 ± 0.85^d | | 55.49 ± 1.09^{ab} | | 62.28 ± 0.79^a | | 47.72 ± 2.41^{bcd} |
| Nonanal | 0.55 ± 0.78^a | | 1.18 ± 0.03^a | | 1.17 ± 0.01^a | | 0.59 ± 0.83^a | | 1.06 ± 0.01^a |
| <i>Total</i> | 42.33 ± 3.72^d | | 40.39 ± 0.87^d | | 56.67 ± 1.10^b | | 62.86 ± 0.04^a | | 48.78 ± 2.41^c |
| <i>Others</i> | | | | | | | | | |
| Styrene | 23.23 ± 10.85^a | | 9.40 ± 0.71^{ab} | | 5.09 ± 0.56^b | | 5.63 ± 0.41^{ab} | | 4.78 ± 0.07^b |
| Cyclohexene | 1.71 ± 0.13^a | | 1.46 ± 0.03^{ab} | | 1.63 ± 0.07^{ab} | | 1.73 ± 0.08^a | | 1.40 ± 0.00^b |
| Naphthalene | 1.48 ± 0.05^b | | 5.21 ± 0.22^a | | 1.65 ± 0.19^b | | 1.72 ± 0.17^b | | 1.56 ± 0.00^b |
| β -Methylnaphthalene | 1.44 ± 0.00^b | | 3.42 ± 0.01^a | | 1.56 ± 0.01^b | | 1.67 ± 0.01^b | | 1.40 ± 0.00^b |
| α -Methylnaphthalene | 0.70 ± 1.00^a | | 1.92 ± 0.04^a | | 0.73 ± 1.03^a | | 1.45 ± 0.03^a | | nd |
| <i>Total</i> | 28.58 ± 9.62^a | | 21.40 ± 1.17^{ab} | | 10.64 ± 2.04^c | | 12.20 ± 0.67^{bc} | | 9.13 ± 0.07^c |

Data are the mean \pm standard deviation ($n = 2$). nd, not detected; tr trace. Means in the same row with different superscript letters (a–d) are significantly different ($p < 0.05$)

Table 5 Odor activity value (OAV) of main aromatic compound in bog bilberry juice with different pH conditions fermented by *Lactobacillus plantarum* B7 and C8-1

| Compound | Threshold ^{[1]*} (µg/L) | Aroma description | Aroma series | Before inoculation | | After fermentation | | | | |
|-------------------------|----------------------------------|----------------------------------|-----------------|--------------------|--------------------|--------------------|--------------------|-------------------|-------------------|--|
| | | | | pH 2.65 | pH 3.50 | pH 2.65 | | pH 3.50 | | |
| | | | | | | B7 | C8-1 | B7 | C8-1 | |
| <i>Alcohols</i> | | | | | | | | | | |
| (Z)-3-Hexen-1-ol | 70 | Grassy-green | Green | 1.08 ^c | 0.91 ^c | 3.69 ^a | 3.79 ^a | 1.82 ^b | 2.03 ^b | |
| 1-Octen-3-ol | 1 | Earthy, mushroom, vegetable-like | Chemical, Green | 1.01 ^a | 0.92 ^b | 0.84 ^c | 0.76 ^d | 0.49 ^c | 0.49 ^e | |
| 1-Heptanol | 3 | Oily, planty | Chemical | 3.66 ^b | 3.26 ^c | 5.38 ^a | 5.07 ^a | 1.52 ^d | 1.53 ^d | |
| Phenylethyl alcohol | 750 | Soft, like roses, jasmine | Floral | 0.88 ^b | 0.82 ^b | 2.31 ^a | 2.37 ^a | 0.61 ^c | 0.61 ^c | |
| <i>Acids</i> | | | | | | | | | | |
| Octanoic acid | 3000 | Faint, fruity-acid, irritating | Chemical | 0.51 ^b | 0.39 ^{bc} | 1.31 ^a | 1.34 ^a | 0.22 ^c | 0.24 ^c | |
| <i>Esters</i> | | | | | | | | | | |
| Ethyl butanoate | 1 | Strawberry, fruity | Fruity | 1.82 ^b | 1.63 ^b | 2.43 ^a | 2.58 ^a | nd | nd | |
| Isopentyl acetate | 2 | Banana, pear, fruity, sweet | Fruity | 3.64 ^b | 3.83 ^b | 15.17 ^a | 13.75 ^a | nd | nd | |
| Ethyl hexanoate | 1 | Fruity, green apple | Fruity, Green | 3.86 ^b | 3.26 ^b | 7.79 ^a | 7.62 ^a | nd | nd | |
| <i>Aldehyde/Ketones</i> | | | | | | | | | | |
| Nonanal | 1 | Fruity | Fruity | 0.55 ^a | 1.18 ^a | 1.17 ^a | 0.59 ^a | 1.06 ^a | 1.04 ^a | |

*Reference [1] <http://www.leffingwell.com/odorthre.htm>. Values in the same row with different superscript letters (a–e) are significantly different ($p < 0.05$). Aroma series is classified according to aroma description. Volatile compound with OAV above 1 is qualified

fermented flavor notes can be incorporated into the overall aroma of the fermented foods to enhance the aromatic complexity (Chen and Liu 2016). In addition, the flavorless bound form volatiles are mainly present in foods. Hydrolysis could result in the release of their aglycones, which could further improve the overall aroma in foods (Ugliano et al. 2003). In this study, *L. plantarum* possesses the β -glucosidase activity, and this could enhance the release of the volatile aglycones from their precursors in the juices. These volatiles can further be metabolized by *L. plantarum* to yield other compounds (Boido et al. 2002). For example, in this study, quercetin-3-*O*-galactoside significantly decreased its concentration in the NJ after the fermentation. Meanwhile, the juice showed a significant increase on the concentration of quercetin. These indicated that quercetin-3-*O*-galactoside was hydrolyzed into quercetin and galactose moiety. This was because that malic acid was completely consumed in the NJ. The *L. plantarum* strains required more reducing sugar and energy compared to the strains in the AJ. As a result, these strains hydrolyzed more glycosidically conjugated phenolic and aromatic compounds to obtain monosaccharide (e.g. galactose) for their energy needs. This could explain why more volatile compounds were yielded in the NJ after the fermentation by these strains. In the AJ, the consumption of reducing sugar was relatively slow by these strains during the fermentation

process. The *L. plantarum* strains did not require monosaccharides as the energy source in the juice, which lowered the hydrolysis process of the glycosidically conjugated phenolic and aromatic compounds. This resulted in the AJ with lower concentration of the volatile compounds after the fermentation.

Conclusion

In conclusion, malic acid and reducing sugar were mainly consumed by two *L. plantarum* strains in juice without pH adjustment after fermentation. These strains preferred to metabolize quinic acid and citric acid in pH adjusted juice, and resulted in more release of shikimic acid, lactic acid, acetic acid, and aromatic amino acids. Phenolic compounds decreased on their concentration in juices after fermentation. Juice without pH adjustment exhibited an enhancement on volatile complexity and concentration.

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References

- Baranowski ES, Nagel CW (1983) Kinetics of malvidin-3-glucoside condensation in wine model systems. *J Food Sci* 48:419–421
- Boido E, Lloret A, Medina K, Farinã L, Carrau F, Versini G, Dellacassa E (2002) Aroma composition of *Vitis vinifera* Cv. tannat: the typical red wine from Uruguay. *J Agr Food Chem* 51:5408–5413
- Boulton RB, Singleton VL, Bisson LF, Kunkee RE (1999) Principles and practices of winemaking. Springer, New York
- Chen D, Liu S (2016) Transformation of chemical constituents of lychee wine by simultaneous alcoholic and malolactic fermentations. *Food Chem* 196:988–995
- Coelho E, Vilanova M, Genisheva Z, Oliveira JM, Teixeira JA, Domingues L (2015) Systematic approach for the development of fruit wines from industrially processed fruit concentrates, including optimization of fermentation parameters, chemical characterization and sensory evaluation. *LWT-Food Sci Technol* 62:1043–1052
- Colak N, Torun H, Gruz J, Strnad M, Hermosín-Gutiérrez I, Hayirlioglu-Ayaz S, Ayaz FA (2016) Bog bilberry phenolics, antioxidant capacity and nutrient profile. *Food Chem* 201:339–349
- De Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. *J Appl Bacteriol* 23:130–135
- Dewick PM (1998) The biosynthesis of shikimate metabolites. *Nat Prod Rep* 15:263–290
- Di Cagno R, Coda R, De Angelis M, Gobbetti M (2013) Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol* 33:1–10
- Di Cagno R, Filannino P, Gobbetti M (2015) Vegetable and Fruit Fermentation by Lactic Acid Bacteria. In: Mozzi F, Raya RR, Vignolo GM (eds) *Biotechnology of lactic acid bacteria: novel applications*, 2nd edn. Wiley-Blackwell, New Jersey, pp 216–230
- Filannino P, Cardinali G, Rizzello CG, Buchin S, De Angelis M, Gobbetti M, Di Cagno R (2014) Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices. *Appl Environ Microbiol* 80:2206–2215
- Filannino P, Bai Y, Di Cagno R, Gobbetti M, Ganzle MG (2015) Metabolism of phenolic compounds by *Lactobacillus* spp. during fermentation of cherry juice and broccoli puree. *Food Microbiol* 46:272–279
- Fugelsang KC, Edwards CG (2006) *Wine microbiology: practical applications and procedures*, 2nd edn. Springer, New York
- Gao Y, Tian Y, Liu D, Li Z, Zhang X, Li J, Huang J et al (2015) Evolution of phenolic compounds and sensory in bottled red wines and their co-development. *Food Chem* 172:565–574
- Ghosh S, Chisti Y, Banerjee UC (2012) Production of shikimic acid. *Biotechnol Adv* 30:1425–1431
- Gobbetti M, Cagno RD, Angelis MD (2010) Functional microorganisms for functional food quality. *Crit Rev Food Sci Nutr* 50:716–727
- Gómez Alonso S, Hermosín Gutiérrez I, García Romero E (2007) Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. *J Agr Food Chem* 55:608–613
- Gonzalez-Bello C (2016) Inhibition of shikimate kinase and type II dehydroquinase for antibiotic discovery: structure-based design and simulation studies. *Curr Top Med Chem* 16:960–977
- Guchte MVD, Serror P, Chervaux C, Smokvina T, Ehrlich SD, Maguin E (2002) Stress responses in lactic acid bacteria. *Anton Leeuwenhoek* 82:187–216
- Helinck S, Bars DL, Moreau D, Yvon M (2004) Ability of thermophilic lactic acid bacteria to produce aroma compounds from amino acids. *Appl Environ Microbiol* 70:3855–3861
- Hulme AC (1958) Quinic and shikimic acids in fruits. *Plant Food Hum Nutr* 3:468–473
- Lee GH, Shin Y, Oh MJ (2008) Aroma-active components of *Lycii fructus* (kukija). *J Food Sci* 73:500–505
- Liu S, Yang H, Li S, Zhang J, Li T, Zhu B, Zhang B (2015) Polyphenolic compositions and chromatic characteristics of bog bilberry syrup wines. *Molecules* 20:19865–19877
- Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F, Veberic R (2012) Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *J Food Sci* 77:1064–1070
- Mousavi Z, Mousavi SM, Razavi SH, Hadinejad M, Emam-Djomeh Z, Mirzapour M (2013) Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. *Food Biotechnol* 27:1–13
- Nilchian Z, Sharifan A, Rahimi E, Mazid Abadi N (2016) Improvement of fermented cucumber characteristics by starter culture of *Lactobacillus plantarum*, *L. bulgaricus* and *S. thermophiles*. *J Food Biosci Technol* 6:31–40
- Pascual-Teresa SD, Sanchez-Ballesta MT, García-Viguera C (2013) Anthocyanins. *Nat Prod* 8:329–332
- Pritchard GG, Coolbear T (1993) The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiol Rev* 12:179–206
- Tabasco R, Sánchez-Patán F, Monagas M, Bartolomé B, Victoria Moreno-Arribas M, Peláez C, Requena T (2011) Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. *Food Microbiol* 28:1345–1352
- Ugliano M, Genovese A, Moio L (2003) Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *J Agr Food Chem* 51:5073–5078
- Wang L, Su S, Wu J, Du H, Li S, Huo J, Zhang Y et al (2014) Variation of anthocyanins and flavonols in *Vaccinium uliginosum* berry in Lesser Khingan Mountains and its antioxidant activity. *Food Chem* 160:357–364
- Wei M, Gu P, Li CJ, Yang H, Liu S, Zhang J, Yan Z et al (2014) Determination of 7 organic acids in *Vaccinium uliginosum* products by HPLC. *China Brewing* 33:145–148 (in Chinese)
- Wen Y, He F, Zhu B, Lan Y, Pan Q, Li C, Reeves MJ et al (2014) Free and glycosidically bound aroma compounds in cherry (*Prunus avium* L.). *Food Chem* 152:29–36
- Whiting GC (1958) The non-volatile organic acids of some berry fruits. *J Sci Food Agric* 9:244–248
- Wu Y, Duan S, Zhao L, Gao Z, Luo M, Song S, Xu W et al (2016) Aroma characterization based on aromatic series analysis in table grapes. *Sci Rep* 6:31116
- Zhang M, Xu Q, Duan C, Qu W, Wu Y (2007) Comparative study of aromatic compounds in young red wines from cabernet sauvignon, cabernet franc, and cabernet gémischet varieties in China. *J Food Sci* 72:C248–C252