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Changes in phenolics and antioxidant property of peach fruit during ripening and responses to 1-methylcyclopropene

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ABSTRACT

This study investigated the postharvest characters, phenolic compounds and total antioxidant activities (TAA) in response to 1-methylcyclopropene (1-MCP) during the ripening process of peach (*Prunus persica* L. cv. Yuhualu) fruit. Peaches were treated with air (control) or 5 μL L^{-1} of 1-MCP for 24 h, followed by storage for up to ten days at 20 °C. 1-MCP treatment best retained firmness, soluble solids, titratable acidity and ascorbic acid. Additionally, ethylene production and respiration rate were delayed. Moreover, treatment with 1-MCP effectively postponed the onset of peak values of phenolic compounds positively identified in the peach fruit. TAA is an important nutritional attribute in the human diet. Our study showed that 1-MCP delayed the increase of antioxidant activity and suppressed TAA during the prolonged ripening period. These results demonstrated that 1-MCP treatment is a good practice for maintaining fruit quality, but may have complex effects on phenolic metabolism and antioxidant activity.

1. Introduction

Peach (*Prunus persica* L.) fruit, belongs to the Rosaceae family, has been indigenous to China over 3000 years and now is cultivated widely in appropriate climates around the world (Lurie and Crisosto, 2005). Peach fruit is considered to be one of the most important and popular commodities consumed worldwide due to its delicious flavor and attractive appearance as well as exhibiting a high nutritional value because of its high levels of major antioxidants and anticarcinogenic compounds, including vitamins (A, C and E), carotenoids and phenolics (Durst and Weaver, 2013; Gil et al., 2002). Among these bioactive compounds, phenolics have gained more attention in recent years due to their remarkable antioxidant properties. Antioxidant phenolics, by virtue of their hydrogen and electron donating abilities and metal chelating effects, exhibit a wide range of biological properties including anti-inflammatory, anti-microbial, anti-allergenicity, anti-thrombotic, cardioprotective and vasodilatory actions (Middleton et al., 2000; Puupponen-Pimiä et al., 2001). Epidemiological studies have elucidated that dietary intake of peach can suppress the generation of reactive oxygen species in human plasma (Sun et al., 2002) and provide protection from certain chronic diseases (Noratto et al., 2009).

Peach fruit, a living organism with high metabolic activity, succumbs to rapid quality decreases after harvest. Ethylene, as one of several plant growth regulators, can affect growth and developmental processes including ripening and senescence. During postharvest storage, ethylene can cause negative effects including senescence, accelerated quality loss, reduced nutrient composition, increased fruit pathogen susceptibility, and physiological disorders in fruit and vegetables (Martínez-Romero et al., 2007). Therefore, the control of ethylene production and action is an important component in postharvest management. 1-Methylcyclopropene (1-MCP) is thought to interact with ethylene receptors and thereby prevent ethylene-dependent responses (Sisler and Serek, 1997). When applied at the correct time, 1-MCP has been demonstrated to protect fruit and vegetables from exogenous and self-produced ethylene, increasing their postharvest life and providing more flexibility during storage, distribution and retail (Watkins, 2006). Recently, a plethora of studies have verified the usefulness of 1-MCP in delaying ripening and extending shelf life of climacteric fruit. In peaches, 1-MCP treatment can effectively delay ripening, lower ethylene production and respiratory rates and maintain good quality (Blankenship and Dole, 2003; Hayama et al., 2008; Liu et al., 2005).

Despite the rapid adoption of 1-MCP based technology, little is known about its effect on the phenolic compounds and antioxidant capacity of peach fruit. Previous work has shown that the peel tissues of 1-MCP treated 'Delicious' apples had higher concentration of total flavonoid and lower value of

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chlorogenic acid than untreated fruit (MacLean et al., 2006). While 1-MCP maintained anthocyanin concentrations, the flavonols and flavan-3-ols were unaffected by treatment (MacLean et al., 2006). The studies by Larrigaudière et al. (2004) and Chiriboga et al. (2013) indicated that the fruit treated with 1-MCP exhibited higher antioxidant potential, but the research was restricted to related enzymes, especially CAT, SOD, POX and ascorbate peroxidase.

The focus of current research in the nutrition literature for peach fruit was mostly toward phenolics. Because of the complexities of phenolic metabolism, reports on relationships of this compound with physiological disorders of 1-MCP treated peach fruit are still limited and inconsistent. This is hampered even further due to variability in the methods which are used to quantify the different phenolic compounds. The objective of the present study, for the first time, was to investigate the effect of a treatment with 1-MCP on the phenolic metabolism and antioxidant potentials of early-maturing melting peach fruit (cv. Yuhualu) during ripening and senescence process.

2. Material and methods

2.1. Fruit material and experimental design

Preclimacteric peach (*P. persica* L. cv. Yuhualu) fruit were obtained from an experimental orchard in Beijing, China. Harvested fruit were immediately delivered to the laboratory and those with uniform size and without visual defects and were randomly divided into two lots of 270 fruit for the following treatments in triplicate (90 fruit per replicate): control or 1-methylcuclopropene-treated (1-MCP). Our preliminary experiments showed that 5 $\mu L\,L^{-1}$ of 1-MCP (EthylBloc, Rohm and Haas China, Inc.) was the optimal concentration for maintaining peach quality. Therefore, in this work, the treated group was fumigated with 5 $\mu L\,L^{-1}$ of 1-MCP in air-tight plastic chambers (120 L) at 20 °C for 24 h in the dark, while the control group was kept under the same condition without 1-MCP. After treatment, all experimental groups were transferred to storage at 20 °C with 90% ± 2% relative humidity (RH) for ten days. The samples were removed on alternate days for further analysis. Some samples were used, freshly, for analyses of firmness, ethylene production, respiration rate, soluble solids and titratable acidity, while the other samples were frozen for lyophilization and storage at −40 °C for further analyses of ascorbic acid, phenolics and antioxidant capacity.

2.2. Chemicals and reagents

Gallic acid, Folin–Ciocalteu's reagent, L-ascorbic acid, β-carotene, dithiothreitol (DTT), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox, a hydrophilic derivative of tocopherol), linoleic acid, Tween 20, neocuproine were purchased from Sigma–Aldrich Chemical Co., (St. Louis, MO, USA). The phenolic standards of protocatechuic acid, chlorogenic acid (5-*O*-caffeoylquinic acid), neo-chlorogenic acid (3-*O*-caffeoylquinic acid), (+)-catechin and quercetin-3-rutinoside were from Fluka-Sigma–Aldrich® (St. Louis, MO, USA). Methanol, acetone, chloroform, acetic and formic acid of HPLC grade were supplied by Merck (Darmstadt, Germany). Potassium persulfate, sodium phosphate, iron (III) chloride, iron (II) chloride tetrahydrate, Iron (II) sulfate heptahydrate and other chemicals used were of standard analytical grade. Deionized and distilled water was used throughout.

2.3. Fruit quality assessment

2.3.1. Ethylene production and respiration rate

To determine the ethylene production and respiration rate, three 1 L airtight glass jars were used. Each subsample of three fruit from each treatment were enclosed in a glass jar hermetically sealed with a rubber stopper for 2 h at 20 °C. Thereafter, one milliliter of the gas sample was withdrawn with a gas syringe from the jar, and was quantified for ethylene using a GC-7890F gas chromatograph (Tianmei, Shanghai) equipped with a FID detector, or quantified for CO_2 with a TCD detector.

2.3.2. Firmness, soluble solids content (SSC) and titratable acidity (TA)

Six fruit for each replicate (each treatment having 3 replicates) were taken at random from each treatment at the sampling date. Fruit firmness was measured on two pared surfaces with a digital force gauge (GY-4, Tuopu Instrument Co., Ltd., Zhejiang, China) with an 11 mm probe. Soluble solids contents (SSC) were determined by extracting and mixing one drop of juice from each fruit by a digital refractometer (Jenway-6405 UV/V) at 20 °C. Titratable acidity (TA) was measured as reported earlier (Terada et al., 1978).

2.3.3. L-ascorbic acid (vitamin C) content

L-ascorbic acid was extracted as method (Yurena Hernández et al., 2006) with some modifications. Freeze-dried peach samples (1.0 g) were extracted with 10 mL cold NaH_2PO_4 buffer solution (20 mM, pH 2.1, containing 1 mM EDTA). The resulting mixture was sonicated for 15 min and centrifuged at 12,000 × *g* for 20 min at 4 °C. This procedure was repeated two times and the resulting supernatants were collected and brought to final volume of 20 mL. Immediately, 800 μL of the filtered extract (0.2 cm cellulose syringe filter) was added to 200 μL of DTT (20 g L^{-1}) as a reducing agent, left for 2 h at 4 °C before analysis. Precautions were made to avoid light exposure throughout the whole procedure. L-ascorbic acid was determined by HPLC method using a C18 column (Shim–pack VP-ODS 15 cm × 4.6 mm ID, 5 μm, Shimadzu Co., Japan) where 10 μL was injected for each sample. The elution was conducted isocratically using a mixture of 90% formic acid (0.1%, v/v) and 10% methanol at a flow rate of 0.8 mL min^{-1}. The quantification was performed from the peak areas recorded at 245 nm with reference to the calibration curve obtained with L-ascorbic acid reference.

2.4. Fruit extraction and sample preparation

Freeze-dried peach flesh was extracted following conditions previously selected (Pérez-Jiménez and Saura-Calixto, 2005) with slight modifications. One gram of the sample was placed in a capped centrifuge tube and 20 mL of acidic methanol-water (50:50, v/v, pH 2) were added, after which the tube was shaken at room temperature for 1 h. The tube was centrifuged at 12,000 × *g* and the supernatant was recovered. Then 20 mL of 70% aqueous acetone were added to the residue, followed by stirring, shaking and centrifugation. The methanolic and acetonic extracts were combined and evaporated to dryness under vacuum at 30 °C. Phenolic compounds extracted were reconstituted in 20 mL of deionized water and stored at −20 °C until used before 24 h.

2.5. Determination of total phenolics and anthocyanins content

Total phenolic content in the extracts was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965). The TPs contents were expressed as mg kg^{-1} on a fresh-weight basis.