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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.

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 et al., 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 and 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 and 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 et al., 2013; Vizzotto et al., 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.

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Many studies investigated the influence of post-harvest treatments on the modulation of metabolite composition in plants and fruit. Zhang and 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 et al., 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 et al., 1998; Kunz et al., 2006). The discovery of a specific mechanism of UV-B perception (Kliebenstein et al., 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 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-turrax (Ika T25, Staufen, Germany). The extracts were centrifuged at 6000 × *g* for 10 min at 4 °C and the resulting solutions filtered using 0.22 µm cellulose syringe filters into amber vials for further use.

The screening of fruit metabolites was carried out by UHPLC liquid chromatographic coupled to a quadrupole-time-of-flight high-resolution mass spectrometer via an electrospray ionization system (UHPLC-ESI/QTOF-MS). More in detail, a 1290 UHPLC and a G6550 QTOF mass spectrometer equipped with a Dual Electrospray JetStream ionization system (all from Agilent technologies, Santa Clara, CA, USA) were used. Instrumental parameters were set up as optimized in previous experiments (Lucini et al., 2015). The instrument operated in positive SCAN mode and was set to acquire spectra in the range of 100–1200 m/z. Reverse phase chromatographic separation was achieved in a methanol gradient using a Knauer BlueOrchid C18 column (100 × 2 mm i.d., 1.8 µm). The LC mobile phase was a water-methanol mixture and the gradient started with 5% B to increase until 90% B within 30 min, then was held for 5 min. The mobile phase temperature was set to 35 °C, the injection volume was 3 µL and the flow rate was 220 µL min^{-1}.

Raw data were processed using the software Profinder B.07 (Agilent Technologies), according to the 'find-by-formula' algorithm. Compounds identification was achieved using the entire isotopic pattern (monoisotopic accurate mass, isotope spacing, and ratio). Data were subsequently mined against the databases exported from (i) Phenol-Explorer 3.6 (Rothwell et al., 2013) and (ii) PlantCyc 9.5 (Plant Metabolic Network, http://www.plantcyc.org; released November 2014). In both cases, identification underwent a recursive analysis workflow having retention time alignment as mandatory in the second ID step.

A filter by frequency was applied after deconvolution and identification, retaining only those compounds being in 100% of replications within at least one treatment.

2.3. Statistical analysis

Interpretation of metabolomic results was carried out using Mass Profiler Professional B.12.06 (from Agilent technologies). Compounds abundance was log2 normalized, normalized at 75th percentile and baselined versus the median of each compound in all samples. A multivariate Partial Least Squares Discriminant Analysis (PLS-DA followed by N-fold validation, with N = 4), was performed to identify differences among treatments. The most discriminant compounds were then exported from PLS-DA covariance structures according to their weight in the loading plot (VIP analysis). Finally, one-way analysis of variance and fold-change (FC) analyses were combined into Volcano plot (FC threshold ≥ 2; p-value ≤ 0.05 following Bonferroni multiple testing correction) to gain differential compounds in pairwise comparisons.

3. Results and discussion

3.1. Influence of UV-B treatments on phenolic profile

Since previous studies highlighted that phenolic compounds are remarkably affected by UV-B radiation (Hagen et al., 2007; Ruiz et al., 2016; Scattino et al., 2014), we first checked possible change in their profile to verify whether and how such metabolites were modulated by the UV-B treatments. To this aim, a phenolics-specific database (Phenol-Explorer) was used to identify the compounds resulting from the UHPLC–ESI/QTOF-MS analysis. The full list of compounds identified is reported as Supplementary data (Table S1).

The effect of UV-B treatments on phenolics accumulation in peach skin was evaluated by the supervised multivariate analysis PLS-DA. The