Nutritional and flavor properties of grape juice as affected by fermentation with lactic acid bacteria

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ABSTRACT
Two lactic acid bacteria (LAB) strains of Lactobacillus plantarum 21802 and Lactobacillus brevis 6239 were selected and combined for fermentation of grape juice. The changes of sugars, organic acids, volatiles, phenolic compounds as well as antioxidant capacity of the grape juice were characterized. Results showed that fermentation with LAB led to a slight decrease in sugar content and increased in organic acid content. The sugars were mainly consumed at the initial stage of fermentation, while the malic acid was consumed completely during the fermentation. The contents of total phenolics and flavonoids in grape juice decreased gradually during the fermentation process. As compared with the initial contents in unfermented grape juice, the contents of proanthocyanin B2, rutin, isoorientin, rhamnose-3-O-glucoside in the final fermented grape juice were increased by 26.83%, 249.24%, 138.48%, 139.60%, respectively, while the contents of gallic acid, proanthocyanin B1, catechin and epicatechin were decreased by 24.49%, 14.85%, 71.68%, 42.52%, respectively. The mice fed with the fermented grape juice showed higher activity of superoxide dismutase and lower level of malondialdehyde both in serum and liver tissue than those fed with the unfermented grape juice. Fermentation with LAB also enhanced the flavor profile by increasing the total number and content of volatile compounds, especially those of monoterpene alcohols, which are the main and characteristic aroma volatile compounds in Muscat aroma grapes. This research confirmed that fermentation with LAB might be a desirable option for improving the flavor and nutritional attributes of grape juice by altering the ratio of sugar to organic acid, improving the phenolic compounds, enhancing the antioxidant capacity, intensifying of the characteristic aroma and pleasant flavor of the grape juice.

INTRODUCTION
In recent years, fermented fruit and vegetable juices with probiotics have attracted considerable attention due to the increasing health awareness of consumers and growing number of people with health risks associated with fermented dairy foods. Fermentation with probiotics has been demonstrated to improve the flavor property and enhance the health benefits of the fruit and vegetable juices by altering the composition and contents of flavor components and bioactive compounds. Furthermore, probiotics themselves have also showed remarkable health benefits to human by competitively inhibiting growth and proliferation of pathogens in gastrointestinal tract, stimulating the immune system, and improving bioavailability of nutrients. As a result, a wide variety of fruit
and vegetable juices have been used to develop probiotic functional beverage by fermentation with various probiotic strains, among which lactic acid bacteria (LAB) is the most appreciated group.\textsuperscript{[11,14]}

Grape is one of the largest fruit crops worldwide and rich in carbohydrates, organic acids, amino acids, vitamins, melatonin and phenolic compounds.\textsuperscript{[15–17]} The grape berry and its derived products have shown potent antioxidant, anticarcinogenic, antibacterial, antidiabetic, and anti-inflammatory activities as well as cardioprotective, hepatoprotective and neuroprotective effects.\textsuperscript{[18]} Owing to this merit, grape and its derived products are promoted as a healthy food in our daily diet.\textsuperscript{[19]} Furthermore, grape and its derived products are also often used as a medium to develop functional foods.\textsuperscript{[20–23]} Previous studies showed that some probiotic lactic acid bacteria could grow and survive in grape juice, suggesting a potential for fortification and fermentation of grape juice with probiotic lactic acid bacteria.\textsuperscript{[24–27]} However, little information on the fermentation of grape juice with LAB is available up to now. Since the effects of fermentation with LAB on the chemical composition and nutritive attributes of juices are primarily dependent on the plant matrices, and the LAB strains used for fermentation are usually substrate-specific, the screening of LAB strains suitable for grape juice fermentation and evaluation of the effects of fermentation with LAB on the flavor, nutritional as well as functional properties are still essential for the development of fermented grape juice.

‘Jumeigui’ is one of the most popular table grape cultivars owing to its rich Muscat flavor and high sugar levels and cultivated widely in China.\textsuperscript{[28]} In our previous research, two Lactobacillus strains of L. plantarum 21802 and L. brevis 6239, were selected as the most suitable probiotic strains for the fermentation of ‘Jumeigui’ grape juice, which yielded a fermented grape juice with satisfactory viable counts of LAB and sensory attribute.\textsuperscript{[29]} However, the sensory evaluation in the above research were based on organoleptic investigation, and no data about the flavor and nutritional components of the grape juice, such as sugar, organic acid, phenolic compound, and volatile flavor compound, was available. In order to get an comprehensive understanding on the effects of fermentation with LAB on the flavor and nutritional properties of grape juice, the changes of sugars, organic acids, volatiles, phenolic compounds as well as antioxidant capacity of the grape juice related to fermentation with the selected Lactobacillus strains were characterized in this work.

**MATERIALS AND METHODS**

**Grape berries**

Grape berries of the variety ‘Jumeigui’ were harvested at maturity from an orchard of Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences. The fresh harvested grape berries without insect damage or mechanical injury were selected for juice making and further studies.

**Lactobacillus strains and culture preparation**

The strains of Lactobacillus plantarum 21802 and Lactobacillus brevis 6239 were obtained from China Center of Industrial Culture Collection (CICC, Beijing, China). The strains were firstly activated and propagated in a MRS broth (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) at 37°C for 48 h. Then the resulting cultures were inoculated into pasteurized grape juice and incubated at 37°C for 48 h to adapt the growth conditions of grape juice. The final cultures of L. plantarum 21802 and L. brevis 6239 were mixed with a ratio of 1:2, and the resulting culture was used as starters for fermentation of grape juice due to the fact that the combination of them could yield a fermented grape juice with satisfactory viable counts of LAB and sensory property.\textsuperscript{[28]}

**Grape juice and fermentation**

The selected grape berries were washed with distilled water, and then were pureed by using a lab-scale food processor (JYL-G12E, Joyoung, China) after the surface water was removed by flow air. After
pasteurization in boiling water for 5 min, the juice was cooled and diluted with distilled water at a ratio of 2:1. The diluted juice was then homogenized, pasteurized, cooled, inoculated with LAB culture (5% v/v) and then incubated at 36°C for fermentation.

**High performance liquid chromatography (HPLC) analysis of soluble sugars**

HPLC analysis of soluble sugars in grape juice was performed by using an e2695 HPLC system coupled with a 2414 refractive index detector (Waters Corp., Wilford, MA, USA) according to the method described in a previous research. A Waters Sugar-Pak I column (6.5 × 300 mm) was used to separate the sugars under the temperature of 80°C with EDTA-Na$_2$-Ca solution (50 mg/L) as elution solvent. Chromatograms were recorded and compared with those of sucrose, glucose, and fructose standards. Contents of sugars were calculated according to the external calibration curves prepared with sugar standards, and the results were reported as gram per liter (g/L) of grape juice.

**HPLC analysis of organic acids**

HPLC analysis of organic acids in grape juices was performed by using an e2695 HPLC system coupled with a 2489 UV/Vis detector (Waters Corp., Wilford, MA, USA) according to the method described in a previous research. A Welch Ultimate AQ-C18 column (4.6 × 250 mm, 5 μm, Welch Science &Technology Co., Ltd, Shanghai, China) was used to separate the organic acids with (NH$_4$)$_2$HPO$_4$ solution (0.02 mol/L, pH 2.4) as elution solvent. Chromatograms were recorded at 210 nm and compared with those of standards. Contents of organic acids were calculated according to the external calibration curves prepared with organic acid standards, and the results were reported as milligram per 100 milliliter (mg/100 mL) of grape juice.

**Total phenolic content**

The total phenolic content (TPC) in grape juice was determined by using the Folin-Ciocalteu phenol reagent following the method described in a previous research with slight modifications. The grape juice was filtered, and 1.0 mL of diluted grape juice was added into 2.5 mL of Folin-Ciocalteu phenol reagent and incubated in the dark at 50°C for 5 min. Then, the mixture was cooled and 2.0 mL of aqueous solution of Na$_2$CO$_3$ (75 g/L) was added and mixed thoroughly. The absorbance of the resulting reaction mixture was measured at 760 nm with a spectrophotometer (Specord 50, Analytik Jena AG, Germany) after standing for 30 min in the dark. Gallic acid was used as standard. The TPC in grape juice was calculated according to the external calibration curve prepared with gallic acid standard, and the results were reported as milligram of gallic acid equivalents per liter (mg GAE/L) of grape juice.

**Total flavonoid content**

The total flavonoid content (TFC) in grape juice was measured by using a colorimetric method as described in a previous research with minor modifications. Briefly, the grape juice was filtered, and 1.0 mL of diluted grape juice was added into 0.3 mL of aqueous solution of NaNO$_2$ (50 g/L). The mixture was incubated for 6 min, and then 0.5 mL of aqueous solution of Al(NO$_3$)$_3$ was added. The resulting mixture was allowed to stand for another 6 min, and then 3.4 mL of aqueous solution of NaOH (1.0 mol/L) was added into the reaction system. The final mixture was incubated for 15 min, and then the absorbance was determined at 510 nm with a spectrophotometer (Specord 50, Analytik Jena AG, Germany). Catechin was used as standard for calibration of the TFC, and the results were reported as milligram of catechin equivalents per liter (mg CE/L) of grape juice.
**HPLC analysis of phenolic compounds**

The grape juice was extracted with ethyl acetate (1:3 v/v) for three times, and the combined organic phases were evaporated under reduced pressure with a rotary evaporator at 37°C. Then the remaining residue was redissolved with methanol solution (80% v/v) and subjected to HPLC analysis after filtrating through a microporous membrane (0.22 µm). The HPLC system (Waters Corp., Wilford, MA, USA) was equipped with an e2695 solvent delivery pump, a 2489 UV/Vis detector, a 2998 diode-array detector, and a Symmetry C18 column (4.6 × 250 mm, 5.0 µm). The mobile phase consisted of acetonitrile (solvent A), aqueous acetic acid solution (2 g/L, solvent B), and methanol (solvent C). The gradient elution was carried out at a constant flow rate of 1.0 mL/min and programmed as follows: 0 min, 5% A, 95% B, 0% C; 11 min, 14% A, 83% B, 3% C; 13 min, 15% A, 82% B, 3% C; 18 min, 23% A, 73% B, 4% C; 21 min, 25% A, 70% B, 5% C; 28 min, 25% A, 60% B, 15% C; 35 min, 0% A, 0% B, 100% C; 40 min, 0% A, 0% B, 100% C; 50 min, 5% A, 95% B, 0% C. Phenolic compounds in grape juice were identified by comparing the chromatographic retention time and UV spectral characteristics with those of authentic standards. For quantitative measurement, external calibration curves were prepared with phenolic standards, and the results were reported as milligram per liter (mg/L) of grape juice.

**Antioxidant capacity**

The antioxidant capacities of fermented and unfermented grape juice were evaluated by using an in vivo test under approval and supervision of the Biomedical Ethical Committee of Henan University. Male BALB/c mice of 4–5 weeks old with an average weight of 20–22 g were acclimatized for 7 days at room temperature (20 ± 2°C) and received a standard pellet diet and distilled water throughout the experiment. Then they were divided into seven groups and subjected to different treatments as depicted in Table 1. The fermented or unfermented grape juice was diluted to 5 mL with normal saline for the treatments of low and middle concentration before administration. All the samples were administered daily by oral gavage for 30 days. After that, the mice were sacrificed and their livers and sera were collected. Before antioxidant assay, the liver was homogenized in normal saline and then centrifuged at 1016 × g for 10 min under a temperature of 4°C, and the serum was centrifuged directly. The resulted supernatants were used for determination of superoxide dismutase (SOD) and malondialdehyde (MDA) by using the commercial SOD assay kit (Product number A001-3-2, Nanjing Jiancheng Bioengineering Institute, China) and MDA assay kit (Product number A003-1-2, Nanjing Jiancheng Bioengineering Institute, China) according to the methods depicted in the specification sheets of the products.

**Volatile compounds**

Headspace-solid phase micro-extraction (HP-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used for analysis of the volatile compounds in grape juice according to the method described in a previous research with minor modifications. Briefly, 7 mL of grape juice together with 2 g of NaCl, 10 µL of 2-octanol (added as an internal standard), were put in a sealed headspace phial and heated at 40°C for 30 min under an agitation speed of 400 r/min for equilibration.

| Table 1. Grouping of mice and treatment for each group. | Treatment |
|--------------------------------------------------------|-----------|
| Group                                                  | Treatment |
| Low concentration of fermented grape juice (A)          | Mice fed with fermented grape juice (1 mL/100 g/d) |
| Middle concentration of fermented grape juice (B)      | Mice fed with fermented grape juice (2.5 mL/100 g/d) |
| High concentration of fermented grape juice (C)        | Mice fed with fermented grape juice (5 mL/100 g/d) |
| Low concentration of unfermented grape juice (D)       | Mice fed with unfermented grape juice (1 mL/100 g/d) |
| Middle concentration of unfermented grape juice (E)    | Mice fed with unfermented grape juice (2.5 mL/100 g/d) |
| High concentration of unfermented grape juice (F)      | Mice fed with unfermented grape juice (5 mL/100 g/d) |
| Normal control group (G)                               | Mice fed with normal saline (5 mL/100 g/d) |
The extraction was initiated when the SPME fiber of 50/30 μm DVB/CAR/PDMS (Supelco, USA) was inserted into the phial and exposed to the headspace and lasted at 40°C for 30 min. The volatile analytes were then thermally desorbed at 250°C for 8 min and subjected to analysis by using a 7890–5975 C GC-MS (Agilent, Santa Clara, California, USA) equipped with a DB-225 MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas at a flow rate of 1.0 mL/min. The temperature of the column was maintained at 40°C for 3 min, and then increased to 160°C at a rate of 3°C/min. After maintaining at 160°C for 2 min, the column temperature was then increased to 220°C at a rate of 8°C/min and held for 3 min. The mass spectrometer (MS) was operated in electron impact mode, and the electron energy and scan range were set at 70 eV and 50–550 m/z respectively. The volatile compounds were identified by comparing their mass spectral data with those in the commercial mass spectral libraries of NIST and Wiley. Semi-quantification of the identified volatile compounds was achieved on the basis of the internal standard of 2-octanol.

**RESULTS**

**Sugars**

As shown in Table 2, glucose and fructose are the main sugars in grape juice, which contribute to 99.23% of the total sugar content in grape juice. The content of sucrose is rather low, which only contributes to 0.77% of the total sugar content. During fermentation with LAB, all the sugars showed declining trends in general, but the changes were different according to the individual sugar and fermentation stage. At the initial stage (within 12 h) of fermentation with LAB, the contents of glucose and fructose as well as the total sugars in grape juice decreased significantly (p < .05), while the content of sucrose remained unaffected by the fermentation. After 12 h of fermentation, the content of sucrose decreased significantly (p < .05), but no significant changes could be observed for the contents of glucose and fructose as well as the total sugars. This indicates that the glucose and fructose were used as the main carbon sources for LAB growth and fermentation at the initial stage, but then other carbon sources took the place of them in the following fermentation process, leading to no obvious change in the total sugar content. At the end of fermentation, the contents of sucrose, glucose, fructose, and total sugars, showed a decrease of 35.19%, 1.22%, 3.97%, and 2.78%, respectively. Yang et al. also reported a significant decrease in sucrose content in a vegetable–fruit beverage after fermentation with *L. plantarum* 115, while the total sugar content showed little change. The amount of total sugars in sea buckthorn juice was not decreased by fermentation with *L. plantarum*, indicating other carbon sources might be utilized for LAB growth and fermentation. Additionally, several researches have revealed the glycosidase activities in LAB, which could hydrolyze the osidic bonds present in polysaccharids or glycosylated compounds. The releasing sugars by hydrolysis of polysaccharids or glycosylated compounds might partly compensate the consumption of sugars, leading to little decrease in total sugar content during LAB growth and fermentation.

**Organic Acids**

By using HPLC analysis, five organic acids, including tartaric acid, malic acid, quinic acid, citric acid, and shikimic acid, were identified in the unfermented grape juice, among which tartaric acid was the

| Time (h) | Sugar | 0 | 12 | 24 | 36 | 48 | 60 |
|---------|-------|---|----|----|----|----|----|
| Sucrose |       | 1.08 ± 0.02 | 1.09 ± 0.04 | 0.97 ± 0.02 | 0.87 ± 0.03 | 0.78 ± 0.01 | 0.71 ± 0.01 |
| Glucose |       | 68.67 ± 0.89 | 65.45 ± 1.14 | 65.80 ± 1.61 | 66.67 ± 2.05 | 66.70 ± 0.42 | 67.83 ± 0.24 |
| Fructose|       | 69.63 ± 0.84 | 66.15 ± 1.18 | 66.45 ± 1.64 | 67.23 ± 2.03 | 66.53 ± 0.40 | 66.96 ± 0.23 |
| Total sugars |     | 139.38 ± 1.77 | 132.69 ± 2.35 | 133.23 ± 3.28 | 134.77 ± 4.10 | 134.01 ± 0.82 | 135.50 ± 0.47 |

Note: Different letters for each sugar indicate the significant differences at p < 0.05
Table 3. Changes of organic acid content during fermentation of grape juice (mg/100 mL).

| Organic acid   | 0   | 12  | 24  | 36  | 48  | 60  |
|----------------|-----|-----|-----|-----|-----|-----|
|                |     |     |     |     |     |     |
| Tartric acid   | 230.30 ± 20.01 ± 60.37 | 346.67 ± 30.97 | 354.76 ± 1.62 | 363.13 ± 0.63 | 367.70 ± 1.81 | 368.53 ± 1.07 |
| Quinic acid    | 68.02 ± 4.90 ± 9.13 | 82.42 ± 1.21 | 83.11 ± 9.13 | 93.76 ± 2.76 | 83.58 ± 7.61 | 81.47 ± 1.83 |
| Malic acid     | 151.48 ± 17.18 ± 9.13 | 125.11 ± 21.3 | 83.05 ± 0.48 | 15.45 ± 1.46 | n.d. | n.d. |
| Shikimic acid  | 0.05 ± 0.00 ± 0.00 | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 |
| Lactic acid    | n.d. | 206.23 ± 6.46 | 235.89 ± 14.35 | 326.91 ± 61.3 | 410.53 ± 84.8 | 594.94 ± 88.24 |
| Acetic acid    | n.d. | 0.25 ± 0.01 | 0.24 ± 0.00 | 0.21 ± 0.01 | 0.24 ± 0.00 | 0.24 ± 0.01 |
| Citric acid    | 82.52 ± 75.65 | 123.25 ± 36.21 | 97.54 ± 2.06 | 107.71 ± 19.93 | 103.38 ± 1.73 | 102.95 ± 0.09 |
| Total organic  | 532.37 ± 49.44 | 843.21 ± 7.77 | 854.64 ± 12.49 | 907.23 ± 42.98 | 965.48 ± 10.48 | 1148.19 ± 2.92 |

Note: n.d. means not detectable. Different letters for each organic acid indicate the significant differences at p < 0.05.

predominant acid, accounting for 43.26% of the total content of organic acids (Table 3). Fermentation with LAB resulted in the formation of large amount of lactic acid in grape juice. As shown in Table 3, no lactic acid could be detected in the unfermented grape juice, but the content of lactic acid was increased to 206.23 mg/100 mL after fermentation for 12 h, which was only lower than that of tartaric acid. During the fermentation process, the content of lactic acid in grape juice increased continuously and exceeded that of tartaric acid after fermentation for 48 h. The content of lactic acid achieved to 594.94 mg/100 mL at the end of fermentation, accounting for 51.82% of the total content of organic acids in the final fermented grape juice. This indicates that lactic acid is the main organic acid metabolite formed in the LAB fermentation of grape juice, which is in agreement with many other researches. [39–42]

There was a remarkable increase in content of tartaric acid at the initial stage (within 12 h) of fermentation, but afterward the content of tartaric acid changed little during the following fermentation process. The contents of citric and quinic acid also showed a great increase during the initial 12 h of fermentation and then underwent slight changes till the end of fermentation. However, the content of malic acid decreased continuously during the fermentation and could not be detected after fermentation for 48 h, indicating that all the malic acid had been completely consumed and converted into lactic acid by LAB. Acetic acid was also not detected in the unfermented grape juice, but it appeared after fermentation for 12 h, and then remained at a low level till the end of fermentation. Only trace amount of shikimic acid was detected in the unfermented grape juice and little change could be observed during fermentation with LAB.

**Phenolic compounds**

As shown in Figure 1, the contents of total phenolics and flavonoids in grape juice decreased gradually during the fermentation with LAB, indicating that oxidation and degradation of phenolic compounds might occur during this process. This result is in agreement with those reported by Hashemi et al., who also observed a continuous decline of TPC in sweet lemon juice during 48 h of fermentation with Lactobacillus plantarum L5S. [43] The TPC in apple juice has also been reported to be decreased by 22.7% after fermentation with Lactobacillus plantarum ATCC14917 at 37°C for 72 h. [44] After 24 h of fermentation with different LAB strains at 37°C, the TPC and TFC in cabbage juice showed a reduction of approx 15%–24% and 15.7%–23.9%, respectively. [45]

Based on available phenolic standards, 10 phenolic compounds were identified in the unfermented and fermented grape juices by using HPLC analysis. As shown in Table 4, the initial unfermented grape juice contained gallic acid, proanthocyanin B1, proanthocyanin B2, catechin, epicatechin, rutin, isoquercitrin, and rhamnose-3-O-glucoside. Contents of all the detected phenolic compounds showed a remarkable increase during the first 12 h of fermentation, indicating an enhanced release of these phenolic compounds from grape pulp by fermentation with LAB. Afterward, the contents of gallic acid, proanthocyanin B1,
catechin, and epicatechin, decreased continuously till the end of fermentation, leading to a reduction of 24.49%, 14.85%, 71.68%, 42.52%, respectively, as compared with the initial contents in unfermented grape juice. The contents of proanthocyanin B2, rutin, isoquercitrin, and rhamnose-3-O-glucoside also showed a declining trend after fermentation for 12 h, but there was a significant increase at the end stage of fermentation (from 48 h to 60 h). As compared with the initial contents in unfermented grape juice, the contents of proanthocyanin B2, rutin, isoquercitrin, and rhamnose-3-O-glucoside in the final fermented grape juice were increased by 26.83%, 249.24%, 138.48%, 139.60%, respectively. Caffeic acid and α-arbutin were not detected in the unfermented grape juice, but appeared at the late or end stage of fermentation, suggesting that biotransformation of other phenolics into these compounds occurred at these stages.

**Antioxidant Capacity**

The SOD activity and MDA level in liver and serum of mice fed with different grape juices were used as the indicators to evaluate their antioxidant capacities. As shown in Table 5, the mice fed with normal saline (group G) showed the lowest SOD activity both in liver tissue and serum. This suggests that feeding with the fermented and unfermented grape juice both could improve the in vivo antioxidant

**Table 4.** Changes of phenolic composition and content during the fermentation of grape juice (mg/L).

| Organic acid       | 0        | 12       | 24       | 36       | 48       | 60       |
|--------------------|----------|----------|----------|----------|----------|----------|
| α-Arbutin           | n.d.     | n.d.     | n.d.     | 0.02 ± 0.03 a | 0.35 ± 0.19 a |
| Gallic acid         | 1.96 ± 0.13 a,b | 2.09 ± 0.11 a | 2.05 ± 0.06 a,b | 1.86 ± 0.04 b,c | 1.75 ± 0.05 c | 1.48 ± 0.12 d |
| Procyanidin B1      | 26.74 ± 2.30 c | 35.96 ± 1.51 a | 34.42 ± 1.38 b | 32.74 ± 1.38 b | 25.87 ± 1.6 d | 22.77 ± 2.16 d |
| Catechin            | 87.35 ± 7.71 b | 99.05 ± 3.81 a | 97.11 ± 3.53 a | 92.76 ± 3.30 ab | 74.87 ± 5.30 c | 24.74 ± 2.37 d |
| Procyanidin B2      | 20.91 ± 2.64 b | 28.78 ± 1.95 a | 27.52 ± 1.27 a | 25.48 ± 2.31 a | 18.61 ± 1.52 b | 26.52 ± 3.24 a |
| Caffeic acid        | n.d.     | n.d.     | n.d.     | n.d.     | n.d.     | 0.72 ± 0.03 a |
| Epicatechin         | 34.48 ± 3.28 b | 44.45 ± 2.66 a | 43.09 ± 2.24 a | 40.67 ± 3.02 a | 30.65 ± 1.98 b | 19.82 ± 5.06 c |
| Rutin               | 2.64 ± 0.15 b | 3.59 ± 0.60 b | 3.37 ± 0.46 b | 3.19 ± 0.75 b | 2.63 ± 0.04 b | 9.22 ± 1.83 a |
| Isoquercitrin       | 4.86 ± 0.09 c | 6.86 ± 1.09 b | 6.44 ± 0.56 b | 6.08 ± 0.81 bc | 5.03 ± 0.16 c | 11.59 ± 1.21 a |
| Isohamnetin-3-O-glucoside | 2.02 ± 0.20 b | 2.48 ± 0.68 b | 2.17 ± 0.21 b | 2.29 ± 0.22 b | 1.75 ± 0.06 b | 4.84 ± 0.87 a |

Total phenols: 180.96 ± 16.13 b 223.26 ± 12.11 a 216.17 ± 9.55 a 205.09 ± 11.15 a 161.18 ± 10.47 b 122.05 ± 16.53 c

Note: n.d. means not detectable. Different letters for each phenolic compound indicate the significant differences at p < 0.05.
level of mice. The mice fed with high concentration of fermented grape juice (group C) showed the highest SOD activity both in liver tissue and serum, and significant difference was observed in liver SOD between this group and the groups fed with unfermented grape juice at different concentrations (group D, E, F). The SOD activity in serum of mice fed with high concentration of fermented grape juice (group C) was also significantly higher than those fed with low and high concentration of unfermented grape juice (group D, F). These results indicate that the fermented grape juice exhibits a higher antioxidant capacity than the unfermented grape juice. In addition, a dose-dependent manner was observed both in liver and serum SOD for the treatments with different concentrations of fermented grape juice, while little dose-dependent effect could be found for the treatments with different concentrations of unfermented grape juice.

The MDA level is commonly known as a marker of lipid peroxidation induced by oxidative stress and the antioxidant status in tissues and cells.\(^{[46]}\) As shown in Table 5, the serum of mice fed with normal saline (group G) showed the highest level of MDA, indicating that feeding with the fermented or unfermented grape juice both could prevent the lipid peroxidation of cell membranes induced by oxidative stress and thereby alleviate the cellular damage in mice. The MDA level in the serum of mice fed with low concentration of fermented grape juice (group A) was significantly lower than those fed with the corresponding concentrations of unfermented grape juice, indicating that the antioxidant capacity of the fermented grape juice was higher than that of the unfermented grape juice. This result is also in accordance with the effects evaluated by using SOD as antioxidant indicator. The liver MDA levels of the mice fed with medium and high concentrations of fermented grape juice (group B, C) were also significantly lower than those of treatments with the unfermented grape juice (group D, E, F) and normal saline (group G). However, no significant difference could be observed between the treatments with the unfermented grape juice (group D, E, F) and normal saline (group G).

**Volatile compounds**

A total number of 70 volatile compounds were identified in the fermented and unfermented grape juices, among which alcohols were the most abundant volatile flavor components. As shown in Table 6, the total number of volatile alcohols identified in the fermented and unfermented grape juices was 26 and 19, respectively, and the total amount of them achieved to 996.95 and 492.68 μg/L, respectively, contributing to 86.66% and 70.99% of the total amount of volatile compounds in each grape juice. Other classes of volatile compounds, including eaters, alkenes, aldehydes, ketones, phenols, and acids, showed a rather low level in both fermented and unfermented grape juices. Fermentation with LAB resulted in an increase of 102.35% in total alcohol content, 83.76% in total ester content, 1681.42% in total volatile acid content, while the contents of total aldehydes, ketones, alkenes, and volatile phenols, were decreased by 45.36%, 9.93%, 79.47%, and 71.82%, respectively. As a result, the content of total volatiles in grape juice also showed an increase of 65.75% after

| Table 5. The SOD activity and MDA level in mice liver and serum of different treatments. |
|-----------------|-----------------|-----------------|---------------|---------------|---------------|
| Group | Liver SOD (U/mg prot) | Serum SOD (U/mg prot) | Liver MDA (nmol/mg prot) | Serum MDA (nmol/mg prot) |
|-------|-------------------|------------------|----------------|------------------|
| A     | 230.85 ± 22.59 \(abc\) | 189.08 ± 8.51 \(abc\) | 8.77 ± 0.78 \(ab\) | 4.87 ± 0.42 \(c\) |
| B     | 242.6 ± 30.5 \(ab\) | 193.11 ± 18.06 \(ab\) | 7.97 ± 0.65 \(b\) | 4.62 ± 1.26 \(c\) |
| C     | 271.91 ± 21.56 \(a\) | 199.57 ± 6.25 \(a\) | 7.99 ± 0.82 \(b\) | 4.44 ± 0.67 \(c\) |
| D     | 226.39 ± 37.91 \(bc\) | 177.73 ± 9.94 \(c\) | 9.57 ± 0.34 \(a\) | 5.71 ± 0.39 \(ab\) |
| E     | 227.59 ± 14.09 \(bc\) | 185.20 ± 9.15 \(abc\) | 9.55 ± 0.86 \(a\) | 5.08 ± 0.72 \(bc\) |
| F     | 227.65 ± 18.32 \(bc\) | 184.20 ± 10.04 \(bc\) | 9.36 ± 0.69 \(a\) | 5.01 ± 0.83 \(bc\) |
| G     | 207.96 ± 40.54 \(c\) | 147.69 ± 16.15 \(d\) | 9.61 ± 0.56 \(a\) | 5.93 ± 0.49 \(a\) |

Note: Group A, B, and C were the low, medium and high dose groups fed with fermented grape juice, group D, E and F were the low, medium and high dose groups fed with unfermented grape juice, and group G was the control group of normal saline. Different letters for each sugar indicate the significant differences at \(p < 0.05\).
| Volatile compound | Unfermented juice | Fermented juice | Volatile compound | Unfermented juice | Fermented juice |
|-------------------|-------------------|----------------|-------------------|-------------------|----------------|
| Alcohols          |                   |                | Alkenes           |                   |                |
| trans-2-Penten-1-ol | 1.64              | n.d.           | Myrcene           | 5.85              | 18.88          |
| trans-2-Hexene-1-ol | 27.00             | 210.55         | (+)-Dipentene     | 3.86              | n.d.           |
| n-Hexanol         | 22.74             | 98.71          | Terpinolene       | 31.98             | n.d.           |
| cis-2-Hexen-1-ol  | 15.84             | 1.80           | 5-Methyl-1-hexene | 22.90             | n.d.           |
| 2-Hexadecanol     | 3.79              | n.d.           | Octene            | 13.86             | n.d.           |
| 2-Ethylhexanol    | 24.05             | 22.64          | Cyclooctene oxide | 13.54             | n.d.           |
| Linalool          | 157.10            | 225.63         |                   |                   |                |
| cis-Verbenol      | 4.21              | 2.93           |                   |                   |                |
| Terpinen-4-ol     | 3.46              | 4.47           |                   |                   |                |
| L-Menthol         | 1.65              | 1.94           |                   |                   |                |
| alpha-Terpineol   | 65.64             | 111.85         |                   |                   |                |
| 2,2,6-Trimethyl-6-vinyltetrahydro-2 H-pyran-3-ol | 8.65 | 4.37 | 3-Methylbenzaldehyde | 4.24 | 6.43 |
| (R)-(+)-β-Citronellol | 13.32 | 20.99 | 2,3,7-Dimethyl-2,6-octadienal | 5.38 | 15.32 |
| Citronellol       | 19.25             | 33.99          | 4-Methylphenylglyoxal hydrate | 0.76 | n.d. |
| Nerol             | 21.66             | 42.32          | Citral            | 4.67              | 8.06           |
| Geraniol          | 98.87             | 127.29         | p-Toluic acid     | n.d.              | 5.37           |
| Lavandulol        | 1.27              | 5.89           | Citronellal       | n.d.              | 2.04           |
| Isopulegol        | 0.65              | 1.37           | 4-Phenylbutanal   | n.d.              | 0.95           |
| 1-Dodecanol       | 1.91              | 4.04           |                   |                   |                |
| trans-3-Hexen-1-ol | n.d.              | 20.24          | Methyl eugenol    | 0.46              | n.d.           |
| 2-Nonanol         | n.d.              | 1.67           | 2,4-Di-tert-butylphenol | 4.76 | 1.00 |
| 1-Octanol         | n.d.              | 2.11           | 2,4-Di-tert-butyl-5-methylphenol | 0.42 | n.d. |
| (-)-Terpinen-4-ol | n.d.              | 5.66           | Isoeugenol        | 0.35              | 0.68           |
| 1-Nonanol         | n.d.              | 1.78           |                   |                   |                |
| (S)-(−)-alpha-Terpineol | n.d. | 1.30 | 2-Octanone        | 18.03             | 12.40          |
| Phenylethyl Alcohol | n.d.              | 40.55          | Damascenone       | 1.51              | 2.74           |
| 6-Methyl-1-heptanol | n.d.              | 2.13           | Geranylacetone    | 0.61              | 0.63           |
| Isoamylol         | n.d.              | 0.75           | Damascenone       | n.d.              | 2.37           |
| Esters            |                   |                | Ketones           |                   |                |
| 1-Methyl-4-(1-methylvinyl) cyclohexyl acetate | 3.57 | 12.70 | 2-Octanone        | 18.03             | 12.40          |
| Linalyl formate   | 0.69              | 1.47           | 5-Aminohexanoic acid | 0.60 | n.d. |
| Isoamyl laurate   | 0.40              | n.d.           | 1-Hexanoic acid  | n.d.              | 14.18          |
| Ethyl phenylacetate | 1.61              | 3.52           | cis-8,11,14-Eicosatrienoic acid | n.d. | 0.88 |
| Tributyl phosphate | 0.86              | n.d.           | Octanoic acid    | n.d.              | 5.74           |
| 1-O-Butyl 2-Octyl benzene-1,2-dicarboxylate | 3.01 | n.d. | Nonanoic acid     | n.d.              | 1.33           |
| Linalyl butyrate  | 0.71              | 0.71           | Acetic acid       | n.d.              | 25.47          |
| Geranyl isovalerate | n.d.              | 0.21           | 2-Methyl-2-pentenoic acid | n.d. | 0.94 |
|                   |                   |                | 3-Hydroxydodecanoic acid | n.d. | 1.43 |

Note: n.d. means not detectable.
fermentation. This indicates that fermentation with selected LAB could enrich the volatile aroma components in grape juice.

Monoterpene alcohols, including linalool, cis-verbenol, terpentin-4-ol, 1-menthol, alpha-terpineol, 2,2,6-trimethyl-6-vinyltetrahydro-2 H-pyran-3-ol, (R)-(+)β-citronellol, citronellol, nerol, geraniol, lavandulol, and isopulegol, were the predominant alcohols in both fermented and unfermented grape juices. The content of total monoterpene alcohols in the unfermented grape juice was 395.73 μg/L, accounting for 80.32% of the total alcohol content in the grape juice. After fermentation with LAB, two new monoterpene alcohols, including (-)-terpentin-4-ol and (S)-(+)α-terpineol, were detected, and the content of total monoterpene alcohols achieved to 590.00 μg/L, showing an increase of 49.09% as compared with the unfermented grape juice. Owing to their very low odor threshold and high content in Muscat grapes, linalool and geraniol are generally considered as the two major flavor contributors in grape berries or juices of Muscat aroma type varieties.\textsuperscript{[15,47]}

In the present research, these two volatiles showed a rather higher level than other volatiles in the unfermented grape juice and were increased by 43.62% and 28.74% respectively after fermentation with LAB. Additionally, nerol, which was identified as the unique volatile in Muscat grapes,\textsuperscript{[48]} was also found to be increased by 95.38% after fermentation. This indicates that fermentation with LAB could intensify the characteristic aroma of grape juice of 'Jumeigui' variety, which belongs to the Muscat aroma type. The contents of α-terpineol, (R)-(+)β-citronellol, citronellol, lavandulol, isopulegol, and terpentin-4-ol, were also increased significantly after fermentation.

The level of trans-2-hexene-1-ol and n-hexanol in grape juice also showed a great increase after fermentation with LAB. As compared to those in the unfermented grape juice, the concentrations of these two compounds in fermented grape juice were increased by 6.80 and 3.34 folds respectively. Additionally, some new alcohols, including trans-3-hexen-1-ol, 2-nonanol, 1-octanol, (-)-terpentin-4-ol, 1-nonenol, (S)-(+)α-terpineol, phenyl ethyl alcohol, 6-methyl-1-heptanol, and isoamyl alcohol, were generated during fermentation. The six-carbon (C6) alcohols had been suggested as the important green leaf volatiles in grape juice and could be formed by enzymatic oxidation of polyunsaturated fatty acids or reduction of aldehydes\textsuperscript{[49,50]} The present results indicate that fermentation with LAB could enhance these conversions.

A total number of 6 volatile esters were identified in the unfermented grape juice, among which 1-methyl-4-(1-methylvinyl)cyclohexyl acetate, 1-O-butyl 2-O-octyl benzene-1,2-dicarboxylate, and ethyl phenylacetate, were the dominant compounds. After fermentation with LAB, the concentrations of 1-methyl-4-(1-methylvinyl)cyclohexyl acetate and ethyl phenylacetate were increased by 2.56 and 1.19 times respectively, while 1-O-butyl 2-O-octyl benzene-1,2-dicarboxylate as well as two other esters, namely isomyl laurate and tributyl phosphate, were decreased to the undetectable level. However, two new esters, namely linalyl butyrate and geranyl isovalerate, were formed with trace amount during fermentation with LAB.

There were 6 alkenens, including myrcene, (S)-(+)-dipentene, terpinolene, 5-methyl-1-hexene, ocimene, and cyclooctene oxide, in the unfermented grape juice with a total amount of 91.98 μg/L. However, only myrcene was detected in the fermented grape juice with a concentration of 18.88 μg/L, which was 2.23 times higher than that of the unfermented grape juice.

Phenylacetaldehyde and 2-hexenal were the predominant aldehydes in the unfermented grape juice, accounting for 73.63% of the total amount of aldehydes. After fermentation, they were decreased to the undetectable level. Additionally, some other aldehydes, including decanal, 2-methylbenzaldehyde, and 4-methylphenylglyoxal hydrate, were also undetectable in the fermented grape juice. However, the concentrations of 3-methylbenzaldehyde, citral, and (E)-3,7-dimethyl-2,6-octadienal, showed an increase of 51.65%, 72.59%, and 184.76% respectively. Three new aldehydes, namely p-toluylaldehyde, citronellal, and 4-phenylbutanal, were formed during fermentation with LAB.

There were 4 ketones in the unfermented grape juice, among which 2-octanone was the predominant compound. Fermentation with LAB caused a decrease of 31.23% in concentration of 2-octanone, while the concentration of damascenone was increased by 81.46% after fermentation. The content of
geranylacetone was rather low and showed no difference between the fermented and unfermented grape juice. However, a new ketone, namely damascenone, was detected in the fermented grape juice.

A total number of 4 volatile phenols were detected in the unfermented grape juice, while in the fermented grape juice, only 2 volatile phenols were detected. As shown in Tables 6, 2, 4-di-tert-butylphenol was the predominant volatile phenol in the unfermented grape juice with a concentration of 4.76 µg/L. After fermentation, it was decreased to 1.00 µg/L. Methyl eugenol and 2,4-di-tert-butyl-5-methylphenol, which existed in the unfermented grape juice with trace amount, were not detected in the fermented grape juice.

In the unfermented grape juice, only geranic acid and 5-aminohexanoic acid were detected with low concentrations. After fermentation, the concentration of geranic acid showed an increase of 2.00 folds, while the 5-aminohexanoic acid was undetectable in the fermented grape juice. However, several new volatile acids, including 1-hexanoic acid, cis-8,11,14-eicosatrienoic acid, octanoic acid, nonanoic acid, acetic acid, 2-methyl-2-pentenoic acid, and 3-hydroxydodecanoic acid, were detected in the fermented grape juice.

Discussion

Sugars and organic acids are the main nutrients and taste components in grape juice, contributing to the main soluble solid content and sensory attributes of grape juice. They also can be used as carbon source and provide energy for LAB growth and fermentation, but there exists great difference according to different strains and fruit juices. Markkinen et al. noticed that fermentation with Lactobacillus plantarum significantly decreased the concentrations of total sugars and fructose in the chokeberry juice with a pH of 3.36, while no significant decrease of total sugars could be observed in the sea buckthorn juice with a pH of 2.86.\textsuperscript{[34]} The Lactobacillus strains used in their experiments seemed to prefer organic acids as carbon source rather than sugars in the sea buckthorn juice, likely due to the low pH of the sea buckthorn juice. Filannino et al. suggested an adaption mechanism in metabolism of LAB in response to the hostile environment of fruit juices.\textsuperscript{[41]} They found that the malolactic specific activity in LAB cells harvested from fermented cherry juice and pineapple juice with higher initial acidity was 10 times higher than those from carrot juice and tomato juice with lower acidity. Accordingly, the concentrations of glucose and fructose of cherry juice and pineapple juice showed no significant change during LAB fermentation, while the contents of glucose and fructose of carrot juice and tomato juice significantly decreased during LAB fermentation. In the present research, the contents of total sugar, glucose, and fructose, decreased significantly at the initial stage of fermentation, indicating that they were used as carbon source for LAB growth and production of acids. But after that period, the LAB used in this experiment shifted to prefer organic acids as carbon source rather than sugars due to the improving acidity of the grape juice, leading to no obvious change in sugar content till the end of fermentation. Other research also indicated the use of organic acids instead of sugars as carbon source by LAB due to the hostile environment of fruit juices.\textsuperscript{[59]}

The malolactic fermentation is a common metabolic process for LAB, which can afford the conversion of malic acid into lactic acid to lower the acidity of growth matrices and provide energy for bacterial growth.\textsuperscript{[51]} In the present research, the content of lactic acid in grape juice increased continuously during fermentation, while the content of malic acid decreased promptly, indicating that strong malolactic fermentation occurred in this process. Similar results were also reported in many other fruit juice fermentation with LAB.\textsuperscript{[34,39,52]} Interestingly, the content of lactic acid still increased substantially after fermentation for 48 h, in which period, there was no malic acid that could be used in the grape juice, and the total lactic acid content in the final fermented grape juice was much higher than that could be achieved by complete conversion of all the malic acid in the grape juice. This suggests that other carbon sources might participate in the formation of lactic acid. As a matter of fact, sugars are also the commonly used carbon source for LAB growth and lactic acid fermentation, and citric acid, tartaric acid, quinic acid as well as some amino acids can also be served as carbon sources for some LAB strains.\textsuperscript{[51,53]}
Phenolic compounds are the essential bioactive components in grape juice and believed to be associated with the multiple health benefits of grape juice. Some researchers have reported that fermentation with LAB could enhance the release of soluble conjugated or insoluble bounded phenolic compounds from plant cell wall, and hence the phenolic content in the fermented fruit juices was increased. However, the phenolics are generally regarded as unstable compounds in fruit juices that are ready to be oxidized and degraded during fermentation, resulting in a decline in contents of these compounds. Furthermore, the LAB has been reported to possess several metabolic pathways to metabolize the plant phenolics, which might cause the degradation and biotransformation of specific phenolics. In the present research, the TPC and TFC in grape juice showed a declining trend during fermentation with LAB, suggesting that the oxidation and degradation of phenolics might occur in this process. Further analysis of individual phenolic compounds by HPLC revealed that most of the detected phenolic compounds had a notable improvement in concentration at the initial stage of fermentation, indicating an enhancement of release of soluble conjugated or insoluble bounded phenolic compounds from grape tissue. The decline in contents of these phenolic compounds after the initial 12 h of fermentation might be ascribed to the more oxidation and degradation of phenolic compounds than enhancement of release of the soluble conjugated or insoluble bounded phenolic compounds from grape tissue. Interestingly, the contents of proanthocyanin B2, rutin, isouercitrin, and rhamnose-3-O-glucoside in the fermented grape juice showed a significant increase at the end of fermentation, and two new phenolic compounds, identified as caffeic acid and α-arbutin, were also generated at that stage. This indicates that biotransformation of other phenolics into these compounds might occur during fermentation with LAB. The metabolism and biotransformation of phenolic compounds by LAB have been documented by several researches. During fermentation of apple juice, the quercetin-3-O-galactoside, quercetin-3-O-glucoside, and phlorizin were greatly metabolized by Lactobacillus plantarum ATCC14917, resulting in the increase in concentrations of 5-O-caffeoylquinic acid and quercetin. The condensed tannin increased during fermentation of cashew apple juice with different Lactobacillus strains, while the hydrolysable tannins decreased. The epicatechin and catechin in pomegranate juice could be almost completely metabolized by several LAB strains, and a new catechin derivative was identified after fermentation. The protocatechuic, caffeic, and p-coumaric acids in cherry juice could be metabolized by LAB to catechol, vinyl catechol, p-vinylphenol, respectively. The accumulation of dihydrocaffeic acid in fermented cherry juice was also suggested as the result of metabolism of caffeic acid by LAB. Fermentation of pomegranate juice with Lactobacillus plantarum resulted in an enhanced release of ellagic acid. However, the effect of fermentation with LAB on the phenolic compounds seems to be diverse, depending on the strains, plant matrices, as well as fermentation conditions and times. Little information about the metabolism of phenolic compounds in grape juice during fermentation with LAB is available up to now. Further research should be done to elucidate the mechanism involved in the metabolism of phenolic compounds in grape juice during fermentation with different LAB strains.

Volatile compounds play an essential role in the flavor attributes of fruit juice. Many studies have documented the enhancement of flavor profile by comparing the volatile compounds of fermented and unfermented fruit juices, in spite of the diverse results related to different fruit matrices, LAB strains, as well as the individual volatile compounds themselves and fermentation conditions. The present research also revealed an increase of the abundance of volatile compounds in grape juice by fermentation with LAB, which is in agreement with the previous studies. The grape juice contains large amount of proteins and amino acids, which could be degraded and converted into aldehydes, alcohols, and acids by a variety of cytoplasmic enzymes (such as aminotransferase, decarboxylase, dehydrogenase, hydrogenase, dehydrogenase, etc.) from LAB. The increased availability of the alcohol precursors in fermented grape juice might be responsible for the increase in the level of esters in juices after fermentation with LAB. However, the level of aldehydes in the fermented grape juice showed a great decrease as compared with the unfermented grape juice. This could be explained by the unstable nature of this class of compounds in food matrices that could be readily reduced to alcohols or oxidized to acids, in particular under microbial activity. Many other researches also reported the decrease of aldehydes and increase of alcohols and acids in juices after fermentation with LAB. High concentration of
aldehydes have been related to off-flavors and poor consumer acceptability for beverages,\textsuperscript{70,71} and decrease of the level of aldehydes and increase of the levels of alcohols as well as esters by fermentation with LAB were regarded as an desirable option for improving the flavor attributes of juices.\textsuperscript{4}

The monoterpene alcohols may also be classified as terpenes together with myrcene, (+)-dipentene, terpinolene, and ocimene, which are listed as alkenes in Table 6. These compounds had been recognized as the primary aromatic compounds responsible for the characteristic ‘floral’ and ‘fruit-like’ aroma of Muscat grapes and their processing products.\textsuperscript{15,47,72} However, the terpenes are more abundantly present as glycosidically conjugated forms in grapes and musts, which could be hydrolyzed by β-glycosidases or acids, leading to the release of the free volatile aroma compounds.\textsuperscript{47,72,73} Several researches have reported the glycosidase activity of LAB and their effects on release of volatile terpenes from grape tissues.\textsuperscript{74,75} In the present research, the increase in levels of monoterpene alcohols and myrcene after fermentation with LAB might be the results of the hydrolysis of their conjugated precursors in grape juice by β-glycosidases from LAB. In a similar research performed with apple juice, fermentation with different LAB strains all resulted in the increase in concentrations of isoprene, myrcene, D-limonene, ocimene, linalool, eugenol, α-terpineol, and geraniol.\textsuperscript{68} Fermentation of tomato juice with \textit{Lactobacillus plantarum} LP54 also led to the notable increase in levels of beta-pinene, beta-myrcene, p-mentha-1,5,8-triene, limonene, alpha-terpinene, cis-ocimene, alpha-terpinolene, and beta-ionone.\textsuperscript{69} The increase in level of terpenes might enhance the ‘floral’ and ‘fruity’ aroma and pleasant flavor of the grape juice, making it more acceptable for the consumers.

\textbf{Conclusion}

Fermentation with LAB caused a slight decrease in sugar content and great increase of organic acid content. Lactic acid was the main metabolite formed during the fermentation, while malic acid was consumed completely during the fermentation. The TPC and TFC declined gradually during the fermentation process, while the individual phenolic compounds showed diverse trends during fermentation. Fermentation with LAB also improve the antioxidant capacity of grape juice and enhance the flavor profile by increasing the total number and content of volatile compounds, especially those of monoterpene alcohols, which are the main and characteristic aroma volatile compounds in Muscat aroma grapes. This research confirmed that fermentation with LAB might be a desirable approach for improving the flavor and nutritional properties of grape juice by altering the ratio of sugar to organic acid, improving the phenolic compounds, enhancing the antioxidant capacity, intensifying of the characteristic aroma and pleasant flavor of the grape juice.

\textbf{Disclosure statement}

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