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Effect of 1-MCP on the production of volatiles and biosynthesis-related gene expression in peach fruit during cold storage

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ABSTRACT

As an effective ethylene action inhibitor, 1-Methylcyclopropene (1-MCP) can delay fruit ripening and senescence. However, little is known about the effect of 1-MCP on peach fruit aroma-related volatiles and their biosynthesis-related gene expression during cold storage. Peaches (cv. Xiahui 6) were treated with 1-MCP (10 μL L^{-1} for 12 h) to investigate the influence of 1-MCP on aromatic volatile biosynthesis and involved genes expression during cold storage. 1-MCP treatment significantly decreased ethylene production and delayed the ripening and senescence of peach at cold storage. A lower production of esters was found in 1-MCP treated fruit, while there were higher alcohol acyltransferase (AAT) activity and *PpaAAT1/2* expression in treated fruit during the early storage. The performance patterns of both aldehydes and alcohols were different between 1-MCP and control fruit accompanied by lower lipoxygenase (LOX), alcohol dehydrogenase (ADH), hydroperoxide lyase (HPL) activities in accordance with the expression of *PpaLOX3*, *PpaADH1* and *PpaHPL1* in 1-MCP treated fruit compared with the control in the late storage. All in all, the role of 1-MCP on the volatiles and its related genes expression in peach fruits was discussed, and 1-MCP affected the formation of aroma volatile by functioning in the expression of pivotal enzyme genes including LOX, HPL, ADH and AAT.

1. Introduction

Peach (*Prunus persica* L.) fruit aroma determined by volatile compounds is one of the most significant indicators of fruit quality. Research has revealed that volatile compounds, which general is recognized as the product of secondary metabolism, were generated from a series of enzymatic reaction and fatty acids, amino acids, carbohydrates and other substances as precursors in the growth and development process of peach fruit. Up to now, more than 100 compounds have been identified in peach fruit (Aubert and Milhet, 2007; Wang et al., 2016). Among the reported volatiles presented in peach fruit, only a few with high odor active values are regarded as dominating volatiles (Eduardo et al., 2010). These volatiles are composed of lactones, basically γ- and δ-decalactones, and C_6 aldehydes and alcohols such as n-hexanal, (E)-2-hexenal, and (E)-2-hexenol (Horvat et al., 1992; Kakiuchi and Ohmiya, 1991).

Peach fruit has been described as a climacteric fruit, since its ripening is accompanied by ethylene and respiration burst and significant softening. It will easily become soft within 2–3 days at room temperature after harvest (Xi et al., 2012). 1-Methylcyclopropene (1-MCP) as an ethylene action inhibitor can bind to ethylene receptors with 10 times more affinity than ethylene itself, and therefore is an effective ethylene-antagonizing compound and is used to prolong storage or shelf-life of various respiratory peak fruit and vegetables (Blankenship and Dole, 2003; Watkins, 2006). Previous studies on peach fruit have shown that 1-MCP is highly effective on maintaining flesh firmness and acidity, decreasing respiration and ethylene production, but it has been also reported to decrease juiciness and to increase the incidence of some physiological disorders (Fan et al., 2002; Ku and Wills, 1999; Li et al., 2001). 1-MCP has also been extensively used on other climacteric and non-climacteric fruits. For instance, the application of 1-MCP on pear showed that 1-MCP could prevent pear from superficial scald, friction browning and impact bruising (Calvo and Sozzi, 2004; Ekman et al., 2004). Various studies of 1-MCP effect on the storage of banana fruit found that 1-MCP played a crucial role in extending the shelf-life of fruit (Baezsañudo et al., 2009; Harris et al., 2000; Golding et al., 1999; Pelayo et al., 2003; Trivedi and Nath, 2004). However, there have also been some unfavorable effect of 1-MCP application such as inhibited the biosynthesis of volatile esters and alcohols on 'Golden Delicious' apple (Fan et al., 2002) and on other fruits (Ortiz et al., 2010).

Fruit volatiles produced by main fatty acid pathway comprised of lipoxygenase (LOX) and β-oxidation pathways. LOX as a catalyst can

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convert C_{18} polyunsaturated fatty acids into hydroperoxides and then flavors such as n-hexanal and (E)-2-hexenal produced from the cleavage of hydroperoxides catalyzed by hydroperoxide lyase (HPL). Finally, alcohol dehydrogenases (ADH) transform C_6 and C_9 aldehydes into corresponding alcohols. Various esters are synthesized by alcohol acyltransferases (AAT) to combine various alcohols and acyl-CoAs (Schwab et al., 2008). Researches have showed that gene expression and enzyme activity has a close relationship with the synthesis of volatiles in fruits (Aharoni et al., 2000; Manríquez et al., 2006; Tijet et al., 2000; Zhang et al., 2009).

Peach aroma has been studied for a long time, some researches mainly aimed at detecting peach aroma content through new technologies and methods (Bianchi et al., 2017; Rizzolo et al., 2013). However, some other researches largely focused on the effects of post or preharvest treatments and postharvest storage methods on peach aroma from different aspects. As one of the most widely used postharvest handling techniques, there have been fewer reports focused on the function of 1-MCP on aroma volatiles of peach fruit than other fruit, and peach aroma on these papers were studied just from related compounds and enzymes level (Ortiz et al., 2010; Qian et al., 2013). Cold storage, a common storage method for peach, has also been studied together with peach aroma from physiological, biochemical and molecular level (Raffo et al., 2008; Shinya et al., 2014; Zhang et al., 2011). However, little is known about the effect of 1-MCP on flavour-related volatiles and the relationship with the expression of relevant genes of involved in fatty acid pathway during the storage of cold. Further more, the use of 1-MCP treatment and cold storage has seldom been documented on the analysis of volatiles in peach fruit. In the present investigation, volatile production and gene expression patterns of LOX, HPL, ADH, AAT based on real-time PCR subjected to 1-MCP treatment were determined using white-fleshed melting peach fruit. Besides, quality indicators including firmness, TSS and volatiles and the activity of involved enzymes were measured during cold storage. On the basis of these findings, the possible roles played by 1-MCP in regulating volatile formation in postharvest peach fruit were assayed.

2. Materials and methods

2.1. Plant materials and treatments and sampling

The experiments were conducted with melting flesh type peach fruit (*Prunus persica* L. cv. Xiahui 6)harvested at commercial maturity from Jiangsu Academy of Agricultural Sciences orchard, Nanjing, Jiangsu Province, China. About four hundred fruits uniform in size and without visible defects or decay were transported to the laboratory on the day of harvest. The fruits were randomly divided into two groups, one group was treated with 10 μL L^{-1} 1-MCP (Sinopharm Chemical Reagent Beijing Co., Ltd, China) at room temperature (25 ± 1 °C) for 12 h as described by Jiang et al. (2014) and another group was meanwhile placed at the same temperature for same time. Both control and treated groups were stored, subsequently, at 4 ± 0.5 °C with 85–90 % relative humidity for 30 days. Evaluated samples (10 fruits for each sample and replicated 3 time) were taken from each group at the interval of every five days for quality, physico-chemical, biochemical and molecular analysis.

2.2. Evaluations of fruit ripening

For measurement of ethylene production, a method described by Khan (2008) was adopted. Three replicates of five fruits each were enclosed in 3.18 L glass jars and capped with rubber stoppers for 1 h at each sampling time. 1 mL of headspace gas was taken out with a syringe from each jar and injected into a gas chromatograph (Agilent Technologies 7890A) fitted with flame ionization detector (FID) and a HP-AL/S column (30 m × 0.53 mm × 15 μm, Agilent, USA). Nitrogen was used as a carrier gas with a flow rate at 3 mL min^{-1}. The temperature settings of oven, inlet and detector were 100, 120 and 200 °C, respectively. Ethylene were identified by the peak time of the ethylene standard and quantified by contrasting to the ethylene standard curve, and the results were expressed as nmol kg^{-1} s^{-1}.

Fruit firmness was measured using a Texture Analyzer (TA.XT. Plus, America) calibrated with a 8.0-mm-diameter probe head at 10 mm final penetration depth. Two measurements were made on the opposite sides at the equator of each of 15 fruits selected randomly from 30 fruits after the removal of a 1 mm-thick slice of skin, and the results were expressed in newtons (N).

Soluble solid content (SSC) was determined with a digital hand-held refractometer (Atago, Tokyo, Japan) to which the juice extracted from the same 5 fruits that had been used to assess firmness was applied, and the data were expressed as percentage (%).

After the measurement of ethylene, firmness and SSC, the flesh from the same samples was gathered and immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

2.3. Fruit volatile analysis

The volatile compounds of peach fruit were determined using GC–MS based on solid-phase micro-extraction (SPME) according to a method of Zhang et al. (2010) with some modifications. At each sampling point of storage, 7 g homogenate taken from sarcocarp of fifteen fruits was transferred to a 20 mL vial and was homogenized with 3 mL saturated sodium chloride. Subsequently, the vials were immediately transferred to -80 °C refrigerator until volatiles analysis. For the assay of volatiles, the vials were taken out of refrigerator and thawed at ambient temperature. After the samples was equilibrated at 40 °C for 20 min, a fiber coated with 65 μm of polydimethylsiloxane and divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, USA) was used for volatile compounds extraction at 40 °C for 30 min. The volatiles adsorbed on the extraction head were then desorbed over 5 min at 240 °C into the splitless injection port of the GC. The volatile compounds of peach fruit were analyzed depending on GC–MS (7890A-5795C, Agilent, USA) fitted with HP-5MS UI column (30 m × 0.25 mm × 0.25 μm, Agilent, USA). The other conditions were set as follows: the oven temperature was firstly held at 40 °C for 3 min, and increased at a rate of 3 °C min^{-1} to 130 °C and kept 2 min, and finally increased to 240 °C by 8 °C min^{-1}. Helium was used as carrier gas at flow rate of 1.0 mL min^{-1}. Electronic ionization was used at 70 eV. Detection was performed in full scan mode, from 29 to 540 amu. Since standards were not available, the identification was determined using the NIST 2008 libraries (the minimum matching requirement was 80%). Peak area percentage was used to describe the results as relative content.

2.4. Enzyme activity analysis

The activity of LOX was measured using the method described by Axerold et al. (1980) with slight modifications. 2 g frozen pulp tissue was milled into fine powder in liquid nitrogen and was blended with 6 mL pH 7.0 50 mM phosphate extraction solution. 10 mM sodium soyate was performed as the substrate. 0.2 mL crude enzyme was added to substrate in 2.7 mL phosphate buffer system, and the increase of one unit in absorbance at 234 nm per minute of per milligram protein was expressed as one U of LOX enzyme activity, and the LOX enzyme activity was expressed as specific activity (U mg $protein^{-1}$).

HPL enzyme activity was analyzed in accordance with Vick (1991) with some modifications. Hydroperoxidelyase assay mixture was consisted of 2 g frozen flesh powder, 6 mL extraction buffer containing 150 mM HEPES-KOH (pH 8.0), 250 mM sorbitol, 10 mM ethylene diamine tetraacetic acid (EDTA), 10 mM $MgCl_2$, 1% V/V glycerin, 4% PVP and 0.1 mM PMSF as a protease inhibitor. Then the homogenate was centrifuged at 15,000 g for 30 min at 4 °C. Linoleate sodium hydroperoxide was considered as the substrate prepared as follows: 10 mL distilled water, 200 μL 10 mM linoleic acid sodium, 400 μL LOX