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Comparative analysis of the volatile organic compounds in mature fruits of 12 Occidental pear (*Pyrus communis* L.) cultivars

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ABSTRACT

Aroma is an appreciated fruit characteristic, and volatile flavor plays a key role in determining the perception and acceptability of fruit products by consumers. Occidental pear (*Pyrus communis*) is an aroma-dense fruit, and evaluation of the fruit aroma of different cultivars is meaningful to pear-breeding programs. In this study, we dissected the aroma composition and concentration of mature fruits from 12 Occidental pear cultivars. A total of 335 volatile organic compounds were identified, which were primarily esters, alcohols, alkanes, acids, ketones, terpenes and aldehydes. The concentration of total aroma was highest in 'Alexandrine Douillard' (18.73 μg/g), whereas the lowest total concentration was in 'Bartlett-Max Red' (0.33 μg/g). The principal aroma biosynthetic pathway of 'Bartlett-Max Red', 'Abate Fetel' and 'Butirra Rosata Morettini' was the fatty acid pathway, whereas the amino acid pathway primarily functioned in 'Bartlett', 'Yubileen Dar', 'Doctor Jules Guyot' and 'Alexandrine Douillard'. Based on the ABC's of Perfumery System, the 12 pear cultivars were divided into two groups, 'La france', 'Abate Fetel', 'Bartlett', 'Beurre Bosc', 'Alexandrine Douillard', 'Doctor Jules Guyot' and 'Yubileen Dar' as the fruit scent type (Group one); and 'Butirra Rosata Morettini', 'beurré Hardy', 'Bartlett-Max Red', 'Clapp Favorite' and 'Red Clapp Favorite' as the aliphatic scent type (Group two).

1. Introduction

Flavor consists of perception in both the mouth (sweetness, acidity or bitterness) and nose (odor), with odor the latter one was produced by amounts of volatile organic compounds. Aroma is a complex mixture product of aromatic volatile organic compounds, which has an important influence on the overall flavor of fruits and vegetables (Baldwin, 2002). As aroma is one of the most appreciated fruit characteristics, volatile organic compounds are likely to play a key role in determining the perception and acceptability of products by consumers (Villatoro et al., 2008).

As an important trait of fruit quality, aroma has received increasing attention in recent years. To date, the aroma of various types of fresh fruits has been evaluated, including watermelon (Lewinsohn et al., 2005), mango (Lalel et al., 2003), melon (Obando-Ulloa et al., 2008), strawberry (Van de Poel et al., 2014), peach (Wang et al., 2009), tomato (Maul et al., 2000) and banana (Hultin and Proctor, 1961). The most important volatile organic compounds include amino acid-derived compounds, lipid-derived compounds, phenolic derivatives, and terpenoids (Schwab et al., 2008). The volatile organic compounds of fruit are complex and vary depending on cultivar, fruit sample (intact fruit, slices, or homogenized samples), ripeness (Song and Bangerth, 1996; Steingass et al., 2015), pre- and post-harvest (Ayala-Zavala et al., 2004), environmental conditions, and analytical methods used (Brückner and Wyllie, 2008). Each fruit species has a distinctive aroma that depends on not only the combination of different volatile organic compounds, but also the concentration and perception threshold of individual volatile organic compounds (Seymour et al., 2012). For example, C6 compounds (hexanol, hexanal) are summarized as 'green leaf volatiles' relating to the 'green grassy' note and also contribute to the herbaceous odor in grape juice (Conde et al., 2007; Ruther, 2000; Watkins and Wijesundera, 2006). High levels of phenyl acetaldehyde contribute to the development of grass aroma of cultivated tomato (Tadmor et al., 2002). In research on avocado, *(E)*-2-pentenal is found only in the cultivar 'Fortuna', which possesses a fruity aroma, whereas a high content of ethyl acetate in the cultivar 'Collinson' produces a fresh fruity flavor (Galvao et al., 2016).

Pear (*Pyrus* spp., Rosaceae) is one of the most important fruit crops with high economic value in the temperate zones, with cultivation in more than 50 countries (Kole, 2011). Based on the geographic

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distribution, the *Pyrus* species are divided into two native groups: Occidental pears and Oriental pears (Janick and Moore, 1996). *Pyrus communis* L. (Occidental pear) is the most commonly cultivated pear species in western countries (Europe, North America, South America, Australia, and Africa) and has been cultivated in Europe since as early as 1000 BCE. By contrast, the Oriental pears are separated into four species: *P. pyrifolia* Nakai., *P. ussuriensis* Maxim., *P.* × *bretschneideri* Rehd., and *P.* × *sinkiangensis* Yu. Several biological features vary between the Occidental pear and Oriental pear, for example, most Occidental pear fruits have melting flesh with buttery, juicy texture, intense flavor and aroma, whereas most Oriental pear fruits have crisp flesh with more juice but less aroma. Thus, the Occidental pear is a valuable resource in which to discover volatile organic compounds compared with the Oriental pear. To date, the primary volatile organic compounds of several pear cultivars have been investigated, including 'La France' (Shiota, 1990), 'Bartlett' (Romani and Ku, 1966), 'Packham's Triumph' (Chervin et al., 2000), 'Anjou', 'Comice', 'Bosc', 'Seckel', 'Vicar of Winkfield' and 'Forelle' (Suwanagul and Richardson, 1998). Ethyl, hexyl acetates are the principal volatile organic compounds in these pears. However, these studies were primarily focused on the influence of various postharvest treatments on the volatile organic compounds of a single pear cultivar. For example, Chervin et al. (2000) reported the influence of low oxygen storage on volatile organic compounds of 'Packham's Triumph' pears (Chervin et al., 2000), and the biosynthesis of volatile organic compounds of 'Comice' stored under long-term controlled-atmosphere conditions has also been investigated (Lara et al., 2003). Rizzolo et al. (1991) revealed that ethylene removal treatment on 'Passa Crassana' pears under a controlled atmosphere changes the volatile organic compounds; for example, pentyl acetate, ethyl propanoate and hexanal disappeared, whereas butyl acetate and hexyl acetate were newly identified in the fruit flesh (Rizzolo et al., 1991). Compared with other Rosaceae species, such as apple, peach and strawberry (Chagné et al., 2014), the aroma traits of pear are less well studied, including the postharvest studies. Thus, elucidating the composition, concentration and distinctive types of the fruit aroma of Occidental pear at the germplasm level is of significant value.

General volatile organic compounds from a few Occidental pear cultivars are well investigated, but limited information is available for many Occidental pear cultivars. To close this gap, we conducted a comparative analysis of the aroma constituents of 12 Occidental pear cultivars. In this paper, headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrophotometry (GC−MS) were used to analyze a wide range of components and concentration of the volatile organic compounds in 12 *P. communis* cultivars. The contributions of this study were to identify the specific volatile organic compounds of each cultivar and based on analysis of the aroma types, to group the 12 pear cultivars into fruit scent and aliphatic scent types. The collective results of our study will provide a foundation for pear breeding aimed at improving the fruit aroma quality.

2. Materials and methods

2.1. Plant materials

The cultivars, abbreviation, origin, producing area, sampling time, skin and taste of 12 Occidental pear (*Pyrus communis* L.) are introduced in Table 1, and photographs of the 12 Occidental pear cultivars are displayed in Fig. S1. Healthy fruit at commercial ripening was harvested from five trees of each cultivar. For each cultivar, maturity was determined using conventional indices, such as fruit life (days after pollination), skin color and size. All fruits were packed and delivered immediately to the laboratory at Nanjing Agricultural University and were stored at 4 °C immediately. Samples were placed to become edible stable at room temperature before testing (approximately five days). For each cultivar, three fruits were combined for each of three replicates.

2.2. Extraction and concentration of volatile organic compounds

HS-SPME was used for the volatile organic compounds isolated extraction. SPME fibres coated with a 65 μm thickness of polydimethylsiloxane–divinylbenzene (65 μm PDMS/DVB; Supelco Co., Bellefonte, PA, USA) were used in this study based on preliminary tests and literature (Qin et al., 2012). The fibers were activated before sampling according to the manufacturer's instructions. The core and exocarp of each pear was removed and discarded, the pulp was juiced with a commercial juice extractor, and the volatile organic compounds of the juice were isolated and was used for concentration analysis. For each extraction, 5 g of pulp were placed into a 20-ml screw-cap headspace brown vial containing 5 ml of NaCl solution (0.36 g/ml) to facilitate the release of volatile organic compounds which were based on reported method of Qin et al. (2012). Before sealing of the vials, 5 μl of a solution of 0.82 g/l 3-nonanone/methanol solution was added as internal standard. The mixture was placed into a constant-temperature water bath at 40 °C and was stirred for 30 min at 30 rpm with a PTFE stirrer bar that was added to each vial. The SPME fiber was exposed to the head space of the sample for 30 min to adsorb the analytes and introduced into the heated injector port of the chromatograph for desorption at 250 °C for 5 min in splitless mode.

2.3. GC–MS conditions

The organic extract was analysed with a Bruker 320 mass selective detector coupled to a Bruker 450 gas chromatograph, equipped with a 30 m × 0.25 um × 0.25 mm BR-5 MS (5% phenyl-polymethylsiloxane) capillary column. Helium was used as the carrier gas at a constant column flow of 1.0 ml/min. The injector and detector temperature were 250 and 280 °C, respectively. The temperature program was as follows: 35 °C for 8 min, raised at 5 °C/min up to 140 °C and hold for 2 min, then raised at 10 °C/min up to 270°C and kept at this temperature for 5 min. Mass spectra were recorded at 70 eV in electron impact (EI) ionization mode. The temperatures of the quadrupole mass detector and ion source were 150 and 230 °C, respectively. The temperature of the transfer line was 280 °C. Mass spectra were scanned in the m/z range 33–350 amu at intervals of 1 s. Tentative identification of the volatile organic compounds was performed by comparing the mass spectra of the samples with the data system library (NIST 2013) (Qin et al., 2012). MS identification was confirmed with authentic references.

2.4. Computing formula

2.4.1. Determination of the volatile organic compounds concentration

Quantification was performed by the internal standard method in which the concentration of each volatile organic compound was normalized to that of 3-nonanone. Peak areas were measured by integration and were normalized against the internal standard substance for samples.

The formula for content quantification was the following: the concentration of components (μg/g) = [peak area of component / peak area of internal standard * the concentration of internal standard (g/ml) * 5 μl] / mass of sample (g). The concentration of the internal standard 3-nonanone was 0.82* 10^{-3} g/ml.

2.4.2. Analysis of aroma characteristics

Analysis of aroma characteristics was using the method of Training the ABCs of Perfumery (Dowthwaite, 1998), which is a method to describe consists of aroma. Author created an odor classification system based on 26 easy key words and raised three value to classify the odor qualitatively and quantitatively. The following formula was used: Aroma Characteristics (%) = (relative molecular mass$_i$ * relative impact value * ABC value) / $\sum_i^n$ (relative molecular mass$_i$ * relative impact value * ABC value). Relative impact value represents the strength of one