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Full Length Research Paper

Characteristics of organic acids in the fruit of different pear species

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The contents of organic acids in the fruit of 40 cultivars of four major pear species, *Pyrus ussuriensis, Pyrus bretschneideri, Pyrus pyrifolia, and Pyrus communis,* were examined using high performance liquid chromatography (HPLC). The results showed that the major components of organic acids present in the pear fruit were malic and citric acids. The total organic acid content and the individual contents of malic, citric, and quinic acids were significantly higher in the fruit of *P. ussuriensis* than in the other three species. Among the ten organic acids examined, both malic and citric acids exhibited highly significant positive correlation with quinic acid, whereas extremely significant negative correlation was observed between acetic and lactic acids, and between succinic and fumaric acids. Furthermore, significant positive correlation was observed between malic and citric acids, and between quinic acid and shikimic acids, whereas significant negative correlation was found between quinic and tartaric acids. The differences in the contents of the 10 individual organic acids and the total organic acid content in the fruit of the 40 pear cultivars reached a significant and strong level.

Key words: Pear, fruit, quality, organic acid, difference, correlation.

INTRODUCTION

Organic acid content is an important indicator of the flavour and nutritional quality of fruits (Davis and Hobson, 1981; Robertson et al., 1989; Bassi and Selli, 1990; Salles, 2003). Some level of organic acids in fruits can enhance appetite and facilitate digestion (Perdok et al., 2003). They lead to the chemical stabilisation of the water-soluble B vitamins and vitamin C in food and reduce their susceptibility to destruction by heat and light.

These organic acids can improve potassium absorption by the body and promote the dissolution and metabolism of copper, zinc, and calcium, which facilitate the utilisation of these elements and improve disease resistance (Russell, 1992; Scheiblew et al., 1997). Hudina and Stampar (2000) have analysed the organic acid composition of 8 cultivars of *Pyrus communis* and 4 cultivars of *Pyrus pyrifolia* and discovered that the ‘Hardy fruits contained tartaric, malic, citric, and fumaric acids. Some studies has reported that the organic acid in the’ fruit included malic, citric, quinic, shikimic, lactic, tartaric, fruit included malic, citric, quinic, shikimic, lactic, tartaric, fumaric, and succinic acids (Gil et al., 2000; Moing et al., 2001; Sturm et al., 2003; Kafkas et al., 2006). However, few studies have investigated the organic acid content in the fruit of different pear species within a single cultivation region.

Pear cultivation in China dates back at least 2,500 years. During the long history of cultivation, many fine cultivars of pear have been generated through continuous breeding and introduction. Resources of over 2,000 germplasm lines have been preserved, including cultivars of major domestic and foreign species such as *Pyrus ussuriensis*, *Pyrus bretschneideri*, *P. pyrifolia,* and *P. communis*. The characterisation of the organic acid composition and content in the fruits of these major species will provide guidelines for the comprehensive evaluation and utilisation of the abundant resources of pear cultivars in China for the breeding of new cultivars that meet consumer demand. A total of 40 cultivars of *P. ussuriensis*, *P. bretschneideri*, *P. pyrifolia,* and *P. communis* were analysed in the present study. The contents of malic, citric, quinic, oxalic, acetic, shikimic, succinic, fumaric, tartaric, and lactic acids were examined in the ripe fruits of these cultivars. The results were also

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Table 1. The cultivars used in this study.

Species	Cultivars code
Pyrus ussuriensis	1. Jingbai Pear, 2. red Nanguo, 3.Xiangjianba, 4.Dananguo Pear, 5.Nanguo Pear, 6.Huagaiwang, 7.Huagai, 8.Jianba, 9.Jianbawang, 10.early-ripening Jianba
Pyrus bretschneideri	11.Wuyuexian, 12.Huasu, 13.Huajin, 14.Dangshansuli, 5.Zaosu, 16.Korlaxiangli, 17.New Zealand Red Pear,18.Xinping, 19.Zheli, 20.Qiyuesu
Pyrus pyrifolia	21.Dayexue, 22.Jinhua 4, 23.Lvbaoshi, 24.Whasan, 25.Wangkeumbae, 26.New star, 27.Wonhwang, 28.Goid-nijisseiki, 29.Atago, 30.Zaomeisu
Pyrus communis	31.Late Bartlett, 32.Clapp′s Favourite, 33.Early Red Du Comice, 34.Red D Anjou, 35.Louis, 36.Italy Black Pear, 37.Starkrimson, 38.Philip, 39.Bartlett, 40.Conferenc

evaluated for reliability.

MATERIALS AND METHODS

Materials

This study was conducted during the 2009 growing seasons in the Pear Cultivar Resource Garden at the Liaoning Institute of Fruit Science located in Xiongyue, Yingkou, Liaoning, China (40° 10' N, 122° 09' E, 20.4 m above sea level). The experimental site had a typical temperate continental monsoon climate with an annual mean temperature of 9.4°C, 614.4 mm of rainfall and 66% of relative humidity, the summers were hot (mean 23.3°C) and short (about 130 days) and winters were cold (mean -6.23°C) and long (about 80 days). In 2009, the mean temperature was 9.7°C, the rainfall was 529.1 mm and the relative humidity was 63%. The soil was a sand loam with a pH of 6.28, 0.54% (w/w) of organic matter, 0.06% of total nitrogen, 0.05% of phosphorus and 1.82% of exchangeable potassium. Ten cultivars were selected for each of the four major species, *P. ussuriensis, P. bretschneideri, P. pyrifolia*, and *P. communis*, providing a total of 40 cultivars (Table 1). In the spring of 2004, the 40 cultivars were collected from some pear orchards and grafted onto the 17 year old 'Nanguo Pear' trees whose rootstocks were sorbs (*P. ussuriensis*) in the Pear Cultivar Resource Garden. Plant spacing was at 4 x 3 m, with a spindly training system. All plants received the standard cultural practices used for local production, including pruning, fertigation, and pest control. Five ripe fruits (the surface of fruit changed from green to yellow or bright red, at the same time, the surface of the seeds of the fruit changed from white to black-brown) were collected for each cultivar. Following the removal of the peel and the core, the pulp of the five fruits were cut into small pieces and mixed together, frozen with liquid nitrogen, and stored at -70°C until use.

Methods

The measurement of organic acids was performed according to the method published by Nisperos-Carriedo et al. (1992). Pear pulp (1 g) was homogenised with 5 ml of ice-cold 0.2% metaphosphoric acid and centrifuged at 10,000 g for 15 min. The precipitates were extracted again with 4 ml of 0.2% metaphosphoric acid. Supernatants from the two extractions were combined, and the volume was adjusted to 10 ml and passed through a 0.45 µm filter before examination. The process was performed for each sample using a Diane U-3000 HPLC system with a UV/VIS detector. The HPLC system exhibited the following characteristics: chromatography column XB-C18 (4.6 × 250 mm), 0.2% metaphosphoric acid as the mobile phase, flow rate 1 ml/min, column temperature 35°C, sample injection volume 10.0 µl, detection wavelength 210 nm. The reagents used included analytical-grade metaphosphoric acid and chromatography-grade standards for malic, citric, quinic, oxalic, acetic, shikimic, succinic, fumaric, tartaric, and lactic acids supplied by Sigma-Aldrich.

Each sample was analysed in triplicate and the figures were then averaged. The results were statistically evaluated by bivariate analysis of correlation and one way analysis of variance (ANOVA). Statistical correlation coefficients with ' ' were considered 5% significant level and ' ' were considered at the 1% significant level, means were tested by the spearman (2-tailed). Statistical differences with p-values under 0.05 were considered significant and means were tested by the least significant difference (LSD). Both of the bivariate correlation analysis and differences analysis using SPSS program version 10.01 (SPSS Inc., Chicago, IL). The graphs were drawn by using Microsoft Excel software.

RESULTS AND ANALYSIS

Organic acid constituents and content in the fruit of the four pear species

P. ussuriensis

The organic acid constituents and content in the fruit of the *P. ussuriensis* varieties are shown in Figure 1. Only the 'red Nanguo' variety which contained all 10 of the organic acids were investigated, and the remaining varieties contained 5 to 9 types of organic acid. Nine individual organic acids were detected in the fruit of 7

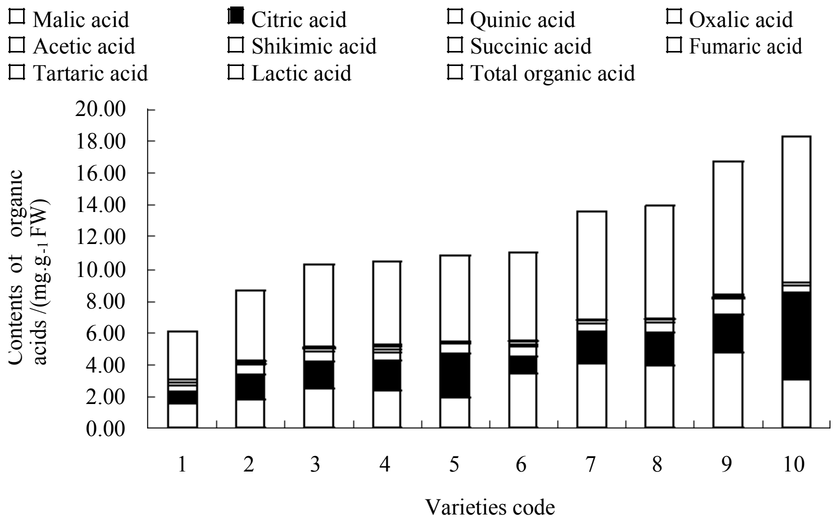

Figure 1. Kinds and contents of organic acids in fruit of *Pyrus ussuriensis.*

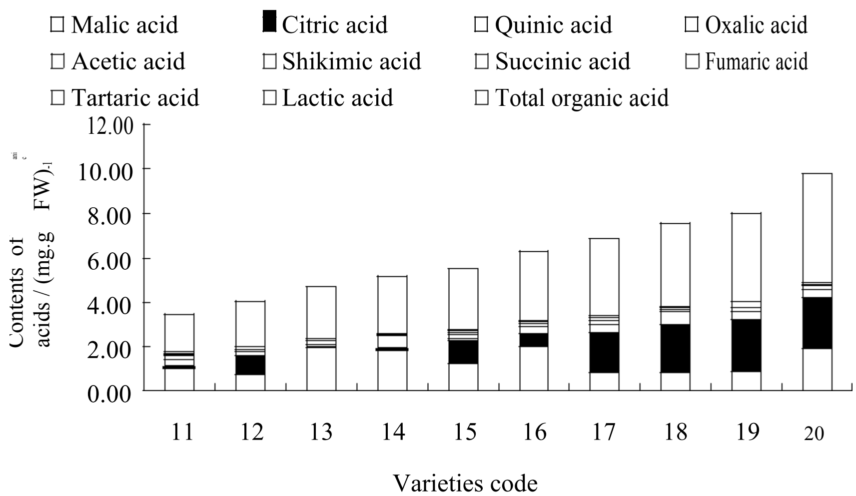

Figure 2. Kinds and contents of organic acids in fruit of *Pyrus bretschneideri.*

varieties including the ‘early-ripening Jianba’, 8 were detected in the fruit of the ‘Jingbai Pear’, and 5 were detected in the fruit of the ‘Nanguo Pear’. The total organic acid content ranged from 3.04 to 9.13 mg·g^{-1} FW. The dominant organic acids in the fruit of *P. ussuriensis* were malic and citric acid. Malic acid exhibited the highest content in most varieties except in two, which showed higher citric acid content.

The malic acid content ranged from 1.51 to 4.78 mg·g^{-1} FW, accounting for 33 to 62% of the total organic acid content. The citric acid content ranged from 0.77 to 5.51 mg·g^{-1} FW, accounting for 20 to 60% of the total organic acid content. The minor organic acids in the fruit were quinic (0.35 to 0.95 mg·g^{-1} FW) and oxalic acid (0.002 to 0. 18 mg·g^{-1} FW), which accounted for 4 to 15% and 0.3 to 5.6% of the total organic acid, respectively. The content of acetic, shikimic, succinic, fumaric, tartaric, and lactic acids were relatively low. Although the differences in the organic acid composition among the varieties were not significant, the content of each individual organic acid varied greatly. The total organic acid content was relatively high in ‘Jianba’ and ‘Huagai’ and relatively low in ‘Nanguo Pear’ and ‘Jingbai Pear’.

P. bretschneideri

Of the 10 organic acids examined in the fruit of the 10 cultivars of the *P. bretschneideri* species, 9 were detected in the fruit of 4 cultivars including ‘Qiyuesu’, 8 were detected in the fruit of 5 cultivars including ‘Xinping’, and 6 were detected in the fruit of ‘Zhe Pear’ (Figure 2). Malic, citric, quinic, oxalic, and shikimic acids were found in the fruit of all 10 cultivars. The total organic acid

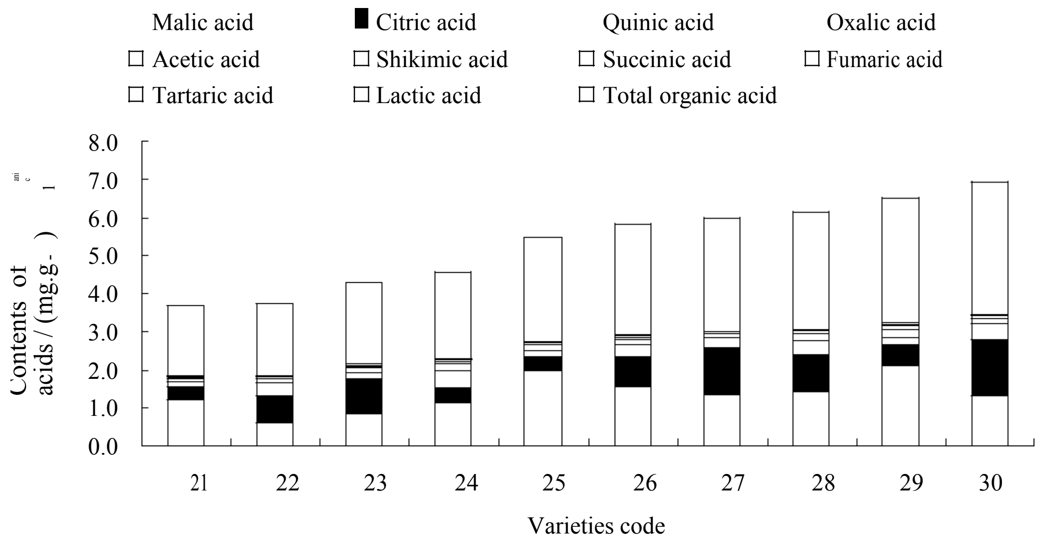

Figure 3. Kinds and contents of organic acids in fruit of *Pyrus pyrifolia.*

contents ranged from 1.74 to 4.88 mg·g^{-1} FW, with malic and citric acids being the major constituents. The citric acid content was higher than the malic acid content in 5 cultivars.

The malic acid content ranged from 0.71 to 1.98 mg·g^{-1} FW, accounting for 22 to 83% of the total organic acid content. The citric acid content ranged from 0.05 to 2.35 mg·g^{-1} FW, accounting for 2 to 58% of the total organic acid content. The minor organic acids in the fruit were quinic and oxalic acids with contents of 0.08 to 0.57 mg·g^{-1} FW (3 to 20% of the total) and 0.002 to 0.19 mg·g^{-1} FW (0.1 to 9% of the total), respectively. The content of acetic, shikimic, succinic, fumaric, tartaric, and lactic acids was relatively low. Again, the organic acid composition did not differ significantly between cultivars, but the contents of the individual organic acids varied greatly. The total organic acid content was relatively high in 'Qiyuesu' and 'Zhe Pear' and low in 'Wuyuexian'.

P. pyrifolia

The organic acid constituents and content in the fruit of the 10 cultivars of the *P. pyrifolia* species are shown in Figure 3. Of the 10 organic acids examined, 9 were detected in the fruit of 'Atago' and 'Huashan', 8 were found in the fruit of 6 cultivars including 'Zaomeisu', and 7 were present in the fruit of 'Yuanhuang' and 'Jinhua No. 4'. Malic, citric, quinic, oxalic, shikimic, and fumaric acids were detected in all of the 10 cultivars, tartaric acid was detected in 2 cultivars, and acetic acid was detected in only 1 cultivar. The total organic acid content ranged from 1.84 to 3.46 mg·g^{-1} FW, with malic and citric acids being the major constituents.

The citric acid content was higher than the malic acid content in 3 cultivars. The malic acid content ranged from 0.61 to 2.11 mg·g^{-1} FW (that is, 32 to 73% of the total) followed by citric acid with a content of 0.36 to 1.48 mg·g^{-1} FW (that is, 13 to 43% of the total). The minor organic acids in the fruit were quinic and oxalic acids with contents of 0.12 to 0.44 mg·g^{-1} FW (that is, 6 to 19% of the total) and 0.01 to 0.17 mg·g^{-1} FW (that is, 0.4 to 8% of the total), respectively. The content of acetic, shikimic, succinic, fumaric, tartaric, and lactic acids were relatively low. Although all of the cultivars showed similar organic acid composition, the total organic acid content varied significantly. The total organic acid content was highest in 'Zaomeisu' and 'Atago' and lowest in 'Jinhua No. 4' and 'Dayexue'.

P. communis

The organic acid constituents and content in the fruit of the 10 cultivars of the *P. communis* species are shown in Figure 4. Of the 10 organic acids examined, 9 were detected in the fruit of 'Clapp's Favourite', and 8 were detected in the fruit of the remaining 9 cultivars including 'Conferenc'. Malic, citric, oxalic, shikimic, and fumaric acids were detected in all 10 cultivars, whereas tartaric and lactic acids were detected in only 4 cultivars. The total organic acid content ranged from 0.86 to 3.51 mg·g^{-1} FW, with malic and citric acids being the major constituents.

The citric acid content was higher than the malic acid content in 1 cultivar. The malic acid content ranged from 0.69 to 2.61 mg·g^{-1} FW (that is, 32 to 82% of the total), followed by citric acid with a content of 0.01 to 1.35 mg·g^{-1} FW (that is, 1 to 52% of the total). The minor organic acids in the fruit were oxalic acid (0.01 to 0.24 mg·g^{-1} FW) and acetic acid (0 to 0.30 mg·g^{-1} FW), accounting for 0.4 to 11% and 0 to 10% of the total

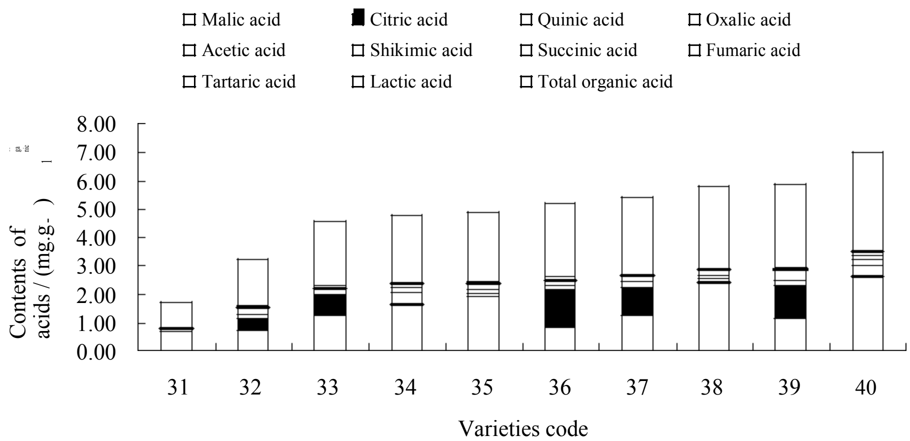

Figure 4. Kinds and contents of organic acids in fruit of *Pyrus communis.*

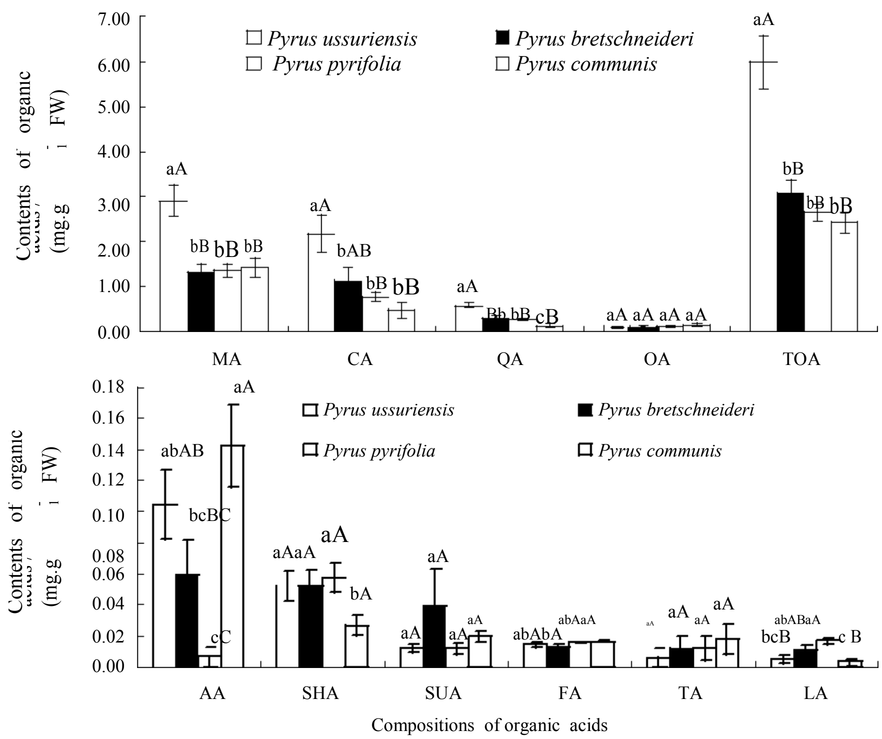

Figure 5. Contents of organic acids in different pear species. Different letters (a-c and A-C) in each column indicated significant differences at $P < 0.05$ and $P<0.01$ level, respectively. TOA: Total organic acid; MA: Malic acid; CA: Citric acid; QA: Quinic acid; OA: Oxalic acid; AA: Acetic acid; SHA: Shikimic acid; SUA: Succinic acid; FA: Fumaric acid; TA: Tartaric acid; LA: Lactic acid. Each point represented the mean of 5 observations and error bars were ±SE of the mean.

organic acid content, respectively. The content of quinic, shikimic, succinic, fumaric, tartaric, and lactic acids were relatively low. The total organic acid content was highest in ‘Conferenc’ and ‘Bartlett’ and lowest in ‘Clapp’s Favourite’ and ‘late Bartlett’. However, the organic acid composition among the cultivars did not differ significantly.

Differences in the organic acid contents in the fruit of the four pear species

The differences among the average content of the 10 organic acids and the total organic acid in the fruit of the four pear species were analysed (Figure 5). The total organic acid content was highest in *P. ussuriensis*

Table 2. Correlation coefficient among the different organic acids.

Organic acid	Malic acid	Citric acid	Quinic acid	Oxalic acid	Acetic acid	Shikimic acid	Succinic acid	Fumaric acid	Tartaric acid
Citric acid	0.34*								
Quinic acid	0.59**	0.46**							
Oxalic acid	-0.07	0.08	-0.13						
Acetic acid	0.24	-0.12	-0.14	0.03					
Shikimic acid	0.08	0.02	0.35*	-0.28	-0.09				
Succinic acid	-0.16	0.13	-0.01	0.17	-0.11	-0.17			
Fumaric acid	0.15	-0.12	-0.12	-0.13	0.30	0.12	-0.55**		
Tartaric acid	-0.25	-0.16	-0.31*	0.15	0.01	-0.09	-0.06	-0.04	
Lactic acid	-0.17	0.02	-0.04	0.24	-0.72**	0.05	-0.16	0.03	0.22

*P<0.05, **P<0.01.

(5.98 mg·g^{-1} FW), followed by *P. bretschneideri* (3.07 mg·g^{-1} FW), *P. pyrifolia* (2.66 mg·g^{-1} FW), and *P. communis* (2.42 mg·g^{-1} FW). Malic and citric acids were the major organic acid constituents in the fruit of all four pear species, with malic acid generally being more abundant than citric acid.

The content of malic and citric acids was significantly higher in the fruit of *P. ussuriensis* than in the remaining three species. The quinic acid content in the fruit of *P. ussuriensis* was 0.59 mg·g^{-1} FW, which was significantly higher than in the other three species. *P. bretschneideri* and *P. pyrifolia* showed similar quinic acid content, which was significantly higher than in *P. communis*. The content of oxalic, succinic, and tartaric acids in the fruit was relatively low and did not differ significantly between the four species. The fruit of *P. communis* showed the highest acetic acid content (0.14 mg·g^{-1} FW), followed by *P. ussuriensis* (0.10 mg·g^{-1} FW), without any significant difference between the two.

The acetic acid content was significantly higher in these species than in *P. pyrifolia*. The highest shikimic acid content was found in the fruit of *P. pyrifolia* (0.06 mg·g^{-1} FW), followed by *P. bretschneideri* and *P. ussuriensis*. Shikimic acid contents of all these three were significantly higher than *P. communis.* The highest fumaric acid content was found in *P. communis* (0.0167 mg·g^{-1} FW), followed by *P. pyrifolia* (0.0162 mg·g^{-1} FW), *P. ussuriensis* (0.0146 mg·g^{-1} FW), and finally *P. bretschneideri* (0.0129 mg·g^{-1} FW). A significant difference was found in the fumaric acid content of *P. communis* and *P. bretschneideri*. *P. pyrifolia* exhibited the highest lactic acid content (0.02 mg·g^{-1} FW), followed by *P. bretschneideri* and *P. ussuriensis. P. communis* showed the lowest lactic acid content (0.003 mg·g^{-1} FW). Significant differences were detected in the lactic acid contents of *P. pyrifolia*, *P. ussuriensis*, and *P. communis*.

Correlations among the different organic acid constituents in pear fruit

Table 2 listed the correlation coefficients among the different organic acids in the fruit of 40 pear cultivars. Both malic and citric acids showed highly significant positive correlation with quinic acid, indicating that fruits with a high content of malic or citric acid also contained high quinic acid content. Significant negative correlation was observed between acetic and lactic acids, and between succinic and fumaric acids, suggesting that the content of lactic or fumaric acid would greatly decrease when the content of acetic or succinic acid in the fruit increased.

Significant positive correlation was observed between malic and citric acid, and between quinic and shikimic acid, indicating that the content of malic or quinic acid would increase when the content of citric or shikimic acid in the fruit increased. Quinic and tartaric acid showed a significant negative correlation, indicating that the content of tartaric acid in the fruits would significantly decrease when the quinic acid content increased. Although some correlation was also observed between the other organic acids, their value was not significant enough to explain any significant impact on the content of each other.

Differences in the organic acid constituents of pear fruit

Differences among the average contents of the 10 organic acids and between the average contents of the individual organic acids and the average total organic acid content were analysed in the fruit of 40 pear cultivars. As shown in Figure 6, the total organic acid content and the contents of malic, citric, and quinic acids exhibited large average values, with highly significant differences observed between them. Oxalic, acetic, shikimic, succinic, fumaric, tartaric, and lactic acids exhibited relatively low average values with no significant differences observed between the individual components.

Significant differences were observed between the average total organic acid content and the averages of the 10 individual organic acid components, indicating that the total organic acid content in pear fruit comprises a

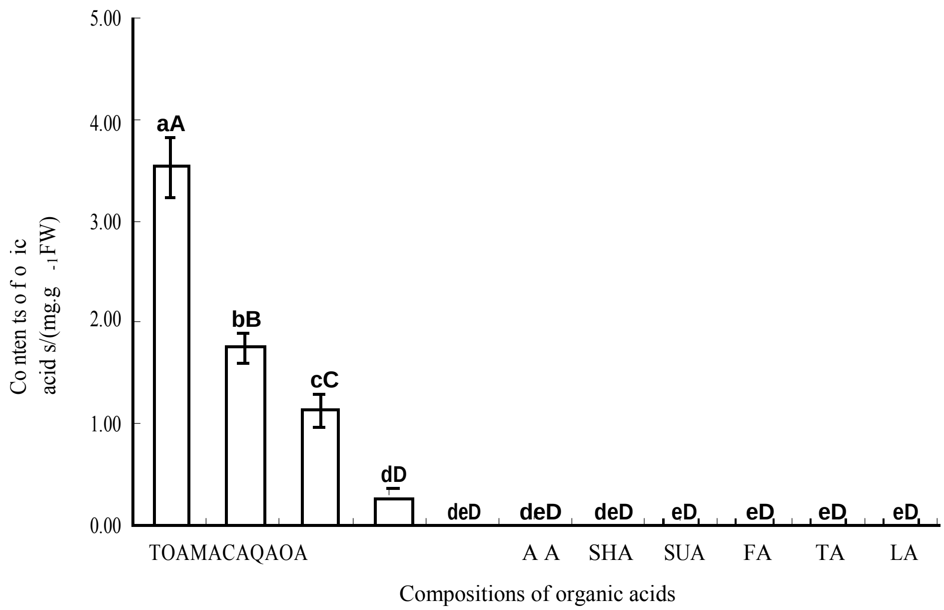

Figure 6. Differences in the organic acid constituents of four pear species. Different letters (a-e and A-D) in each column indicated significant differences at $P < 0.05$ and $P<0.01$ level, respectively. TOA: Total organic acid; MA: Malic acid; CA: Citric acid; QA: Quinic acid; OA: Oxalic acid; AA: Acetic acid; SHA: Shikimic acid; SUA: Succinic acid; FA: Fumaric acid; TA: Tartaric acid; LA: Lactic acid. Each point represented the mean of 5 observations and error bars were ±SE of the mean.

combination of multiple organic acids, not only one or two. The average malic acid content was significantly higher than the citric acid content. This observation indicates that most of the cultivars are malic acid-dominated.

DISCUSSION

Characteristics of the organic acid composition of the fruit of different pear species

The composition and content of organic acid in fruits are comprehensive traits that constitute multiple factors. Our study examined the contents of 10 organic acids in the fruit of 40 pear cultivars of four species collected from the same resource garden. Although the composition, the total organic acid content, and the individual organic acid content differed among different species, malic and citric acids were consistently found to be the major constituents of the organic acid, with other acids present in relatively low quantities. These results are consistent with the findings of Kallio and Hakala (2000), who reported that most fruits usually contained one or two major organic acid components, whereas other organic acids are only present in small, trace quantities. In the composition, the fruit of most cultivars of *P. ussuriensis* species contained a higher content of malic than citric acid.

In contrast, while 5 cultivars of *P. bretschneideri* contained mostly malic acid, the remaining 5 cultivars exhibited higher contents of citric than malic acid. Among the 10 cultivars of *P. pyrifolia*, 7 cultivars contained a higher content of malic than citric acid, whereas the citric acid content was higher in the remaining 3 cultivars. The fruit of 9 cultivars of *P. communis* contained higher contents of malic acid, whereas only one variety showed that a malic acid content was lower than the citric acid. These results indicate there is a significant difference in the contents of malic and citric acids in the fruit of different pear species. Arfaioli and Bosetto (1993) also pointed out that, pear fruits usually contained malic acid as a major component, followed by citric acid. However, we found that the content of citric acid was higher than the malic acid concentration in some pear cultivars.

The flavour and quality of pear fruits are related to their organic acid compositions and contents, as well as to the ratio of sugar to organic acid (Visser, 1986). The ratio of sugar to organic acids in pear fruits is primarily determined by the organic acid content because the organic acid content exhibits a wider range in the fruit than the sugar content (He, 2002). Malic acid has a mild sour flavour with a refreshing taste, and compared to citric acid, it produces a slow but long-lasting taste stimulation. Thus, fruits with high malic acid content usually possess excellent flavour. Among the 40 pear cultivars tested in our study, 11 cultivars exhibited excellent flavour despite containing citric acid as the dominant organic acid.

Therefore, high citric acid content should also be considered a valuable resource and breeding objective to achieve flavour diversity among fine pear cultivars. In addition, 8 to 9 different organic acids were detected in the fruit of most pear cultivars. Hudina and Stampar (2000) detected 4 different organic acids in the fruit of 12 cultivars of *P. ussuriensis* and *P. pyrifolia*. These results indicate the presence of multiple organic acids in pear fruits with differences in organic acids compositions among different cultivars. Due to the differences in the organic acid composition and content in the fruits, different cultivars of pears show different quality and flavour.

The relationship among different organic acid constituents in pear fruit

Significant correlations were found among the different organic acid constituents of pears with differences observed in the correlations between different organic acids. Among these 10 organic acids, both malic and citric acid showed highly significant positive correlation with quinic acid. Significant negative correlations were observed between acetic and lactic acid, and between succinic and fumaric acid. Significant positive correlations were found between malic and citric acid, and between quinic and shikimic acid, whereas quinic and tartaric acid showed highly significant negative correlation. Gao et al. (2004) examined the organic acid content in the juice of 8 pear cultivars and found different degrees of correlation among the organic acids. In her study, the correlation coefficient between succinic and lactic acid was highly significant (0.8750), highly significant correlations were also detected between the following pairs of organic acids: succinic and fumaric acid, citric and shikimic acid, quinic and shikimic acid, citric and quinic acid, fumaric and lactic acid, and succinic and quinic acid.

Although content and composition of organic acids differ across species and cultivars, few studies have analysed the significant differences in the organic acid content of fruits. Li et al. (2007) found the same organic acids secreted by apple rootstocks and *Malus robusta* roots, but detected significant differences in the total secretion levels. Our study revealed highly significant differences in the total organic acid content and the content of malic, citric, and quinic acid in pear fruits. The content of oxalic, acetic, shikimic, succinic, fumaric, tartaric, and lactic acids, exhibited highly significant difference from the total acid content, indicating that the total organic acid content constitutes a variety of organic acids.

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