Effects of co-fermentation and sequential malolactic fermentation on antioxidant activities and phenolic components of red-fleshed apple cider

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Abstract. Red-fleshed apple (Malus niedzwetzkyana dieck) was used as the fermentation material, and the fermentation mode of red-fleshed apple cider was studied by using alcohol fermentation as control (CK), simultaneous malolactic fermentation (SIM) and sequential MLF (SEQ). The main chemical parameters, antioxidant activities and sensory quality were analyzed to evaluate the quality of red-fleshed apple cider. Compared with the cider without lactic acid bacteria, the total phenolics contents (TPC) and total anthocyanins contents (TAC) in MLF apple cider were significantly decreased, while the total flavonoids content (TFC) were significantly increased. Among them, the TPC and TAC in apple cider obtained by SIM were the highest, while the TFC in apple cider obtained by SEQ. In addition, compared with the control group, the pH of cider obtained by SEQ and SIM was significantly increased by about 0.25 units, while the total acidity was significantly decreased. Therefore the taste of cider obtained from SIM and SEQ were significantly improved, and the cider obtained by SIM got the highest score by sensory analysis, which scored the highest for floral and fruitiness notes. These results indicated that SIM was an effective way to produce red-fleshed apple cider.

1. Introduction

Red fleshed apple (Malus niedzwetzkyana Dieck) is the fruit of Malus in Rosaceae. The existing red-fleshed apple resources can be divided into two categories, one is the red-fleshed variety of cultivated apple (M. domestica var. niedzwetzkyana), the other is the red-fleshed variety of sevice apple (M. sieversii f. niedzwetzkyana) [1]. Red-fleshed apple variety (M. domestica var. niedzwetzkyana) is red in peel and flesh, which contains a large number of anthocyanins and other flavonoids and has been proved that M. domestica var. niedzwetzkyana has strong antioxidant and anticancer cell proliferation ability in vitro [2-5]. Therefore, it is favored by consumers.

Cider is a kind of fruit wine produced by fermentation of fresh apple juice or concentrated apple juice under the action of microorganisms. In addition, apple cider is the second largest fruit wine in the world, which has rich nutritional components and great health value. Apple cider brewing usually includes two kinds of biological fermentation: alcoholic fermentation (AF) and malolactic acid fermentation (MLF), which are driven by yeast and lactic acid bacteria (LAB) respectively. The quality of cider mainly depends on the apple raw materials and fermentation technology. As the saying goes “seven parts of raw materials and three parts of technology”, the quality of raw materials directly affects the quality of
the final cider. At present, there are no processing varieties specialized in brewing cider in China, mainly using edible varieties such as ‘Fuji’, ‘Guoguang’ and ‘Xinhongxing’. Red-fleshed apple is rich in phenolics, which can increase the structure of cider. Therefore, cider brewed with red-fleshed apple has good taste and nutritional value. However, there are few reports on the red-fleshed apple cider at home and abroad.

The proper amount of organic acids in cider can bring refreshing and fresh feeling to cider. Once the content is too high, it will give people a rough and stiff feeling, which will seriously affect the sensory quality of cider. Generally speaking, the malic acid in red-fleshed apple is higher, which will greatly reduce the taste of cider. Therefore, it is very important to control the concentration of organic acid in cider reasonably. MLF is usually used for deacidification of wine, enhancing microbial stability and producing ideal flavor compounds [6]. MLF is a kind of secondary biological fermentation by lactic acid bacteria. The common lactic acid bacteria used for malolactic acid fermentation mainly include O. oeni, Lactobacillus, Leuconostoc mesenteroides and Pediococcus [7]. However, Oenococcus oeni (O. oeni) is the most suitable LAB for malolactic fermentation because of its strong tolerance to harsh wine fermentation environment (low pH, high alcohol and SO₂) and low levels of biogenic amine metabolites [8]. During MLF, O. oeni can convert L-malic acid (a sour acid) into L-lactic acid (a softer acid) and CO₂ under the malate lactase, so as to improve the quality of wine.

There are two main types of phenolic compounds in cider, one is neutral phenol (flavonoids), the other is acid phenol (phenolic acid). These polyphenols come from apples, which have great changes in the apple processing (such as crushing and fermentation) [9, 10]. On the one hand, they inhibit the growth of microorganisms, on the other hand, they participate in the fermentation process, and are used by microorganisms to increase the aroma of cider. Phenolic compounds are secondary metabolites of apple, which directly affect the color, the balance between bitterness and astringency, and determine the overall quality of cider [11]. Phenolic compounds in cider were closely related to the antioxidant activities, antiproliferative and prevention of cardiovascular and cerebrovascular diseases [12, 13], which directly reflect the nutritional quality of cider. Lea (1990) also proposed that polyphenols in cider can bind with protein to enhance the stability of colloid [14]. MLF and LAB could affect the concentration of phenolic compounds in wine, and the phenolic compounds also affected the growth of LAB and the fermentation process of MLF [15-17].

Traditionally, MLF was carried out sequentially after the end of AF, or malolactic fermentation bacteria initiate malolactic fermentation by spontaneous or inoculation after the end of AF [18]. However, in recent years, many researchers had completed the fermentation of wine, cherry wine and lychee wine through simultaneous inoculation of Saccharomyces cerevisiae and LAB [19-21]. The co-fermentation greatly shortened the fermentation time and improved the production efficiency. The objective of this study was to compare the chemical compositions, antioxidant activities, phenolic components and sensory quality of red-fleshed apple cider obtained from alcohol fermentation, sequential fermentation and simultaneous malolactic fermentation, so as to provide the best fermentation mode for red-fleshed apple cider.

2. Material and methods

2.1. Material

Apple material: The F2 hybrid population of Xinjiang red-fleshed apple used in this study grew in Guanxian fruit tree breeding base of Shandong Agricultural University (N 36° 29′ E 115° 27′). The same agricultural management mode was adopted and harvested at commercial maturity stage.

Saccharomyces cerevisiae: Excellence XR was purchased from Lamothe Abiet Company (France) in the form of active dry powder. The activation method was carried out according to the product instructions provided by the manufacturer.

O. oeni PG-16: An autochthonous strain O. oeni which was isolated from red-fleshed apple cider after alcoholic fermentation and stored in 30% glycerol at -20 °C until use. O. oeni PG-16 was inoculated into MRS (pH 4.8) broth medium supplemented with 2% tomato juice and 0.05g/L nystatin (Acofarma;
Terrassa, Spain) at 28°C for about 72 h, centrifuged at 10000 rpm for 10 min, and then resuspended after cleaning twice with 0.9% sterile NaCl.

2.2. Chemicals and reagents
Methanol, acetonitrile, rutin, catechin, epicatechin, gallic acid, procyanidin B2, guava glucoside, Hyperoside, Isoquercitrin, quercetin, chlorogenic acid, cinnamic acid, coumarin, ferulic acid, vanillin, syringic acid and vanillic acid were purchased from sigma Aldrich Company (Shanghai, China). All reference materials of phenolic were dissolved in chromatographic methanol and stored at -80°C. The other reagents were all domestic analytical grade.

2.3. Preparation of apple cider
Red-fleshed apple juice without mildew was prepared by wall breaking machine (MJ-BL1214A, Midea, China) after being cleaned with tap water, drained, and seeded manually. Additionally, the SO₂ (50 mg/L) was added in apple juice, and then it was evenly distributed into a 2 L glass bottle for three fermentation treatments, namely control group (CK, inoculated with yeast only), co-fermentation MLF group (SIM; inoculated with O. oeni after 24 hours of yeast inoculation) and sequential MLF group (SEQ, inoculated with O. oeni on the 8th day after yeast inoculation and AF completion) [22]. Saccharomyces cerevisiae and O. oeni were inoculated at the concentration of 10⁷ CFU/mL, and the fermentation temperature was 18 ~ 20°C. After the beginning of alcohol fermentation (AF), sucrose (food grade) was added to adjust the final cider alcohol content to 11%. After fermentation, samples were collected and stored at -20°C until analysis. Three independent biological repeats were set for each treatment.

2.4. Extraction of phenolic compounds
The extraction of phenolic compounds from cider was carried out according to the method described in Li et al. [23] with slight modification. Briefly, 20 mL apple cider was mixed with 20 mL ethyl acetate, and incubated in a shaker for 30 min at room temperature. The organic phase was collected and repeated three times to extract acid phenol. After that, the pH of the remaining cider sample were adjusted to 7.0 with 1mol/L NaOH, then mixed with 20mL ethyl acetate and incubated in shaking table for 30min at room temperature. The organic phase was collected and repeated three times to extract neutral phenol. Finally, all the collected organic phases were combined and evaporated to dryness at 37°C, and dissolved in 4mL chromatographic methanol.

2.5. Chemical analysis
Residual sugar, alcohol, pH, total acid and volatile acid were determined according to standardized methods [24, 25]. Malic acid was determined by HPLC according to Lee et al. [26].

2.6. Determination of phenolics
The total phenol content (TPC) of cider were determined by Folin-Ciocalteu method and with slight modification [27]. Briefly, 1mL cider sample was fully mixed with 1mL Folin-Ciocalteu reagent. After incubation at room temperature for 1 min, 3 mL 7% NaCO₃ solution and 5 mL ddH₂O were added successively. The reaction was conducted in dark at room temperature for 30min and determined at 765 nm.

The determination of total flavonoid content (TFC) in cider was carried out according to the method described in Wang et al. [28], with slight modification. Briefly, 1 mL cider sample was fully mixed with 1 mL Folin-Ciocalteu reagent. After incubation at room temperature for 1 min, 3 mL 7% NaNO₂, 1 mL 10% Al(NO₃)₃, and 4 mL 2 M NaOH were added into 0.5 mL cider sample in turn. The reaction was conducted in dark at room temperature for 15 min and determined at 510 nm. The results were expressed as rutin equivalent (RE)/L.

The determination of anthocyanin content (TAC) in cider was carried out according to the pH differential method described in Wrolstad [29], with slight modification. Briefly, 1 mL cider sample was mixed with 4 mL KCl buffer (pH = 1.0) or 4 mL NaAc buffer (pH = 4.5), and incubated in dark at 4°C for 15min. At each pH, the absorbance difference (OD₅₁₀-OD₇₀₀) of the reaction solution was determined.
by UV-vis spectrophotometer at 510nm and 700nm, respectively. TAC was calculated by the following formula: 
\[
TAC = \frac{(a \times MW \times DF \times V \times 1000)}{(\varepsilon \times M)}
\]
where the \( a = (A_{510} - A_{700}) \) pH 1.0 - (A_{510} - A_{700}) pH 4.5; MW is the relative molecular weight of Cy-3-G (449g / mol), DF is the dilution multiple; V is the extraction volume; \( \varepsilon \) is the molar extinction coefficient of Cy-3-G (26900); M is the extraction quality. TAC value is expressed as Mg Cy-3-G equivalent/L or Mg C3GE/L.

2.7. Determination of individual phenolic

The flavonoids and phenolic acids were determined according to the methods described by Li et al. and Yin et al. respectively [30, 31]. The flavonoids were analyzed by UPLC-MS/MS and acquity UPLC system (Waters) equipped with Acquity BEH C18 (Waters, 100 mm×2.1 mm, 1.7 μm) column. And the phenolic acid were analyzed by HPLC equipped with Acclaim 120 C18 (150 mm×3 mm, 3 μm, Dionex, USA). The qualitative and quantitative analysis of individual phenolic was based on the corresponding external standard.

2.8. Measurement of antioxidant activities

2.8.1. DPPH assay. The DPPH free radical scaling activity of apple cider were determined according to the method described by Blois [32], with slight modification. Briefly, 20 μL cider and 2 mL 6.5 × 10^{-5} M DPPH (dissolved in methanol) were mixed in a 10 mL centrifuge tube, and the absorbance value (OD_{517}) of the reaction solution were determined at 517 nm after incubating in dark for 30 min at room temperature. The DPPH values were expressed as trolox equivalent (TE)/L.

2.8.2. ABTS assay. The ABTS free radical scavenging capacity of cider were determined by ABTS total antioxidant capacity test kit according to the instructions provided by the manufacturer. The ABTS values of cider were expressed as TE/L.

2.8.3. FRAP assay. The ferric reducing antioxidant power (FRAP) of the apple cider were determined by the previously method described by Benzie and Strain [33], with slight modification. Briefly, 30 mM acetic acid buffer (pH = 3.6), 20 mM ferric chloride and 10 mm TPTZ (dissolved in 40 mM HCl) were mixed (10:1:1, v/v/v) and incubated in water bath at 37 °C for 5 min to prepare FRAP working solution. Then 10μL apple cider was mixed with 1mL ddH₂O and 1.8mL FRAP working solution, and the absorbance value (OD_{593}) of the reaction solution were determined at 593 nm after incubating in dark at 37 °C for 10 min. The results were expressed as TE/L.

2.9. Sensory analysis

Sensory evaluation was performed on the wine samples following the method proposed by Sun et al. [34]. The quantitative descriptive analysis (QDA) was carried out by a panel consisted of 11 tasters (6 females and 5 males, ranging in ages from 24 to 45). During the sensory experiment, Thirty millilitres of each sample was served at 18°C in duplicates in standard wine-testing glasses (ISO 3591, 1997) covered with a piece of glass sheet prior to analysis, Panelists were requested to describe the wine aroma with the seven odorant classes (floral, fruity, sweet, green/acid, solvent, fatty and global aroma) and to assess the intensity using a five point scale, where 1=very weak, 2=weak, 3=medium, 4=intensive and 5=very intensive.

2.10. Statistical analysis

SPSS16.0 statistical package for Windows (SPSS; Chicago, Illinois) was used for statistical analysis. The significant difference (P< 0.05) between cider samples was analyzed by ANOVA and Duncan test. Data were presented as mean ± standard errors (SD) from three independent experiments.
3. Results and discussion

3.1. Treatment effect on main chemical parameters

Cider fermentation mainly includes two types, one is alcohol fermentation, and the other is malolactic fermentation. Alcohol fermentation is a process in which sugar is metabolized into alcohol and CO₂ is released under the yeast. Malic acid fermentation is a process in which malic acid is converted into lactic acid by lactic acid bacteria. The main chemical parameters of different cider are shown in Table 1. There was no significant difference in alcohol content of cider obtained from different fermentation modes, which was consistent with the research results of Benito et al. [35], who also reported that there was no significant difference in alcohol content of wine obtained by AF and MLF. The total residual sugar in all red-fleshed apple cider was less than 4g/L, and the total residual sugar in cider obtained by SIM was the highest, which was 3.25g/L. There was no significant difference between the control group and SEQ. The reason for this result may be due to the interaction between Saccharomyces cerevisiae and LAB during the fermentation. The co-inoculation of yeast and Oenococcus oeni affected the balance of organic acids in cider. Compared with the control group, the pH of cider obtained by MLF increased significantly from the initial 3.44 to 3.69 in SEQ or 3.68 in SIM. This is consistent with the results of previous studies reported by Taniasuri et al. who reported that pH can be increased by 0.1-0.3 units after MLF [36]. In addition, MLF significantly reduced the total acid content in cider, but the inoculation time did not cause significant difference among MLF samples. Previous studies had shown that O. oeni can generate acetic acid (the main volatile acid) through sugar metabolism or citric acid metabolism, which can be used as a substrate to synthesize acetate (with fruit flavor characteristics) through acetyl coenzyme A [37, 38]. Our results showed that MLF increased the volatile acids in cider, but there was no significant difference between SEQ and SIM. Among them, the volatile acids in cider obtained from SIM was the highest (1.03 g/L). Malic acid (a sharp acid) is the main organic acid component in apple cider. It can be converted into lactic acid (a soft acid) by Oenococcus oeni, which plays an important role in improving the quality of cider [7].

| Parameters                  | Control       | SEQ           | SIM           |
|-----------------------------|---------------|---------------|---------------|
| Alcohol (%vol)              | 11.12 ± 0.01a | 11.32 ± 0.02a | 11.08 ± 0.04a |
| Total reducing sugar (g/L)  | 1.31±0.05b    | 1.05±0.03b    | 3.25±0.09a    |
| pH                          | 3.44 ± 0.01b  | 3.69 ± 0.01a  | 3.68 ± 0.01a  |
| Titratable acidity (g/L)    | 6.31± 0.22a   | 5.03± 0.32b   | 5.11± 0.27b   |
| Volatile acidity (g/L)      | 0.41 ± 0.01b  | 0.79 ± 0.04ab | 1.03 ± 0.02a  |
| Malic acid (g/L)            | 5.81 ± 0.34 a | 0.55 ± 0.27b  | 0.65 ± 0.19b  |

Note: Different letters in the same row indicate significant differences (P<0.05)

3.2. Treatment effect on total phenolic, flavonoids and anthocyanins

TPC, TFC and TAC in cider obtained from different fermentation are shown in Table 2. The highest TPC was found in control (600.24 mg GAE/L), followed by SIM (510.13 mg GAE/L), then SEQ (471.14 mg GAE/L). There were significant differences in the TPC among the three fermentation modes (P<0.05). The highest TFC was observed in SEQ (292.75 mg RE/L), followed by SIM (262.06 mg RE/L), and then control (200.13 mg RE/L). The ranking of TAC in cider was control (72.04mg C3GE/L) > SIM (63.74 mg C3GE/L) > SEQ (60.51 mg C3GE/L). The phenolic compounds in cider mainly come from apple materials, which can inhibit the growth of yeast and lactic acid bacteria, and in turn can be metabolized and utilized by yeast and lactic acid bacteria. Microbial species and inoculation methods have an important impact on the phenolic compounds in cider. Compared with the control group, the TPC and TAC in apple cider after MLF were significantly decreased, but the TFC were significantly increased. In addition, the TPC in cider obtained by SEQ was significantly lower than that of SIM, but
the TFC was on the contrary, and the TAC had no significant difference ($P<0.05$), which was consistent with the results of Hernández et al. [16] and Versari et al. [39]. The former reported that MLF caused the decrease of TPC in cider, and the latter reported that SIM caused a greater reduction of TAC in cider than that in SEQ, which may be that the biodegradation of pyruvic acid and glyoxylic acid had an effect on the formation of polymeric pigment. Besides, anthocyanins are unstable, which are affected by SO$_2$, temperature and pH. With the increase of pH, MLF will lead to the degradation of anthocyanins.

### Table 2. Total phenolic, flavonoids, anthocyanins of different apple ciders

| Apple ciders | Phenolics (mgGAE/L) | Flavonoids (mgRE/L) | Anthocyanins (mgC3GE/L) |
|--------------|---------------------|---------------------|------------------------|
| Control      | 600.24±23.11a       | 200.13±5.12c        | 72.04±6.02a            |
| SEQ          | 471.14±10.40c       | 292.75±6.01a        | 60.51±7.43b            |
| SIM          | 510.13±12.02b       | 262.06±12.52b       | 63.74±3.08b            |

Note: Different letters in the same column indicate significant differences ($P<0.05$)

3.3. Treatment effect on antioxidant activities

Microorganisms and inoculation time had an important impact on the antioxidant activities of apple cider. The antioxidant activities of different ciders are shown in Table 3. The antioxidant capacities of different ciders were evaluated by DPPH ranging from 2758.61 to 3188.96 um TE/L, by ABTS from 5108.54 to 6442.34 um TE/L, and by FRAP from 3405.69 to 3936.99 um TE/L. Among them, compared with the control group, the antioxidant abilities of cider after MLF were significantly increased ($P<0.05$), and the antioxidant activities of cider obtained by SIM was significantly higher than that of SEQ. The difference in the TPC, TFC, TAC and phenolic compounds in different cider may be the main reason for the difference in antioxidant activities, which was similar to the results previously reported [40].

### Table 3. Antioxidant activities (DPPH, ABTS and FRAP) of different apple ciders

| Apple cider | DPPH uM TE/L  | ABTS uM TE/L | FRAP uM TE/L |
|-------------|---------------|--------------|--------------|
| Control     | 2758.61±104.85c | 5108.54±122.11c | 3405.69±55.47c |
| SEQ         | 3028.75±92.88b   | 5867.12±88.44b   | 3660.12±96.84b  |
| SIM         | 3188.96±127.14a  | 6442.34±73.38a   | 3936.99±56.11a  |

Note: Different letters in the same column indicate significant differences ($P<0.05$)

3.4. Treatment effect on phenolic compounds

In this study, a total of 16 main individual phenolics were analyzed qualitatively and quantitatively (Table 4), including 9 flavonoids (procyanidin B2; epicatechin; rutin; hyperin; Isoquercitrin; guava glucoside; phloridin; quercetin; kaempferol) and 7 phenolic acids (chlorogenic acid; cinnamic acid; coumarin; ferulic acid; syringic acid; vanillic acid; gallic Acid).

Our results showed that procyanidin B2, ferulic acid and cinnamic acid in cider after MLF were significantly lower than those in the control. The procyanidin B2 in cider after MLF was significantly reduced, which was mainly because procyanidin B2 existed in the form of oligomer after MLF [41]. As ferulic acid is the precursor of aromatization, it can be degraded by _O. oeni_ to produce vanillin [42], which results in the decrease of ferulic acid in cider after MLF. Cinnamic acid, as a precursor of hydroxycinnamic acid derivatives, which was metabolized and utilized by microorganisms during cider fermentation, resulting in the decrease of it. Our results showed that the quercetin in cider significantly decreased after MLF, and the quercetin in cider obtained by SIM was significantly lower than that in SEQ, which was consistent with the results of Rodríguez et al. [43] and Versari et al. [39]. In addition, _O. oeni_ can be inhibited by phenolics such as flavanol, flavonol, chlorogenic acid and ferulic acid in cider, and can also improve the cider aroma by degrading phenolics [42, 44, 45]. Different microorganisms and inoculation time caused the difference of phenolic compounds in cider during
fermentation, which may be due to the different metabolic pathways of lactic acid bacteria under different inoculation modes (co-fermentation mainly involved in sugar heterologous metabolism and organic acid metabolism; sequential fermentation mainly carried out organic acid metabolism) or adaptability with \textit{Saccharomyces cerevisiae}.

### Table 4. Analysis of the major phenolics of apple ciders

| Phenolic components       | Control       | SEQ           | SIM           |
|--------------------------|---------------|---------------|---------------|
| Procyanidin B2           | 3.51±0.02a    | 1.48±0.01b    | 1.04±0.04c    |
| Epicatechin              | 0.72±0.08b    | 1.16±0.06a    | 1.12±0.13a    |
| Rutin                    | 20.18±1.25c   | 23.40±1.03a   | 21.30±0.39b   |
| Quercetin-3-galactoside  | 1.47±0.25a    | 0.57±0.09b    | 0.41±0.11b    |
| Isoquercetin             | 3.01±0.11a    | 1.33±0.06b    | 1.28±0.08b    |
| Quercetin-3-arabinoside  | 0.39±0.05a    | 0.14±0.03c    | 0.16±0.04b    |
| Phlorizin                | 2.82±0.12a    | 1.67±0.13b    | 1.94±0.15b    |
| Quercetin                | 10.71±0.33a   | 7.54±0.62b    | 7.16±0.39c    |
| Kaempferol               | 0.12±0.02a    | 0.07±0.02b    | 0.06±0.02c    |
| Chlorogenic acid         | 16.62±0.53a   | 14.61±0.45b   | 11.71±0.26c   |
| Cinnamic acid            | 0.31±0.01a    | 0.22±0.01b    | 0.11±0.01c    |
| Coumarin                 | 1.28±0.10a    | 1.05±0.12b    | 1.31±0.19a    |
| Ferulic acid             | 3.34±0.02a    | 2.72±0.03c    | 3.04±0.04b    |
| Syringate                | 2.61±0.02c    | 3.02±0.04b    | 3.91±0.11a    |
| Vanillic acid            | 5.30±0.14a    | 4.01±0.22b    | 4.93±0.33a    |
| Gallic acid              | 16.21±0.35c   | 21.54±1.27a   | 18.53±1.06b   |

Note: Different letters in the same row indicate significant differences ($P<0.05$)

### 3.5. Quantitative descriptive analysis (QDA)

A quantitative descriptive analysis (QDA) was used for the sensory evaluation of the different samples. Red-fleshed apple cider resulting from AF was characterized by increased intensities of ‘green’ and ‘acid’ descriptors, and slight notes of ‘floral’, ‘solvent’, ‘sweet’ and ‘fatty’. Ciders obtained at the end of MLF had many significant changes in the sensory profiles; in particular, the ‘acid’ descriptor. Reduced acidity of the apple cider was consistent with the degradation of titratable acidity and malic acid, while increases in the acetic acid had no adverse effects on the cider quality (Fig. 1). Ciders made from MLF had increased intensities of ‘fruity’, ‘solvent’ and ‘fatty’ descriptors than the yeast-only control; in particular, those produces using SIM. Together, these results suggest that MLF and inoculation time have a remarkable effect on the sensory qualities of apple ciders and that there are specific differences between SIM and SEQ. For ciders produced using SIM, the use of PG-16 enhanced the ‘fruity’, ‘floral’ and ‘fatty’ descriptors of the cider and reduced the ‘green’ and ‘acid’ descriptors such that the overall sensory quality of the cider was improved. These results suggest that when the same strain was used, the diverse sensory profiles across different ciders are primarily dependent on the inoculation method, with the SIM improving the quality of red-fleshed cider better.
4. Conclusion

In conclusion, microbial species and inoculation time had an important impact on the phenolic compounds and sensory quality of cider. Compared with cider inoculated only with yeast, *O. oeni* significantly reduced the acidity of cider, enhanced the antioxidant activities of the apple cider, and improved the quality of the cider. In addition, sensory analysis showed that the cider obtained from co-inoculated with yeast and *O. oeni* kept the unique style of red-fleshed apple and scored highest fruit and floral characteristics. Therefore, co-inoculation of Excellence XR and *O. oeni* PG-16 played an important role in improving the quality of red-fleshed apple cider and can be widely applied in red-fleshed apple cider brewing.

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