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Improvement of flavour quality and consumer acceptance during postharvest ripening in greenhouse peaches by carbon dioxide enrichment

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ABSTRACT

In this study we assessed the impact of carbon dioxide enrichment (CDE) on flavour quality development of peach fruit, using peach trees grown in a greenhouse with a carbon-dioxide-enriched atmosphere. Fruit sugar, organic acids, volatiles contents and consumer acceptability were investigated, focusing on the period of postharvest ripening. Higher levels of sucrose, lactones, norisoprenoids, and lower levels of malic acid were found in CDE-treated fruit than those in the control fruit grown under normal conditions. We also measured significantly elevated amounts of pyruvic acid, precursors of volatile compounds, linoleic acid and linolenic acid as a result of CDE. Additionally, CDE-treated fruit were relatively well accepted by consumers compared to the control fruit. These results suggested that CDE can markedly improve the flavour quality and consumer acceptance of greenhouse-grown peaches. The possible mechanism could be that CDE increased precursors available for the biosynthesis of flavour compounds through regulation of photosynthesis.

1. Introduction

Peach (*Prunus persica* L. Batsch) fruit flavour is a consequence of the biosynthesis of a diverse set of phytochemicals, including sugars, acids, and numerous volatiles (Kader, 2008; Klee, 2010). The sweetness of peaches results from the accumulation of sucrose, sorbitol, glucose, and fructose, with sucrose being predominant at fruit maturity, followed by glucose and fructose, and lower levels of sorbitol (Wu, Quilot, Kervella, Génard, & Li, 2003). The main acids in peach fruits are malic, citric, and quinic acid, which contribute to the fruit sourness (Wu et al., 2003), while peach aroma depends on more than 100 volatile compounds, of which about 25 contribute to the typical peach aroma. In particular, γ- and δ-decalactone play a key role, together with C6 compounds, alcohols, esters, terpenoids, and phenolic volatiles (Sánchez et al., 2013).

The biosynthesis of flavour compounds is reliant on primary and secondary metabolites derived from photosynthesis (Carrari & Fernie, 2006). In this regard, pyruvic acid, a central carbon compound in primary metabolism, is usually considered an important precursor of sugars, organic acids, and fatty acids (Jardine et al., 2010), while the fatty acids, such as linoleic (18:2) and linolenic acid (18:3), represent precursors of aroma compounds. Thus, levels of pyruvic acid, linoleic acid and linolenic acid in fruit can affect the biosynthesis of flavour compounds and have a major influence on flavour quality development (Carrari & Fernie, 2006). To date, numerous studies have described the influence of factors, such as genetic variation, maturation and postharvest handling on fruit flavour quality (Eduardo et al., 2013; Illa et al., 2011; Ortiz, Graell, López, Echeverría, & Lara, 2010; Wang et al., 2009; Xi et al., 2012). However, while the effect of fertilisation (Jia, Hirano, & Okamoto, 1999) and bagging (Jia, Araki, & Okamoto, 2005) on flavour quality have been described, cultivation practices and technologies that affect flavour quality during fruit development are rarely reported.

Greenhouses are widely used for peach cultivation in northern China due to the protection that they provide from low temperatures and their promotion of early fruit maturation. However, peach fruit cultivated in greenhouses generally exhibit poor flavour and often lack significant aroma. It has been observed that

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at night, CO_2 levels in greenhouses generally increase above average values, but decrease below 200 ppm during the day, especially from 10:00 to 14:00 (Castilla, 2013); thus low CO_2 levels may be a limiting factor for the productivity of fruit trees cultivated in greenhouses. To counter such effects, carbon dioxide enrichment (CDE) has been used for many horticulture crops, such as strawberry, grape, tomato and peach (Castilla, 2013); however, little is known about the effect of this technology on the development of fruit flavour quality, which involves the synthesis of carbon compounds that act as precursors for the volatile aroma constituents.

The objective of this current study was to investigate the effectiveness of CDE for improving fruit flavour and customer acceptance of greenhouse-grown peaches. The findings presented here provide insights into the importance of greenhouse carbon dioxide regimes for fruit quality and will help provide additional options to enhance peach flavour quality.

2. Material and methods

2.1. Carbon dioxide enrichment

The experimental orchard was located in Yanji County, Xinjiang Autonomous Region, Kuerle, China (45° 19′ N, 86° 03′ E). 'Zaolupantao' flat peach trees were planted in 2007 in rows in a north–south orientation, with a distance of 2–3 m between rows. Fertilisation management and pest control were carried out according to standard practices and drip irrigation was used to supply fruit trees with water (Xi, Cui, Ya, & Yu, 2007). A hermetic barrier wall was used to divide the greenhouse into two parts: one area with a CO_2-enriched atmosphere, and the other with ambient air as a control. The barrier wall can prevent gas exchange between the two areas. An automatic MIC-300-CO_2-IR CO_2 compensation system (Shenzhen Alenson Electronic Co., Ltd., China) was used to enrich the atmosphere in CO_2 from 12:00 to 16:00 each day and the CO_2 levels of the greenhouse atmosphere were maintained at 360 ppm during the main CO_2 shortage period (from the first day of April to the end of April, 2009, Fig. S1). To keep CO_2 concentration homogeneity within the greenhouse, the CO_2 was transported to every tree top by plastic pipe pore.

2.2. Chemicals

Citric acid, malic acid, quinic acid, shikimic acid, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, γ-hexalactone, γ-heptalactone, γ-octalactone, δ-octalactone, γ-jasmolactone, γ-decalactone, δ-decalactone, γ-dodecalactone, β-damascone, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were obtained from Sigma (St. Louis, MO). Pyruvic acid, *n*-hexanal, (*E*)-2-hexenal, (*Z*)-2-hexenal, hexyl acetate, 3-hydroxy-β-ionone, 3-hydroxy-7,8-dihydro-β-ionone, 3-hydroxy-5,6-epoxy-β-ionone, 3-hydroxy-β-damascone, β-damascenone were obtained from Fluka (Buchs, Switzerland). Fructose, sucrose, glucose, sorbitol and other reagents were analytical grade and purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China).

2.3. Plant materials

Fruit were harvested at the commercial maturation stage. Fruit maturity was evaluated based on skin colour analysed using a MiniScan XE plus (Hunter Associates Laboratory Inc., Reston, VA). Fruit with colour values of $L = 73.3 \pm 3.18$, $H_{ab} = 62.66 \pm 4.11$, $C^* = 59.14 \pm 1.42$ were transported to the laboratory; fruit with uniform size, and without visible disease or damage were selected for use in the experiment. The control and CDE-treated groups with 500 fruit each group were stored at 20 °C for seven days. The relative humidity was kept at 92–98% using a CS-20Z digital automatic humidity controller (HangZhou ZhengDao Electrical Appliance Co. Ltd., China). During storage period (0–7 days), 40 fruit were taken out from the storage room every day for determining ethylene, firmness, total soluble solids (TSS) and titratable acidity (TA). Ninety fruit from at harvest (0d) and the fifth day after harvest (5d) were used to determine flavour compounds, enzyme assays, pyruvic acid and fatty acid analysis and sensory measurement.

2.4. Determination ethylene, firmness, total soluble solids and titratable acidity

The production of ethylene by the fruit was measured by placing ten fruits as one replicate in a sealed 1-L glass container for one hour and three replicates were used for every time point. Gas samples (1 mL) were then extracted from the containers and injected into an Agilent 7820A gas chromatograph (GC; Agilent Technologies, Inc., Santa Clara, CA) fitted with a stainless steel Supelco Porapak-Q column (2 m in length, o.d., 3.175 mm; mesh size, 80/100; Supelco, Bellefonte, PA) and a flame ionization detector (FID). Nitrogen was used as the carrier gas. The column temperature was maintained at 80 °C, the injector temperature at 150 °C, and the detector temperature at 200 °C.

Fruit firmness was measured at the equator of the fruit using a penetrometer (Model: HL-300, Xianlin Non Detection Device Co. Ltd, Nanjing, China) with an 8-mm diameter head. Ten fruit were used as one replicate and two measurements were made on opposite sides of each fruit after removal of a 1-mm thick slice of peel, and three replicates were used for every time point.

TSS and TA measurements were conducted on juice samples collected from ten fruit for one replicate, and three replicates were used. TA measurements were performed by titrating 10 mL of peach juice with 0.2 M NaOH until reaching a pH of 8.2, and TA values were expressed as mmol L^{-1} H^+. TSS values on the opposite parts of each fruit were measured with a hand-held refractometer (Model: B32T Brix Meter, Guangzhou Ruiqi Trade Co. Ltd, Guangdong, China).

2.5. Measurements of sugars, organic acids and aroma volatiles

Sugars and organic acids were extracted as described by Zhang et al. (2005). For each sample, 2 g of frozen peach mesocarp were powdered in liquid nitrogen, and then 5.0 mL of cold ethanol (80%) were added to the powder. The sample was then incubated for 20 min in a 35 °C water bath and centrifuged at 10,000*g* for 10 min. This extraction procedure was repeated three times and the supernatants were combined. The total volume was then adjusted to 25 mL with 80% ethanol. From this, 1 mL was dried under vacuum (Eppendorf Concentrate Plus, Germany) at 45 °C, the residue was resuspended in 0.5 mL distilled water and filtered through a 0.22 μm, 13 mm diameter syringe filter (Shanghaixingya Purification Material Factory, China). The filtered solution was then used for sugar and organic acid analysis.

Chromatographic separation of sugars involved acetonitrile:water (80:20, v/v) as the mobile phase with a flow rate of 1.0 mL min^{-1} with a 5.0 μm NH_2 (4.6 mm × 250 mm) column (GL Sciences Inc., Torrance, CA). Eluted peaks were detected with an RI-1530 refractive index detector (JASCO International Co. Ltd, Tokyo, Japan). Chromatographic separation of organic acids involved $(NH_4)_2HPO_4$ (50 mM, pH 2.7) as the mobile phase, with a flow rate of 0.5 mL min^{-1}, and samples was injected onto an ODS C18 (4.6 mm × 250 mm) column (Beckman Coulter Inc., Brea, CA). Organic acids were detected with a 166 UV–Vis detector (Beckman Coulter Inc.) at a wavelength of 210 nm. Soluble sugars and organic acids were quantified according to standard curves of authentic compounds. Extracts from three triplicate tissue samples were analysed.