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Ethylene effects on apple fruit cuticular wax composition and content during cold storage

Fujun Li, Dedong Min, Baicheng Song, Shujun Shao, Xinhua Zhang*

School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, 255049 Shandong, PR China

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ABSTRACT

To investigate ethylene effects on fruit cuticular wax during cold storage, apple fruit (*Malus domestica* Borkh. c.v. Starkrimson) were treated with 500 mg L^{-1} ethephon and 1 μL L^{-1} 1-methylcyclopropene (1-MCP), and the composition and contents of the cuticular wax were analyzed at 20 d intervals during cold storage. The results showed that the prevailing cuticular wax components were acids, 1-alcohols and n-alkanes. Ethephon accelerated fruit senescence, positively regulated total wax quantity and its alcohol, olefin, alkane, and acid constituents during cold storage, especially 1-pentacosanol, 1-octacosanol, α-farnesane, n-octacosanoic acid, stearyl acid butyl ester, stearic acid hexyl ester and n-tetradecanal, while 1-MCP inhibited these processes. It can be concluded that ethylene altered the fruit cuticular wax contents and constituents, which may alter the subsequent postharvest qualities of the fruit and the susceptibility of the fruit to disorders during cold storage.

1. Introduction

Studies on apple (Belding et al., 1998; Ju and Bramlage, 2001; Dong et al., 2012), cherry (Belge et al., 2014a), peach (Belge et al., 2014b), citrus (Cajuste et al., 2010; Wang et al., 2014), pear (Li et al., 2012), have demonstrated that the horticultural fruit cuticular wax composition is continuously changing during development and after harvest. Additionally, its composition is affected by environmental factors, such as wind, temperature, light, pre-harvest bagging, controlled atmosphere (Li et al., 2012; Dong et al., 2012).

A few studies have reported that ethylene is involved in the changes in cuticular wax composition. Ju and Bramlage (2001) found that preharvest treatment with 200 mg L^{-1} ethephon increased internal ethylene concentrations, and accelerated wax and α-farnesene accumulation. The latter is thought to be responsible for the occurrence of superficial scald of apple fruit during cold storage (Lurie and Watkins, 2012). Cajuste et al. (2010) also found that ethylene might play a protective role in reducing non-chilling peel pitting by inducing structural changes in surface wax and that ethylene improved physical barriers to *Penicillium digitatum* penetration by inducing the formation of new waxes in 'Navelate' orange fruit during cold storage.

1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene, was reported to change fruit postharvest traits by affecting the composition and structure of apple fruit waxes. Curry (2008) found that 1-MCP strongly delayed development of certain wax constituents thought responsible for fruit greasiness during cold storage. Dong et al. (2012) found that 1-MCP changed the wax composition and delayed the decline of total wax, indicating that ethylene strongly influences wax composition in 'Red Fuji' apple fruit.

However, the details regarding the effects and potential mechanisms of ethylene on wax synthesis and degradation are still unclear. In addition, the relationship between ethylene and wax composition also need further investigation. Starkrimson (*Malus domestica* Borkh. c.v. Starkrimson), a well-known apple cultivar worldwide, is rich in wax content (Belding et al., 1998) and susceptibility to disorders related to the cuticle, such as superficial scald (Lurie and Watkins, 2012). Hence, it is appropriate to use as a material to study the effect of ethylene on cuticular wax during fruit postharvest. In this paper, the cuticular wax composition and responses in apple fruit to ethylene and 1-MCP were analyzed. The main objective was to further clarify the role of ethylene in regulating cuticular wax composition during apple fruit cold storage.

2. Materials and methods

2.1. Materials

Apple fruit were harvested at commercial maturation from a commercial orchard located in Zhaoyuan city, Shandong province, PR China. Fruit were placed on trays and transported to the laboratory, where 15 fruit were selected randomly to measure soluble solid content (SSC), titratable acid (TA), firmness, cell membrane permeability, respiration rate and ethylene production. Another 15 fruit were used to

* Corresponding author.
E-mail address: zxh@sdut.edu.cn (X. Zhang).

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estimate fruit surface area, for wax extraction and to analyze wax constituents.

2.2. Treatments

The remaining fruit were divided randomly into three groups of 240. The first group served as ethephon and were treated with 500 mg L^{-1} ethephon by submerging fruit for 5 s and then sealing them in a 400 L chamber for 18 h. The second group (~240 fruit) served as 1-MCP and were treated with 1 μL L^{-1} 1-MCP (0.14%, a.i., SmartFresh™, AgroFresh Inc., Rohm and Haas) according to user instructions in a 400 L chamber for 18 h at 20 °C, after being immersing in deionized water for 5 s. The third group (~240 fruit) served as control and were submerged in deionized water for 5 s and then sealed in a 400 L chamber for 18 h.

2.3. Storage and sampling

After treatment, all the fruit were air dried and subsequently stored at 0 ~ 1 °C and 85%-95% humidity for measurement at 20 d intervals. Thirty fruit per treatment were used in each measurement, 15 fruit were divided randomly into 3 groups as replicates for the measurements of SSC, TA, firmness, cell membrane permeability, respiration rate and ethylene production, and the other 15 fruit were divided randomly into three groups as replicates for the fruit surface area and wax extraction measurements.

2.4. Determination of fruit SSC, TA, firmness and cell membrane permeability

5 fruit per replicate were used to determine the fruit SSC, TA, firmness and cell membrane permeability according to Li et al. (2013). For TA content, the fruit was homogenized with a blender, and 20 mL portion of the juice was diluted to 100 mL with distilled water. A 50 mL portion of the juice was titrated with 0.1 mol L^{-1} NaOH to a pH of 8.1. Result was expressed as% malic acid. SSC was measured using a manual refractometer (WYT-J , Chengdu Optical Apparatus Co. Ltd., China) in juice pressed from a sample of homogenized fruit slices. Fruit flesh firmness was measured twice using a handheld hardness (GY-1, Mudan River, China) on opposing sides of the each fruit equator after a 10-mm diameter skin was removed. The cell membrane permeability was measured according to Zhou et al. (2008) with minor modifications. Flesh from 5 fruit per replicate was collected using a cork borer with a diameter of 10 mm from equatorial region, and cut it into slices of 2 mm thickness. 15 slices samples per replicate were randomly selected and rinsed with distilled deionized water three times and immersed in 100 mL distilled deionized water for 1 h. Subsequently, the initial electrical conductivity was recorded using a conductivity meter (DDS-307A, Shanghai Leici Apparatus, Shanghai, China). The samples were then boiled for 5 min, and the total electrical conductivity was recorded after the sample reached room temperature. The membrane permeability was expressed by the relative leakage rate, which was calculated as the percentage of the total electrical conductivity. The measurements of SSC and firmness were repeated 12 times and the measurements of TA and Cell membrane permeability were repeated three times.

2.5. Determination of ethylene production and respiration rate

For ethylene determination, 5 fruit per replicate were enclosed in a 4-L airtight jar for 0.5 h at 20 °C. The ethylene production was then measured using a gas chromatograph (Agilent 6890N, USA) equipped with a flame ionization detector (FID) and a HP-5 column. The injector, oven, and detector temperatures were 50, 110, and 150 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 40 mL min^{-1}. The ethylene production was expressed in ng $kg^{-1}s^{-1}$.

After sampling for ethylene, the CO_2 concentration was measured using a gas analyzer (CheckMate 9900; PBI Dansensor, Denmark) by a syringe through a rubber septum on each jar cap. The respiration rate of the fruit was expressed as the CO_2 production in μg $kg^{-1}s^{-1}$. Both the measurements were repeated 3 times.

2.6. Fruit surface area estimations and wax extraction

The estimations of fruit surface area and wax extraction were according to Belding et al. (1998) and Parsons et al. (2012), with minor modifications. The wax extraction was performed by submerging each fruit in clean chloroform for 30 s at room temperature and then drying under a stream of N_2. The residues were re-dissolved in 10 mL clean chloroform and transferred into chloroform-resistant vials. Tetracosane was added to all extracts as an internal standard, and the solvents were then dried under a stream of N_2 with anhydrous Na_2SO_4. The dry mass was stored at −20 °C until GC and GC–MS analysis.

2.7. Wax derivatization and GC–MS or GC analysis

Prior to the GC analysis, the hydroxyl-containing compounds were transformed to the corresponding trimethylsilyl derivatives by adding 75 μL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Supelco, Sigma-Aldrich Co. LLC, USA) and 25 μL pyridine at 50 °C for 1 h (Belding et al., 1998). Then, the samples were again dried and redissolved in 1 mL clean chloroform.

Quantitative analysis was performed using a gas chromatograph equipped with a flame ionization detector (Agilent 6890N, USA). A capillary column (RTX-5, 30 m, 0.25 mm i.d., 0.25 μm film) was used for the separation of compounds using N_2 as a carrier gas. 1 μL of each sample was injected into the column. The column temperature was programmed with an initial temperature of 120 °C for 2 min. The temperature was raised by 10 °C min^{-1} to 190 °C, raised by 2 °C min^{-1} to 216 °C, held for 5 min at 216 °C, raised by 3 °C min^{-1} to 300 °C, and finally held for 5 min at 300 °C. The detector gases were hydrogen and air at flows of 50 and 400 mL min^{-1}, respectively. The temperature of FID was 320 °C. Quantification was based on flame ionization detector peak areas and the internal standard tetracosane.

Qualitative analysis was carried out with a GC–MS (QP-2010 plus, Shimazu, Tokyo, Japan) under the same conditions as GC except that helium was used as carrier gas and the inlet pressure constant was 30 kPa. Wax compounds were identified by matching their electron ionization mass spectra (EI-MS) (70 eV, m/z 50–850) with those from the NIST 08 MS library.

Wax quantitative and qualitative analysis were both repeated 3 times.

2.8. Statistical analysis

Results are given as the mean ± standard error. All statistical analyses were performed with SPSS 16.0. A mean comparison using Duncan's multiple comparison procedure was performed to determine significant differences among means at $P < 0.05$.

3. Results

3.1. Evaluation of respiration rate, ethylene production and fruit quality parameters

Ethephon increased the respiration and ethylene production peak and accelerated the respiration and ethylene production, whereas 1-MCP inhibited and retarded them (Fig. 1A and Fig. 1B).

Similarly, ethephon treatment accelerated softening, as well as the increasing firstly and then decreasing of SSC and TA. Cell membrane permeability also increased in the presence of ethephon and decreased with 1-MCP. These results show that ethylene accelerated fruit senescence and 1-MCP retarded this process (Table 1).