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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

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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.

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

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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

assay, 100 mL of peach extract was mixed with 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} 2,4,6-Tripyridyl-s-Triazine (TPTZ) and 40 mmol L^{-1} $FeCl_3$ $10H_2O$) and was incubated at 37 °C for 4 min and the absorbance was measured at 593 nm (Drogoudi et al., 2016). Standard curves were obtained by using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard solution, and accordingly results were expressed as mmol Trolox equivalents per 100 g^{-1} FW for both assays.

Subsequently, peach extracts were analyzed on a Waters HPLC system comprising of a binary pump, a vacuum degasser, an autosampler, a thermostatted column compartment and a photodiode array (PDA) detector. After filtration on syringe-filter (0.22 μm), 20 μL of each extract were injected into a Waters Sherisorb®, phase ODS 2 (250 mm × 4.6 mm i.d.). The mobile phase consisted of solvents A (10 mL L^{-1} acetic acid in water) and B (acetonitrile). The following elution conditions were applied: 0–5 min, 3–9% B; 5–15 min, 9–16% B; 15–45 min, 16–50% B; 45–50 min, 50% isocratic. The flow rate was 1 mL min^{-1}. According to Orazem et al. (2011), the phenolic acids and flavan-3-ols were analyzed at 280 nm and flavonols were analyzed at 350 nm. A six-level calibration curve was constructed for each of the studied phenolic compounds, with triplicate determinations at each level. The chromatographic peaks were identified by the retention times of the standard compounds.

2.4. The inhibitory effect of peach phenolic extracts on supercoiled DNA breakage induced by hydroxyl radical (•OH) and by peroxyl radical (ROO•)

Mesocarp tissue of the examined cultivars at LM stage were analyzed at harvest and at ripe stage, following a 3-d shelf life after harvest or after removal from 2 and 4 weeks of cold storage. Such material was extracted with 80% methanol aqueous solution (1:3, v/v) to recover phenolic compounds. The homogenate was centrifuged (20000×*g*, 20 min, 4 °C) and the soluble phenolic content was measured at the supernatant, using the Folin-Ciocalteu reagent and expressed as GAE (Scalbert et al., 1989; Asami et al., 2003).

The ability of peach phenolic extracts (100 μM GAE) to protect supercoiled pBR322 plasmid DNA against •OH generated by Fenton's reaction was estimated as described by Hu and Kitts (2001). After reaction, the mixture was analyzed on a 0.7% (w/v) agarose gel on a Sub-Cell GT Electrophoresis System (Bio-Rad, Hercules,CA, USA) in a 1xTAE buffer (40 mM Tris-HCl, 2 mM EDTA, pH 8.5). Intact supercoiled fast migrating DNA was separated from fragmented open circular and from fragmented linear slow migrating DNA (single-strand break) by electrophoresis at 90 V for 1 h. Gels were stained with ethidium bromide (0.5 mg/mL) and DNA bands were visualized on a UV transilluminator. Images were scanned by a Bio-Rad imaging calibrated densitometer (GS-800) and quantified by Quantity One software (version 1.3, Bio-Rad). All tests were run in three biological replications, normalized and averaged.

Similarly, in order to determine the inhibitory effect of peach phenolic extracts on Supercoiled DNA breakage induced by peroxyl radical (ROO•), intact pBR322 plasmid DNA (0.5 μg) was mixed with 2.4 mM 2,2′-azobis (2-amidinopropane) dihydrochloride and 100 μM GAE of phenolic extract in a total volume of 25 μL. The mixture was incubated for 2 h at 37 °C before being applied to 0.7% (w/v) agarose gel (Lim et al., 2001). DNA bands were separated and analyzed as previously described.

2.5. Gene expression analysis

Mesocarp tissue of the examined cultivars, harvested at LM stage, were analyzed at harvest and following a 3-d shelf life after harvest or after removal from 2 and 4 weeks of cold storage. Total RNA was extracted from three biological replications of 100 mg of peach fruit according to the protocol developed by Gambino et al. (2008). RNA integrity was confirmed [spectrophotometrically (Nanodrop 1000 Spectrophotometer, Thermo Scientific) and with gel electrophoresis] and subsequently samples were treated with RNase-free DNase (Cat. No. NU01a, HT Biotechnology LTD, England), to remove total gDNA, as elsewhere described (Georgiadou et al., 2016). First-strand cDNA synthesis was performed from 0.5 μg of total RNA and was reverse transcribed using the PrimeScript™ RT reagent kit (Takara Bio, Japan), according to the manufacturer's instructions. The transcript levels were analyzed in a Biorad IQ5 real-time PCR cycler (Biorad, USA). In total, three independent biological replicates were analyzed from each peach cultivar under each treatment. The reaction mixture consisted of 4 μL cDNA in reaction buffer (10-fold diluted first-strand cDNA), 0.5 μL of each primer (10 pmoL/μl; Supplementary Table 1) and 5 μL SensiFAST™ SYBR® & Fluorescein mix 2x (Bioline). The total reaction volume was 10 μL. The initial denaturation stage was at 95 °C for 5 min, followed by 40 cycles of amplification [95 °C for 30 s, annealing temperature (Tm °C) for 30 s, and 72 °C for 30 s] and a final elongation stage at 72 °C for 5 min. Gene amplification cycle was followed by a melting curve run, carrying out 61 cycles with 0.5 °C increment between 65 and 95 °C. The annealing temperatures of the oligonucleotide sequences of previously published peach antioxidant primers were 56–58 °C. Peach actin gene was used as a housekeeping reference gene (Meisel et al., 2005).

The relative quantification of gene expression levels and statistical analysis using pairwise fixed reallocation randomization test were performed using the REST-XL software according to Pfaffl et al. (2002). Gene expression levels were normalized against actin peach housekeeping reference gene and the harvest treatment of each peach cultivar was used for calibration.

2.6. Statistical analysis

Qualitative and phytochemical data were subjected to MANOVA analyses, using the maturity stage, cold storage and shelf life period as treatments and least significant differences (LSD) were calculated. Pearson's correlation analysis was performed between ripening related traits and antioxidant parameters. Data on the inhibitory effect of peach phenolic extracts on supercoiled DNA breakage induced by hydroxyl radical (•OH) and peroxyl radical (ROO•) were analyzed by one-way analysis of variance ($P < 0.05$). Different letters indicate statistically significant differences among the treatments within each cultivar according to Duncan's multiple range test. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

3.1. Non-destructive characterisation of maturity stage

Over the recent years, visible/near infrared (vis/NIR) spectroscopy has been proven to be a useful tool for non-destructive evaluation of maturity stage and/or internal quality fruit parameters (Nicolaï et al., 2007), including peaches (Herrero-Langreo et al., 2011; Vanoli and Buccheri, 2012; Drogoudi et al., 2016). In particular, the I_{AD} index has been shown to successfully determine maturity stage and/or to sort fruit into categories of maturity. Fruits of the examined cultivars were harvested based on size and background color and subsequently were categorized as LM and HM based on their I_{AD} index. The mean values and distribution range of the I_{AD} index found in a total of 700–1250 fruits per cultivar differed significantly (Fig. 1). Therefore, I_{AD} index is cultivar-

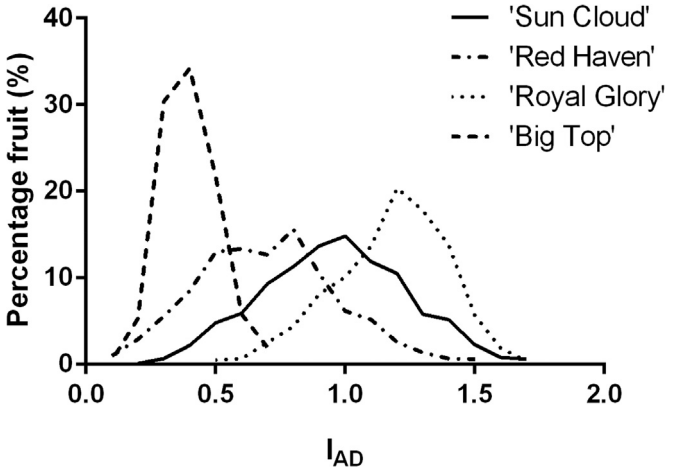

Fig. 1. Distribution of I_{AD} index of commercially harvested 'Big Top' (a), 'Royal Glory' (b), 'Red Haven' (c) and 'Sun Cloud' (d) fruits.

dependent, and specific values per cultivar should be found in order to be commercially applied. Moreover, it has been proven particularly important in cultivars where external color cannot define *per se* the maturity stage; this is the case for 'Royal Glory' (Supplementary Fig. 1). The I_{AD} index went descending with the progress of shelf life period both after harvest or after removal from cold storage (Supplementary Fig. 2), reflecting fruit physiological development. 'Big Top' fruit had the lowest I_{AD} reduction, compared with the remaining cultivars for both maturity stages (32% vs 64–88% in the HM fruit and 62% vs 74–92% in the LM fruit). Significant correlations were found between changes in I_{AD} and various ripening-related parameters measured during storage and shelf life (data not shown). Indicatively, highly positive correlation coefficients (r) were found between I_{AD} and FF values of the examined cultivars (r = 0.826–0.909), except for the slow-softening cultivar 'Big Top' (r = 0.720). In agreement with a companion study of our group (Drogoudi et al., 2016), I_{AD} index seems not to follow FF changes in slow-softening type cultivars.

3.2. Qualitative attributes

Flesh firmness at harvest was in the range 45.2–57.4 N in LM, and 38.0–45.0 N in the HM fruit for all examined cultivars (Fig. 2). A substantial decrease in FF during shelf life was monitored in all cultivars and especially in the HM fruit, demonstrating their melting-flesh character and their decreased storage ability. Particularly, 'Red Haven' and also 'Sun Cloud' demonstrated a substantial decrease in FF with the progress of shelf life period, reaching a melting stage at 3 d after harvest for both maturity stages. Notably, flesh firmness after 4 weeks of cold storage was similar or higher compared with 2-week cold storage for all cultivars and maturity stages: although no results regarding juiciness extractability were obtained, such data may be attributed to the development of chilling disorders and/or further weight loss. Significant, but not strong, positive correlations were found between firmness loss and weight loss in 'Big Top', 'Royal Glory' and 'Sun Cloud' (r = 0.625, 0.728 and 0.705, respectively), but not in 'Red Haven'. Weight loss varied between 9 and 12% in all cultivars after 2 weeks cold storage and additional ripening at room temperature for 5 d (Supplementary Fig. 3).

Besides FF and WL, SSC and TA are subjected to considerable changes during peach fruit ripening (Crisosto and Crisosto, 2005; Crisosto et al., 2006). At harvest, SSC was higher in Big Top' and 'Royal Glory' fruits compared with 'Red Haven' and 'Sun Cloud' fruits both in LM (11.9, 11.2, 10.8 and 10.9%, respectively) and HM stages (13.6, 12.2, 11.9 and 11.5%, respectively). An increment in SSC was noticed mostly during shelf life, which was more pronounced in 'Sun Cloud' fruits (data not shown). At harvest, TA was lower in 'Big Top' and 'Royal Glory' compared with 'Red Haven' and 'Sun Cloud' fruit (0.51, 0.62, 0.93 and 0.97 g 100^{-1} mL in LM and 0.50, 0.59, 0.85 and 0.92 g 100^{-1} mL in HM, respectively), concomitant with a higher ripening index (SSC/TA) of the aforementioned cultivars ('Big Top' and 'Royal Glory') (Fig. 3).

3.3. Ethylene production and respiration rate

Ethylene production indicated a sharp increase during ripening after removal from cold storage in all examined cultivars, being more pronounced in 'Big Top' and 'Royal Glory' fruits (Fig. 4). Advanced maturity fruits at harvest (HM) were generally characterized by higher ethylene contents; being more pronounced after 2 weeks of cold storage. Notably, flesh firmness reduction was less pronounced after 4 weeks compared with two weeks, coupled with a depression in ethylene production, possibly due to the incidence of chilling injury. Elevated levels of ethylene production after extended cold storage can be considered as an indicator of normal ripening, but no direct correlation with FF values can be established (Figs. 2 and 4). While basal levels of ethylene production were monitored during ripening after harvest, respiration rate registered a different pattern. All cultivars presented similar respiration rates at harvest being fluctuated in a different manner depending on the cultivar and storage regime considered (Fig. 5). Overall, ethylene production and respiration rate can be considered cultivar-dependent traits, not necessarily affected by fruit maturity stage at harvest.

3.4. Phytochemical content

The combined effects of genotype and postharvest ripening on phytochemical content of peach and nectarines have not been elucidated in detail. This issue is becoming more complicated if an additional cold storage regime, prior to shelf life ripening, is considered. A recent study from our group documented that ripening at room temperature just after harvest affected the antioxidant capacity of peach and nectarine cultivars in a different manner (Drogoudi et al., 2016). Based on this data, we have selected cultivars of significant economic importance and distinct phytochemical properties, i.e. containing low/intermediate ('Big Top', 'Red Haven', 'Royal Glory') and high ('Sun Cloud') antioxidant contents.

Although differences may occur from year to year due to orchard factors, agronomical practices (Iglesias and Echeverria, 2009) and experimental conditions, results of the current study demonstrated the superiority of 'Sun Cloud' cultivar regarding its total phenolic content. Such fruit contained 70–90 mg of gallic acid equivalents 100 g^{-1} FW for both maturity stages and all examined storage times (Supplementary Table 2). 'Red Haven' presented intermediate values; while substantially lower contents were monitored in 'Big Top' and 'Royal Glory' fruits. It is already well documented that great differences in total phenol content exist among different peach and nectarine cultivars with values ranging between 11.7 and 157.4 mg 100 g^{-1} GAE (Drogoudi et al., 2016, 2017, 45 cultivars, 26 cultivars; Cantín et al., 2009, 218 genotypes). It should also be considered that the experimental procedures applied may be subjected to slight modifications; thus, results are directly comparable for fruit analyzed under similar experimental conditions. Results also revealed that cold-stored fruit may possess higher amounts of phenolic compounds, particularly after 4 weeks of cold storage. In certain cases, extended storage lead to an increase of

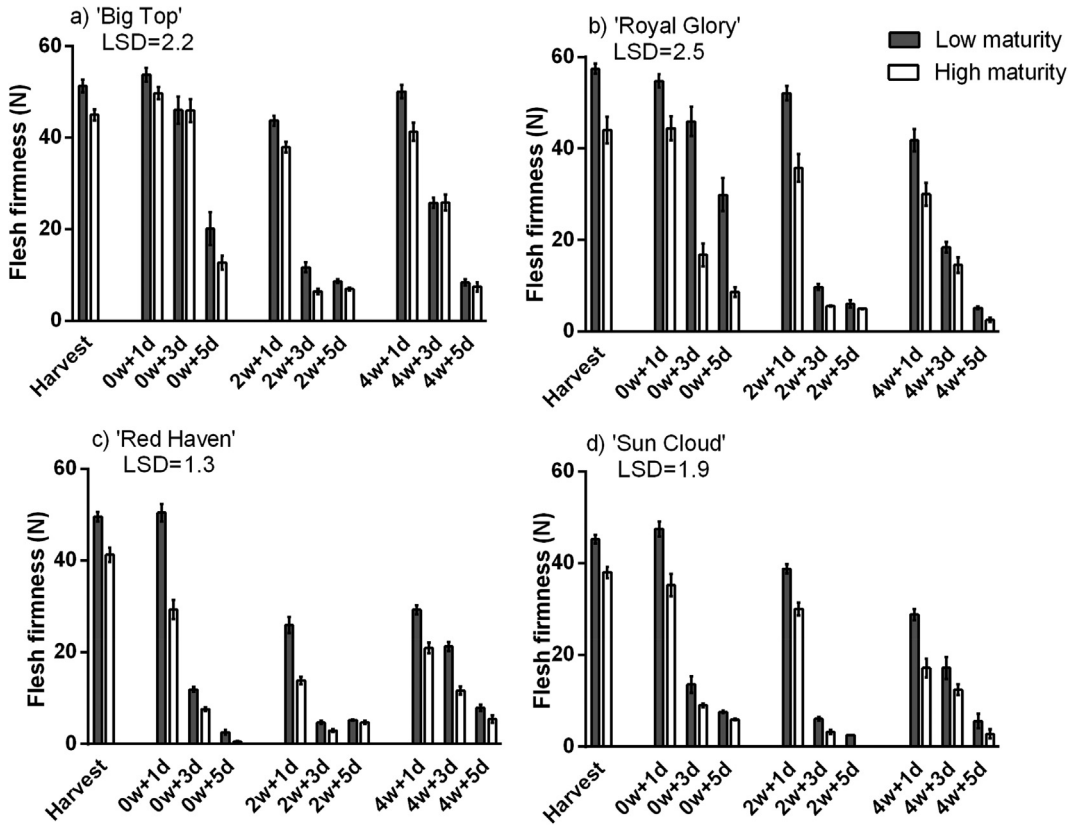

Fig. 2. Flesh firmness (N) in 'Big Top' (a), 'Royal Glory' (b), 'Red Haven' (c) and 'Sun Cloud' (d) fruits, harvested at high and low maturity stage (defined as low and high I_{AD} index, respectively). Measurements were made after harvest (0 w), 2 weeks (2 w) or 4 weeks (4 w) cold storage (0 °C, R.H. ~90%) and additional ripening for 1, 3 and 5 d at room temperature (20 °C), respectively. The lines represent the standard error of mean (±SE). LSD = Least significant differences at $p < 0.05$.

phenolic composition and antioxidant capacity on a fresh weight basis; this increase is mainly attributed to ripening/softening changes (weight loss) and increased extractability of phenolic compounds resulting from changes in the fruit cell wall rather than stimulation in the phenolic metabolic pathways (Supplementary Fig. 3, Supplementary Table 2). Previous studies also reported a similar effect of low-temperature storage on peach polyphenolic content (Asami et al., 2003; Di Vaio et al., 2008). Phenolic content in peaches was decreased and/or remained relative stable during maintenance at room temperature (shelf life). The latter is possibly linked with the activity of polyphenol oxidase that is higher at 20 °C than at cold storage (Lurie and Crisosto, 2005). In the present study, maturity stage had no effect on total phenolic content for all examined cultivars. Antioxidant capacity, evaluated with both assays, followed a similar trend with TPs content (Supplementary Table 2). A correlation between antioxidant capacity and TPs for all cultivars ($0.762 < r < 0.842$ with DPPH assay) and ($0.606 < r < 0.965$ with FRAP assay) was monitored, since phenolic compounds are the main group of natural antioxidants in polar peach extracts.

In addition, a reference HPLC fingerprint, consisted of 7 phenolic compounds (chlorogenic, neochlorogenic acid, catechin, epicatechin, rutin, quecetin-3-*O*-glucoside, procyanidin B1), was developed to study phenolic composition of peach fruit. Different HPLC fingerprints were found for each cultivar: qualitative and quantitative changes regarding hydroxycinammic acids and flavonols were monitored in the examined cultivars, suggesting that the polyphenolic profile was cultivar-dependent (Table 1). Notably, procyanidin B1 was the major flavonol in 'Sun Cloud', while it was absent in the other three cultivars. In the present study, 'Sun Cloud' had always the highest TPs content due to its high hydrocycinnamates content (Supplementary Table 2). 'Sun Cloud' also contained the highest amounts of flavan-3-ols, whereas the highest concentration of flavonols was found in 'Big Top'. Previous studies on polyphenolic composition of peach fruit showed that they are rich in hydroxycinnamic acids, flavonols and flavan-3-ols and great diversity in polyphenolic composition of peach fruits exist (Tomas-Barberan et al., 2001; Andreotti et al., 2008; Liu et al., 2015).

Results also demonstrated that the concentration of the examined phenolic compounds was independent from the maturity stage at harvest. Nevertheless, a significant role of cold storage was disclosed for certain compounds, such as catechin and rutin contents. The documented substantial weight loss can partially explain such differences. This accumulation of phenolic compounds after cold storage has also been observed in other peach cultivar (Meng et al., 2009), whereas extended cold storage and CI symptoms has been reported to be accompanied by enzymatic oxidation of simple phenols (Lurie and Crisosto, 2005). Maintenance at room temperature for 5 d led to a significant increase of hydroxycinnamates and flavan-3-ols, but had no effect on the flavonol contents. It is unclear whether the aforementioned increase in the phenolic content was due to stimulation in the phenolic metabolic pathways related with ripening/softening changes or increased extractability of phenolic compounds resulting from changes in the fruit cell wall.

3.5. The effect of maturity stage and cold storage on biomolecules that protect DNA form free radicals

While studies on antioxidant activity of herbaceous plant extracts against DNA damage have been reported (Lin et al., 2013),

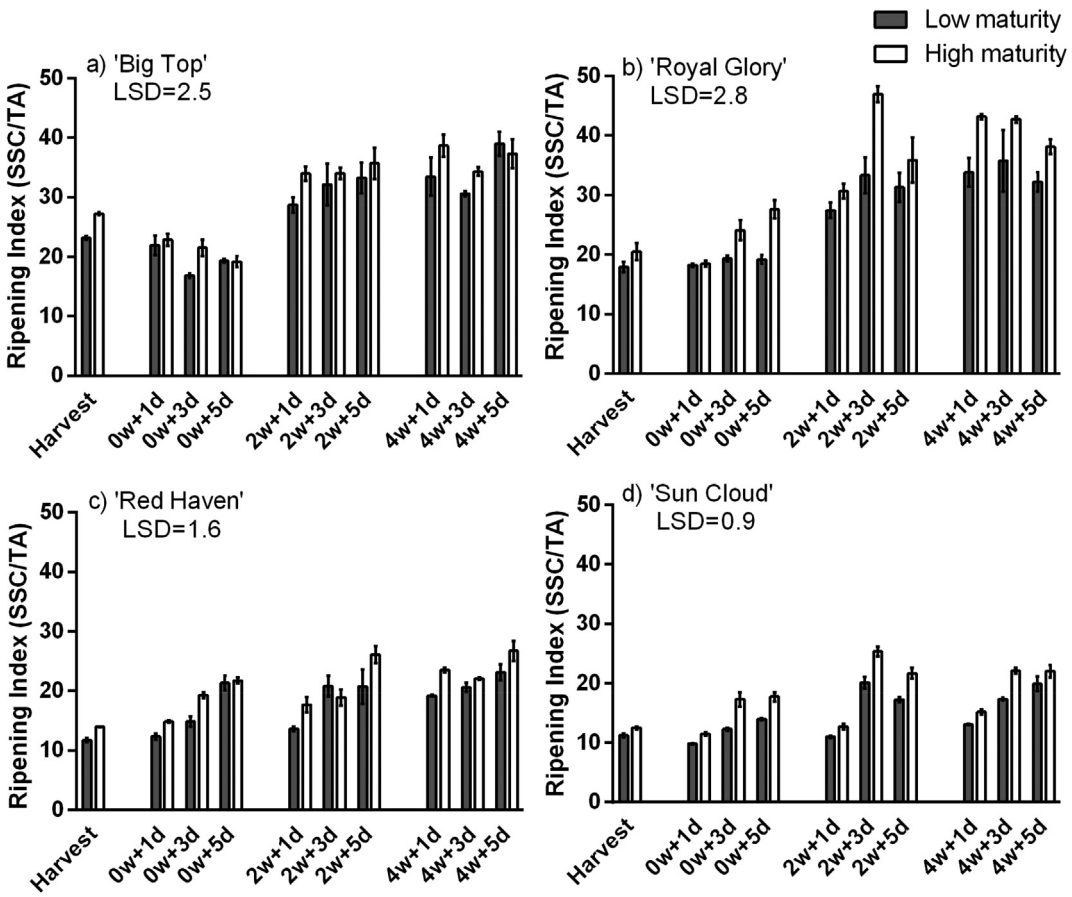

Fig. 3. Ripening index (SSC/TA) in 'Big Top' (a), 'Royal Glory' (b), 'Red Haven' (c) and 'Sun Cloud' (d) fruits. Treatments and abbreviations are explained in Fig. 1.

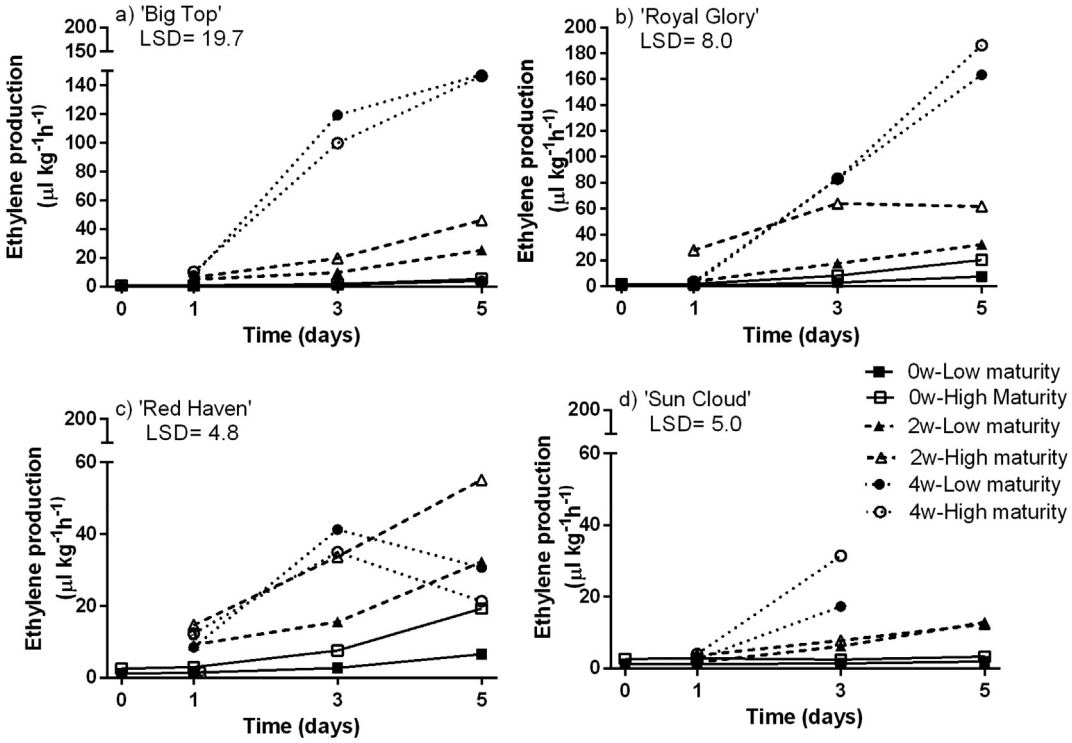

Fig. 4. Ethylene production in 'Big Top' (a), 'Royal Glory' (b), 'Red Haven' (c) and 'Sun Cloud' (d) fruits. Treatments and abbreviations are explained in Fig. 1.

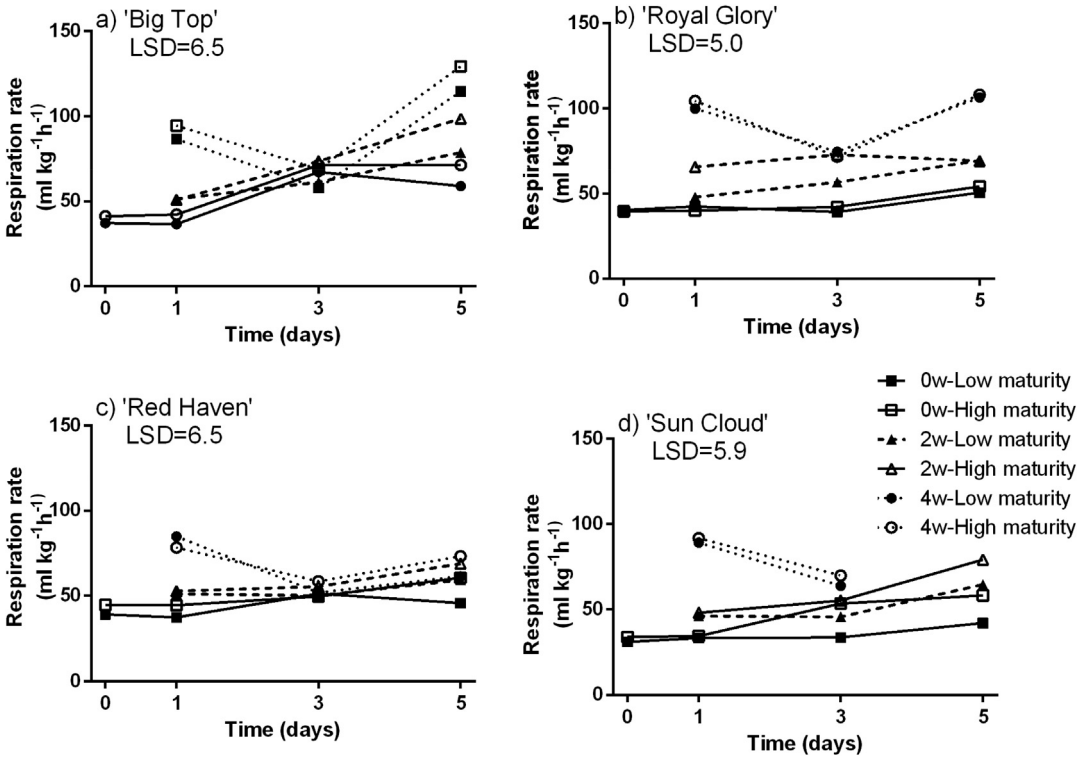

Fig. 5. Respiration rate in 'Big Top' (a), 'Royal Glory' (b), 'Red Haven' (c) and 'Sun Cloud' (d) fruits. Treatments and abbreviations are explained in Fig. 1.

studies on the protective effects of fruit extracts against DNA nicking from ROS/RNS are extremely limited. The antioxidant ability of peach and nectarine mesocarp phenolic extracts to scavenge •OH and ROO• was estimated based on their ability to protect plasmid pBR322 DNA from fragmentation induced by the aforementioned free radicals. Fig. 6 depicts the separation of the supercoiled plasmid DNA form I which is prone to oxidation, from open circular fragmented form II and linear fragmented form III. At lane A the migration of plasmid pBR322 DNA (form I) in the absence of any oxidant is depicted (Fig. 6A and B). Lane B shows the migration of plasmid pBR322 DNA after its incubation with either •OH or ROO• in the absence of phenolic extract, whereas the remaining lanes show the migration on the agarose gel of pBR322 DNA after its incubation with either •OH or ROO• and phenolic extract, as described in the methodology.

At harvest, 'Royal Glory', 'Big Top' and 'Sun Cloud' phenolic extracts exhibited similar ability to protect plasmid pBR322 DNA from oxidation induced by •OH with intact supercoiled form I and fragmented open circular form II being apparent at approximately the same intensity (Fig. 6A). Lower protective ability characterized the phenolic extracts of 'Red Haven' at harvest, since the presence of linear fragmented form III was obvious in contrast with the rest cultivars that continued to be visualized also at all examined stages (0 w + 3 d, 2 w + 3 d, 4 w + 3 d) (Fig. 6A). The linear fragmented form III at 'Royal Glory' and 'Big Top' was detected only at 4 w + 3 d, whereas at 'Sun Cloud' was transiently detected at 0 w + 3 d, showing its increased ability to protect plasmid DNA after 2 and 4 weeks cold storage and additional ripening at room temperature for 3 d (Fig. 6A).

In regard with peach and nectarine phenolic extracts' ability to protect plasmid DNA from ROO• induced oxidation, it was observed that 'Sun Cloud' extracts were more efficient in protecting pBR322 DNA, followed by the extracts of the rest cultivars that were characterized by similar protective ability (Fig. 6B). Form III was detected at 'Royal Glory', 'Red Haven' and 'Big Top' with the same intensity after 3 d shelf life, following harvest and 2 and 4-week cold storage, while it was absent in 'Sun Cloud' fruit (Fig. 6B).

In line with our observations, no direct correlation was observed between the ability of phenolic extracts to inhibit DNA breakage and the phenols content quantity. However, it is clear that phenolic extracts of the tested fruits are able to prevent radical-mediated DNA damage denoting their importance at the battle against ROS/RNS attack. This ability is variably dependent on the genotype, environmental growing conditions and maturity stage considered (Ziogas et al., 2010; Prakash et al., 2013; Karagiannis et al., 2016).

3.6. Expression analysis of antioxidant genes

In the present work, antioxidant metabolism was monitored over a time-course exposure of peach fruits to a temperature stress combination, following a combined physiological, biochemical and molecular approach. Due to the differences found among classical postharvest criteria, including phytochemicals, phenolic compounds and antioxidant capacity, a detailed study at gene expression level was carried out in order to acquire new insights on how enzymatic antioxidant biosynthetic pathways were regulated under cold conditions and storage. In this sense, a heat map of the relative expression levels of major antioxidant genes (*CAT, POD, Cu/Zn SOD2, Cu/Zn SOD3, MnSOD, perAPX1, perAPX2, perAPX3, cAPX, chlorAPX, chlorGR, GPX6, GPX8*) for each peach cultivar and storage treatment is presented in Fig. 7.

Most of the antioxidant genes were generally down-regulated under cold storage, in line with a previous report demonstrating down-regulation of antioxidant components in cultivars sensitive to chilling injury (Pons et al., 2014). Furthermore, the transcriptomic analysis in peach cultivars subjected to cold storage showed that genes related to antioxidant systems and the biosynthesis of metabolites with antioxidant activity correlates with

Table 1
Content of individual flavonols and hydroxycinamic acids of Big Top', 'Royal Glory', 'Red Haven' and 'Sun Cloud' peach fruits, harvested at two distinct maturity stages (advanced maturity, HM and less pronounced maturity, LM) and additional ripening for 1 and 5 d at room temperature (20 °C) after harvest or 2 and 4 weeks (w) cold storage (0 °C, R.H. ~90%), respectively. Data are the means of 3 replications ± S.E.

Cultivar/Treatment	Neochlorogenic acid	Chlorogenic acid	Procyanidin B1	Epicatechin	Catechin	Rutin	Quercetin-3-*O*-glucose
Big Top [LM]							
1 d SL	18.5 ± 2.8c,d	1.6 ± 0.2a*	nd**	6.0 ±0.4a	8.4 ± 0.2c	16.5 ± 1.1a	4.0 ± 0.4a,b
5 d SL	22.7 ± 2.9a,b,c	2.1 ± 0.4a	nd*	6.1 ± 0.5a	9.1 ± 0.3a,b,c	17.7 ± 0.4a	4.7 ± 0.2a
2 w CT +1 d SL	19.6 ± 2.2b,c,d	1.9 ± 0.2a	nd*	5.9 ± 0.8a	8.0 ± 0.7c	17.6 ± 2.2a	3.7 ± 0.2b,c
2 w CT +5 d SL	21.1 ± 1.6a,b,c,d	1.8 ± 0.1a	nd*	6.0 ± 0.1a	8.8 ± 1.2b,c	18.8 ± 1.4a	4.1 ± 0.3a,b
4 w CT +1 d SL	16.6 ± 1.1d	1.7 ± 0.2a	nd*	6.5 ± 0.3a	11.7 ± 1.1a	16.7 ± 0.4a	4.2 ± 0.2a,b
4 w CT +5 d SL	21.8 ± 1.4a,b,c,d	1.9 ± 0.2a	nd*	6.8 ± 0.3a	11.8 ± 1.0a	18.5 ± 1.1a	4.2 ± 0.4a,b
Big Top [HM]							
1 d SL	24.2 ± 1.7a,b	1.9 ± 0.2a	nd*	5.8 ± 0.4a	8.1 ± 0.7c	17.0 ± 1.0a	4.0 ± 0.2a,b
5 d SL	20.3 ± 0.9b,c,d	1.8 ± 0.1a	nd*	5.7 ± 0.8a	7.9 ± 1.0c	17.0 ± 0.7a	3.8 ± 0.2b,c
2 w CT +1 d SL	21.1 ± 1.1a,b,c,d	1.9 ± 0.3a	nd*	6.5 ± 0.7a	8.2 ± 0.9c	18.3 ± 1.5a	2.7 ± 0.1d
2 w CT +5 d SL	20.3 ± 0.9b,c,d	1.8 ± 0.1a	nd*	6.8 ± 0.6a	8.0 ± 0.9c	17.6 ± 1.2	2.7 ± 0.1d
4 w CT +1 d SL	26.5 ± 1.1a	2.2 ± 0.2a	nd*	7.4 ± 0.9a	11.3 ± 0.5a,b	18.1 ± 0.2a	2.9 ± 0.4c,d
4 w CT +5 d SL	23.4 ± 0.2a,b,c	2.0 ± 0.1a	nd*	7.5 ± 0.7a	11.4 ± 1.4a,b	18.6 ± 2.0a	2.7 ± 0.3d
Royal Glory [LM]							
1 d SL	14.9 ± 2.0a	4.5 ± 0.2a	nd*	7.4 ± 0.3a,b	17.5 ± 0.4a	10.1 ± 2.0a	6.8 ± 0.3a
5 d SL	14.7 ± 2.9a	4.4 ± 0.5a	nd*	7.0 ± 0.4a,b	17.8 ± 0.6a	9.8 ± 0.2a	6.7 ± 0.5a
2 w CT +1 d SL	15.0 ± 1.6a	4.1 ± 0.2a	nd*	7.9 ± 0.1a,b	16.9 ± 1.6a	10.7 ± 0.4a	6.6 ± 0.4a
2 w CT +5 d SL	16.3 ± 2.2a	3.9 ± 0.3a	nd*	7.6 ± 0.0a,b	16.5 ± 0.2a	10.3 ± 0.3a	6.0 ± 0.3a
4 w CT +1 d SL	15.5 ± 1.7a	3.8 ± 0.3a	nd*	7.9 ± 0.5a,b	18.8 ± 1.0a	10.2 ± 0.4a	6.5 ± 0.3a
4 w CT +5 d SL	15.9 ± 2.3a	3.9 ± 0.3a	nd*	7.7 ± 0.7a,b	18.8 ± 2.9a	10.1 ± 1.6a	6.2 ± 0.4a
Royal Glory [HM]							
1 d SL	15.9 ± 1.7a	4.1 ± 0.5a	nd*	7.4 ± 0.3a,b	16.9 ± 2.7a	9.3 ± 0.8a	6.2 ± 0.6a
5 d SL	16.6 ± 0.4a	4.5 ± 0.4a	nd*	8.3 ± 0.3a	20.5 ± 0.9a	10.6 ± 0.8a	7.2 ± 0.4a
2 w CT +1 d SL	15.6 ± 0.5a	4.3 ± 0.1a	nd*	7.1 ± 0.1,b	17.0 ± 0.3a	9.2 ± 0.2a	6.4 ± 0.6a
2 w CT +5 d SL	16.9 ± 2.5a	4.6 ± 0.4a	nd*	8.4 ± 0.9a	20.2 ± 1.7a	11.3 ± 1.0a	6.9 ± 0.1a
4 w CT +1 d SL	17.9 ± 0.8a	4.6 ± 0.3a	nd*	6.8 ± 0.4	16.5 ± 1.9a	9.6 ± 0.5a	6.6 ± 0.3a
4 w CT +5 d SL	18.9 ± 2.4a	5.0 ± 0.4a	nd*	7.3 ± 0.3a,b	18.4 ± 1.0a	10.2 ± 0.3a	6.5 ± 0.3a
Red Haven [LM]							
1 d SL	44.6 ± 3.0a,b	3.9 ± 0.2a	nd*	3.8 ± 0.2a	16.8 ± 0.9c,d	7.9 ± 1.1b,c	4.2 ± 0.2b
5 d SL	47.2 ± 3.2a,b	3.5 ± 0.2a	nd*	3.6 ± 0.6a	17.8 ± 1.3c,d	7.7 ± 1.2c	4.2 ± 0.1b
2 w CT +1 d SL	43.2 ± 2.2a,b	3.8 ± 0.2a	nd*	4.1 ± 0.2a	21.2 ± 1.6a,b,c	7.1 ± 0.4c	4.1 ± 0.2b
2 w CT +5 d SL	54.1 ± 4.0a	4.1 ± 0.2a	nd*	4.7 ± 0.5a	24.4 ± 1.2a	8.1 ± 0.9b,c	5.0 ± 0.3a,b
4 w CT +1 d SL	43.3 ± 3.3a,b	3.3 ± 0.5a	nd*	4.3 ± 0.4a	21.1 ± 1.3a,b,c	11.9 ± 1.3a	4.5 ± 0.3a,b
4 w CT +5 d SL	47.2 ± 6.0a,b	3.8 ± 0.4a	nd*	4.3 ± 0.4a	22.8 ± 1.5a,b	12.5 ± 0.9a	4.9 ± 0.4a,b
Red Haven [HM]							
1 d SL	46.0 ± 2.1a,b	4.0 ± 0.4a	nd*	3.8 ± 0.3a	16.0 ± 2.5d	7.3 ± 0.3c	4.7 ± 0.5a,b
5 d SL	44.3 ± 3.8a,b	3.7 ± 0.1a	nd*	4.3 ± 0.6a	15.9 ± 1.5d	7.9 ± 1.0b,c	4.3 ± 0.2b
2 w CT +1 d SL	43.4 ± 4.0a,b	3.7 ± 0.4a	nd*	4.1 ± 0.2a	16.8 ± 0.7c,d	7.5 ± 0.7c	4.4 ± 0.2a,b
2 w CT +5 d SL	49.2 ± 3.5a,b	4.2 ± 0.3a	nd*	4.3 ± 0.3a	18.4 ± 0.7b,c,d	8.3 ± 0.6b,c	5.3 ± 0.0a
4 w CT +1 d SL	40.8 ± 2.8b	3.7 ± 0.2a	nd*	4.2 ± 0.2a	18.8 ± 1.1b,c,d	11.5 ± 0.7a	4.8 ± 0.1a,b
4 w CT +5 d SL	44.5 ± 1.7a,b	3.7 ± 0.3a	nd*	4.3 ± 0.1a	19.4 ± 2.0b,c,d	10.6 ± 0.8a,b	4.7 ± 0.4a,b
Sun Cloud [LM]							
1 d SL	57.6 ± 2.0c,d,e	16.9 ± 1.8a	27.9 ± 1.3a	6.8 ± 0.7a	9.0 ± 0.5a	14.3 ± 0.5b	1.2 ± 0.1a.b
5 d SL	59.1 ± 6.4c,d,e	17.1 ± 1.6a	27.5 ± 2.5a	6.6 ± 0.4a	10.5 ± 1.4a	15.1 ± 2.8a,b	1.5 ± 0.1a
2 w CT +1 d SL	54.7 ± 3.1d,e	15.6 ± 0.5a	27.4 ± 5.6a	6.9 ± 0.8a	7.8 ± 0.6a	16.4 ± 0.5a,b	1.1 ± 0.3a,b
2 w CT +5 d SL	74.5 ± 2.8a,b	19.8 ± 0.4a	28.1 ± 1.4a	6.7 ± 0.4a	9.9 ± 0.6a	19.3 ± 2.7a,b	1.1 ± 0.2a,b
4 w CT +1 d SL	67.1 ± 7.1a,b,c,d	19.1 ± 0.1a	29.2 ± 2.4a	6.7 ± 0.1a	7.9 ± 0.7a	17.0 ± 0.3a,b	1.1 ± 0.3a,b
4 w CT +5 d SL	69.4 ± 5.9a,b,c	17.4 ± 1.7a	26.6 ± 0.9a	7.4 ± 0.4a	8.4 ± 1.0a	14.8 ± 0.7a,b	1.0 ± 0.1a,b
Sun Cloud [HM]							
1 d SL	61.8 ± 3.6b,c,d,e	16.4 ± 1.5a	25.6 ± 1.4a	6.9 ± 0.5a	9.0 ± 1.0a	16.6 ± 1.4a,b	1.1 ± 0.1a,b
5 d SL	53.7 ± 3.0d,e	16.5 ± 1.9a	23.3 ± 1.3a	7.2 ± 0.1a	10.6 ± 1.0a	17.1 ± 0.4a,b	0.9 ± 0.3b
2 w CT +1 d SL	48.4 ± 1.3e	15.8 ± 1.1a	23.9 ± 0.8a	6.7 ± 0.4a	8.8 ± 0.9a	18.9 ± 0.9a,b	1.1 ± 0.0a,b
2 w CT +5 d SL	56.8 ± 0.1c,d,e	17.3 ± 2.2a	28.5 ± 2.6a	6.9 ± 0.1a	9.4 ± 0.4a	20.3 ± 3.6a	1.2 ± 0.2a,b
4 w CT +1 d SL	67.4 ± 2.2a,b,c,d	15.6 ± 1.0a	23.4 ± 1.1a	7.4 ± 0.6a	9.3 ± 0.5a	16.7 ± 1.4a,b	1.5 ± 0.1a
4 w CT +5 d SL	77.0 ± 7.9a	17.1 ± 1.1a	27.5 ± 2.4a	7.6 ± 0.5a	9.6 ± 0.7a	18.3 ± 1.9a,b	1.5 ± 0.1a

*Different letters within columns indicate statistically significant differences in each peach cultivar for both maturity stages ($P < 0.05$, Duncan's test), **nd = not detected.

chilling tolerance (Pons et al., 2015). *CAT* and *cAPX* were significantly down-regulated in most of storage treatments and cultivars. In general, the transcript expression profile in 'Sun Cloud' fruit under cold storage demonstrated lower overall suppression levels compared with the other cultivars, correlating with improved phytochemical content and antioxidant capacity (Table 1, Supplementary Table 2, Fig. 7). *Cu/ZnSOD2*, *perAPX3* and *GPX8* were also significantly down-regulated in most of the storage treatments

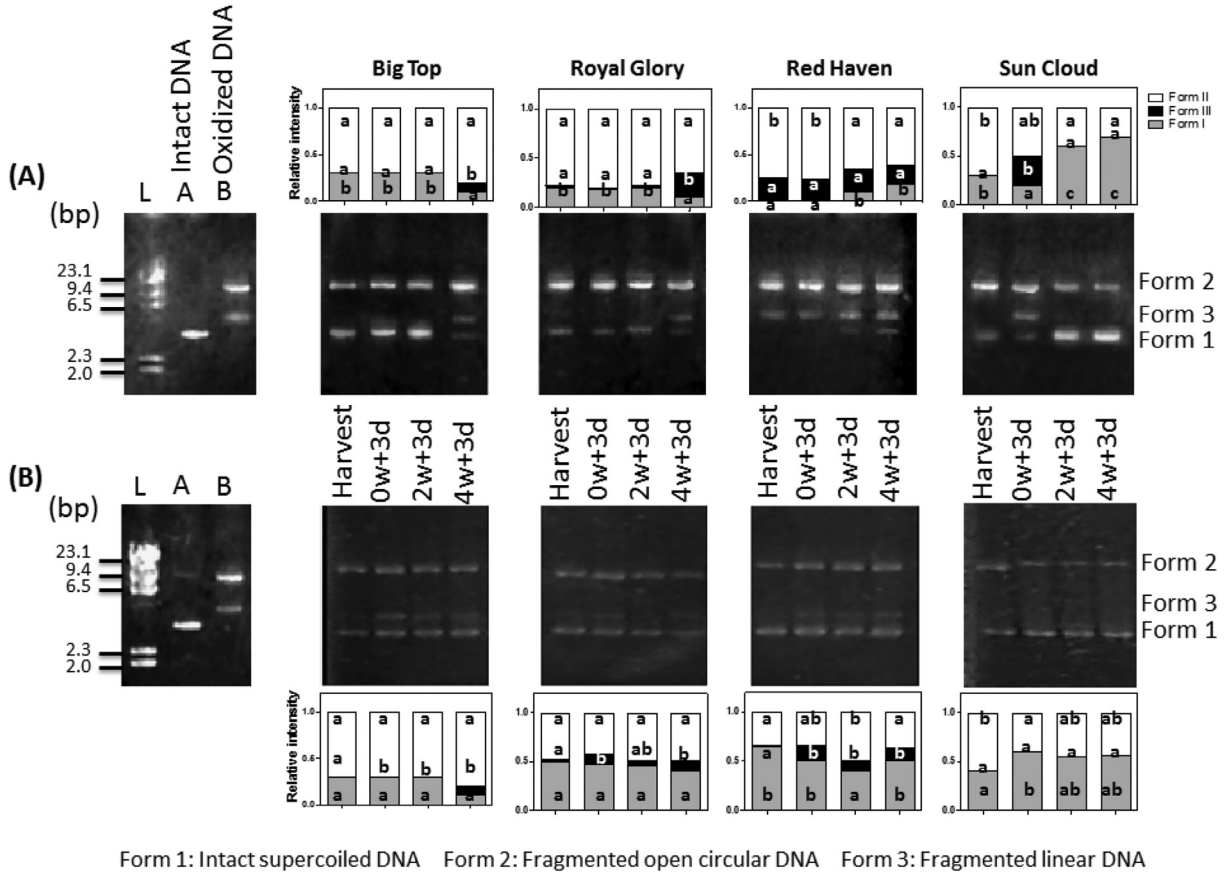

Fig. 6. Protective effect of peach and nectarine fruit-extracted phenols from, 'Big Top', 'Royal Glory', 'Red Haven' and 'Sun Cloud' fruits at harvest and after additional ripening at room temperature for 3 d after harvest or 2 and 4 w of cold storage (Harvest, 0w+3d, 2w+3d, 4w+3d) against pBR322 DNA fragmentation induced by A) •OH or B) ROO•. Lane L, *Hin*dIII lambda digest DNA size markers; Lane A = intact pBR322 DNA (no oxidant or protective factor); lane B = oxidized plasmid pBR322 DNA (incubation with either •OH or ROO• in the absence of phenolic extract). Densitometric quantification of the form I, II, III intensity (arbitrary units relative intensity); Data represent mean for n = 3. Comparisons conducted among the different maturity stages within each cultivar for each form separately. Values followed by the same letter are not significant different according to LSD test for $P \leq 0.05$.

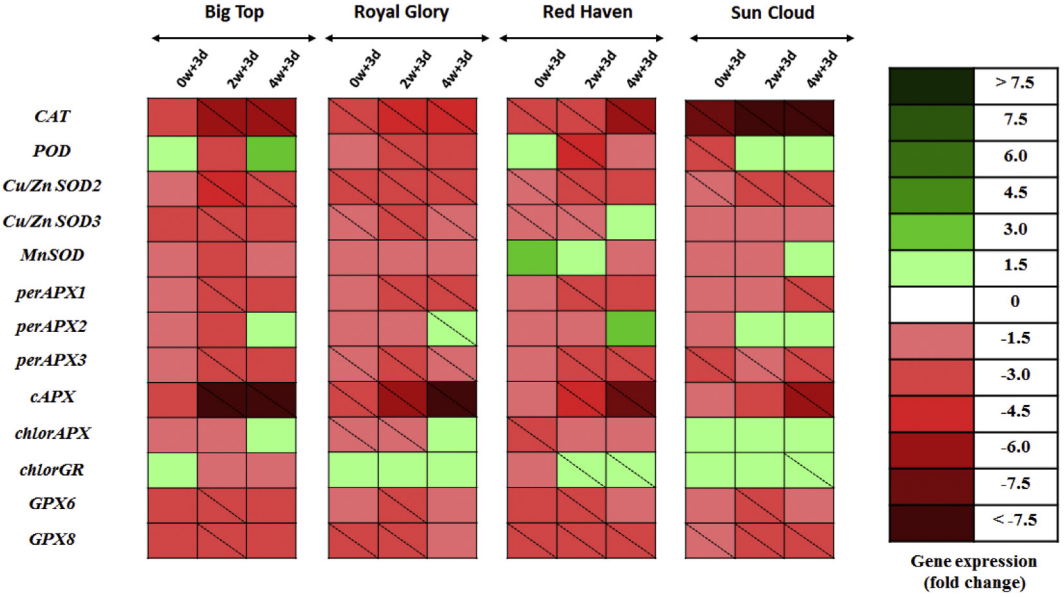

Fig. 7. Heat map of the relative transcript levels of enzymatic antioxidants (*CAT, POD, Cu/Zn SOD2, Cu/Zn SOD3, MnSOD, perAPX1, perAPX2, perAPX3, cAPX, chlorAPX, chlorGR, GPX6, GPX8*). Relative mRNA abundance was evaluated by real-time RT-qPCR using three biological repeats. Up-regulation is indicated in green; down-regulation is indicated in red. A diagonal line in a box indicates a statistically significant value ($P \leq 0.05$). A scale of color intensity is presented as a legend. The harvest point per cultivar was used for calibrating gene expression values. Actual relative expression levels are shown in Supplementary Tables (3-6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and cultivars. On the other hand, the remaining genes were down-regulated to a lower extent or not significantly changed. Contrarily, a significant induction was observed in *chlorGR* expression during cold storage in 'Red Haven' and 'Sun Cloud' cultivars, while *perAPX2* was induced in 'Royal Glory' cultivar. It is interesting to note that other reports demonstrated that *CAT1* and *GPX6* up-regulation in particular was linked with increased tolerance to cold stress (Dagar et al., 2013; Huan et al., 2016); such induction was not observed in any of the presently tested cultivars, therefore suggesting that this regulation could be genotype-specific or that improved antioxidant capacity in 'Sun Cloud' in particular could be linked with compounds other than enzymatic antioxidants.

4. Conclusions

The current study tried to shed light and address real-time metabolic changes in the interplay among genotype, maturity stage, room temperature maintenance and cold storage period on phenolic composition and antioxidant potency. Results indicated a substantial decrease in FF during shelf-life in all examined cultivars demonstrating their melting-flesh character with more pronounced changes in HM compared with LM fruit. 'Big Top' and 'Royal Glory' demonstrated less pronounced changes in fruit firmness than the other cultivars. Such data could not be directly correlated with weight loss and ethylene evolution. Notably, a differential accumulation of individual phenolic compounds was monitored in the examined cultivars under different storage regimes, which suggests that phenolics may have different roles in various physiological pathways. The increase in phytochemical content after extended cold storage may partially be attributed to extensive weight loss that concentrate its content and/or a protective mechanism against oxidative damage, induced by temperature stress. Therefore, it can be inferred that antioxidant genes regulate peach fruit development and softening by influencing specific polyphenolic signal transduction pathways. Overall, phytochemical data indicated the superiority of 'Sun Cloud', as validated by an array of biochemical and molecular assays, nevertheless it postulated increased softening rate being a major drawback for its cultivation. Noteworthy, other agronomical traits should also be considered for the promotion of a cultivar, as an added value product, while the specific regulatory mechanisms of antioxidant genes that affect peach fruit ripening also require further investigation.

Authors' contributions

GAM conceived the project and designed the experiments. VF, ECG and GT undertook the molecular experiments. VG and GAM undertook the analytical experiments. PD, GP and GT undertook the experiment and qualitative measurements. PD, ECG, VG, VF and GT were involved in the data analysis. GAM, PD, VG, VF, KP, AM wrote the paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2017.08.022.

References

Abidi, W., Cantín, C.M., Jiménez, S., Giménez, R., Moreno, M.A., Gogorcena, Y., 2015. Influence of antioxidant compounds, total sugars and genetic background on the chilling injury susceptibility of a non-melting peach (*Prunus persica* L.) Batsch) progeny. J. Sci. Food Agric. 95, 351–358.

Andreotti, C., Ravaglia, D., Rgaini, A., Costa, G., 2008. Phenolic compounds in peach (*Prunus persica*) cultivars at harvest and during fruit maturation. Ann. Appl. Biol. 153, 11–23.

Asami, D.K., Hong, Y.J., Barrett, D.M., Mitchell, A.E., 2003. Processing-induced changes in total phenolics and procyanidins in clingstone peaches. J. Sci. Food Agric. 83, 56–63.

Aubert, C., Bony, P., Chalot, G., Landry, P., Lurol, S., 2014. Effects of storage temperature, storage duration, and subsequent ripening on the physicochemical characteristics, volatile compounds, and phytochemicals of western red nectarine (*Prunus persica* L. Batsch). J. Agric. Food Chem. 62, 4707–4724.

Brummell, D.A., Dal Cin, V., Lurie, S., Crisosto, C.H., Labavitch, J.M., 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55, 2041–2052.

Cantín, C.M., Moreno, M.A., Gogorcena, Y., 2009. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. J. Agric. Food Chem. 57, 4586–4592.

Cantín, C.M., Gogorcena, Y., Moreno, M.A., 2010. Phenotypic diversity and relationships of fruit quality traits in peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. Euphytica 171, 211–226.

Crisosto, C.H., Crisosto, G.M., 2005. Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine [*P. persica* (L.) Batsch] cultivars. Postharvest Biol. Technol. 38, 239–246.

Crisosto, C.H., Crisosto, G.M., Echeverria, G., Puy, J., 2006. Segregation of peach and nectarine (*Prunus persica* (L.) Batsch) cultivars according to their organoleptic characteristics. Postharvest Biol. Technol. 39, 10–18.

Dagar, A., Pons Puig, C., Marti Ibanez, C., Ziliotto, F., Bonghi, C., Crisosto, C.H., Friedman, H., Lurie, S., Granell, A., 2013. Comparative transcript profiling of a peach and its nectarine mutant at harvest reveals differences in gene expression related to storability. Tree Gen. Genom 9, 223–235.

Di Vaio, C., Graziani, G., Marra, L., Cascone, A., Ritieni, A., 2008. Antioxidant capacities, carotenoids and polyphenols evaluation of fresh and refrigerated peach and nectarine cultivars from Italy. Eur. Food Res. Technol. 227, 1225–1231.

Drogoudi, P., Pantelidis, G.E., Goulas, V., Manganaris, G.A., Ziogas, V., Manganaris, A., 2016. The appraisal of qualitative parameters and antioxidant contents during postharvest peach fruit ripening underlines the genotype significance. Postharvest Biol. Technol. 115, 142–150.

Drogoudi, P., Gerasopoulos, D., Kafkaletou, M., Tsantili, E., 2017. Phenotypic characterization of qualitative parameters and antioxidant contents in peach and nectarine fruit and changes after jam preparation. J. Sci. Food Agric. http://dx.doi.org/10.1002/jsfa.8188.

Font i Forcada, C., Gradziel, T.M., Gogorcena, Y., Moreno, M.A., 2014. Phenotypic diversity among local Spanish and foreign peach and nectarine [*Prunus persica* (L.) Batsch] accessions. Euphytica 197, 261–277.

Gambino, G., Perrone, I., Gribaudo, I., 2008. A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. Phytochem. Anal. 19, 520–525.

Georgiadou, E.C., Goulas, V., Ntourou, T., Manganaris, G.A., Kalaitzis, P., Fotopoulos, V., 2016. Regulation of on-tree vitamin E biosynthesis in olive fruit during successive growing years: the impact of fruit development and environmental cues. Front. Plant Sci. 7, 1656.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Kader, A.A., 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin c contents of nectarine, peach, and plum cultivars from California. J. Agric. Food Chem. 50, 4976–4982.

Goulas, V., Minas, I.S., Kourdoulas, P., Vicente, A.R., Manganaris, G.A., 2014. Phytochemical content, antioxidants and cell wall metabolism of two loquat (*Eriobotrya japonica*) cultivars under different storage regimes. Food Chem. 155, 227–234.

Herrero-Langreo, A., Fernández-Ahumada, E., Roger, J.M., Palagós, B., Lleó, L., 2011. Combination of optical and non-destructive mechanical techniques for the measurement of maturity in peach. J. Food Eng. 108, 150–157.

Hu, C., Kitts, D.D., 2001. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems *in vitro*. Mol. Cell. Biochem. 218, 147–155.

Huan, C., Jiang, L., An, X., Yu, M., Xu, Y., Ma, R., Yu, Z., 2016. Potential role of reactive oxygen species and antioxidant genes in the regulation of peach fruit development and ripening. Plant Physiol. Biochem. 104, 294–303.

Iglesias, I., Echeverria, G., 2009. Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. Sci. Hort. 120, 41–50.

Karagiannis, E., Tanou, G., Samiotaki, M., Michailidis, M., Diamantidis, G., Minas, I.S., Molassiotis, A., 2016. Comparative physiological and proteomic analysis reveal distinct regulation of peach skin quality traits by altitude. Front. Plant Sci. 7, 1689.

Lim, K., Hu, C., Kitts, D.D., 2001. Antioxidant activity of a *Rhus verniciflua* stokes ethanol extract. Food Chem. Toxicol. 39, 229–237.

Lin, K.H., Yang, Y.Y., Yang, C.M., Huang, M.Y., Lo, H.F., Liu, K.C., Lin, H.S., Chao, P.Y., 2013. BMC Res. Notes 6, 490.

Liu, H., Cao, J., Jiang, W., 2015. Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. LWT-Food Sci. Technol. 63, 1042–1048.
Lurie, S., Crisosto, C.H., 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. 37, 195–208.
Meisel, L., Fonseca, B., Gonzalez, S., Baeza-Yates, R., Cambiazo, V., Campos, R., Gonzalez, M., Orellana, A., Retamales, J., Silva, H., 2005. A rapid and efficient method for purifying high quality total RNA from peaches (*Prunus persica*) for functional genomics analyses. Biol. Res. 38, 83–88.
Meng, X., Han, J., Wang, Q., Tian, S., 2009. Changes in physiology and quality of peach fruits treated by methyl jasmonate under low temperature stress. Food Chem. 114, 1028–1035.
Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol. Technol. 46, 99–118.
Orazem, P., Stampar, F., Hudina, M., 2011. Fruit quality of redhaven and royal glory peach cultivars on seven different rootstocks. J. Agric. Food Chem. 59, 9394–9401.
Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST(C)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 30, e36.
Pons, C., Martí, C., Forment, J., Crisosto, C.H., Dandekar, A.M., Granell, A., 2014. A bulk segregant gene expression analysis of a peach population reveals components of the underlying mechanism of the fruit cold response. PLoS One 9, e90706.
Pons, C.P., Dagar, A., Marti Ibanez, C., Singh, V., Crisosto, C.H., Friedman, H., Lurie, S., Granell, A., 2015. Pre-symptomatic transcriptome changes during cold storage of chilling sensitive and resistant peach cultivars to elucidate chilling injury mechanisms. BMC Genomics 16, 245.
Pons, C., Marti, C., Forment, J., Crisosto, C.H., Dandekar, A.M., Granell, A., 2016. A genetic genomics-expression approach reveals components of the molecular mechanisms beyond the cell wall that underlie peach fruit woolliness due to cold storage. Plant Mol. Biol. 92, 483–503.
Prakash, D., Upadhyay, G., Gupta, C., 2013. Total phenol and antioxidant activity of some fruits and their under-utilized parts. Int. Food Res. J. 20, 1717–1724.
Reig, G., Iglesias, I., Gatius, F., Alegre, S., 2013. Antioxidant capacity, quality, and anthocyanin and nutrient contents of several peach cultivars [*Prunus persica* (L.) Batsch] Grown in Spain. J. Agric. Food Chem. 61, 6344–6357.
Scalbert, A., Monties, B., Janin, G., 1989. Tannins in wood: comparison of different estimation methods. J. Agric. Food Chem. 37, 1324–1329.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1998. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Met. Enzymol. 299, 152–178.
Tomas-Barberan, F.A., Gil, M.I., Gremin, P., Waterhouse, A.L., Hess-Pierce, B., Kader, A.A., 2001. HPLC–DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J. Agric. Food Chem. 49, 4748–4760.
Vanoli, M., Buccheri, M., 2012. Overview of methods for assessing harvest maturity. Stewart Postharvest Rev. 8, 1–11.
Vizzotto, M., Cisneros-Zevallos, L., Byrne, D.H., Ramming, D.W., Okie, W.R., 2007. Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. J. Amer. Soc. Hort. Sci. 132, 334–340.
Ziogas, V., Tanou, G., Molassiotis, A., Diamantidis, G., Vasilakakis, M., 2010. Antioxidant and free radical-scavenging activities of phenolic extracts of olive fruits. Food Chem. 120, 1097–1103.