Manuscript Details

Manuscript number	POSTEC_2017_766_R1
Title	Post-harvest UV-B radiation modulates metabolite profile in peach fruit
Article type	Research Paper

Abstract

The possibility to modify plant metabolic profile of plants and fruit to improve their healthy properties using eco-friendly tools, rather than transgenic approaches, gained interest in the last decades. Ultraviolet-B (UV-B) radiation, at low levels, thanks to its ability to influence plant secondary metabolism, could be successfully used to achieve this goal. However, few studies have been conducted so far on the effects of post-harvest UV-B treatments on fruit metabolomics. The present research, aimed to evaluate the impact of UV-B on peach metabolites profile through non-targeted metabolomics (UHPLC-ESI/QTOF-MS) coupled with multivariate chemometrics, provided evidence that 10 and 60 min of post-harvest UV-B irradiation influenced several classes of metabolites. Most phenolics were down-accumulated 24 h after both UV-B treatments, though, after 36 h, anthocyanins, flavones and dihydroflavonols increased (2.06-, 1.92-, 1.68-fold with 10 min UV-B; 6.65-, 2.53-, 2.05-fold with 60 min UV-B, respectively). UV-B reduced carotenoids and most lipids and increased some biosynthetic intermediates and degradation products, some of them known for their positive role in human health. Among alkaloids, some pteridines accumulated, likely derived from folates degradation, while indole alkaloids decreased. Despite the decrease of some bioprotective metabolites as carotenoids, the UV-B-induced up-accumulation of many antioxidant phenolics after 36 h from the exposure suggests an improvement of the healthy properties of peach fruit and reinforces the potential of UV-B controlled irradiation as a nutraceuticals-increasing tool in fruit.

Keywords	Phenolics, Peach fruit, Prunus persica L., Metabolomics, Terpenoids, UV-B radiation

Corresponding Author	Antonella Castagna
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Suggested reviewers	Javier Martinez-Abaigar, Susanne Neugart, Francisco Tomas-Barberan

Submission Files Included in this PDF

File Name [File Type]

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Fig. 1.docx [Figure]

Fig. 2.docx [Figure]

Fig. 3.docx [Figure]

tab 1.docx [Table]

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Tab S1 Dataset Phenol-Explorer.xlsx [Table]

tab S2 Discriminant_Phenol-Explorer.xlsx [Table]

Tab S3 Dataset PlantCyc.xlsx [Table]

Tab S4 Discriminant_PlantCyc.xlsx [Table]

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Pisa, 24 January 2018

Dear Professor Tonutti
Associate Editor
Postharvest Biology and Technology

Please find here enclosed the revised version of the manuscript "Post-harvest UV-B radiation modulates metabolite profiling in peach fruit", authors: Marco Santin, Luigi Lucini, Antonella Castagna, Giulia Chiodelli, Marie-Theres Hauser, Annamaria Ranieri.

The manuscript was revised according your requirements and the suggestions made by the reviewers, as detailed in the specific file.

All authors agree with the contents of the manuscript and its submission to the journal. All Authors listed have contributed significantly to the work and agree to be in the author list.

The research is original, was carried out by the authors and no part of it has been published in any form elsewhere.

Concerning the options for reproducing color illustrations in the article, I choose the color reproduction only in the online version, and the black and white reproduction in the printed version.

Hoping that the revised manuscript will be suitable for publication in Postharvest Biology and Technology, I send my best regards.

Yours sincerely
Antonella Castagna

Ref: POSTEC_2017_766
Title: Post-harvest UV-B radiation modulates metabolite profiling in peach fruit
Journal: Postharvest Biology and Technology

Dear Dr. Castagna,

Thank you for submitting your manuscript to Postharvest Biology and Technology. I have completed the review of your manuscript and a summary is appended below. The reviewers recommend reconsideration of your paper following major revision.

In addition to addressing all reviewers comments (in particular concerning tab. 1 data, FC values and how they have been calculated and reported in figures and tables), please consider also the followings:

- provide a better and nore descriptive legend of tab. 1

Reply: The caption of table 1 was entirely revised to be more descriptive and self-explanatory.

- check English language throughout the manuscript, as pointed out by Reviewer 2
Reply: Following the request of Reviewer 2, the manuscript was carefully checked for English grammar and style

- Change 'metabolite profiling' in the title with 'metabolite profile' (profiling is the act of measuring the profile)

Reply: the word “profiling” was substituted with “profile” in the title and text

- Add space before %

Reply: As required, space was added before %

- change "fruits" with "fruit" in the 1st and 5th highlight
Reply: the word was corrected as requested.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, mark every change made to the manuscript in color, and provide suitable rebuttals for any comments not addressed.

To submit your revised manuscript:

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article upon acceptance. Both articles will be linked on ScienceDirect. Please use the MethodsX template available here when preparing your article: https://www.elsevier.com/MethodsX-template. Open access fees apply.

I look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Professor Tonutti
Associate Editor
Postharvest Biology and Technology

Comments from the editors and reviewers:
-Reviewer 1

-
Dear Editor!
Here are my commnets regarding manuscript POSTEC_2017_766,

The manuscript aim to evaluate the effect of post-harvest UV-B radiation on metabolite profiling in peach fruit. Introduction part is focused on the paper, methodologies are given in detail, and results presentation, interpretation and discussion is satisfactory. In the text below, is a number of points that need attention (see some comments to the authors).

line 101: please specify wether 1 single fruit represents biological replicate
Reply: Each individual fruit represented a biological replicate. This information was inserted in the revised text (line116)

line 111: my questions: Groups of five peaches = five biological control
Reply: Yes, this is true. For any treatment (i.e. control at 24 h, UV-B 10 min at 36 h, etc.), 5 individual fruit were sampled representing 5 biological replicates.

line 116: Five individual replicates; is that technical replicate (was each fruit sampled 5 times) or five individual replicates refer to biological replicates
Reply: five individual replicates refer to biological replicates. To avoid misunderstandings, the sentence was simplified as follows:" Samples were extracted in…"

line 185: Are the most significant parameters that contribute to cstering
Reply: Indeed, they are those phenolics better discriminating among treatments. A sentence has been added, and the sentence already present amended, to make this clearer.

line 198: hydroxycoumarins instead of hydroxycumarins
Reply: the word was corrected

Figures 1, 3
for how much of the total variability acounts both functions (t0, t1), I suggest that data should be inserted within both axes on Figures 1 and 3
Reply: Unlike PCA, the PLS-DA is a supervised multivariate statistic, for which the total variability explained by first and second component (here defined as latent vectors) is not calculated. In order to provide with information having a comparable meaning, we specified in the text the overall accuracy of the PLS-DA class prediction models (following N-fold validation).

Tab. 1 column FC (abs)

the values are separated by comma instead of full stop (.) some results are expressed in exponential way i.e. 4,66E+07. I suggest all the values should be expressed in exponential way
Reply: We apologise for the use of comma, due to the Italian language of the keyboard. Values are now expressed in exponential way, except numbers to 0 and 1 power

values under 36 h and 10 min are exactly the same: 726869,25 four times, 16 two times, 821619,75 three times, 4,66E+07 two times, 133196,55 two times, 3,5389855 two times. Is it a coincidence?
Reply: Unfortunately, they are multiple IDs for isobaric compounds, that cannot be discriminated neither when the highest mass resolutions (e.g. Orbitrap) is used. In the former revision we decided to keep a line for each compound; however, we realized this might be misinterpreted and therefore we are now reporting them in the same table line. As they are multiple IDs, we separate each possible compound by a "/".

I suggest all values can be expressed with two digits after full stop
Reply: values of revised supplementary tables are reported in exponential way with two digits after full stop

My question: on which basis is the Table 1 constructed in sense that compounds listed are different according to sampling time and time od exposure?
Reply: Volcano analysis (FC threshold = 2; p value threshold = 0.05) revealed that 10 or 60 min UV-B exposure, as well as the two recovery times (24 or 36 h) significantly influenced a different number and a different kind of compounds. E.g. in the 60 min-UVB-treated samples, after 36h, we observed a much longer list of compounds, meaning that more compounds were affected in respect to the 10 min-UVB exposure or the 24 h-recovery time.

-Reviewer 2

The paper reports post-harvest UV-B radiation modulates metabolite profiling in peach fruit. The study contains some interesting data and enhances the previous reports in this research area. The authors should consider the following points before the paper is considered for publication.

1. In the abstract section, the message is too general. The reviewer suggests that the authors should provide more specific information, particular in the objective of the health-promoting value of plant food. The UV-B radiation can be used postharvest treatment to increase the nutritional quality of peach fruit?

Reply: the abstract was re-written adding some quantitative data and better explaining the potential positive effect of UV-B treatments to improve the healthy properties of peach fruit.

2. In the materials section, why the authors use the two doses of UV-B radiation?

Reply: The choice of a short (10 min) and a long (60 min) UV-B treatment was done on the basis of a preliminary study revealing that such doses were effective in modulating the transcript levels of several UV-B-related and flavonoid-related genes (Santin et al. unpublished). In the present research, aimed to increase nutraceutical quality of peaches, we did not try UV-B irradiations longer than 60 min, since these treatments could be too time-consuming and expensive for industrial application and commercial purposes.

3. In the results section, did the UV-B radiation affect storage life?
The authors should provide the relevant data.

Reply: This point is undoubtedly interesting and deserves further investigation. However, this study was addressed to investigate the metabolic changes following UV-B exposure and we did not test parameters linked to the storage life.

4. In general, the paper is well written, but some errors of English in this manuscript could be improved further.

Reply: Following the suggestion of the reviewer the manuscript language was checked and corrected throughout the manuscript.

Highlights

- Decreased levels of most phenolics were detected in UV-B-treated fruit after 24 h
- Accumulation of phenolics was observed 36 h after UV-B irradiation
- Down-accumulation of carotenoids was detected regardless of UV-B dose
- Lipids decreased but their biosynthetic intermediates increased after UV-B exposure
- Pteridins increased and indole alkaloids decreased in 60 min UV-B-treated fruit

Post-harvest UV-B radiation modulates metabolite profile in peach fruit

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ABSTRACT

The possibility to modify plant metabolic profile of plants and fruit to improve their healthy properties using eco-friendly tools, rather than transgenic approaches, gained interest in the last decades. Ultraviolet-B (UV-B) radiation, at low levels, thanks to its ability to influence plant secondary metabolism, could be successfully used to achieve this goal. However, few studies have been conducted so far on the effects of post-harvest UV-B treatments on fruit metabolomics. The present research, aimed to evaluate the impact of UV-B on peach metabolites profile through non-targeted metabolomics (UHPLC-ESI/QTOF-MS) coupled with multivariate chemometrics, provided evidence that 10 and 60 min of post-harvest UV-B irradiation influenced several classes of metabolites. Most phenolics were down-accumulated 24 h after both UV-B treatments, though, after 36 h, anthocyanins, flavones and dihydroflavonols increased (2.06-, 1.92-, 1.68-fold with 10 min UV-B; 6.65-, 2.53-, 2.05-fold with 60 min UV-B, respectively). UV-B reduced carotenoids and most lipids and increased some biosynthetic intermediates and degradation products, some of them known for their positive role in human health. Among alkaloids, some pteridines accumulated, likely derived from folates degradation, while indole alkaloids decreased. Despite the decrease of some bioprotective metabolites as carotenoids, the UV-B-induced up-accumulation of many antioxidant phenolics after 36 h from the exposure suggests an improvement of the healthy properties of peach fruit and reinforces the potential of UV-B controlled irradiation as a nutraceuticals-increasing tool in fruit.

Keywords:

Phenolics, Peach fruit, *Prunus persica* L., Metabolomics, Terpenoids, UV-B radiation

1. INTRODUCTION

Peach (*Prunus persica* L.), one of the most economically important stone fruit worldwide, is widely cultivated and consumed throughout Europe. Peach fruit is particularly popular in the Mediterranean diet (Konopacka et al., 2010) and perfectly matches the consumers' increasing demand of healthy and health-promoting foods. Among the phytochemicals that can be detected in peach, phenolics, carotenoids and ascorbic acid play a predominant role as antioxidants (Gil, Tomás-Barberán, Hess-Pierce, & Kader, 2002). Phenolic compounds, which are often found as glycoside derivatives, represent a wide class of secondary metabolites generally synthesized by plants in response to biotic and abiotic stresses (Zhang & Tsao, 2016). A comprehensive classification of polyphenols was made by Neveu et al. (2010), who divided them into flavonoids, lignans, phenolic acids and stilbenes. Phenolics fulfill important functions for both plant and human metabolism, especially due to their metal chelating activity and their ability to neutralize the reactive oxygen species (ROS), naturally produced by cell metabolism and enhanced by environmental stresses (Zhang & Tsao, 2016).

Besides their health-promoting properties, phenolic compounds contribute to give the fruit hedonistic and organoleptic properties, thus representing a valuable parameter to evaluate the fruit quality (Tomás-Barberán et al., 2001).

Peach fruit contains high levels of phenolic compounds (Aleixandre, Aleixandre-Tudó, Bolaños-Pizzaro, & Aleixandre-Benavent, 2013; Vizzotto, Cisneros-Zevallos, & Byrne, 2007), whose profile strictly depends on different factors such as cultivar (Mokrani et al., 2016), climatic conditions, rootstock and ripening stage (Tavarini et al., 2011). The prevalent compounds detected are flavonols, flavan-3-ols, anthocyanins, and hydroxycinnamic acids (Tomás-Barberán et al., 2001), although many other phenols are present at lower concentrations.

Another important class of metabolites is represented by terpenoids, among which carotenoids deserve particular attention due to their photoprotective role and antioxidant action toward a variety

of environmental stresses. Moreover, as they contribute to the color of many fruit and vegetables, carotenoids have a strong impact on produce quality, especially from a commercial point of view. Many studies investigated the influence of post-harvest treatments on the modulation of metabolite composition in plants and fruit. Zhang & Tian (2009) found altered plasma membrane composition in peaches stored at 0 °C, with increased membrane fluidity due to a higher presence of unsaturated membrane lipids and N-acylphosphatidylethanolamine. Post-harvest treatments with 1-methylcyclopropene, carbon dioxide and nitrogen, followed by low temperature storage, were found to be effective in modulating the carotenoid profile, as well as the content of abscisic acid and ethylene (Caprioli, Lafuente, Rodrigo, & Mencarelli, 2009).

Recently, ultraviolet-B (UV-B) radiation (280-315 nm), at low and ecologically-relevant levels, was recognized to be able to stimulate the secondary metabolism of plants, possibly increasing the health-promoting value of deriving food (Schreiner et al., 2012). Nevertheless, the great potential of UV-B radiation has been investigated for a relative short time, since in the past it was instead considered as a stress factor (Jansen, Gaba, & Greenberg, 1998; Kunz, Cahill, Mohr, Osmond, & Vonarx, 2006). The discovery of a specific mechanism of UV-B perception (Kliebenstein, Lim, Landry, & Last, 2002) and the subsequent signal transduction pathway paved the way to investigate the possibility to exploit UV-B radiation to improve the nutraceutical properties of plant food. Scattino et al. (2014) showed that UV-B radiation can influence the concentration of several polyphenols in peach, through a molecular regulation on their biosynthetic genes. Also carotenoids were found to be affected by UV-B radiation, although the studies were carried in tomato (Castagna, Chiavaro, Dall'Asta, et al., 2013; Lazzeri et al., 2012). Besides genetic variability, UV-B effects on plant metabolism depends on duration and intensity of UV-B radiation (Liu et al., 2011; Scattino et al., 2014). Based on these considerations, the present research aimed to evaluate the impact of two different doses of UV-B radiation on the metabolite profile of peach fruit through non-targeted metabolomics coupled with multivariate chemometrics such as Partial Least Squares Discriminant Analysis (*PLS-DA)*. While most previous studies aimed to evaluate the impact of UV-

B radiation on specific compounds or specific metabolite classes, the current work was addressed to investigate the effect of UV-B radiation on peach metabolism with a holistic approach, trying to achieve a more complete overview on a wide range of metabolic classes.

2. MATERIALS AND METHODS

2.1 Plant material and UV-B treatment

Organic peach fruit (*Prunus persica* L., cv Fairtime) were purchased from a local biological supermarket and rapidly delivered to the laboratory of the Department of Applied Genetics and Cell Biology of BOKU University in Vienna (Austria). All peaches were accurately checked and only undamaged fruit with homogeneous dimension and color were used. Five peaches, sampled immediately after their arrival in the laboratory, represented the time 0 (T_0). The other fruit were randomly divided into three groups and assigned to control or UV-B treatments as described below. Peaches were placed inside proper chambers, each equipped with three UV-B lamp tubes (Philips Ultraviolet-B Narrowband, TL 20W/01 – RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands). The UV-B treatment was performed at room temperature (24 °C), with a UV-B irradiation of 2.3134 W m^{-2} at fruit height. White light was also ensured in each chamber, providing a total irradiation of 10.7026 W m^{-2}. Fruit were exposed to two different UV-B treatments, lasting 10 or 60 min respectively, and only the irradiated side of the fruit was sampled and stored for analysis. Control fruit were kept under the same conditions but received only white light. Groups of five peaches per treatment (control, UV-B 10 min and UV-B 60 min) were sampled at 24 and 36 h after the UV-B exposure. Each individual fruit represented a biological replicate. Skin was accurately peeled with scalpel and tweezers, then samples were immediately dipped into liquid nitrogen, freeze-dried, and kept at -80 °C until analyses.

2.2 Extraction and metabolomic analysis

Samples were extracted as previously set up (Borgognone et al., 2014). Five individual replicates from each sample were extracted in 10 volumes of 0.1 % HCOOH in 80 % ethanol using an Ultra-

122 turrax (Ika T25, Staufen, Germany). The extracts were centrifuged at 6000 x *g* for 10 min at 4 °C
123 and the resulting solutions filtered using 0.22 µm cellulose syringe filters into amber vials for
124 further use.
125 The screening of fruit metabolites was carried out by UHPLC liquid chromatographic coupled to a
126 quadrupole-time-of-flight high-resolution mass spectrometer via an electrospray ionization system
127 (UHPLC-ESI/QTOF-MS). More in detail, a 1290 UHPLC and a G6550 QTOF mass spectrometer
128 equipped with a Dual Electrospray JetStream ionization system (all from Agilent technologies,
129 Santa Clara, CA, USA) were used. Instrumental parameters were set up as optimized in previous
130 experiments (Lucini et al., 2015). The instrument operated in positive SCAN mode and was set to
131 acquire spectra in the range of 100–1200 m/z. Reverse phase chromatographic separation was
132 achieved in a methanol gradient using a Knauer BlueOrchid C18 column (100 × 2 mm i.d., 1.8 µm).
133 The LC mobile phase was a water-methanol mixture and the gradient started with 5 % B to increase
134 until 90 % B within 30 min, then was held for 5 min. The mobile phase temperature was set to 35
135 °C, the injection volume was 3 µL and the flow rate was 220 µL min^{-1}.
136 Raw data were processed using the software Profinder B.07 (Agilent Technologies), according to
137 the 'find-by-formula' algorithm. Compounds identification was achieved using the entire isotopic
138 pattern (monoisotopic accurate mass, isotope spacing, and ratio). Data were subsequently mined
139 against the databases exported from (i) Phenol-Explorer 3.6 (Rothwell et al., 2013) and (ii)
140 PlantCyc 9.5 (Plant Metabolic Network, http://www.plantcyc.org; released November 2014). In
141 both cases, identification underwent a recursive analysis workflow having retention time alignment
142 as mandatory in the second ID step.
143 A filter by frequency was applied after deconvolution and identification, retaining only those
144 compounds being in 100 % of replications within at least one treatment.
145 **2.3 Statistical analysis**
146 Interpretation of metabolomic results was carried out using Mass Profiler Professional B.12.06
147 (from Agilent technologies). Compounds abundance was log2 normalized, normalized at 75th

148 percentile and baselined versus the median of each compound in all samples. A multivariate Partial
149 Least Squares Discriminant Analysis (PLS-DA followed by N-fold validation, with N=4), was
150 performed to identify differences among treatments. The most discriminant compounds were then
151 exported from PLS-DA covariance structures according to their weight in the loading plot (VIP
152 analysis). Finally, one-way analysis of variance and fold-change (FC) analyses were combined into
153 Volcano plot (FC threshold ≥ 2; p-value ≤ 0.05 following Bonferroni multiple testing correction) to
154 gain differential compounds in pairwise comparisons.
155
156 ## 3. RESULTS AND DISCUSSION
157 ### 3.1 Influence of UV-B treatments on phenolic profile
158 Since previous studies highlighted that phenolic compounds are remarkably affected by UV-B
159 radiation (Hagen et al., 2007; Ruiz et al., 2016; Scattino et al., 2014), we first checked possible
160 change in their profile to verify whether and how such metabolites were modulated by the UV-B
161 treatments. To this aim, a phenolics-specific database (Phenol-Explorer) was used to identify the
162 compounds resulting from the UHPLC–ESI/QTOF-MS analysis. The full list of compounds
163 identified is reported as Supplementary data (Tab. S1).
164 The effect of UV-B treatments on phenolics accumulation in peach skin was evaluated by the
165 supervised multivariate analysis PLS-DA. The PLS-DA score plot (Fig. 1) showed a clear
166 separation within the groups groups (overall class prediction accuracy = 100%), demonstrating that
167 UV-B radiation influenced phenolics concentration. In particular, after 24 h of recovering (Fig. 1A),
168 the 60 min UV-B treated group clearly separated from the other two treatments (10 min UV-B and
169 control) on the first latent vector (t0 axis), while, on the second latent vector (t1 axis), control group
170 was distinctly separated from the UV-irradiated samples, irrespective of the duration of the UV-B
171 treatment. Briefly, the PLS-DA score plot revealed a quantitative separation on the t0 axis (60 min
172 UV-B treated group against 10 min UV-B treated and control groups) and a qualitative one on the
173 t1 axis (10 and 60 min UV-B treated groups against the control group). Flavonoid compounds

belonging to flavanols, flavones, dihydroflavonols and flavonols subclasses were the most significant parameters contributing to clustering, although several other compounds from different classes could be identified (e.g., hydroxycoumarins, hydroxybenzoic and hydroxycinnamic acids) (Tab. S2).

After 36 h from the UV-B treatment, the PLS-DA score plot showed a more distinct grouping among the treatments (Fig. 1B), with overall class prediction model accuracy reaching 100 %. An evident separation was noticeable on the t0 axis between the 10 min UV-B treated group and the control group, while the 60 min UV-B treated samples were spread along the axis, partially overlapping the other groups. However, on the t1 axis, the 60 min UV-B treated samples were distributed on the lower portion of the plot, clearly separated from the control and the 10 min UV-B treated groups, that were plotted on the higher sector. Starting from the loading plot underlying the PLS-DA prediction model, the compounds having the highest score in first and/or second latent vectors (*i.e.*, those with the highest discrimination potential) were selected. At 24 h recovery time, the most discriminant phenolics were ascribed mainly to flavonoids (anthocyanins, flavones, flavonols, etc.), followed by hydroxycinnamic acids, isoflavonoids, lignans, tyrosols and others contributed to discriminate the treatments (Tab. S2). Furthermore, the number of discriminant compounds highlighted from PLS-DA was higher at 36 h after the UV-B treatment (45 compounds) as compared to 24 h after (20 compounds). Detailed information about the discriminant compounds of PLS-DA, including their score in first and second latent vectors, is reported as Supplementary data (Tab. S2).

An increase or a decrease in metabolites accumulation following 10 min UV-B treatment was observed after 24 h recovery, depending on the different subclasses considered. The highest accumulation was observed for alkylphenols (1.40-fold), hydroxycoumarins (1.42-fold) and hydroxyphenilacetic acids (1.30-fold), while subclasses that decreased the most were anthocyanins (0.46-fold), dihydroflavonols (0.50-fold) and flavones (0.60-fold) (Fig. 2 A).

199 The 60 min UV-B treatment had an overall negative effect on metabolites accumulation after 24 h
200 recovery, as indicated by the negative fold-change values exhibited by most phenolics (Fig. 2 B).
201 Only alkylphenols, hydroxycoumarins and hydroxybenzoketones were up-accumulated, although
202 only slightly (about 1.13-, 1.02- and 1.00-fold, respectively). The subclasses displaying the greatest
203 decrease were dihydroflavonols (0.38-folds as compared to control), anthocyanins (0.49-fold) and
204 tyrosols (0.50-fold).
205 After 36 h recovery, the situation changed drastically. In both the 10 min and 60 min UV-B treated
206 groups, the metabolites of almost all the phenolic classes generally increased, revealing an overall
207 positive effect of UV-B radiation (Fig. 2 C, D). Dihydroflavonols, anthocyanins and flavones were
208 the subclasses undergoing the major increase following UV-B treatment (2.06-, 1.92-, 1.68-fold
209 after 10 min UV-B; 6.65-, 2.53-, 2.05-fold after 60 min UV-B, respectively). Their chemical
210 structures give these subclasses a high antioxidant activity, which could play a key role not only for
211 peach defense but also for human health. Among the few subclasses that were negatively affected
212 by UV-B radiation after 36 h recovery, the alkylmethoxyphenols and the tyrosols displayed the
213 highest reduction in both the UV-B treated groups. However, due to their relatively low abundance
214 in peach fruit, their decrease is not expected to alter peach properties extensively.
215 The overall reduction in almost all the phenolics detected 24 h after UV-B irradiation, and the
216 following general increase after 36 h, was observed for both 10 min and 60 min UV-B treated
217 groups (Fig. 2).
218 Scattino et al. (2014) observed that peaches irradiated continuously for 12 h underwent a decrease
219 in hydroxycinnamic acids, flavonols and in the anthocyanin cyanidin-3-glucoside. However, after
220 36 h of UV-B exposure, the concentration of such phenolics significantly increased. In the study by
221 Ruiz et al. (2016), a significantly higher concentration of several flavonoid subclasses (flavanones,
222 dihydroflavonols, flavones, flavonols and anthocyanins) was detected 48 h after 3 min UV-B
223 treatment in lemon skin.

224 The fluctuating trend observed in our study might be due to a defensive response of the fruit
225 towards UV-B radiation, which is well-known to be an abiotic stressor for plants (Jansen, Hectors,
226 O'Brien, Guisez, & Potters, 2008). We hypothesize that, in the first hours after UV-B treatment, the
227 phenolic compounds already present in the skin tissue might have started to counteract the
228 potentially disruptive effects of UV-B radiation (and/or UVB-induced ROS) within the cell. This
229 may explain the initial decrease in phenolics detected 24 h after the UV-B treatment. Meanwhile,
230 transcription of several biosynthetic genes of the phenylpropanoid pathway may have increased,
231 since UV-B radiation is known to induce expression of genes involved in phenolic biosynthesis
232 (Liu, Gregan, Winefield, & Jordan, 2015; Scattino et al., 2014). This, in turn, could account for the
233 accumulation of metabolites detected after 36 h from the treatment. This behavior might be an
234 acclimation response to UV-B: the existing UV-B-protective compounds work as a defensive line
235 against UV-B, and are therefore degraded, while their *de-novo* synthesis is stimulated at
236 transcriptional level through the UVR8 pathway. Preliminary results on the expression of flavonoid
237 biosynthetic and regulatory genes, as well as of UVR8 pathway-related genes, support this
238 hypothesis (Santin et al., unpublished).

239 **3.2 UV-B radiation-induced changes on other metabolic classes**

240 To detect whether UV-B exposure influenced metabolic classes other than phenolics, the QTOF-
241 MS data were run against PlantCyc, an extensive database containing plant compounds from both
242 primary and secondary metabolism. The full list of compounds identified is reported as
243 Supplementary data (Tab. S3).

244 The PLS-DA score plot displayed a clear clustering of the three treatments, after both 24 h and 36 h
245 of recovery (Fig. 3). Indeed, N-fold validation led to an overall class prediction accuracy of 100 %.
246 At the shorter recovery time (24 h, Fig. 3A), the control group was separated from both the UV-B
247 treated groups on the first latent vector (t0 axis), being located in the positive and negative halves of
248 the plot, respectively. However, on the second latent vector (t1 axis), discrimination was visible

249 only between the 10 min-UV-B-treated samples (upper, positive) and the 60 min-UV-B-treated
250 samples (lower, negative), while the control group partially overlapped with the other ones.
251 After 36 h from the UV-B treatment (Fig. 3B), the differently UV-B treated groups could be well
252 clustered in the score plot from PLS-DA covariance structure. On the first latent vector, both the 10
253 and 60 min UV-B treated samples clustered in the left (negative) portion of the hyperspace, while
254 the controls were all located in the right (positive) region. However, on the second latent vector, the
255 discrimination was visible only between the 10 min UV-B-treated group (lower, in the negative
256 half), and the 60 min UV-B treated samples and controls (both upper, in the positive half). Looking
257 at the PLS-DA score plot from both irradiation times, it appears that peach fruit metabolic profile
258 changed in response to treatment in a dose-dependent and time-dependent way. Moreover, being the
259 treatments discriminated when two latent vectors are considered, it can be postulated that
260 differences at metabolome level were represented in the dataset. On this basis, the most
261 discriminating compounds were exported from loading plots according to their weight in the class
262 prediction model, and then used to shed light on the metabolic changes occurred in response to UV-
263 B treatment.
264 The discriminant compounds that maximized the differences among the groups in the PLS-DA
265 analysis are reported in Table S4. As given, the majority of them were lipids or lipids-related (lipid
266 peroxidation products or biosynthesis intermediates) molecules, but also several terpenoids and
267 phenolics could be found.
268 Since the PLS-DA analysis confirmed an effect of UV-B radiation on several metabolites of
269 different classes, a Volcano analysis (FC threshold ≥ 2; p-value ≤ 0.05) was performed to identify
270 the most affected compounds (Tab. 1), starting from the molecules identified from the PlantCyc
271 database. Since a phenolics-specific database was previously used to detect changes in phenolic
272 profile, phenolics (still confirmed as differential compounds) were not further taken into
273 consideration in this analysis. The highest number of differential metabolites was detected
274 following 60 min UV-B treatment and 36 h of recovery, confirming a dose-dependent effect (60

min is more effective than 10 min; Tab. 1). Moreover, the delayed response (more metabolites after the longest recovery time) is likely due to the need to perceive UV-B radiation, transmit the signal, activate specific responses and then start to accumulate the newly-synthetized metabolites. It is therefore likely that the effects of UV-B treatment in terms of metabolic changes after 24 h from exposure are still not as visible as after 36 h.

Differential metabolites were then grouped in relatively homogenous biochemical class to facilitate the discussion on the actual metabolic changes observed in response to UV-B.

3.2.1 Terpenoids

Terpenoids are able to counteract the harmful effect of several abiotic stresses, such as UV-B radiation, mainly by neutralizing ROS (Loreto & Velikova, 2001; Affek & Yakir, 2002) and an increase in their content after UV-B treatment has been reported for several plants (Blande, Turunen, & Holopainen, 2009; Johnson, Kirby, Naxakis, & Pearson, 1999). In our study, several carotenoids were found to be down-accumulated 36 h after the UV-B irradiation (Tab. 1). Particularly, the samples treated for 10 min showed a decrease in isozeaxanthin, lutein, lactucaxanthin and β-carotene. The quenching capacity of carotenoids towards different ROS has been widely described (Fiedor & Burda, 2014), as well as their modulation under UV-B radiation (Liu et al., 2011; Castagna et al., 2013). Since UV-B radiation is a potential source of oxidative stress (Czégény, Le Martret, Pávkovics, Dix, & Hideg, 2016), it might be possible that carotenoids were consumed to counteract the potentially damaging ROS. This possibility is in line with the results observed for the phenolic compounds. In fact, almost all the phenolics that were modulated by the UV-B treatments were firstly down accumulated after 24 h from the irradiation. However, differently from phenolics, the down-accumulation of carotenoids was still detectable after 36 h, maybe due to a longer turnover time of these metabolites. The down accumulation of several carotenoids was accompanied by an increase in *all-trans*-10'-apo-beta-carotenal, an apocarotenoid, 36 h after both 10 min and 60 min UV-B treatments. Apocarotenoids are well-known products of oxidative cleavage of carotenoids (Havaux, 2014). However, apocarotenoids are not simply

301 degradation products, but some of them, acting as hormones, signals and volatiles, could have a
302 functional role for the plant cell (Hou et al., 2016). Moreover, they have been reported to inhibit
303 cancer cell proliferation and to be biologically active in cellular signalling related to cancer (Sharoni
304 et al., 2016), thus suggesting a positive role in human health and physiology.
305 *3.2.2 Lipids*
306 It is well-known from literature that UV-B radiation can cause lipid peroxidation by the production
307 of oxygen radicals (Demidchik, 2015). Welti et al. (2002) showed that the cell membranes
308 composition in *Arabidopsis thaliana* after an abiotic stress, such as freezing, is highly susceptible to
309 alteration due to an increase in lipolytic activities. In our study, a modulation in several lipids was
310 detected, especially in the 60 min-UV-B treated group after 36 h of recovery (Tab. 1). The lipid
311 subfamilies which were mostly affected by the UV-B treatment were structural lipids
312 (phospholipids, sphingolipids, glycolipids) and brassinosteroids. The first three subclasses represent
313 important constituents of plant cell membranes. Among them, several molecules shared by lipid
314 biosynthetic- and degrading-pathways were found, such as 1-18:1-2-16:0-phosphatidate, which was
315 significantly up accumulated following UV-B irradiation. These intermediate lipids may derive
316 either from a newly UV-B-induced synthesis of membrane components, necessary to replace the
317 oxidized molecules after the UV-B peroxidation, or from the degradation of the existing membrane
318 lipids, producing such cleavage compounds.
319 Other than the membrane constituents, also a few brassinosteroids were found to be affected by
320 UV-B treatment, particularly only in the 60 min-UV-B-treated samples after 36 h from the
321 irradiation.
322 *3.2.3 Alkaloids*
323 Although less efficient than phenolics, also alkaloids were reported to counteract the oxidative
324 stress from UV-B exposure in plants (Larson, 1988). In the present research, the effect of UV-B
325 radiation was mainly visible after 36 h from UV-B treatment in the 60 min-UV-B treated samples
326 (Tab. 1). Among the different alkaloids influenced by UV-B exposure, two pteridines, namely 7,8-

dihydroneopterin and 7,8-dihydromonapterin, were up accumulated. Pteridines, together with p-aminobenzoate and glutamate, are essential constituent of folates and play an important role in folates biosynthesis (Hanson & Gregory, 2002). Furthermore, due to molecular instability of plant folates and their high susceptibility to oxidation, pteridines could also accumulate as oxidative cleavage products (Scott, Rébeillé, & Fletcher, 2000). In our work, it may be possible that the oxygen radicals produced by the UV-B treatment were counteracted not only by phenolics and terpenoids, but also by folates, resulting in increased pteridines concentration.

Differently from pteridines, two indole alkaloids (paspaline and 3'-*O*-demethyl-staurosporine) were down accumulated in the 60 min-UV-B-treated group (Tab. 1). In *Catharanthus roseus*, UV-B exposure for up to 20 min was found to increase the content of several indole alkaloids 72 h after irradiation (Binder, Peebles, Shanks, & San, 2009). In the same species, Ouwerkerk & Memelink (1999) found that UV-B radiation is able to stimulate the expression of genes involved in the early stages of indole alkaloids biosynthesis.

We hypothesize that, in peach, the lower content of indole alkaloids detected 36 h after UV-B exposure was due to their consumption following reaction with the UV-B-induced ROS. However, as hypothesized for phenolics, at the same time, UV-B radiation could have triggered the expression of biosynthetic genes. For this reason, a delayed accumulation of such alkaloids might be detectable only later than 36 h, as shown by Binder et al. (2009).

4. CONCLUSIONS

Despite the effect of UV-B radiation on specific metabolic classes has been previously faced, few studies investigated the impact of UV-B radiation on a wide range of metabolites in fruit. This work provides evidence that UV-B radiation is able to affect several classes of metabolites in peach skin. For any class considered, UV-B influence was more pronounced after 36 h of recovery than after 24 h. After an initial general decrease of most phenolics subclasses (24 h after irradiation), likely due to their degradation during detoxification of UV-B-induced ROS, an overall increase was visible 36

353 h after treatment, especially for dihydroflavonols, anthocyanins, and flavones, suggesting higher
354 transcription of biosynthetic genes. The accumulation of such antioxidant compounds might open
355 the possibility to exploit UV-B radiation as a nutraceuticals-increasing tool in fruit. Besides
356 phenolics, the metabolic response to UV-B radiation involved other biochemical classes such as
357 terpenoids, lipids and alkaloids, with possible effects on health-promoting properties of peach. The
358 ROS-mediated oxidative stress induced by UV-B might have played a prominent role, particularly
359 in the non-phenolic metabolite families. However, further investigations are needed to study the
360 molecular mechanisms underlying the differential effects played by UV-B radiation on the diverse
361 metabolites and to understand the role played by ROS-mediated or UV-B specific signalling routes.
362 Moreover, considering the wide range of metabolites responding to UV-B treatments, researches on
363 possible UV-B-driven modifications of organoleptic quality of peach fruit are highly recommended.
364
365
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370
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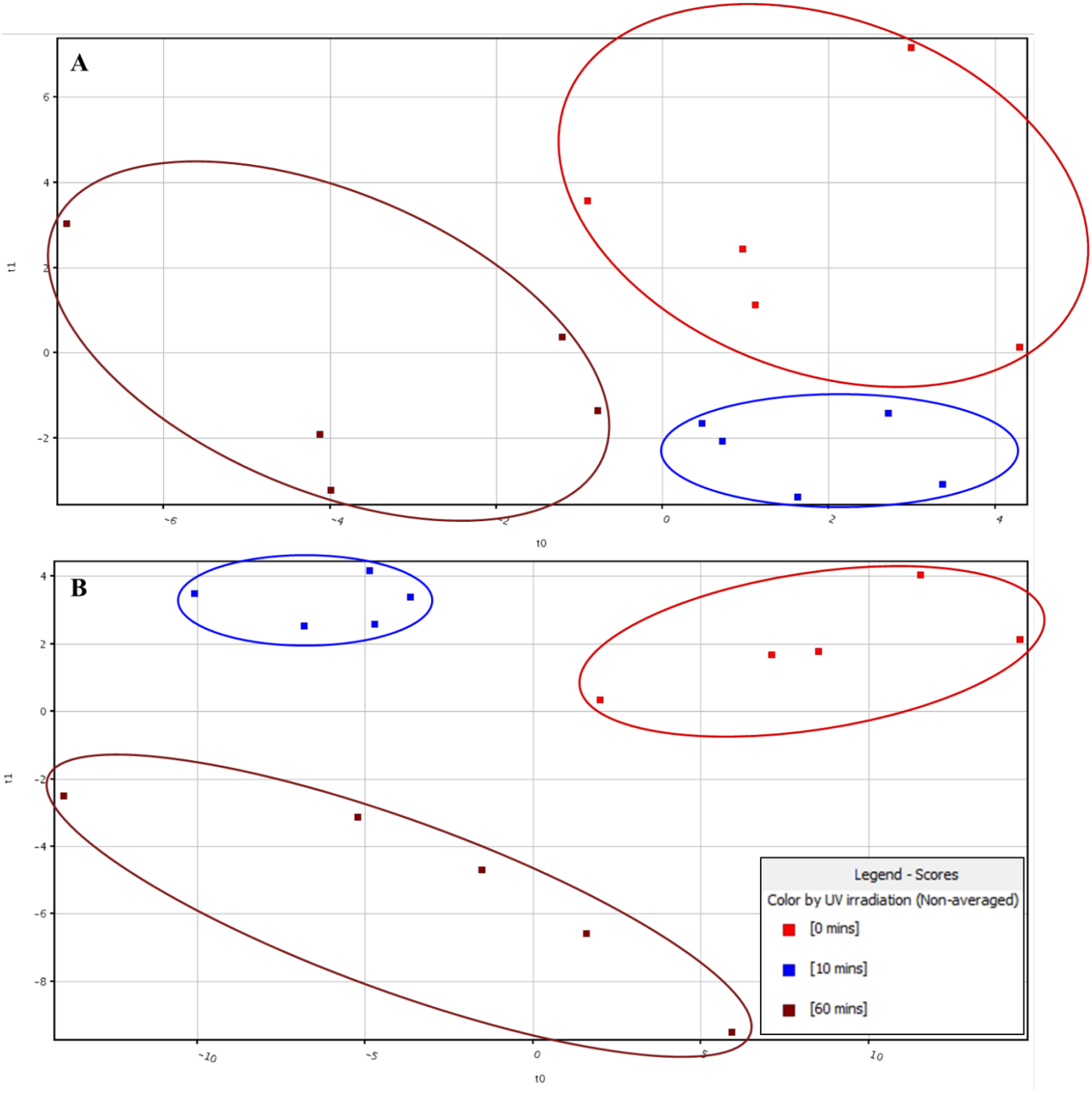
Figure captions

Figure 1. PLS-DA loading plot hyperspace carried out from the UHPLC-ESI/QTOF-MS phenolic profile in the samples investigated. Each point represents a biological replicate. Red, 0 min UV-B; blue, 10 min UV-B; brown, 60 min UV-B.

Figure 2. Fold change in phenolics accumulation following 10 min (A, C) or 60 min (B, D) UV-B exposure and 24 h (A, B) or 36 h (C, D) recovery. For each phenolic class, control (0 min UV-B) value was set to 1. Long names of phenolic classes are abbreviated as follows: HC acids, Hydroxycinnamic acids; DH flavonols, Dihydroflavonols; HB acids, Hydroxybenzoic acids; H coumarins, Hydroxycoumarins; HPheA acids, Hydroxyphenylacetic acids; HPheP acids, Hydroxyphenylpropanoic acids; Phe terpenes, Phenolic terpenes; HPhe propenes, Hydroxyphenylpropenes; HB aldehydes, Hydroxybenzaldehydes; DH chalcones, Dihydrochalcones; HB ketones, Hydroxybenzoketones; HC aldehydes, Hydroxycinnamaldehydes; AM phenols, Alkylmethoxyphenols; Others, Other polyphenols

Figure 3. PLS-DA loading plot hyperspace carried out from the UHPLC-ESI/QTOF-MS metabolite profile in the samples investigated. Each point represents a biological replicate. Red, 0 min UV-B; blue, 10 min UV-B; brown, 60 min UV-B.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t0
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t1
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Legend - Scores
Color by UV irradiation (Non-averaged)
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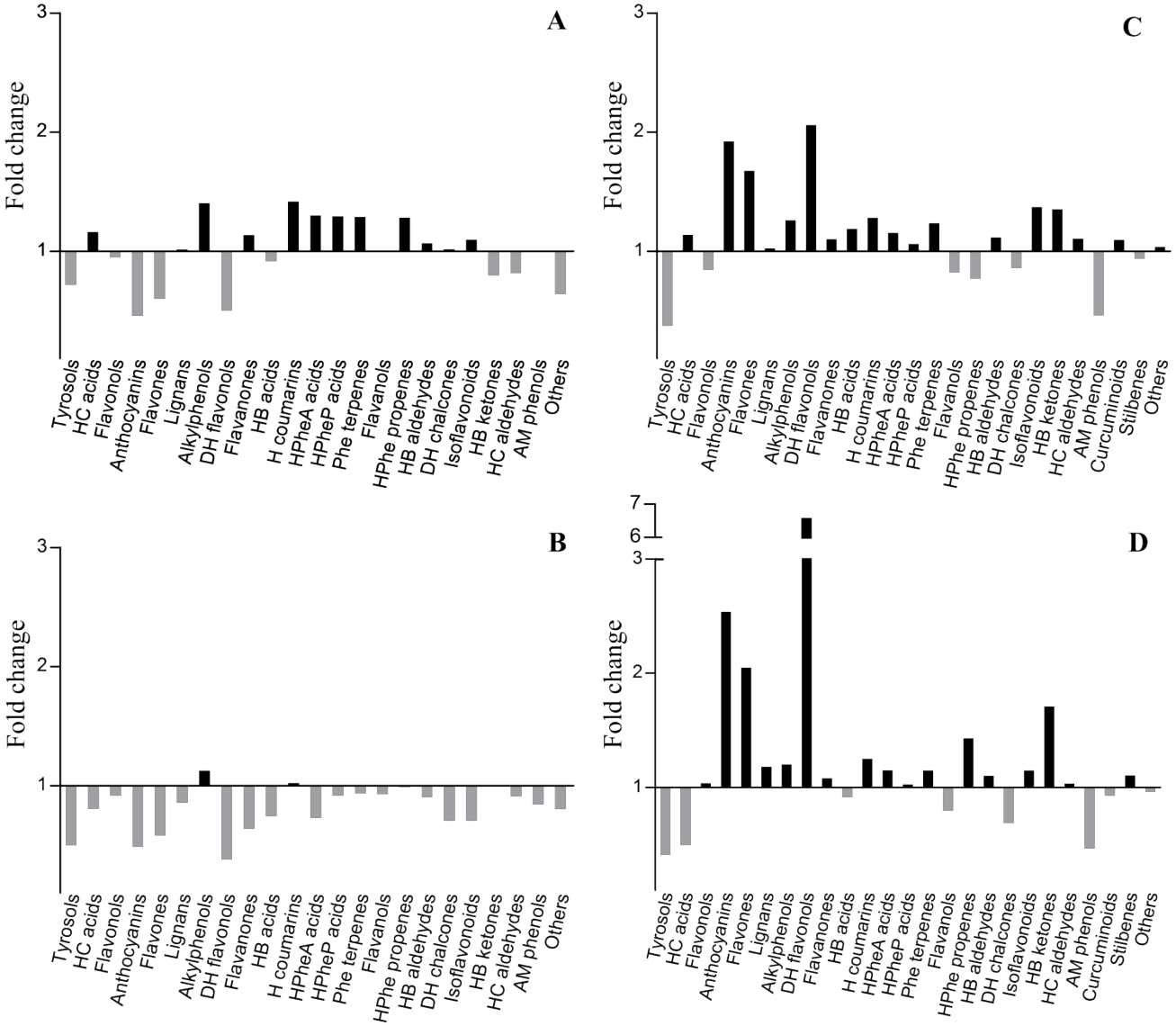
[60 mins]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C
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Fold change
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HC acids
Flavonols
Anthocyanins
Flavones
Lignans
Alkylphenols
DH flavonols
Flavanones
HB acids
H coumarins
HPheA acids
HPheP acids
Phe terpenes
Flavanols
HPhe propenes
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DH chalcones
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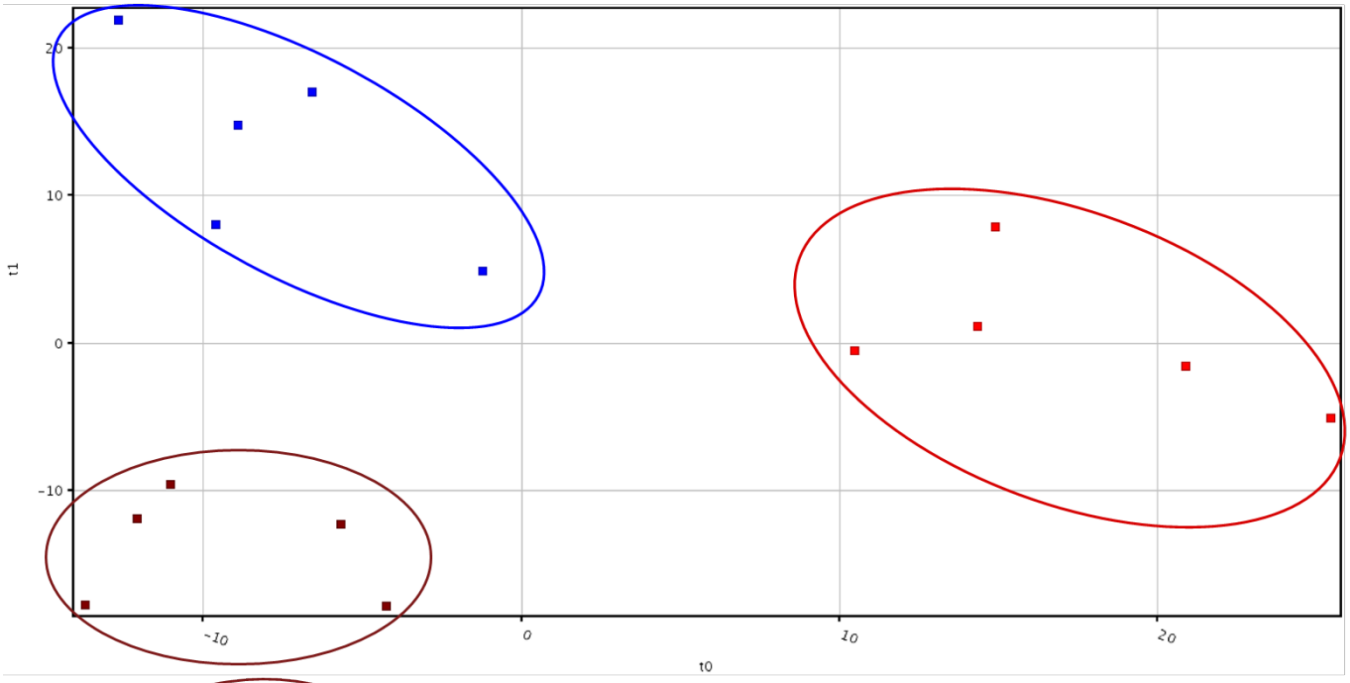
HC aldehydes
AM phenols
Curcuminoids
Stilbenes
Others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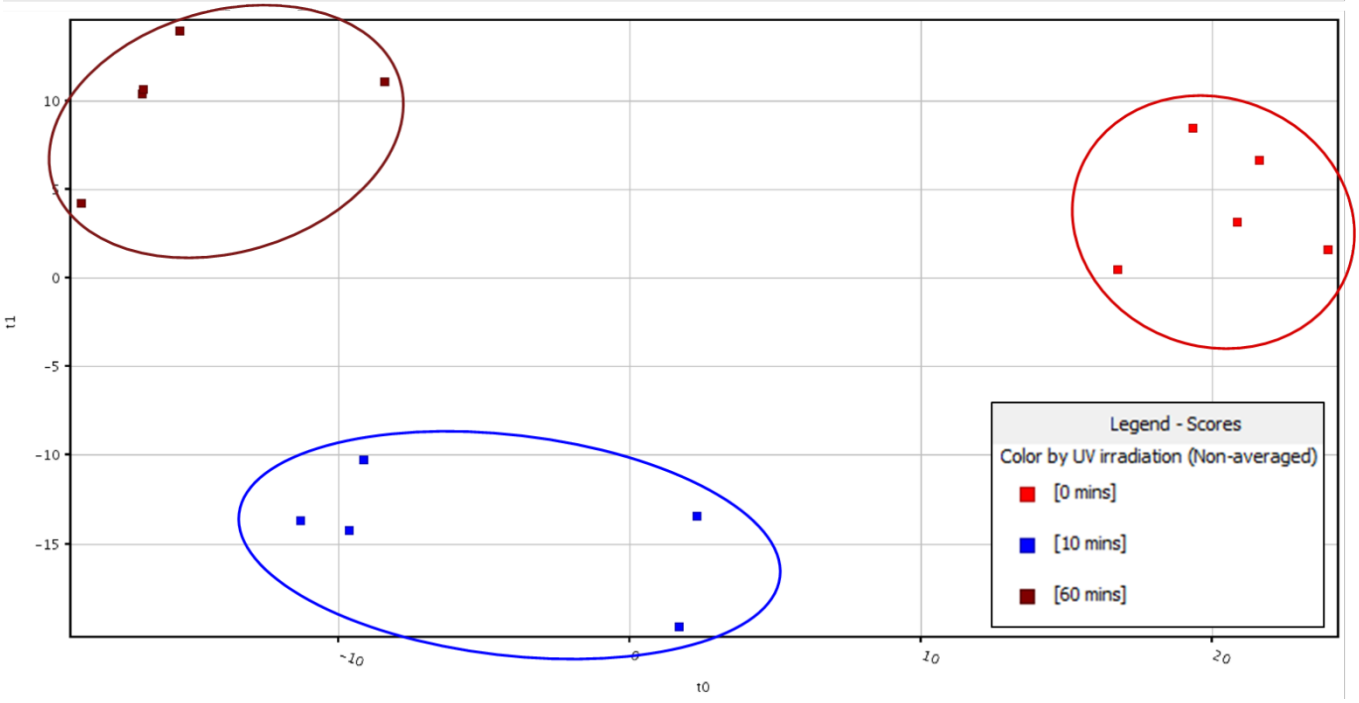
10
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10
20
t0
Legend - Scores
Color by UV irradiation (Non-averaged)
[0 mins]
[10 mins]
[60 mins]

Tab. 1. Different metabolites changing in peach fruits following either 10 or 60 min of post-harvest UV-B treatment. Compounds were selected by combining analysis of variance and fold-change into Volcano Plot (Bonferroni multiple testing correction, P<0.05; fold-change cut-off = 2; n = 5 per treatment). According to the output of software Mass Profiler Professional, p values = 0 denote highly significant differences, whereas FC = 16 identify very high fold-change values.

Sampling time after UV-B exposure	UV-B exposure time	Compound	Family	Superfamily	p (Corr)	FC (abs)	Regulation
24 h							
	10 min	4α-formyl-5α-cholesta-8,24-dien-3β-ol	Steroids	Lipids	0	3.14	up
		a 2-acyl-sn-glycero-3-phosphoethanolamine (n-C14:1)	Phospholipids	Lipids	0	2.76	up
		indole-3-acetonitrile-cysteine conjugate	Nitrile		6.47E-09	4.69E+06	down
	60 min	naphthylisoquinoline	Isoquinoline alkaloids	Alkaloids	0	4.86E+03	up
36 h							
	10 min	dihydromacarpine	Benzophenanthridine alkaloids	Alkaloids	0	65.57	down
		1-18:2-2-16:0-phosphatidylglycerol	Phospholipids	Lipids	0	16	up
		dihydroxy-*all-trans*-β-carotene / lutein / isozeaxanthin / lactucaxanthin	Carotenols	Terpenoids	3.84E-10	7.27E+05	down
		all-trans -10'-apo-β-carotenal	Apocarotenoids	Terpenoids	0	16	up
	60 min	3'-*O*-demethyl-staurosporine	Indolocarbazole alkaloids	Alkaloids	9.70E-11	5.29E+05	down
		dihydromacarpine	Benzophenanthridine alkaloids	Alkaloids	0	5.72E+02	down
		laudanosine	Isoquinolines, benzopyridines	Alkaloids	4.07E-12	6.83E+04	up
		hydroxycampestanol / deoxo-epicathasterone	Sterols	Lipids	3.13E-13	8.22E+05	down
		1-18:2-2-18:2-sn-glycerol-3-phosphocholine	Phospholipids	Lipids	2.36E-05	4.66E+07	down
		1-18:2-2-16:2- / 1-18:1-2-16:3-monogalactosyldiacylglycerol	Galactolipids	Lipids	1.29E-11	1.33E+05	down
		1-18:1-2-18:3-phosphatidylcholine	Phospholipids	Lipids	2.36E-05	4.66E+07	down
		a sphinga-4,8-dienine-18:0-ceramide	Sphingolipids	Lipids	2.88E-02	4.72E+04	up
		1-18:0-2-18:1-phosphatidylethanolamine	Phospholipids	Lipids	8.71E-13	7.45E+05	up
		1-18:1-2-*trans*-16:1-phosphatidylglycerol	Phospholipids	Lipids	0	16	down

	1-18:2-2-18:2-monogalactosyldiacylglycerol	Glycolipids	Lipids	3.13E-13	8.50E+06	up
	1-18:1-2-16:0-phosphatidate	Phospholipids	Lipids	6.12E-13	2.84E+07	up
	1-18:2-2-16:0-phosphatidylglycerol	Phospholipids	Lipids	0	16	up
	7-methylinosine	Inosines	Nucleosides	4.45E-04	5.55	up
	glutathione	Thiols	Peptides	7.48E-03	2.36	down
	p-nitrophenyl-β-D-xylobioside	Glycosides	Sugars	2.20E-12	1.51E+05	down
	tirucalla-7,24-diene-3β-ol	Triterpenoids	Terpenoids	0	2.37	up
	apo-β-carotenal	Apocarotenoids	Terpenoids	0	16	down
	all-trans-4,4'-diapophytofluene	Apocarotenoids	Terpenoids	4.39E-02	3.17	down
	paspalinine	Indoles	Alkaloids	3.91E-02	7.24	down