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Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines

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ABSTRACT

In the present study the possibility of enhancing phenolic compound contents in peaches and nectarines by post-harvest irradiation with UV-B was assessed. Fruits of 'Suncrest' and 'Babygold 7' peach and 'Big Top' nectarine cultivars were irradiated with UV-B for 12 h, 24 h and 36 h. Control fruits underwent the same conditions but UV-B lamps were screened by benzophenone-treated polyethylene film.

The effectiveness of the UV-B treatment in modulating the concentration of phenolic compounds and the expression of the phenylpropanoid biosynthetic genes, was genotype-dependent. 'Big Top' and 'Suncrest' fruits were affected by increasing health-promoting phenolics whereas in 'Babygold 7' phenolics decreased after UV-B irradiation. A corresponding trend was exhibited by most of tested phenylpropanoid biosynthesis genes.

Based on these results UV-B irradiation can be considered a promising technique to increase the health-promoting potential of peach fruits and indirectly to ameliorate the aesthetic value due to the higher anthocyanin content.

1. Introduction

In the last decades consumers have become more aware of the relationships between diet and diseases. High quality products which associate health, safety and convenience accomplish consumer preferences. For satisfying this current demand, increasing attention for functional foods is growing up in fruit and vegetable market (Schreiner, Korn, Stenger, Holzgreve, & Altmann, 2013). Technologies able to ensure high quality products with high levels of the desired compounds are needed in order to enhance the health benefits and create new opportunities for growers and processors (Cisneros-Zevallos, 2003). The health-promoting properties of fruits and vegetables are due to the presence of some vitamins (A, C, E, and folates), dietary fibres and secondary plant metabolites. Polyphenols, which are prevailed by flavonoids, are one of the main group of secondary plant metabolites. Flavonoids are the most common phenolics obtained from the everyday plant-source diet (Chun, Chung, & Song, 2007) and have aroused substantial attention due to their protective potential against chronic diseases (Weng & Yen, 2012).

Among the different fruit species grown in Europe, apples and peaches are the most popular. Though peaches and nectarines have lower total antioxidant capacity than apples, they are nutritionally important since they represent one of the most important fruit commodities consumed worldwide. Phenolic compounds are the major sources of antioxidant capacity in peaches (Gil, Tomás-Barberán, Hess-Pierce, & Kader, 2002) also involved in fruit visual appearance (pigmentation and browning) and taste (astringency) (Tomás-Barberán et al., 2001). Peaches have been reported to contain flavonols, anthocyanins, flavan-3-ols and hydroxycinnamates (Tomás-Barberán et al., 2001) but their contents vary in relation to different influencing factors, such as cultivar, rootstock, water supply and ripening stage at harvest (Tavarini et al., 2011).

Considerable efforts are being made to increase the level of health-promoting compounds of plant food by both molecular and non-molecular tools, because of the still too low consumption

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of fruits and vegetables despite the well-known beneficial effects on human health. Light intensity and quality are recognised as one of the most effective factors which influence the biosynthesis of secondary plant metabolites. It has been demonstrated that phenolic compounds are very strong absorbers of UV-B radiation and their accumulation may occur as consequence of UV-B eliciting effect (Eichholz et al., 2012). Although it has been reported that UV-B radiation may cause damage to DNA, proteins, and membrane lipids and the inhibition of protein synthesis and photosynthetic reactions (Jenkins, 2009), UV-B radiation (280–315 nm) has been applied during postharvest of fruits and vegetable to trigger changes in the phenol metabolism (e.g. Hagen et al., 2007; Ubi et al., 2006 on apples; Liu et al., 2011 on tomatoes; Interdonato et al., 2011 on lemons; Eichholz et al., 2012 on white asparagus) in order to increase the content of health-promoting compounds. The most damaging effects, in facts, are generally observed in plants exposed to above-ambient levels of UV-B, where the light quality differ substantially from the natural environment (Jenkins, 2009).

Information on UV-B-mediated changes on polyphenol content and biosynthesis in peach fruit is scanty.

On this basis, the study was aimed to investigate the effectiveness of post-harvest UV-B irradiation on improving the health-promoting compound content in peach fruits of three different genotypes (cvs Suncrest, Big Top and Babygold 7). Moreover, the research considered in detail the response of individual hydroxycinnamic acid derivatives, flavonols and anthocyanins present and evaluated the expression levels of the corresponding main genes involved in the phenylpropanoid pathway.

2. Materials and methods

2.1. Plant material and UV-B treatment

Fruits of 'Suncrest' and 'Babygold 7' peach and 'Big Top' nectarine cultivars were harvested in Italy, in correspondence of the commercial maturity, the developmental stage at which physiological maturity has been reached and fruit can be marketed for fresh consumption. A hundred fruits for each cultivar, without defects, selected for uniform size and appearance, were collected and quickly transported to the laboratory at the Department of Agriculture, Food and Environment (DAFE), University of Pisa (Italy).

A group of fruits for each cultivar was immediately sampled after arrival at the laboratory, representing the t_0 of the experiment. The remaining fruits were distributed into climatic chambers (20 °C; R.H. 85%), each equipped with three UV-B lamp tubes (Philips Ultraviolet B, TL 20W-12RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands), providing at fruit height 1.69 W/m^2. To ensure an uniform UV-B dose, fruits were aligned parallel to the lamp tubes. Peaches and nectarines were placed with their peduncle facing down, approximately 40 cm under the lamps, so that only the distal part of each fruit was irradiated, sampled and analysed.

The UV-B irradiation was carried out by treatments of 12 h (73 kJ/m^2), 24 h (146 kJ/m^2) and 36 h (219 kJ/m^2). Control fruits underwent the same conditions but UV-B lamps were screened by benzophenone-treated polyethylene film, known to block UV-B radiation. For any time of sampling, groups consisting of fourteen fruits were collected from control and UV-B chambers. After 36 h, flesh firmness values of 'Babygold 7' and 'Big Top' fruits were in the "ready to buy" range (26.5–35.3 N), while 'Suncrest' peaches were "ready to eat" (8.8–13.2 N), according to Crisosto (2002).

Fruits were carefully peeled using a scalpel. Skin and flesh samples were inserted in falcon tubes kept in dry ice, immediately frozen in liquid nitrogen and stored at −80 °C for the biochemical and molecular analyses.

2.2. Extraction and quantification of total phenols, flavan-3-ols and proanthocyanidins

Freeze-dried skin and flesh samples (0.5 g) were ground with liquid nitrogen to fine powder. The plant material was extracted in triplicate according to the method described by Becatti et al. (2010).

Total phenols were determined on both flesh and skin using the Folin–Ciocalteu colorimetric method. Amounts of 1.85 mL of distilled water, 0.125 mL of Folin–Ciocalteu reagent (Sigma Aldrich Chemical Co., St. Louis, MO) and 0.5 mL of a 20% sodium carbonate solution were added to 25 μL of liquid extract sample in a test tube. The solution was homogenised and left to stand for 30 min. The absorbance was determined versus a blank at 750 nm at room temperature. The total phenol content was expressed as mg of gallic acid/100 g fresh weight (Sigma Aldrich Chemical Co., St. Louis, MO).

Flavan-3-ols content was determined using p-(dimethylamino)cinnamaldehyde (DMACA) reagent (Sigma Aldrich Chemical Co., St. Louis, MO). The reaction mix contained 10 μL of the sample extract, 340 μL of methanol, 250 μL of HCl (0.24 N in MeOH), 250 μL of DMACA solution (0.2% in MeOH). The absorbance was determined at 640 nm, and the total amount of flavan-3-ols was expressed as mg of catechin/100 g fresh weight (Sigma Aldrich Chemical Co., St. Louis, MO).

Proanthocyanidins were quantified in skin samples using a butanol reagent obtained by mixing 128 mg of $FeSO_4{\cdot}7H_2O$ with 5 mL of concentrated HCl in a final volume of 100 mL of *n*-butanol. An aliquot of 50 μL of extract sample was mixed with 700 μL of butanol reagent and heated at 95 °C in a water bath for 45 min. The sample was cooled, 250 μL of *n*-butanol were added and the absorbance was measured at 550 nm. The total amount of condensed tannin was expressed as mg of cyanidin/100 g fresh weight (Sigma Aldrich Chemical Co., St. Louis, MO).

All assays were performed by using an Ultrospec 2100 pro UV–visible spectrophotometer (Amersham Biosciences).

2.3. Extraction, identification and quantification of hydroxycinnamic acids, flavonols and anthocyanins by HPLC–DAD–ESI–MS^n

Skin samples, previously freeze-dried, were ground in fine powder. The plant material (0.02 g) was extracted three times in a final volume of 1.2 mL of 60% aqueous methanol for 90 min in total. The extract was filtered through Corning® Costar® Spin-X® plastic centrifuge tube filters (Sigma Aldrich Chemical Co., St. Louis, MO), subsequently evaporated to dryness and resuspended in 200 μL of distilled water. Each extraction was carried out in triplicate.

A HPLC series 1100 from Agilent (Waldbronn, Germany) consisting of a degaser, binary pump, autosampler, column oven and photodiode array detector was used to determine the hydroxycinnamic acid derivatives and glycosides of flavonols and anthocyanins. The extracts were separated on a Phenomenex Prodigy column (125 × 3.0 mm, ODS 3.5 μm, 100 Å) with a security guard C18 (4 × 3.0 mm, ODS 3.5 μm, 100 Å) at a temperature of 30 °C using a water/acetonitrile gradient. Solvent A consisted of 99.5% water and 0.5% acetic acid; solvent B was 100% acetonitrile. The following gradient was used for Eluent B: 5–7% (0–12 min), 7–9% (12–15 min), 9–12% (15–45 min), 12–15% (45–100 min), 15–75% (100–105 min), 75% isocratic (105–115 min), 75–5% (115–120 min), 5% isocratic (120–123 min). The flow rate was 0.4 mL·min^{-1}, and the detector wavelengths were set at 330, 360 and 520 nm. The hydroxycinnamic acid derivatives and glycosides of flavonols and anthocyanins were identified as deprotonated

molecular ions and characteristic mass fragment ions by HPLC–DAD–ESI–MS^n using an Agilent series 1100 ion trap mass spectrometer in negative ionisation mode. Nitrogen was used as the dry gas (10 L·min^{-1}, 325 °C) in addition to nebuliser gas (40 psi) with a capillary voltage of −3500 V. Helium was used as the collision gas in the ion trap. The mass optimisation for the ion optics of the mass spectrometer was performed for quercetin m/z 301. The MS^n experiments were performed in auto up to MS^3 in a scan from m/z 200–2000. The standards, chlorogenic acid, quercertin-3-glucoside, kaempferol-3-glucoside, isorhamnetin-3-glucoside and cyanidin-3-glucoside (Roth, Karlsruhe, Germany) were used for external calibration curves.

2.4. qRT-PCR analysis

RNA was extracted from freeze-dried skin tissues using E.Z.N.A.® SQ Total RNA Kit. Following RNA isolation, samples were concentrated into a volume of 20 μL using the RNA Clean & Concentrator™-5 (Zymo Research; Orange, CA).

The RNA quality was checked by the 1% agarose gel verification method for intact isolated RNA (MOPS Buffer/Formaldehyde Protocol; Maniatis, Fritsch, & Sambrook, 1982) and using Eppendorf BioPhotometer (Eppendorf, Hamburg, Germany) for getting the concentration and the purity of RNA samples.

First strand cDNA was synthesised from 1 μg of total RNA with the QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany).

Quantitative PCR was performed on the StepOnePlus™ (Applied Biosystems, Foster City, CA), using SYBR® Green. A total reaction volume of 15 μL was used. Reaction included 2 μL template, 0.5 μL of reverse primer, 0.5 μL of forward primer, 7.5 μL iTaq SYBR® Green Supermix with ROX and 4.5 μL RNAfree water.

The qPCR assay was performed using the following conditions: 95 °C for 10 min follow by 40 cycles of 95 °C for 30 s and 60 °C for 30 s. The $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) was used to normalise and calibrate transcript values relative to the endogenous actin gene (actin-forward 5′-CTGGACCTTGCTGGTCGT-3′; actin-reverse 5′-ATTTCCCGCTCAGCAGTG-3′), whose expression did not changed across different genotype, developmental stage and/or treatment.

Primer set used for phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX, also called anthocyanidin synthase, ANS; Appelhagen et al., 2011) gene expression analysis were designed on the sequences reported by Dardick et al. (2010). Chalcone isomerase (CHI) gene specific primers were designed on the sequences reported by Zhou et al. (2013). Average expression for each RNA was determined from the highly

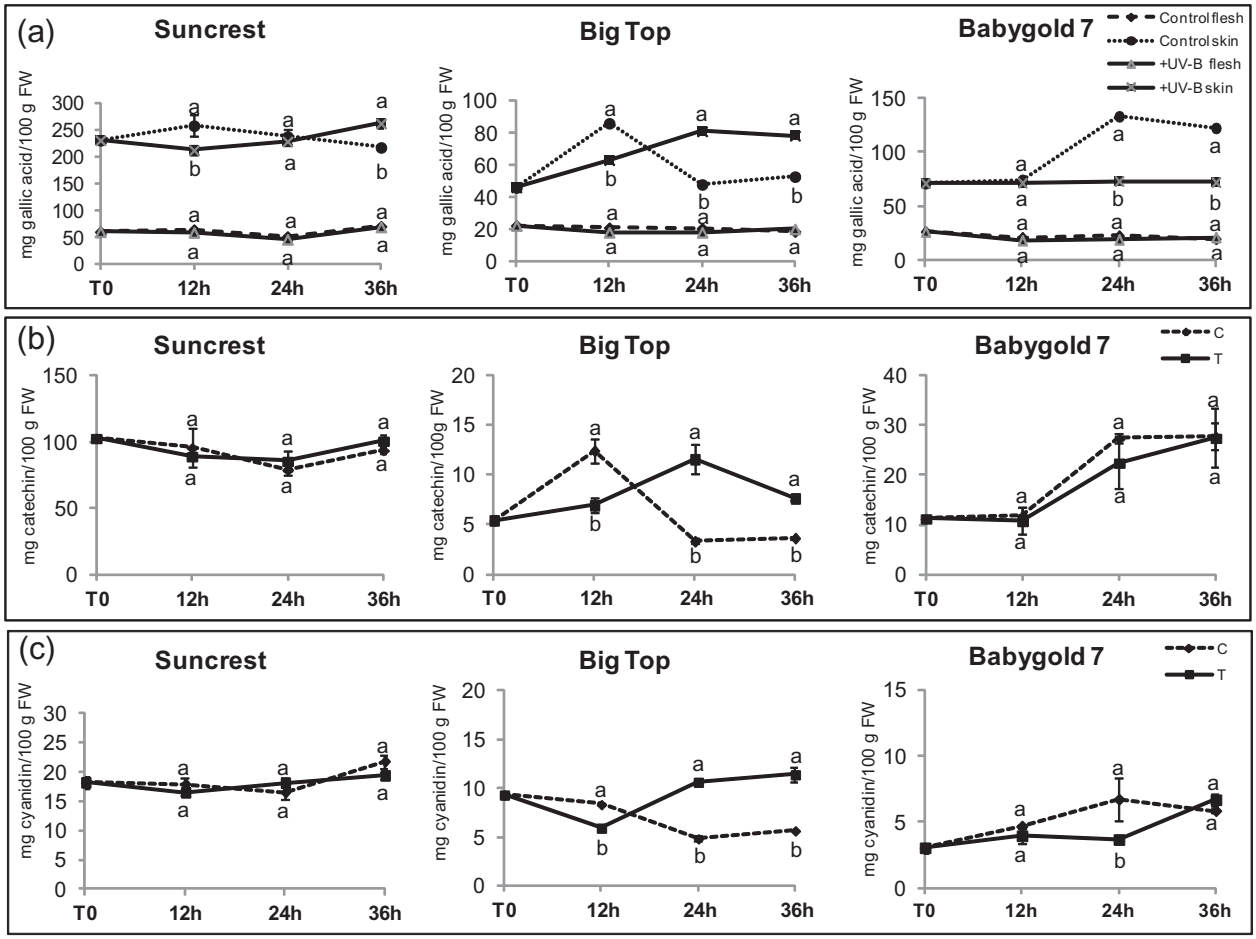

Fig. 1. Concentration of total phenols (a, flesh and skin, expressed as mg of gallic acid/100 mg of FW), catechins (b, skin, expressed as mg of catechin/100 mg FW) and proanthocyanidins (c, skin, expressed as mg of cyanidin/100 mg FW) of peach and nectarine fruits of 'Suncrest', 'Big Top' and 'Babygold 7' cultivars. Fruits were maintained within climatic chambers in the presence (treated fruits, T) or absence (control fruits, C) of UV-B radiation for intervals of 12 h, 24 h and 36 h. Fruits immediately sampled after arrival at the laboratory represent the "starting point" of the experiment (t_0). Data represent the mean of 3 replicates ± SE. Different letters indicate significantly different values according to one-way ANOVA followed by Tukey's test ($P \leqslant 0.05$).

consistent triplicate reactions, with the range of the reactions never more than 0.5 threshold cycle (Ct, a measure of the time at which the PCR product reaches a fixed threshold). Molecular analysis were on samples collected at t_0 and after 36 h for both UV-B treated and control fruits.

2.5. Statistical analysis

Data were subjected to one-way ANOVA for means comparison. Significant differences between UV-B treated and control fruits for each time of sampling were calculated according to Tukey's test ($P \leqslant 0.05$).

3. Results and discussion

3.1. Effect of UV-B radiation on the amount of total phenolics, flavan-3-ols and proanthocyanidins

The effect of UV-B radiation on the total phenolic concentration was studied both in skin and flesh of peach tissues. The total phenolic content was higher in the skin than in the flesh in all cultivars. Our results are consistent with what is reported by Tomás-Barberán et al. (2001) and Remorini et al. (2008). They showed that the total phenolic content of the skin generally was 2–3 times higher than in the flesh.

Total phenol contents from untreated and UV-B treated fruits, expressed as mg of gallic acid/100 g FW, are reported in Fig. 1a. 'Suncrest' fruits were the richest in both skin and flesh phenolics among the cultivar examined.

In 'Suncrest' and 'Big Top' fruits, after an early lower concentration of total phenols in UV-B treated fruits for 12 h, a stimulation of phenol accumulation occurred after 24 h in 'Big Top' (+69%) and 36 h in 'Suncrest' (+21%). The response of 'Babygold 7' fruits to UV-B led to a lower phenolic amount in fruits treated for 24 h and 36 h (Fig. 1a).

The UV-B irradiation influenced the phenol accumulation only in the skin tissue, in agreement with the findings of Hagen et al. (2007), who observed that postharvest irradiation with UV-B radiation enhanced the total phenols in the skin of shade-grown apples. UV-B radiation was previously demonstrated to stimulate the phenol accumulation of fruits albeit at generally lower doses as compared to our experiment. Interdonato et al. (2011) observed that 2 and 3 min of UV-B exposure (0.43 W/m^2) increased the level of total phenolics in flavedo of lemons. In blueberries, UV-B exposure of 0.075 and 0.15 Wh/m^2 resulted in an increase in phenolic compounds (Eichholz et al., 2011). However, UV-B post-harvest treatment with 80 kJ/m^2, a dose similar to that we reached after 12 h, did not affect the total phenolic content of tomatoes (Liu et al., 2011). Conversely, UV-B radiation (1 h a day, 6.08 kJ/m^2) applied to tomato fruits at mature green or turning stage until full ripening, was generally effective in increasing phenolic, flavonoid and flavonol concentration in both skin and flesh (Castagna, Dall'Asta, Chiavaro, Galaverna, & Ranieri, 2013).

The flavan-3-ols and proanthocyanidin contents in the skin (Fig. 1b and c) were positively affected by UV-B treatment only in 'Big Top' fruit. Both classes of compounds responded exhibiting an early decrease after 12 h of UV-B irradiation in comparison to control while a significant increase in their accumulation was observed starting from 24 h of irradiation. The stimulating effect of UV-B on flavan-3-ols led to about 2.5- and 1-fold increase after 24 h and 36 h of irradiation, respectively. The proanthocyanidin content was approximately 1-fold higher in the skin of treated fruits for 24 h and 36 h as compared to untreated samples. Differently from our findings of a stimulating effect of UV-B radiation on skin concentration of flavan-3-ols and proanthocyanidins of 'Big Top' fruits, did not find increased levels of proanthocyanidins in the skin of five apple cultivars after irradiation with UV-B and visible light (0.16–0.2 W/m^2). The amounts of flavan-3-ols and proanthocyanidins in the flesh were not affected by UV-B radiation in the cultivars tested with the exception of 'Babygold 7' where flavan-3-ols decreased in fruits treated for 24 h (−34%, data not shown). Similarly, in 'Aroma' apples the content of epicatechin and proanthocyanidins was minimally influenced by the irradiation treatments with UV-B or similar (0.17 W/m^2) only in the skin (Hagen et al., 2007).

3.2. HPLC–DAD–ESI–MS^n analysis of hydroxycinnamic acids, flavonols and anthocyanins

The methanol extracts obtained from the skin of the three cultivars were separated by HPLC, and the UV and MS spectra of the different peaks were recorded. Table 1 reports the skin concentration of hydroxycinnamic acids, flavonols and anthocyanins of 'Big Top', 'Suncrest' and 'Babygold 7' fruits at t_0. The chromatograms recorded at 330 nm allowed the identification of a main peak coinciding with chlorogenic acid (5-caffeoylquinic acid), and of two minor compounds identified as neochlorogenic acid (3-caffeoylquinic acid) and cryptochlorogenic acid (4-caffeoylquinic acid). These caffeoylquinic acid isomers were detectable in all three cultivar analysed. Conversely, cryptochlorogenic acid was not detected in previous analysis carried out on 25 peach, nectarine and plum cultivars from California (Tomás-Barberán et al., 2001). Chlorogenic acid is the main hydroxycinnamate of peaches, detected in significant amount in all samples tested. Total hydroxycinnamate content of 'Suncrest' fruit was about 3.7- and 6-fold that of 'Big Top' and 'Babygold 7', respectively (Table 1).

Chromatograms recorded at 360 nm from both peach and nectarine samples allowed to detect four quercetin-derivatives

Table 1
Hydroxycinnamic acids, flavonols and anthocyanins (mg/100 g fresh weight) in the fruit skin of peaches and nectarines at t_0.

	Suncrest	Big Top	Babygold 7
Hydroxycinnamic acids[a]			
Chlorogenic acid	58.93 (1.90) a	14.72 (0.43) b	8.91 (0.10) c
Neochlorogenic acid	9.79 (0.24) a	2.75 (0.10) b	2.78 (0.57) b
Cryptochlorogenic acid	3.01 (0.15) a	2.13 (0.18) b	0.32 (0.10) c
Total	71.73 (2.29) a	19.60 (0.71) b	12.01 (0.77) c
Flavonols[b,c,d]			
Quercetin-3-diglucoside[b]	1.93 (0.12) a	n.d. c	1.12 (0.24) b
Quercetin-3-galactoside[b]	6.34 (0.20) a	5.65 (0.55) a	1.68 (0.05) b
Quercetin-3-rutinoside[b]	1.31 (0.04) c	1.69 (0.12) b	2.70 (0.08) a
Quercetin-3-glucoside[b]	5.61 (0.10) b	8.90 (0.79) a	1.19 (0.28) c
Kaempferol-3-galactoside[c]	0.55 (0.02) a	0.48 (0.02) b	0.13 (0.04) c
Kaempferol-3-rutinoside[c]	0.88 (0.02) b	0.37 (0.01) c	2.39 (0.03) a
Kaempferol-3-glucoside[c]	1.77 (0.04) a	1.26 (0.08) b	0.51 (0.13) c
Isorhamnetin-3-galactoside[d]	1.85 (0.03) a	n.d. c	0.54 (0.12) b
Isorhamnetin-3-rutinoside[d]	2.99 (0.01) b	n.d. c	7.42 (0.04) a
Isorhamnetin-3-glucoside[d]	4.41 (0.03) a	n.d. c	1.11 (0.30) b
Total	27.29 (0.61) a	18.35 (1.57) b	18.79 (1.31) b
Anthocyanins[e]			
Cyanidin-3-glucoside	5.63 (0.01) b	24.41 (2.31) a	n.d. c

Mean value ($n = 3$) and standard deviations in parentheses. For each metabolite values followed by different letters are significantly different according to one way ANOVA ($P \leqslant 0.05$) followed by Tukey's test.
[a] Hydroxycinnamic acids quantified as chlorogenic acid.
[b] Flavonols quantified as quercetin-3-glucoside.
[c] Kaempferol-3-glucoside.
[d] Isorhamnetin-3-glucoside.
[e] Anthocyanins quantified as cyanidin-3-glucoside.

Table 2
Hydroxycinnamic acid derivatives, flavonols and anthocyanins (mg/100 g fresh weight) in the skin of 'Suncrest' peaches.

		12 h	24 h	36 h
Hydroxycinnamic acids[a]				
Chlorogenic acid	Vis	62.04 (12.21) a	41.83 (2.32) a	46.22 (1.15) b
	UV-B treated	35.79 (6.16) b	32.97 (1.36) b	50.06 (0.14) a
Neochlorogenic acid	Vis	11.97 (1.98) a	8.34 (0.53) a	9.72 (0.37) b
	UV-B treated	6.52 (1.06) b	6.45 (0.22) b	12.16 (0.02) a
Cryptochlorogenic acid	Vis	3.92 (0.31) a	2.53 (0.08) a	2.27 (0.03) b
	UV-B treated	2.30 (0.40) b	2.05 (0.02) b	3.12 (0.24) a
Total	Vis	77.93 (14.50) a	52.70 (2.93) a	58.21 (1.55) b
	UV-B treated	44.61 (7.62) b	41.47 (1.60) b	65.34 (0.40) a
Flavonols[b,c,d]				
Quercetin-3-diglucoside[b]	Vis	1.19 (0.20) a	0.66 (0.02) b	0.82 (0.01) b
	UV-B treated	0.89 (0.13) b	1.03 (0.05) a	0.90 (0.02) a
Quercetin-3-galactoside[b]	Vis	5.23 (0.81) a	2.98 (0.10) b	3.89 (0.28) b
	UV-B treated	2.97 (0.55) b	4.70 (0.40) a	4.36 (0.07) a
Quercetin-3-rutinoside[b]	Vis	1.09 (0.20) a	0.64 (0.01) b	0.99 (0.06) a
	UV-B treated	0.61 (0.08) b	0.97 (0.04) a	0.97 (0.02) a
Quercetin-3-glucoside[b]	Vis	5.15 (0.80) a	2.66 (0.05) b	3.75 (0.28) b
	UV-B treated	3.07 (0.53) b	4.44 (0.38) a	5.09 (0.03) a
Kaempferol-3-galactoside[c]	Vis	0.42 (0.07) a	0.31 (0.03) b	0.33 (0.01) b
	UV-B treated	0.37 (0.05) a	0.43 (0.04) a	0.40 (0.03) a
Kaempferol-3-rutinoside[c]	Vis	0.82 (0.14) a	0.72 (0.03) a	0.77 (0.05) a
	UV-B treated	0.72 (0.10) a	0.70 (0.06) a	0.78 (0.02) a
Kaempferol-3-glucoside[c]	Vis	1.38 (0.25) a	0.89 (0.04) b	1.08 (0.04) b
	UV-B treated	1.54 (0.54) a	1.34 (0.15) a	1.30 (0.01) a
Isorhamnetin-3-galactoside[d]	Vis	1.39 (0.28) a	0.79 (0.02) b	0.89 (0.06) a
	UV-B treated	1.17 (0.03) a	0.98 (0.12) a	0.90 (0.04) a
Isorhamnetin-3-rutinoside[d]	Vis	2.45 (0.43) a	2.12 (0.10) a	2.24 (0.09) a
	UV-B treated	1.99 (0.11) a	1.90 (0.18) b	2.07 (0.11) a
Isorhamnetin-3-glucoside[d]	Vis	3.26 (0.67) a	2.03 (0.04) b	2.66 (0.05) a
	UV-B treated	2.97 (0.47) a	2.72 (0.26) a	3.01 (0.40) a
Total	Vis	22.38 (3.85) a	13.80 (0.44) b	17.42 (0.95) b
	UV-B treated	16.30 (2.59) a	19.21 (1.68) a	19.78 (0.75) a
Anthocyanins[e]				
Cyanidin-3-glucoside	Vis	7.83 (1.22) a	3.04 (0.08) b	3.87 (0.05) b
	UV-B treated	2.90 (0.48) b	5.43 (0.14) a	7.89 (0.10) a

Mean value ($n = 3$) and standard deviations in parentheses. For each metabolite values followed by different letters are significantly different according to one way ANOVA ($P \leqslant 0.05$) followed by Tukey's test.
[a] Hydroxycinnamic acids quantified as chlorogenic acid.
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[d] Isorhamnetin-3-glucoside.
[e] Anthocyanins quantified as cyanidin-3-glucoside.

(quercetin-3-diglucoside, quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside) and three kaempferol- (kaempferol-3-galactoside, kaempferol-3-rutinoside, kaempferol-3-glucoside) and isorhamnetin-derivatives (isorhamnetin-3-galactoside, isorhamnetin-3-rutinoside, isorhamnetin-3-glucoside), respectively. 'Big Top', 'Suncrest' and 'Babygold 7' fruits had the same individual flavonol-glycosides, with the exception of quercetin-3-diglucoside and the three isorhamnetin-glycosides, which were not present in 'Big Top' (Table 1). The occurrence of kaempferol and isorhamnetin in 'Suncrest' and 'Babygold 7' peach fruits confirmed the findings obtained by Scordino, Sabatino, Muratore, Belligno, and Gagliano (2012), reporting the presence of these flavonol-glycosides in Sicilian peach cultivar. On the contrary, kaempferol and isorhamnetin were not present in Californian peaches (Tomás-Barberán et al., 2001). 'Babygold 7' exhibits a higher content of all flavonols glycosylated with rutinose if compared to 'Suncrest' and 'Big Top' fruits. The contents of quercetin-3-glucoside and quercetin-3-galactoside and the corresponding kaempferol glycosides in 'Babygold 7' appeared significantly lower than 'Big Top' and 'Suncrest'. The total content of glycosylated flavonols in 'Suncrest' was about 1.5 times higher than that of the other cultivars tested (Table 1) suggesting a cultivar-specific effect.

As expected complete lack of anthocyanins was recorded at 520 nm in 'Babygold 7' extracts while in 'Big Top' the cyanidin-3-glucoside content was detected in a 5 times higher amount than in 'Suncrest' (Table 1). Differently from our findings, previous studies found also cyanidin-3-rutinoside as anthocyanin pigments in peaches (Tomás-Barberán et al., 2001; Wu & Prior, 2005).

Comparing the results on phenolic profile of the three cultivars, it is evident that the most abundant phenolic substance in the skin tissue of 'Suncrest' and 'Babygold 7' fruits was chlorogenic acid, while in 'Big Top' it was cyanidin-3-glucoside. It is interesting to underline how 'Big Top', the richest cultivar in cyanidin-3-glucoside (3.3-fold higher than 'Suncrest'), showed lower amounts or even a complete lack of some flavonols, suggesting a possible different regulation of the flavonoid biosynthesis leading to anthocyanin accumulation.

After an UV-B induced decrease at 12 and 24 h, the concentration of the three hydroxycinnamic acids underwent a significant increase in 'Suncrest' skin following 36 h irradiation (8, 25 and 37% for chlorogenic, neochlorogenic and cryptochlorogenic acid, respectively) (Table 2). Stimulation of flavonol accumulation by UV-B treatment occurred earlier, being already evident at 24 h for all glycosides (except kaempferol-3-rutinoside and isorhamnetin-3-rutinoside) (Table 2). As a consequence, at this time, the concentration of total flavonol glycosides was about 39% higher in UV-B-treated samples as compared to the respective controls. Cyanidin-3-glucoside underwent a significant increase after 24 h (79%) and 36 h (104%) of UV-B irradiation as well. 'Big Top' skin accumulated cryptochlorogenic acid following 24 h UV-B irradiation

Table 3
Hydroxycinnamic acid derivatives, flavonols and anthocyanins (mg/100 g fresh weight) in the skin of 'Big Top' nectarines.

		12 h	24 h	36 h
Hydroxycinnamic acids[a]				
Chlorogenic acid	Vis	25.75 (0.70) a	23.92 (2.03) a	15.91 (0.02) b
	UV-B treated	21.48 (0.76) b	26.75 (1.72) a	20.67 (1.75) a
Neochlorogenic acid	Vis	3.72 (0.21) a	4.29 (0.32) a	2.95 (0.33) a
	UV-B treated	3.38 (0.06) b	3.39 (0.21) b	2.98 (0.24) a
Cryptochlorogenic acid	Vis	3.08 (0.19) a	2.18 (0.12) b	1.80 (0.29) b
	UV-B treated	2.90 (0.08) b	4.49 (0.20) a	4.22 (0.42) a
Total	Vis	32.55 (1.10) a	30.39 (2.47) a	20.66 (0.64) b
	UV-B treated	27.76 (0.90) b	34.63 (2.13) a	27.87 (2.41) a
Flavonols[b,c,d]				
Quercetin-3-diglucoside[b]	Vis	n.d.	n.d.	n.d.
	UV-B treated	n.d.	n.d.	n.d.
Quercetin-3-galactoside[b]	Vis	7.16 (0.20) a	3.35 (0.51) b	3.23 (0.39) b
	UV-B treated	4.82 (0.13) b	7.14 (0.26) a	6.47 (0.75) a
Quercetin-3-rutinoside[b]	Vis	1.58 (0.06) a	1.18 (0.17) b	1.06 (0.11) b
	UV-B treated	1.09 (0.04) b	2.11 (0.09) a	1.86 (0.20) a
Quercetin-3-glucoside[b]	Vis	10.45 (0.19) a	6.51 (0.05) b	5.76 (0.67) b
	UV-B treated	8.51 (0.14) b	11.98 (0.50) a	10.90 (1.24) a
Kaempferol-3galactoside[c]	Vis	0.49 (0.02) a	0.31 (0.05) b	0.23 (0.01) b
	UV-B treated	0.33 (0.01) b	0.43 (0.03) a	0.41 (0.02) a
Kaempferol-3-rutinoside[c]	Vis	0.25 (0.02) a	0.23 (0.04) b	0.19 (0.01) b
	UV-B treated	0.14 (0.01) b	0.31 (0.02) a	0.30 (0.02) a
Kaempferol-3-glucoside[c]	Vis	1.08 (0.03) a	0.99 (0.16) a	0.71 (0.01) b
	UV-B treated	0.91 (0.01) b	1.03 (0.08) a	1.01 (0.03) a
Isorhamnetin-3-galactoside[d]	Vis	n.d.	n.d.	n.d.
	UV-B treated	n.d.	n.d.	n.d.
Isorhamnetin-3-rutinoside[d]	Vis	n.d.	n.d.	n.d.
	UV-B treated	n.d.	n.d.	n.d.
Isorhamnetin-3-glucoside[d]	Vis	n.d.	n.d.	n.d.
	UV-B treated	n.d.	n.d.	n.d.
Total	Vis	21.01 (0.52) a	12.57 (0.98) b	11.18 (1.20) b
	UV-B treated	15.80 (0.34) b	23.00 (0.98) a	20.95 (2.26) a
Anthocyanins[e]				
Cyanidin-3-glucoside	Vis	25.70 (1.33) b	20.87 (4.59) b	15.30 (2.20) b
	UV-B treated	28.65 (0.20) a	32.42 (2.93) a	38.70 (6.63) a

Mean value ($n = 3$) and standard deviations in parentheses. For each metabolite values followed by different letters are significantly different according to one way ANOVA ($P \leqslant 0.05$) followed by Tukey's test.
[a] Hydroxycinnamic acids quantified as chlorogenic acid.
[b] Flavonols quantified as quercetin-3-glucoside.
[c] Kaempferol-3-glucoside.
[D] Isorhamnetin-3-glucoside.
[e] Anthocyanins quantified as cyanidin-3-glucoside.

(+106%) and cryptochlorogenic and chlorogenic acid after 36 h treatment (+144% and +30%, respectively, in comparison to untreated skin) (Table 3). Such a positive effect of post-harvest UV-B treatment occurred earlier for flavonols, which, with the exception of kaempferol-3-glucoside, showed a significant increase starting from 24 h UV-B exposure, leading to a total increase of about 83% and 87% at 24 h and 36 h, respectively (Table 3).

Cyanidin-3-glucoside was the only phenolic compound positively affected by UV-B irradiation already after 12 h, although the effect was more pronounced after 24 h and, particularly, after 36 h of treatment, when its concentration was about 1.5-fold higher than in untreated sample (Table 3).

The stimulating effect of UV-B radiation on phenolic compounds was less evident in 'Babygold 7' peaches, where an increase in the three hydroxycinnamic concentration was transiently induced by 24 h UV-B irradiation (+45, +99 and +50%, for chlorogenic, neochlorogenic and cryptochlorogenic acid, respectively), the effect being negative at 36 h (Table 4). Individual flavonols were only slightly influenced by the treatment, and this resulted in a decrease of total flavonol glycosides at both 24 h and 36 h (Table 4).

Opposite responses were observed for hydroxycinnamic acids and flavonols after 24 h, depending on the cultivar considered. In 'Suncrest' and 'Big Top' treated fruits, the amount of individual and total flavonol glycosides was significantly higher as compared to controls, while in 'Babygold 7' the effect of UV-B irradiation was null or even negative. As far as hydroxycinnamic acids are concerned, the opposite behaviour could be observed. The amount of these compounds was indeed generally reduced or unaffected by 24 h UV-B treatment in 'Suncrest' and 'Big Top' skin, but increased in UV-B treated 'Babygold 7' samples. Such a different behaviour shown by the three cultivars was also evident following 36 h UV-B irradiation. In fact, while "Suncrest" and "Big Top" fruits underwent a generalised significant increase in the skin concentration of hydroxycinnamic acids, flavonol glycosides and anthocyanins, the treatment induced a negative effect on phenolic accumulation of 'Babygold 7' fruits.

According to Solovchenko and Schmitz-Eiberger (2003), quercetin-glycosides in apples appear to have a primary function in UV-B protection of fruit peel.

In 'Big Top' fruits, the quercetin/kaempferol ratio, and in 'Suncrest' also the quercetin/isorhamnetin ratio was higher in UV-B exposed fruits, consistent with the hypothesis that quercetin flavonols have a better ability for free radical scavenging than kaempferol and isorhamnetin flavonols (Harborne & Williams, 2000). In this context, the observed increase in quercetin derivatives represent a major response to UV-B treatments (at least in two cvs) where they act as efficient sunscreens.

In 'Babygold 7' fruits the same flavonol glycosides were not effectively involved in the "hormetic" response (i.e. the application of potentially harmful radiation at low doses to induce beneficial stress responses) but, on the contrary, their contents dropped after 24 h and 36 h of UV-B treatment. In particular, in 'Babygold 7' fruit irradiated with UV-B for 36 h, a lower amount of all the flavonols glycosylated with rutinose was observed, while little increases were registered in case of glycosylation with galactose and glucose.

Our results confirmed that postharvest irradiation with UV-B had a marked influence on the concentration of chlorogenic acid, and other hydroxycinnamates, according to the findings of Hagen et al. (2007) and Lancaster, Reay, Norris, and Butler (2000) on apples, and Huyskens-Keil, Eichholz, Kroh, and Rohn (2007) on black currant. Moreover, the present study is in accordance with previous reports showing a positive effect of postharvest UV-B irradiation on anthocyanin levels in peaches, apples and strawberries (Higashio, Hirokane, Sato, Tokuda, & Uragami, 2005; Kataoka & Beppu, 2004; Marais, Jacobs, & Holcroft, 2001). The accumulation of anthocyanins in the skin of peach fruits started earlier (12 h) and increased to a higher level than the accumulation of flavonols (24 h) and hydroxycinnamates (36 h). In 'Suncrest', anthocyanin accumulation occurred prior to the increase of hydroxycinnamates (36 h) but at the same time of flavonols. This evidence suggests that, though flavonols, hydroxycinnamates and anthocyanins originate from the same biosynthetic pathway (Treutter, 2001), their specific synthesis in the peach skin is influenced by UV-B radiation in a time-dependent way.

3.3. Gene expression

To clarify the molecular regulation of the differences observed in flavonoid and hydroxycinnamates accumulation following UV-B irradiation, analysis of *PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H*, *DFR*, and *LDOX* gene expression was carried out by qRT-PCR. The results are shown in Fig. 2. The analysis has been carried out on 'Suncrest', 'Big Top' and 'Babygold 7' skin samples collected at t_0 and after 36 h, in correspondence of UV-B dose inducing significant increases in phenolic accumulation in the two cultivars ('Suncrest' and 'Big Top') which reacted positively to UV-B. Taking into account the expression levels of the phenylpropanoid genes, the three cultivars exhibited different time course expression patterns in untreated fruits. In 'Suncrest', after 36 h, a general suppression of transcript levels occurred. On the contrary, the expression of *C4H*, *CHS*, *DFR*, *LDOX* was up-regulated in untreated fruits of 'Big Top'. Also in 'Babygold 7' the untreated fruits after 36 h showed an increase of the expression levels of all the genes tested in comparison to t_0, with the exception of C4H and 4CL which did not change.

Considering the effect of the UV-B radiation after 36 h, the expression of *PAL*, *4CL*, *CHS*, *CHI* genes was up-regulated in both

Table 4
Hydroxycinnamic acid derivatives, flavonols and anthocyanins (mg/100 g fresh weight) in the skin of 'Babygold 7' peaches.

		12 h	24 h	36 h
Hydroxycinnamic acids[a]				
Chlorogenic acid	Vis	7.90 (0.52) a	10.13 (1.95) b	8.43 (0.41) a
	UV-B treated	9.51 (1.66) a	14.70 (0.10) a	3.49 (0.10) b
Neochlorogenic acid	Vis	1.79 (0.25) a	1.62 (0.28) b	1.81 (0.10) a
	UV-B treated	2.18 (0.41) a	3.22 (0.02) a	1.18 (0.44) b
Cryptochlorogenic acid	Vis	0.50 (0.13) a	0.40 (0.11) b	0.29 (0.02) a
	UV-B treated	0.41 (0.11) a	0.60 (0.01) a	0.17 (0.01) b
Total	Vis	10.19 (0.90) a	12.15 (2.34) b	10.53 (0.53) a
	UV-B treated	12.10 (2.18) a	18.52 (0.13) a	4.84 (0.55) b
Flavonols[b,c,d]				
Quercetin-3-diglucoside[b]	Vis	0.93 (0.13) a	1.78 (0.40) a	1.21 (0.01) a
	UV-B treated	1.14 (0.23) a	1.47 (0.01) b	0.92 (0.01) b
Quercetin-3-galactoside[b]	Vis	2.77 (0.78) a	4.26 (0.96) a	2.64 (0.02) b
	UV-B treated	3.27 (0.53) a	3.62 (0.04) a	2.69 (0.01) a
Quercetin-3-rutinoside[b]	Vis	2.51 (0.32) a	4.33 (0.98) a	2.73 (0.02) a
	UV-B treated	3.03 (0.55) a	3.40 (0.02) a	2.10 (0.02) b
Quercetin-3-glucoside[b]	Vis	2.33 (0.31) a	3.31 (0.75) a	2.16 (0.02) a
	UV-B treated	2.73 (0.45) a	2.47 (0.02) b	2.19 (0.01) a
Kaempferol-3-galactoside[c]	Vis	0.19 (0.05) a	0.27 (0.10) a	0.15 (0.01) b
	UV-B treated	0.17 (0.04) a	0.22 (0.01) a	0.19 (0.01) a
Kaempferol-3-rutinoside[c]	Vis	2.58 (0.10) a	2.67 (0.49) b	2.51 (0.02) a
	UV-B treated	2.91 (0.60) a	3.28 (0.02) a	1.87 (0.02) b
Kaempferol-3-glucoside[c]	Vis	0.54 (0.10) a	0.84 (0.21) a	0.61 (0.01) b
	UV-B treated	0.68 (0.14) a	0.88 (0.02) a	0.86 (0.01) a
Isorhamnetin-3-galactoside[d]	Vis	0.58 (0.13) a	1.06 (0.25) a	0.66 (0.01) b
	UV-B treated	0.64 (0.16) a	0.95 (0.03) a	0.70 (0.01) a
Isorhamnetin-3-rutinoside[d]	Vis	8.58 (0.73) a	9.10 (0.88) a	7.28 (0.06) a
	UV-B treated	8.67 (1.81) a	9.33 (0.09) a	5.27 (0.06) b
Isorhamnetin-3-glucoside[d]	Vis	0.98 (0.12) a	1.64 (0.33) a	1.23 (0.02) b
	UV-B treated	1.20 (0.31) a	1.65 (0.07) a	1.51 (0.01) a
Total	Vis	21.99 (2.77) a	29.26 (5.35) a	21.18 (0.20) a
	UV-B treated	24.44 (4.82) a	18.27 (0.33) b	18.30 (0.17) b
Anthocyanins[e]				
Cyanidin-3-glucoside	Vis	n.d.	n.d.	n.d.
	UV-B treated	n.d.	n.d.	n.d.

Mean value ($n = 3$) and standard deviations in parentheses. For each metabolite values followed by different letters are significantly different according to one way ANOVA ($P \leqslant 0.05$) followed by Tukey's test.

[a] Hydroxycinnamic acids quantified as chlorogenic acid.
[b] Flavonols quantified as quercetin-3-glucoside.
[c] Kaempferol-3-glucoside.
[d] Isorhamnetin-3-glucoside.
[e] Anthocyanins quantified as cyanidin-3-glucoside.

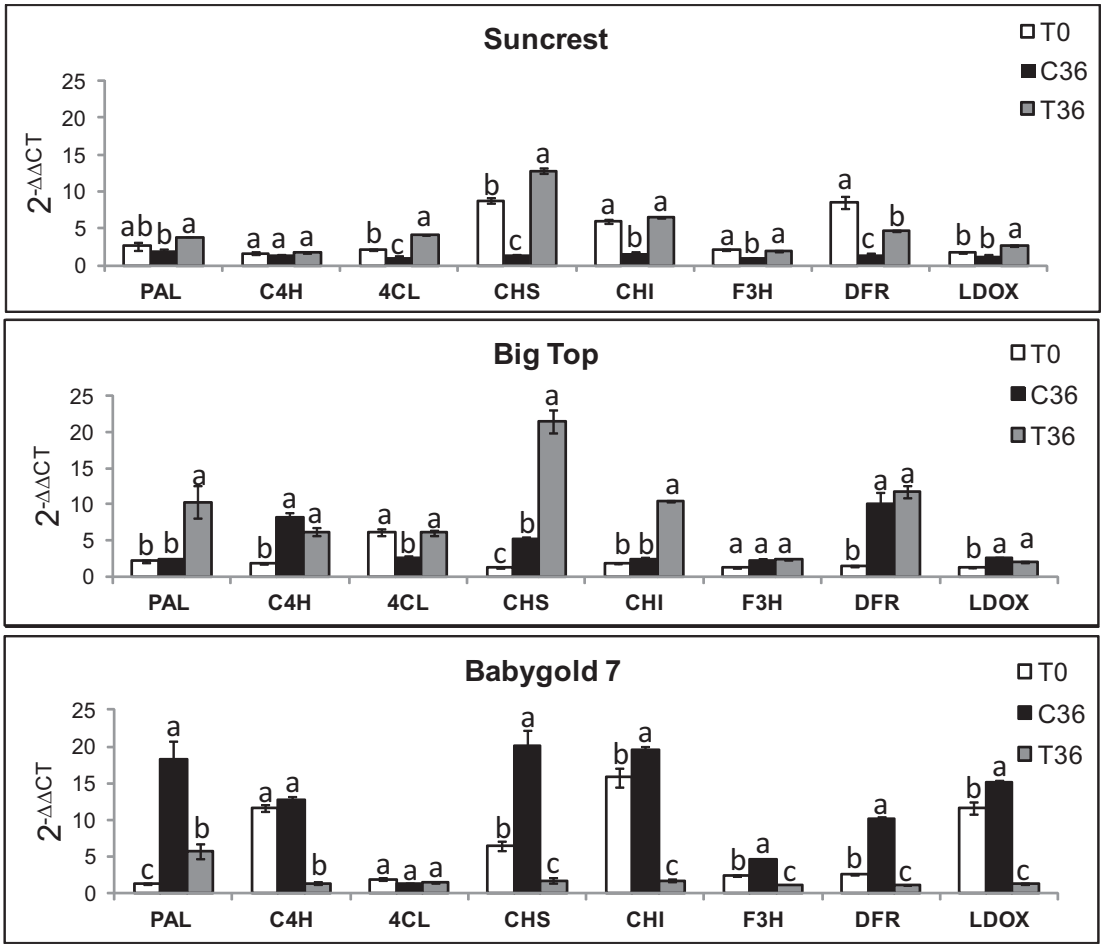

Fig. 2. Expression patterns of phenylpropanoid biosynthetic genes determined by qRT-PCR in the skin of 'Suncrest', 'Big Top' and 'Babygold 7' peach and nectarine fruits maintained within climatic chambers in the presence (treated fruits, T) or absence (control fruits, C) of UV-B radiation for 36 h. Fruits immediately sampled after arrival at the laboratory represent the "starting point" of the experiment (t_0). Data represent the mean of 5 replicates ± SE. Different letters indicate significantly different values according to one-way ANOVA followed by Tukey's test ($P \leqslant 0.05$).

'Big Top' and 'Suncrest' UV-B treated fruits compared to the correspondent control. The expression of *F3H*, *DFR* and *LDOX* was also up-regulated in 'Suncrest' fruit. These results are in agreement with the findings previously reported in the present study about hydroxycinnamic acids and flavonols which increased in 'Big Top' and 'Suncrest' after UV-B irradiation for 36 h. However, even if a marked accumulation of proanthocyanidins and anthocyanins occurred in 'Big Top' no significant difference in the expression was induced by UV-B treatment on *DFR* and *LDOX* genes. Recently, Ravaglia et al. (2013) reported that, during fruit development, anthocyanin concentration of nectarines cv. 'Stark Red Gold' only correlated with the expression of *UDP-glucose-flavonoid-3-O-glucosyltransferase* (*UFGT*), but not with *DFR* and *LDOX* transcription, confirming our results on the different patterns shown by anthocyanin levels and the expression of these two genes. Differently, in the skin of 'Flavortop' nectarine, the anthocyanin accumulation correlated also with the transcript levels of *F3H*, *LDOX* and *CHI* (Tsuda, Yamaguchi, Honda, & Moriguchi, 2004). In our study all these three genes were up-regulated by UV-B in 'Suncrest' fruits, while only the expression of *CHI* was enhanced in 'Big Top' fruits. In 'Babygold 7', all genes tested were down-regulated by UV-B treatment if compared to untreated samples, with the exception of *4CL* gene. The decrease of hydroxycinnamic acid derivatives and flavonols after 36 h of treatment is in agreement with the gene expression of the phenylpropanoid biosynthesis (down-regulation of *PAL*, *C4H*, *CHS*, *CHI*, *F3H*, *DFR*, *LDOX*). The lower expression level of *DFR* and *LDOX* genes did not bring to a significantly decreased amount of proanthocyanidins, while anthocyanins were not present in 'Babygold 7' skin samples.

With exception of few reports exploring the effect of UV-B deprivation on phenylpropanoid biosynthetic genes of fruits (Calvenzani et al., 2010; Giuntini et al., 2008), to the best of our knowledge only few papers deal with the effects of post-harvest UV-B irradiation on gene expression (Ban, Honda, Bessho, Pang, & Moriguchi, 2007; Peng et al., 2013; Ubi et al., 2006).

Ubi et al. (2006) observed an increase in the expression levels of five biosynthetic genes in apple skin, including *CHS*, *F3H* and *DFR*, paralleled by an increase in final anthocyanin concentration. Similarly, Ban et al. (2007) showed that the expression of *CHS*, *F3H* and *FLS* genes was induced by UV-B irradiation in apple skin.

UV-B induced monomerisation of the UV-B receptor UVR8 (UV RESPONSE LOCUS 8) leading to subsequent direct interaction with the multifunctional E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) mediating the translocation of the UVR8 protein from the cytosol into the nucleus. In the nucleus, UVR8 associates with COP1 and the chromatin regions of several UV-B activated transcription factors (HY5: ELONGATED HYPOCOTYL5; a bZIP transcription factor; HYH: HY5 HOMOLOG) (Favory et al., 2009). In turn, HY5 and HYH control expression of a range of key elements for UV-B acclimation, including genes encoding enzymes of the phenylpropanoid pathway such as *CHS* (Oravecz et al., 2006).

Thus, up-regulation of *CHS*, in particular, has been used as one of the major markers of UV-B stress (Tohge, Kusano, Fukushima, Saito, & Fernie, 2011). Peng et al. (2013) suggested that UV-B irradiation would also induce anthocyanin accumulation in apple skin via the COP1/HYH/HY5 signaling pathway.

Our findings confirmed the involvement of *CHS* in the peach skin response to UV-B (*CHS* about 8 and 3-fold higher expressed in 'Suncrest' and 'Big Top' treated fruit). These results let us to suppose that the biosynthesis of metabolites which can protect against UV-B damage have a transcriptional linkage to the UV-B photomorphogenic response, probably regulated in a similar way than what assumed in apple. However, the opposite behaviour observed in 'Babygold 7', where *CHS* expression was about 11-fold lower than in untreated samples, indicated that a genotype-dependent mechanism was involved in UV-B response of peach fruits.

4. Conclusions

The consumption of plant-based food products with elevated concentration of specific secondary metabolites may have protective effects on the human health. The induction of selected plant metabolites accumulation can be obtained by changing parameters in post-harvest but a detailed knowledge of key regulatory steps within the biosynthetic pathways is required to optimise the yield of desired compounds.

The results presented in this work indicate that irradiation with UV-B in post-harvest is an effective tool to modulate the concentration of health-promoting compounds in peach and nectarine fruits and it is able to induce modifications at gene expression level. The skin, the external tissue directly affected by UV-B exposure, was mainly influenced. The use of an appropriate UV-B dose induced positive effects on polyphenol accumulation but attention should be paid to the choice of the genotype since the metabolic response was different depending on the cultivar considered. A concurrent increase in aesthetic value due to the higher anthocyanin content of treated fruits could also be attributed to UV-B post-harvest treatments, but, again, this seems a variety-dependent effect.

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