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# Effects of 1-MCP on volatile production and transcription of ester biosynthesis related genes under cold storage in 'Ruanerli' pear fruit (*Pyrus ussuriensis* Maxim.)

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ABSTRACT

Fruit of *Pyrus ussuriensis* Maxim. produces an intense aroma accompanied by elevated ethylene levels and fruit firmness loss. Although 1-methylcyclopropene (1-MCP) treatment is an effective method to delay fruit ripening, the effect on aroma of pear fruit remains unknown so far. In this study, fruit of 'Ruanerli' pear (*P. ussuriensis*) were treated by 1-MCP under room temperature, and then transferred to cold storage. Changes in the total volatile concentrations and the volatile composition were detected during storage. Although the amount of total volatiles increased during storage in 1-MCP treated fruit, concentrations of both total volatiles and esters were lower in 1-MCP treated fruit compared with control. The effect of 1-MCP on ester production was stronger than on aldehydes. Different transcript patterns of ester biosynthesis related genes were observed in 1-MCP treated fruit. During the storage period, the transcript levels of *PuLOX*s and *PuAAT* were inhibited dramatically in 1-MCP treated fruit. However, the transcript levels of *PuADH*s were not suppressed in treated pear fruit during storage. Treatment with 1-MCP effectively prolonged the storage time and delayed the fruit firmness loss in pear fruit. The lower content of total volatiles and esters may be ascribed to the suppression of *PuLOX*s and *PuAAT* genes.


## 1. Introduction

Pear is one of the most important fruit crops in China, and there are four groups of commercial pear cultivars native to China, including *Pyrus pyrifolia* Nakai, *Pyrus ussuriensis* Maxim., *Pyrus sinkangensis* Yu, and *Pyrus pyrifolia* Chinese white pear group (Teng and Tanabe, 2004). Unlike other species of Asian pear, *P. ussuriensis* is a typical climacteric fruit species producing increased amounts of ethylene and intense aroma during fruit ripening. The consumption of *P. ussuriensis* has increased in recent years in China, mainly because of its unique flavor and strong aroma. Unfortunately *P. ussuriensis* fruit is characterized by high perishability, owing to its rapid firmness loss during postharvest storage which restricts its marketing. Proper harvest time may allow longer storage, but storage under cold conditions has been proven to delay the storage/postharvest ripening of *Pyrus communis* fruit (Villalobos-Acuna et al., 2011).

Fruit quality is generally determined by color, shape, texture, titratable acidity, and aroma. Aroma is an important factor affecting the final sensory quality of fruit and consequently, consumer satisfaction. The fruit aroma represents a complex mixture of many volatile compounds including terpenes, alcohols, aldehydes, esters, acids, ketones, and hydrocarbons (Lara et al., 2003). More than 300 volatile compounds have been identified in pear fruit, mainly European pear (Rapparini and Predieris, 2003). According to our previous study, the different concentrations of volatile esters in different cultivars determined its organoleptic attributes and consumer preference (Li et al., 2012). Volatile esters are the most significant contributors to aroma in various fruit, such as apple (Ban et al., 2010), strawberry (Dong et al., 2013), and banana (Imahori et al., 2013). Esters are generated by the action of alcohol acyltransferases (AATs), which transfer acyl-CoA onto the corresponding alcohol (Brückner and Wyllie, 2008). The availability of substrates, the results of breakdown of unsaturated fatty acids and amino acids, and the properties of alcohol acyltransferases (AATs) determine the composition of volatile esters in fruit (Rapparini and Predieris, 2003). It has been suggested that restricted supply of substrates for esterification may be the major limiting factor for ester

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production in immature apple fruit (Song and Bangerth, 1993). Unsaturated fatty acid, linoleic acid and linolenic acid, are the main substrate and are catabolized through β-oxidation and lipoxygenase pathway (LOX pathway). The C6 aldehyde, alcohols and esters are generated by LOX pathway. In pear fruit, AAT activity increased as the alcohol carbon number increased from one to six, and then decreased (Rapparini and Predieris, 2003). Genes encoding LOX and AAT have been proven to be ethylene-dependent and regulated by ethylene in apple fruit (Defilippi et al., 2005; Souleyre et al., 2005).

1-Methylcyclopropene (1-MCP), which binds to ethylene receptors with 10 times more affinity than ethylene itself, is an effective ethylene-antagonising compound and is used to prolong the storability of various fruit. In climacteric fruits, 1-MCP delays the fruit senescence which results in a longer storage period/shelf-life (Blankenship and Dole, 2003). Aroma biosynthesis is an ethylene dependent process and inhibited in 1-MCP treated apple fruit. Total volatiles and total esters were inhibited in 'Fuji' and 'Gala' apples, while alcohols were not affected by 1-MCP (Fan and Mattheis, 1999). Lipoxygenase and hydroperoxide lyase activities involved in the ester biosynthesis from fatty acid were inhibited in 1-MCP-treated peach fruit (Ortiz et al., 2010). It is interesting that the expression and activity of AAT are highly regulated by ethylene in apple fruit, and a genomics study suggested that ester production in apple was controlled by initial and final enzymatic steps of the LOX pathway (Defilippi et al., 2005; Li et al., 2006).

As mentioned above, esters are the major contributors to fruit aroma, and treatment with 1-MCP is an effective method to prolong the storage potential of *P. communis* fruit. In this study, we systematically investigated the changes of fruit aroma and gene transcription related to ester-biosynthesis in 'Ruanerli' pear fruit treated by 1-MCP. Although some studies have investigated the effect of 1-MCP on volatile compounds produced by climacteric fruit, there is no literature focused on *P. ussuriensis* fruit. Ample evidence pointed out that the effect of 1-MCP on different cultivar/species was different (Blankenship and Dole, 2003). In this work, we analyzed the changes of total volatiles in 1-MCP treated fruit and the transcriptional levels of various genes encoding enzymes related to the biosynthesis of esters in 'Ruanerli' pear (*P. ussuriensis*). The main aim of this study was to analyze the effect of 1-MCP on aroma, mainly volatile esters, in *P. ussuriensis* fruit during storage.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Fruit of 'Ruanerli' pear (*P. ussuriensis* Maxim.) were picked from a commercial orchard in Gansu Province, Northwest China (36°06′N, 103°43′E) on the 4th of October in 2010. Pears were harvested according to the size and color of fruit peel. After harvest, the fruit were transported to the laboratory for selection and storage. Uniform fruit without visible signs of defects or decay were selected as the experimental materials and randomly divided into two groups for different treatments. Control fruit were carefully put into a plastic box and then sealed tightly with the cover for 12 h at room temperature (about 20 °C). A second group was treated with 1.0 μL $L^{-1}$ 1-MCP (Rohm and Haas Company, AgroFresh™ Technology) for 12 h at room temperature in an air-tight container. After treatment, all of the fruit were transferred to 8 °C for storage. During the storage, 15 fruit per treatment, 5 from each of 3 replicates, were sampled at day 3, 6, 9, 14, 19, 24, 29 and 34, respectively. Fruit flesh sliced from fruit was immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

### 2.2. Determination of fruit ripening related indexes

Pear fruit color was determined at four evenly distributed equatorial sites by a colorimeter (MiniScan XE Plus, HunterLab, USA), which provided CIE L*, a*, and b*. Hue angle degree and chroma values were calculated from CIE L*, a*, and b* (McGuire, 1992). Fifteen fruit were used for each treatment.

For the ethylene determination, five fruit (approx. 0.5 kg) of similar size were placed in a 2-L flask which was sealed using a rubber stopper for 1 h at 20 °C. A 1 mL sample of the headspace gas was withdrawn by a gas-tight syringe from each flask and injected into a gas chromatograph model SP 6800 (Lu'nan Chemical Engineering, Shandong, China) as described by Zhang et al. (2010). The GC was equipped with a GDX-502 column and a flame ionization detector (FID). The injector, detector, and oven temperatures were 110 °C, 140 °C, and 90 °C, respectively. The carrier gas was $N_2$ and a rate of 40 mL $min^{-1}$, and the rate of ethylene production was expressed as nL $g^{-1}$ $h^{-1}$.

A TA-XT2i Plus texture analyzer (Stable Micro Systems, Surrey, United Kingdom) was applied to measure fruit firmness. A 7.9-mm diameter probe was chosen for our measurement. The penetration depth and penetration rate were 5 mm and 1 mm $s^{-1}$. Measurements were made at the equator of the fruit after removal of a 1 mm thick slice of peel and data were expressed as Newtons (N).

### 2.3. Determination of aroma volatiles

Determination of volatiles was carried out according to the method used in our previous studies (Li et al., 2014). Ten gram of frozen flesh sample was ground to fine powder in liquid nitrogen and then transferred to a 20-mL vial. Four milliliters of saturated sodium chloride solution was added to minimize the loss of volatile components and avoid browning. Before sealing the vials, 5 μL of 2-octanol was added as an internal standard. A vortex oscillator was used to homogenize the mixture and a stirring bar was used to maximize volatile emission. A fiber coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB, Aldrich, Bellefonte, PA, USA) was selected for manual solid-phase micro-extraction (SPME). According to the manufacturer's instructions, fibers were conditioned at 250 °C for 30 min to prevent contamination before the use. The fiber was exposed to the headspace of the vials for 45 min at room temperature.

A gas chromatograph–mass spectrometer (GC6890/MS9370, Hewlett-Packard, Palo Alto, CA, USA) was used to separate and identify the volatile compounds. The GC–MS system was equipped with an Innowax capillary column (30 m × 0.32 mm × 0.5 μm film thickness). The SPME fiber was exposed in the GC injector port for 5mins at 250 °C with a splitless mode. The temperature of the column began at 40 °C for 2 min, raised to 220 °C at 4 °C $min^{-1}$, and then increasing to 250 °C at 15 °C $min^{-1}$ and held for 2 min. Helium was used as the carrier gas at a constant flow-rate of 1 mL $min^{-1}$. MS was scanned at 70 eV with a scan range of 30–500 amu. The mass spectrometer was operated in the full scan. The temperatures of the ion source and connecting parts were set at 230 °C and 280 °C, respectively. Compounds were identified using NIST/WILEY MS Search 2.0 mass spectra libraries. Most compounds were confirmed by comparison of their linear retention indices and EI mass spectra with those of reference compounds (Sigma–Aldrich, USA). All chemicals used were of reagent grade. Semi-quantitative determination of volatile compounds was carried out using the internal standard method, where the concentrations of various volatile compounds were normalized to that of 2-octanol.