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Research article

Deciphering the interplay among genotype, maturity stage and low-temperature storage on phytochemical composition and transcript levels of enzymatic antioxidants in *Prunus persica* fruit

George A. Manganaris [a, *], Pavlina Drogoudi [b], Vlasios Goulas [a], Georgia Tanou [c], Egli C. Georgiadou [a], George E. Pantelidis [b, d], Konstantinos A. Paschalidis [d, e], Vasileios Fotopoulos [a], Athanasios Manganaris [d]

[a] Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology & Food Science, 3603, Lemesos, Cyprus
[b] Institute of Plant Breeding and Genetic Resources, Department of Deciduous Fruit Trees, Hellenic Agricultural Organization 'Demeter', 38 R.R. Station, 59035, Naoussa, Greece
[c] Aristotle University of Thessaloniki, School of Agriculture, 54124, Thessaloniki, Greece
[d] Alexander Technological Educational Institute, Department of Agricultural Technology, 57400, Sindos-Thessaloniki, Greece
[e] Technological Educational Institute of Crete, Department of Agricultural Technology, 71004, Estavromenos, Heraklion, Crete, Greece

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ABSTRACT

The aim of this study was to understand the antioxidant metabolic changes of peach (cvs. 'Royal Glory', 'Red Haven' and 'Sun Cloud') and nectarine fruits (cv. 'Big Top') exposed to different combinations of low-temperature storage (0, 2, 4 weeks storage at 0 °C, 90% R.H.) and additional ripening at room temperature (1, 3 and 5 d, shelf life, 20 °C) with an array of analytical, biochemical and molecular approaches. Initially, harvested fruit of the examined cultivars were segregated non-destructively at advanced and less pronounced maturity stages and qualitative traits, physiological parameters, phytochemical composition and antioxidant capacity were determined. 'Big Top' and 'Royal Glory' fruits were characterized by slower softening rate and less pronounced ripening-related alterations. The coupling of HPLC fingerprints, consisted of 7 phenolic compounds (chlorogenic, neochlorogenic acid, catechin, epicatechin, rutin, quecetin-3-*O*-glucoside, procyanidin B1) and spectrophotometric methods disclosed a great impact of genotype on peach bioactive composition, with 'Sun Cloud' generally displaying the highest contents. Maturity stage at harvest did not seem to affect fruit phenolic composition and no general guidelines for the impact of cold storage and shelf-life on individual phenolic compounds can be extrapolated. Subsequently, fruit of less pronounced maturity at harvest were used for further molecular analysis. 'Sun Cloud' was proven efficient in protecting plasmid pBR322 DNA against ROO• attack throughout the experimental period and against HO• attack after 2 and 4 weeks of cold storage. Interestingly, a general down-regulation of key genes implicated in the antioxidant apparatus with the prolongation of storage period was recorded; this was more evident for *CAT*, *cAPX*, *Cu/ZnSOD2*, *perAPX3* and *GPX8* genes. Higher antioxidant capacity of 'Sun Cloud' fruit could potentially be linked with compounds other than enzymatic antioxidants that further regulate peach fruit ripening.

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1. Introduction

Peach market life is affected by fruit type/cultivar, maturity stage, qualitative attributes, sensorial properties and storage behaviour (Brummell et al., 2004; Cantín et al., 2009; Drogoudi et al., 2016, 2017; Font i Forcada et al., 2014). The term 'quality' implies a grade of excellence and the main attributes that define it on peaches are textural properties, flavor, juiciness, aroma and phytochemical content (Aubert et al., 2014); the latter is now considered as an extra criterion to define quality (Abidi et al., 2015). However, consumption of fresh peaches and nectarines is negatively affected by a series of reasons, such as inappropriate maturity stage at harvest, extensive softening, as well as the incidence of

* Corresponding author.
E-mail address: george.manganaris@cut.ac.cy (G.A. Manganaris).

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chilling related disorders due to extended exposure to low-temperature storage, evident mainly as lack of juiciness and aroma (Brummell et al., 2004; Abidi et al., 2015; Pons et al., 2016). Recent studies identified a possible role of phenolic compounds on cell wall metabolism of chilling-injured peaches (Aubert et al., 2014).

Peach breeding programs additionally focus on chilling resistant cultivars that seems to be positively connected with enhanced antioxidant profile (Abidi et al., 2015). However, many peach cultivars of economic importance are largely unexplored regarding both their qualitative attributes and phytochemical profile. Considering that numerous peach cultivars exist and many are released on a yearly basis (Reig et al., 2013), relatively few studies dealt with phytochemical analysis and are usually restricted to analysis at commercial maturity stage, while limited information about the effect of ripening and/or storage treatments exists (Cantín et al., 2009; Di Vaio et al., 2008; Gil et al., 2002; Tomas-Barberan et al., 2001; Reig et al., 2013). Notably, such studies highlighted the existence of large variation in the phytochemical content and antioxidant capacity of peach germplasm (Cantín et al., 2010; Reig et al., 2013; Vizzotto et al., 2007).

Peach fruit physiology has been extensively studied both during on-tree maturation and during postharvest ripening after harvest or after cold storage. The existence of peach and nectarine cultivars with distinct ripening properties, softening rates and/or storage potential renders it essential to further define their antioxidant potential. To date, most classical or molecular studies on peach fruit have been performed via the application of a single stress condition. The current study tried to dissect the interplay among genotype, maturity stage at harvest and postharvest performance after cold storage on cultivars with distinct ripening and phytochemical properties. Towards this aim, an array of physiological, analytical, biochemical and molecular assays, with special reference to antioxidant genes were employed.

2. Materials and methods

2.1. Fruit material and experimental design

Fruits of three peach cultivars ('Royal Glory', 'Red Haven', 'Sun Cloud') and one nectarine cultivar ('Big Top') were examined. Cultivars were selected based on their economic importance, as well as for their distinct differences in phytochemical status, as elsewhere determined by our group (high antioxidant capacity for 'Sun Cloud', and intermediate for 'Royal Glory', 'Red Haven' and low for 'Big Top', Drogoudi et al., 2016) and interesting ripening traits (i.e. low-softening rate in 'Big Top' cultivar, intense red-blushed color of the exocarp in 'Royal Glory' even from the immature stage).

Fruits from each cultivar were selected the day of harvest upon arrival at the Agricultural Cooperative of Naoussa, based on size and external background color and subsequently transferred to the Department of Deciduous Fruit Trees (Naoussa, Greece). Fruits were separated non-destructively at two distinct maturity stages for each cultivar with the employment of a DA Meter (Sinteleia, Bologna, Italy) and categorized per cultivar as having 'low I_{AD} index' ('Big Top', 0.1–0.3; 'Royal Glory' 0.8–1.0; 'Red Haven' 0.3–0.6; 'Sun Cloud' 0.7–0.9), corresponding to advanced maturity (high maturity, HM) and 'high I_{AD} index' ('Big Top', 0.5; 'Royal Glory', 1.2–1.4; 'Red Haven', 0.8–1.1; Sun Cloud, 1.1–1.4), corresponding to less pronounced maturity (low maturity, LM) stage. Thirteen lots of 24-fruits per cultivar and maturity stage were used. Ten lots were analyzed at harvest or after harvest and additional ripening for 1, 3 and 5 d at room temperature (20 °C, shelf life), as well as after 2 and 4 weeks (w) commercial cold storage (0 °C, 90% R.H.) plus 1, 3 and 5 d shelf life. The remaining three lots were used for non-destructive assessment of color parameters, weight loss, ethylene production and respiration rate at harvest and after removal from 2 to 4 week of cold storage.

Each lot was divided into three eight-fruit sub-lots corresponding to the three biological replications, and subsequently analyzed for qualitative traits [% weight loss (WL), flesh firmness (FF), soluble solids content (SSC) and titratable acidity (TA)]. Intact fruit tissue, derived from wedged-shaped slices was immediately frozen into liquid nitrogen, ground with a pestle to fine powder, and kept at −80 °C for further analyses (phytochemical content, antioxidant capacity, gene expression analysis), as described below.

2.2. Quality attributes, ethylene production and respiration rate

Weight loss (%) was determined by following the formula: (A–B)/A· 100, where A was the fruit weight at harvest and B was the fruit weight after the storage period. Flesh firmness was determined on opposite sides of the equator of each fruit with a penetrometer (Effegi, Ravenna, Italy) fitted with an 8 mm plunger and results expressed in Newtons. Soluble solids content (SSC) and titratable acidity (TA) were determined as elsewhere described (Drogoudi et al., 2016).

Ethylene and CO_2 production rate were measured on a sample of 10 fruit (5 replications x 2 fruit each) per cultivar and measurement date. Two fruits were enclosed into 2 L airtight jars and left at room temperature for 2 h. An 1 mL gas sample was taken from the exit air flow of the jars and injected into a gas chromatograph (model Varian 3300, Varian Instruments, Walnut Cree, CA) equipped with a flame ionization detector and a stainless column to determine ethylene. Another 1 mL gas sample was directed to an infrared CO_2 analyzer (model Combo 280, David Bishop Instruments, UK) for the CO_2 measurement. The results were converted into μL C_2H_4 kg^{-1} h^{-1} and mL CO_2 kg^{-1} h^{-1} for ethylene production and respiration rate, respectively.

2.3. Phytochemical analysis

Five grams of powdered tissue was homogenized in a Polytron with 10 mL extraction solution, comprising of water-methanol (2:8, v/v) and 2 mM NaF to inactivate polyphenol oxidases and prevent phenolic degradation due to browning. Homogenates were kept on ice until centrifuged at 4000 g for 15 min at 4 °C. The supernatant was recovered carefully to prevent contamination from the pellet (Tomas-Barberan et al., 2001). Phytochemical analysis were carried out for both maturity stages at harvest and additional ripening for 1 and 5 d at room temperature (20 °C) after harvest or 2 and 4 weeks cold storage. All results were expressed on fresh weight (FW) basis.

Total phenolics (TPs) content was measured using a modified Folin–Ciocalteu colorimetric method (Singleton et al., 1998). The reaction mixture consisted of 0.5 mL of diluted extract, 5 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. The tubes were vortexed and then allowed to stand at room temperature for 3 min when one mL of saturated sodium carbonate solution was added. The solution was diluted to 10 mL and after 1 h at room temperature the absorbance was measured at 725 nm against a blank solution. Each measurement was repeated in triplicate and total phenolic content was expressed as gallic acid equivalents (GAE).

Total antioxidant capacity (TAC) was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. For the DPPH assay, the procedure followed the method described in Goulas et al. (2014). Briefly, 2 mL of diluted extract were mixed with 1 mL of 0.3 mmol L^{-1} solution of DPPH in methanol, incubated in the dark for 30 min and the absorbance of the mixture was monitored at 517 nm. For FRAP

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