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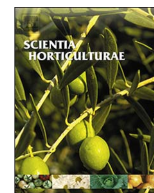

Regulation of apricot ripening and softening process during shelf life by post-storage treatments of exogenous ethylene and 1-methylcyclopropene

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ABSTRACT

Post-storage applications of exogenous ethylene and 1-methylcyclopropene (1-MCP) were performed in cold-stored apricot to regulate the ripening and softening process during shelf life. The results showed that post-storage ethylene treatment could accelerate SSC accumulation, organic acids degradation, color change and ripening process of cold-stored apricot, and could effectively improve the consumer acceptability of the fruit. Post-storage 1-MCP treatment strongly suppressed ethylene production in apricot during shelf life, causing the suppression of expression levels of pectin related genes encoding for pectin degraded enzymes, including polygalacturonase, β-galactosidase and pectin methyl esterase. Microscopic observation provided a direct evidence that post-storage treatment of 1-MCP delayed pectin degradation and middle lamella dissolution of apricot, and delayed the softening process during shelf life. The regulation of ripening and softening process of apricot with post-storage treatment of ethylene or 1-MCP could be effective for improving consumer acceptability or delaying fruit softening of the fruit.

1. Introduction

Apricot (*Prunus armeniaca* L.) is a climacteric fruit and is prone to rapid ripening after harvest, leading to limited shelf life. Large numbers of postharvest losses exist, when apricots are in abundant supply on the market during the short harvest season. Thus, apricots are usually harvested at low maturity, held in cold storage for some time and then moved to market, to extend the supplying period. Apricot can be stored at 0 °C for more than 1 month and still maintains acceptable firmness (Stanley et al., 2013). However, once removed from cold storage to room temperature, the fruit softens rapidly and becomes unedible (Infante et al., 2008).

Climacteric fruit usually exhibits a rapid increase in ethylene production rate when removed from cold storage to room temperature, which may accelerate the softening process or lead to physiological disorders of the fruit during shelf life (Kan et al., 2011). For apricot, delayed softening process during cold storage followed by rapid fruit softening out of storage, has been previously reported in ‘CluthaGold’ and ‘Larclyd’ cultivars. Physiological disorders, such as juiciness loss and mealiness formation, happened when apricot were held at 20 °C after cold storage at 0 °C for 6 weeks (Stanley et al., 2013). Until now, few knowledge is known about the regulation of ripening and softening process of apricot during shelf life after cold storage by controlling the production of ethylene in apricot.

1-Methylcyclopropene (1-MCP) can bind to ethylene receptors in plant cells and inhibit ethylene action of fruit by suppressing the genes expressions related to ethylene biosynthesis, such as ETR, CTR, ERS, ACO, so on. (Cheema et al., 2013; Tatsuki et al., 2009). It has been widely demonstrated that the postharvest treatment of 1-MCP can effectively delay fruit ripening and softening process, and prolong shelf life of various fruits (Bulens et al., 2012; Pereira et al., 2015; Rizzolo et al., 2015). The effect of postharvest 1-MCP treatment on fruits is involved in many factors, including fruit cultivars (Pan et al., 2016), harvest maturity (Rupavatharam et al., 2015), concentration (Manganaris et al., 2007) and exposure time (Cao et al., 2009) of 1-MCP, storage conditions (Tatsuki et al., 2011), which has also been widely reported. However, few literatures have paid attention to the post-storage application of 1-MCP on shelf life of fruits. Pan et al. (2016) reported that the ethylene treatment could improve the marketing acceptance of 0 °C stored plum during the subsequent shelf life at 25 °C, and post-storage 1-MCP treatment effectively inhibited fruit softening. Until now, no one has tried to suppress fruit softening of cold-stored apricot during shelf life by post-storage treatment of 1-MCP.

As is well known, less mature fruit following cold storage usually exhibits poor sensory quality and negative consumer acceptability during marketing because of the slow and delayed ripening process under low temperature. For apricot, the unripe fruit also appears to show low acceptability and quality attributes, after cold storage for

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more than 28 d (Infante et al., 2008). Ethylene, as one of the most important phytohormones, is recognized to play an essential part in fruit ripening (Zhu et al., 2015) and some reports exhibited that small amounts of exogenous ethylene could rapidly accelerate ripening process even at low temperature (Hertog et al., 2016). The ripening capacity development of 1-MCP treated pears could be stimulated by post-storage ethylene adjustment (Xie et al., 2016). Therefore, the problem of poor marketing quality in cold-stored apricot caused by slow ripening process is likely to be solved by post-storage treatment of exogenous ethylene.

The aim of this study was to improve the marketing quality of cold-stored apricot by post-storage treatment of exogenous ethylene, and to regulate the softening process by post-storage treatment of 1-MCP. The softening mechanism of apricot during shelf life was further investigated as affected by post-storage treatments of 1-MCP and exogenous ethylene.

2. Materials and methods

2.1. Fruit material and post-storage treatments

Apricots (*Prunus armeniaca* L. cv. Shushanggan) were harvested at June 29, 2016 at commercial maturity (firmness: 8.8–12.0 N; soluble solid content: 13.7-15.8%) from local orchard in Yining, Xinjiang, China. Sixty kg similar fruit without physical injuries were picked for uniformity in shape, color, and size. After pre-cooling at 5 °C for 24 h, the fruits were placed in plastic baskets and packed with polyethylene bags (thickness: 40 μm). All fruits were stored at a near freezing temperature range of -1.9 ± 0.2 °C. After cold storage for 30 d, the apricots were transferred to 25 °C for 6 h for warming-up, and divided into three groups of control, 1-MCP and ethylene treatment. According to previous method (Pan et al., 2016), the three groups of apricots were sealed in three 150 L stainless steel containers, and respectively exposed to air, 1.0 $\mu L\,L^{-1}$ of 1-MCP (Cao et al., 2009) and 500 $\mu L\,L^{-1}$ of ethylene (Pan et al., 2016). After treated for 12 h in the dark, all groups of apricots were transferred to shelf life at 25 °C.

2.2. Measurement of ethylene production and respiration rate

Ethylene production and respiration rate were determined according to previous study (Fan et al., 2018). Apricots were sealed in a 2 L glass container for 2 h at 25 °C. One mL of headspace gas inside the glass container was collected for injection into a gas chromatograph (GC-7890F, Shanghai Techcomp Bio-equipment Ltd, China), which was equipped with a flame ionization detector (FID), a methane conversion oven and a stainless steel column (internal diameter 3 mm × 2 m length) packed with activated alumina (80/100 mesh). Temperatures of injector, column and detector were set as 60 °C, 120 °C, 150 °C for ethylene measurement and 60 °C, 120 °C, 360 °C for CO_2 measurement, respectively. Ethylene production was calibrated with authentic ethylene gas standards and the results were expressed as $\mu L\,kg^{-1}\,h^{-1}$. For CO_2 measurement, CO_2 was firstly converted to CH_4 and then measured by FID. Respiration rate was calculated using a CO_2 calibration curve and expressed as mg CO_2 $kg^{-1}\,h^{-1}$ (mg $kg^{-1}\,h^{-1}$). Measurements were conducted in three biological replicates.

2.3. Measurement of firmness, soluble solids content (SSC), color change, sugars, organic acids and ripening index

Firmness of apricot flesh was measured on 2 equidistant peeled sites on the equatorial axis of each fruit using a sclerometer (GY-2, Zhejiang Top Instrument Co., Ltd, China) with a diameter 3.5 mm probe. Color parameters *L* (lightness), *a* ('-green' to ' + red') and *b* ('-blue' to ' + yellow') of apricot pulp were determined by a precision colormeter (NR110, 3NH Technology Co., Ltd, China). Color change$\triangle E = \sqrt{(\triangle L)^2 + (\triangle a)^2 + (\triangle b)^2}$. SSC was performed by a digital saccharimeter (PAL-1, Zhejiang Top Instrument Co., Ltd, China).

The sensory analysis was performed according to Echeverría et al. (2015) with some modifications. A taste panel, consisting of 100 consumers, was asked to score how they perceived visual appearance, sweetness, sourness, flavour, firmness and juiciness of apricots using a nine-point hedonic scale (1 = very low intensity; 3 = low intensity; 5 = medium, 7 = high intensity; 9 = very high intensity). At last, consumers were asked to score the overall acceptability of apricots as consumer acceptability. Consumers were students from the College of Food Science and Nutritional Engineering, China Agricultural University, China. The whole process was performed at room temperature (20 °C) in individual booths under white illumination.

Sugar compositions were determined by HPLC method (Wang et al., 2016) with Shimadzu LC-20AB pumps, RID-10A refractive index detector, and an amino column (Innoval Durashell NH_2, 4.6 mm × 250 mm ID, 5.0 μm, Agela Technologies Inc., China). Organic acids were determined by ion chromatography method (Geng et al., 2008) with a Thermo Fisher ICS-1100 system, a conductivity detector, a guard column (IonPac AG23, 4 mm × 50 mm), an anion exchange analytical column (IonPac AS23, 4 mm × 250 mm), an electrolytically regenerated suppressor (AERS 500, 4 mm) and RFC-30 reagent-free controller with ECG-MSA Cartridge. The ripening index was expressed as the ratio of total sugars/total acids.

2.4. Measurement of pectin fractions

Apricot pulp tissues were firstly homogenized in liquid nitrogen by a grinding equipment (A 11 basic, Guangzhou IKA works, China) and the alcohol insoluble residue was extracted according to previous method (Xie et al., 2017). 0.5 g The alcohol insoluble residue was mixed with 200 mL deionized water, followed by shaking for 4 h and centrifugation at 12,000 g for 30 min. The extraction was repeated for two times and the supernatant was collected as water-soluble pectin (WSP). Subsequently, the residue was extracted twice with 200 mL of 50 $mmol\,L^{-1}$ CDTA solution (containing 100 $mmol\,L^{-1}$ NaAc), followed by shaking and centrifugation. The supernatant was collected as CDTA-soluble pectin (CSP). The final residue was extracted twice with 200 mL of 50 $mmol\,L^{-1}$ Na_2CO_3 (containing 20 $mmol\,L^{-1}$ $NaBH_4$) for Na_2CO_3-soluble pectin (NSP). Each pectin fraction was adjusted to pH 6.5 and dialyzed against distilled water (molecular cut-off of 3000 Da) three times for 24 h. The solutions of pectin fraction were concentrated by a rotary vacuum evaporator (RE-52, Shanghai Yarong Biochemistry Instrument Factory, China) at 30 °C and diluted with deionized water to 200 mL. The pectin contents were determined by carbazole colorimetry (Bu et al., 2013) and the absorbance was read at 530 nm by a UV–vis spectrophotometer (T6-1650E, Beijing Puxi instrument Ltd, China). The pectin content was calculated by a standard curve of D-(+)-galacturonic acid.

2.5. Activities of pectin degrading enzymes

Polygalacturonase (PG) and β-Galactosidase (β-GAL) were extracted and determined according to previous method (Phetsirikoon et al., 2016). 1 g of homogenized apricot sample was ground with 8 mL of 40 $mmol\,L^{-1}$ cold NaAc (pH=5.5) and 2 mL of 0.2 $mol\,L^{-1}$ NaCl (containing 2% PVPP, w/v), followed by standing at 4 °C for 15 min and centrifugation at 12,000 g for 30 min. The supernatant was collected as enzyme extract.

For PG activity, 0.1 mL of the enzyme extract was mixed with 0.5 mL of 0.2 $mol\,L^{-1}$ cold NaAc (pH=4.5) and 0.4 mL of 1% (w/v) pectin solution, followed by standing at 37 °C for 1 h. 1 mL of DNS reagent added and the mixture was then boiled for 5 min. After cooling down, the mixture was read at 540 nm. For β-GAL activity, 1 mL of the extract was mixed with 1 mL of cold 50 $mmol\,L^{-1}$ NaAc (pH=5.0) and

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