Phenolic enrichment in apple skin following post-harvest fruit UV-B treatment

Carolina Fagundes Assumpção[a], Vanessa Stahl Hermes[a], Carlos Pagno[a], Antonella Castagna[b], Alessia Mannucci[b], Cristina Sgherri[b], Calogero Pinzino[c], Annamaria Ranieri[b], Simone Hickmann Flôres[a], Alessandro de Oliveira Rios[a,*]

[a] Institute of Food Science and Technology, Federal University of Rio Grande do Sul, Bento Gonçalves, 9000, Porto Alegre, Brazil

[b] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

[c] Institute of Chemistry of Organometallic Compounds (ICCOM), Italian National Research Council (CNR), Area Della Ricerca Del CNR di Pisa, Via G. Moruzzi 1, I-56124
Pisa, Italy

[*] Corresponding author.

E-mail addresses:
carolufmt@gmail.com (C.F. Assumpção),
van.hermes@gmail.com (V.S. Hermes),
cpagno@gmail.com (C. Pagno),
antonella.castagna@unipi.it (A. Castagna),
alessia_mannucci@hotmail.it (A. Mannucci),
cristina.sgherri@unipi.it (C. Sgherri),
rino@pi.iccom.cnr.it (C. Pinzino),
anna.maria.ranieri@unipi.it (A. Ranieri),
simone.flores@ufrgs.br (S.H. Flôres),
alessandro.rios@ufrgs.br (A.d.O. Rios).

ABSTRACT

In apple fruit, phenolic compounds are the major sources of antioxidants, which are particularly concentrated in the skin. In the present experiment apples (cv. Red Delicious) were analyzed for their phenolic composition after the exposure to UV-B for 36 h (219 kJm^{-2}) and during storage (7, 14 and 21 d after the end of the treatment) in order to assess if UV-B treatment could improve marketability of the products as well as shelf-life. Since UV-B irradiation is also known to induce the generation of reactive oxygen species (ROS), the spin-trapping technique was applied to monitor the generation of free radicals under UV-B. The UV-B for 36 h treatment induced the generation of carbon-centered radicals in the skin, the tissue more exposed to radiation, but fruit quality parameters were not affected. Even if firmness progressively decreased and an increasing weight loss occurred during storage, differences between treated and control fruit were not observed. The different phenolic classes of apple skin reacted differently to the UV-B for 36 h irradiation, hydroxycinnamic acids increasing and flavonols decreasing. However, during storage, hydroxycinnamic acids and anthocyanins increased in UV-B-treated samples, as well as flavonols at the end of the storage period. As a consequence, the fruit skin showed a higher antioxidant activity in all the treated samples during storage, increasing the healthy properties of the fruit. This suggests that UV-B technique results in a valid strategy to induce antioxidant production in apple, increasing their nutraceutical value, thus allowing the attainment of phenolic-enriched fruit.

1. Introduction

Ultraviolet-B radiation (UV-B) is intrinsic to sunlight and reaches the earth's surface and has major biological effects on plant growth and development. In Arabidopsis, UV-B light regulates several important photo-morphogenic responses, including stomatal opening, phototropic curvature, and biosynthesis of anthocyanins and other flavonoids (Suesslin and Frohnmeyer, 2003). In addition to its effects on the model plant Arabidopsis, UV-B radiation can increase flower development and fruit color in many fruit trees, such as grape and apple (Zhao et al., 2016). The effectiveness of UV-B radiation has been demonstrated in stimulating secondary metabolism, which influences the nutraceutical value and sensorial quality of fruit (Castagna et al., 2013; Castagna et al., 2014; Liu et al., 2011; Scattino et al., 2014). However, at high doses, UV-B radiation causes similar conditions to oxidative stress, resulting from additional reactive oxygen species (ROS) generation (Hideg et al., 2013) and it was demonstrated that Withania somnifera plants experienced lipid peroxidation causing damages to the cell (Takshak and Agrawal, 2014). In fact, the level of carbon-centered free radicals is the result of an equilibrium between free radical production and their neutralization by antioxidants. In a previous paper carried out on peaches (Sgherri et al., 2015) authors demonstrated that UV-B technique is a good approach to induce antioxidant production in peach fruit, increasing their nutraceutical properties. Indeed, cyanidin-3-Oglucoside, the main cyanidin component, was capable of radicalization in the place of other organic molecules, thus protecting cells from oxidation.

Apple fruit is rich in health-promoting antioxidants such as anthocyanins and other phenolic compounds (Allan et al., 2008). It is reported that, due to their high antioxidant capacity, phenolics offer protection from cancer, cardiovascular conditions and some age-related diseases (Sun et al., 2014). The red coloration of apple skin derives from anthocyanins, whose accumulation is influenced by light, temperature, nutrition as well as by genetic factors (Lin-Wang et al., 2011). The anthocyanins in apples are predominantly glycosylated cyanidin. According to Peng and Moriguchi (2013), cyanidin-3-O-glycosides (cy3-gly) are the main forms of anthocyanins in apple skin, and cyanidin 3-O-galactoside (cy3-gal) covers 80% of the total cy3-gly, being higher in red cultivars as Red Delicious.

Anthocyanin biosynthesis in apple fruit is developmentally regulated and occurs at two stages. The first peak of production occurs at the fruitlet stage in both red and non-red cultivars and it is not economically important (Lancaster and Dougall, 1992). The second peak occurs at the ripening fruit stage in red cultivars like Red Delicious. The anthocyanin accumulation at the second peak is affected by environmental factors, including temperature and light and impact greatly on the marketable value of the product (Ubi et al., 2006).

In the present paper, Red Delicious apple fruit was subjected to UVB for 36 h in order to evaluate the ability of supplementary UV-B radiation to increase the health-promoting potential of apple tissues and, at the same time, to improve shelf-life and quality.

2. Material and methods

2.1. Chemicals

Acetonitrile HPLC grade (assay 99.9%) was purchased from Panreac Química S.A. (Barcelona, Spain); trifluoroacetic acid for HPLC (assay 99%) and formic acid for HPLC (assay 98%) were purchased from Sigma–Aldrich (Madrid, Spain). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

2.2. Plant material and treatment

Fruit of 'Red Delicious' apple cultivar were produced by local company (Illuminati Frutta Soc. Cons. a r.l., Civitella in Val di Chiana, Arezzo, Italy. Latitude: 43.2772° and longitude: 11.8294°) using integrated pest management practices. Apples were used at commercial maturity. Ninety fruit were selected for size and appearance and were transported to the laboratory at the Department of Agriculture, Food, and Environment, University of Pisa (Italy). The experiment was performed once. A group of ten fruit were immediately sampled at the laboratory, representing the time zero (0 h) of the experiment. The remaining fruit was distributed into two 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) which provided 1.69Wm^{-2} at fruit height. The apples were placed with their peduncle facing up (approximately 0.40m under the lamps) and were aligned parallel to the lamp tubes in order to ensure a uniform UV-B dose. The treatment lasted 36 h (219 kJm^{-2}). Control fruit (Vis) were placed for the same time in the climatic chamber where UV-B lamps were screened by benzophenone-treated polyethylene film. This kind of compound is known to block UV-B radiation (Calvenzani et al., 2010). After the UV-B treatment, a group of ten apples was sampled (36 h) while the remaining fruit were stored at room temperature (20 °C) in the dark.
Groups of ten fruit from control and treated apples were sampled at day 7, 14 and 21 d. The apples were carefully peeled using a scalpel and skin samples (thickness of 0.2 mm, approximately) were immediately frozen in liquid nitrogen and stored at −80 °C for further analyses.

2.3. Determination of fruit quality parameters

The texture of apple samples was analyzed on the equatorial of two opposite sides of each fruit after removing a small disc of skin using a penetrometer with 8mm probe (Model 53205; TR, Forlì, Italy). Six measurements were carried out on each fruit. Values were expressed in Newton (N). Total soluble solid content (TSS) was measured in the apple juice by a digital refractometer (Digital Brix Refractometer DBR 35) and expressed as%. Titratable acidity (TA) was determined by titration of 0.01 L of juice with 0.1 mol L^{-1} NaOH to an endpoint of pH 8.2 by automatic titrator (Model T80/20; Schott, Mainz, Germany), and expressed as percentage of malic acid (%). The percentage of weight loss was calculated in comparison with initial weight. Ten fruit were tested for each group.

2.4. Phenolics extraction and determination of total phenols and flavonoids

Frozen dried samples of control and treated apple skin (2 10^{-4} kg dry weight) were ground with liquid nitrogen to a fine powder. The plant material was extracted in triplicate essentially following the method described by Becatti et al. (2010).

Total phenols were determined in control and treated samples of skin according to the Folin–Ciocalteu colorimetric method. Amounts of 1.85 mL of distilled water, 1.25 10^{-4} L 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 10^{-6} L of extract. The solution was homogenized and left to stand for 30 min. The total phenol content was expressed as g kg^{-1} gallic acid dry weight (DW) (Sigma-Aldrich Chemical Co., St. Louis, MO). Absorbance was read at 750 nm at room temperature.

Total flavonoids were quantified following the method reported by Kim et al. (2003). Absorbance was read at 525 nm at room temperature. Results were expressed as g kg^{-1} catechin of DW. Both analyses were performed using an Ultrospec 2100 pro-UV–vis spectrophotometer (Amersham Biosciences).

2.5. Determination of antioxidant activity

A spectrophotometric analysis of antioxidant activity was performed following the method described by Pellegrini et al. (1999), using the discoloration of the radical cation 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS+) by skin apple extracts. The antioxidant capacity was expressed as gmol kg^{-1} Trolox equivalent antioxidant capacity (TEAC) DW.

2.6. UPLC–MS analysis method

UPLC–MS analysis was carried out on phenolic extracts using an Agilent 1290 Infinity II LC system (Agilent Technologies Italia S.p.A., Cernusco Sul Naviglio, Italy) consisting of a degasser, a binary pump, an autosampler, a column oven and equipped with an Agilent 6495A triple quadrupole.

A C18 column, 2.1×50 mm, 1.8 μm (Agilent Zorbax Eclipse Plus, Santa Clara, CA, USA) was used for separation of phenolic compounds. Solvent A consisted of 0.2% formic acid in water whereas solvent B was 0.2% formic acid in acetonitrile. The elution gradient was: 6% B (3 min), from 6 to 30% B in 11 min, from 30 to 100% B in 2 min, 100% B (2 min). The column temperature was 35 °C, the flow rate was 0.3 mL min−1, and the injection volume was 2 10−6 L. Supplementary table reports m/z and Multiple Reaction Monitoring (MRM) transitions of polyphenolic compounds identified in apple skin. MS parameters employed were as follow in ESI(+): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 3500 V, HPRF funnel: 120; LPRF funnel: 40; in ESI(−): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 1500 V; HPRF funnel: 120; LPRF funnel: 80. For quantification, an external standard method was used. A calibration curve in at least five different concentrations from 1 to 500 μg L^{-1} was constructed for each compound analyzed. From these concentrations, as equation of lines (y=a+bx) was constructed that allowed to quantify each compound. Data are expressed as g kg^{-1} DW. For a better understanding of the data, the compounds identified and quantified were separated into groups according to Wildman (2006).

2.7. Electron paramagnetic resonance (EPR) measurements: detection of stable free radicals

Water was Millipore MilliQ grade which was further distilled through a 1-m long Todd column and then saturated with dioxygen or argon at 20 °C. The use of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent further minimizes the artifacts resulting from trace metal impurities in the buffers. The DEPMPO was used as a spin trapping agent. Spin trapping agents are diamagnetic compounds which rapidly scavenge transient and/or stable radicals to form stable paramagnetic spin adducts for different types of radicals (ROS, carboncentered radicals, etc.). Because these are secondary radicals that retain an unpaired electron, they can be detected by EPR (Sgherri et al., 2017). Spectra were recorded at room temperature (25 °C) using a Varian E112 spectrometer equipped with a Varian variable temperature accessory. The spectrometer was interfaced to a PC 486/100 via an acquisition board and a software package designed for EPR measurements (Pinzino and Forte, 1992). EPR assays were carried out in 1mm quartz sample tubes sealed at one end. All experiments were repeated at least three times and in the dark. Computer-based simulations of EPR spectra were performed using the Winsim software (Duling, 1994).

The EPR parameters used were: microwave power, 20 mW; microwave frequency, 9.16 GHz; modulation amplitude, 2.5 G; time constant, 0.125 s.

Freeze-dried control and treated samples of apple skin (ca 0.1 g) were extracted with 10mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)/KOH (pH 7.4) containing 0.05 10^{-3}M

DTPA. Reaction mixtures contained 10mM DEPMPO in ethanol and sample extracts, obtained from the skin of apples previously treated with UV-B for 36 h and analyzed during the storage at 7, 14 and 21 d from the end of the treatment. EPR spectra were also monitored in the absence of DEPMPO and registering the signal in the dark. A sample containing 2mM H_2O_2, 2mM DTPA and 10mM DEPMPO was irradiated for 5 min to monitor the appearance of the hydroxyl radical ($^{\cdot}OH$). In fact, the ultraviolet (UV)/ H_2O_2 system is an advanced oxidation process in which H_2O_2 is added in the presence of UV light to generate hydroxyl radicals ($^{\cdot}OH$) (Sgherri et al., 2015). This procedure was done to test the efficacy of the spin trap for the measurements on biological samples. Three replicates were analyzed for each material and results were expressed as spin g^{-1} DW.

2.8. Statistical analysis

Data were subjected to one-way ANOVA using Statistica 11.0 software. Significant differences between treated and control fruit at each sampling time were calculated using at least three replicates according to Tukey's test ($p \leq 0.05$).

3. Results and discussion

3.1. Effects of UV-B on quality parameters

The analyses of fruit quality parameters, carried out on the whole fruit, showed unaltered behavior in UV-B for 36 h treated apples, in comparison to the controls, in relation to the firmness (Table 1). Following storage, firmness progressively decreased in both treated and control fruit whereas an increasing weight loss occurred (Table 1), the latter being significantly different after 14 d (−20% in treated samples compared to controls). The texture is a physical attribute resulting from the structural constituents of the product, providing an idea of the transformations in the cellular structure, cell cohesion and biochemical alterations (Chitarra and Chitarra, 2005).

Titratable acidity was not affected by UV-B irradiation, each treated sample showing the same value as control during the whole storage period (Table 1). Conversely, both pH and TSS exhibited reduced values in the 36-h-treated samples in comparison with the controls, indicating an acceleration of the ripening process (Majidi et al., 2011). However, at the end of the storage period (21 d), no great difference in any qualitative attributes was shown between the treated and untreated samples, suggesting that the UV-B treatment did not negatively affect fruit shelf-life (Table 1).

Hagen et al. (2007) found that postharvest irradiation of Malus domestica cv. Aroma for ten days improved the apple skin color without influencing the level of soluble solids or titratable acidity, thus indicating that application of UV-B irradiation could contribute to the maintenance of apple properties. However, the maintenance of firmness depends, for each fruit considered, on the ripening

stage, storage conditions as well as on the UV-B dose applied. In our conditions, Red Delicious apples were not affected when irradiated with 219 KJm^{-2} whereas Liu et al. (2011) found that mature-green tomato fruit maintained a high level of firmness if exposed to 20 and 40 kJm^{-2} UV-B and then stored in the dark at 14 °C for up to 37 d. However, the highest dose of 80 kJm^{-2} resulted in higher bioactive compound content but showed negative effects on texture, color, and other antioxidants.

3.2. Effects of UV-B on total phenols, total flavonoids and antioxidant activity in apple skin

Total phenols (Fig. 1A), as well as total flavonoids (Fig. 1B) of apple skin, showed a significant reduction after UV-B treatment (Fig. 1) even if at the end of the storage period (21 d) an accumulation of total phenols had occurred in comparison with the untreated sample. The lower content of total phenols and flavonoids after the 36-h-treatment could be explained by the increased generation of stable carbon radicals in the skin of apples (Fig. 4), and then with their inactivation, which minimized the oxidative stress induced by UV-B in the fruit tissues (Hideg et al., 2013). In fact, it is well known that phenolic compounds are potent antioxidants, directly or indirectly removing ROS and free radical species (Rice-Evans et al., 1997).

As phenolics biosynthesis varies according to the developmental stage, genotype, and environmental factors (Saure, 1990; Treutter, 2001), the increase in total phenols monitored in the treated apples at the end of the storage period could be ascribed to induction by UV-B treatment (Fig. 1). According to Du et al. (2014) stress-mediated changes in phenol content, as well as the physiological status of specialty crops, are dependent on the exposure (adaptation) time and in turn on the dose of UV.

In addition to their ability to provide human daily requirements of antioxidants from fresh consumption, phenolics present antimicrobial properties and color potential (in the case of anthocyanins). For all these characteristics, they appear very attractive as a dietary supplement, pharmaceutical components, and preservatives during food processing and conservation (Du et al., 2014).

In contrast with the decrease in total phenols after treatment for 36 h, antioxidant activity in apple skin extracts was not affected, but rather it increased in the treated samples during storage (Fig. 1C). This discrepancy could be explained considering that each phenolic compound contributed to the total antioxidant activity differently, depending on the number of hydroxylations and methoxylations on their aromatic rings (Rice-Evans et al., 1996). Besides, another compound other than polyphenolics could be contributing to antioxidant capacity as ascorbic acid (Hernández-Herrero and Frutos, 2014).

3.3. Effects of UV-B on main polyphenolic classes in apple skin

Hydroxycinnamic acids (Fig. 2A), flavan-3-ols (Fig. 2B), flavonols (Fig. 2C), anthocyanins (Fig. 2D) and dihydrochalcones (Fig. 2E) represent the main polyphenolic classes identified in apple skin after UPLC–MS analysis. Flavan-3-ols and flavonols were the most representative groups whereas the class of dihydrochalcones includes some compounds such as phloretin and phloridzin, typically found in apple skin (Treutter, 2001) (Table 2). Flavonoid-rich foods exert cardio- and cerebro-protective effects by decreasing oxidative damage to LDL and vascular cells (Lotito and Frei, 2004). Since apple skin is richer than flesh in these nutraceutical compounds, it is recommended that apple be eaten unpeeled, thus getting as many benefits as possible (Scattino et al., 2014).

Similarly to what was observed by Ryan et al. (2002) in the case of plants adaptation to strong sunlight, the different classes of phenolic compounds showed a different behavior when submitted to UV-B radiation (Fig. 2). In particular, hydroxycinnamic acids showed an increase by 38% following 36 h of treatment and maintained higher values in the treated samples during storage as well as anthocyanins (Fig. 2D). Treutter (2001) also observed an accumulation of anthocyanins in apples exposed to UV-B, demonstrating this enhancement to be related to an increased expression of biosynthetic anthocyanin genes.

On other hand, dihydrochalcones remained unaltered at any period analyzed (Fig. 2E), while flavonols were negatively affected by the treatment, showing a reduced content at the end of the exposure (−45%) and after 7 d (−31%) in comparison with the respective control (Fig. 2C). However, at the end of the period (21 d) flavonols were 64% higher in the UV-B-treated sample than control level, suggesting that UV-B treatment slowed down flavonoid loss during storage (Fig. 2). Moreover, reduced values were also observed after 7 d as in the case of flavan-3-ols.

3.4. Effects of UV-B on phenolic composition in apple skin

Twenty-four phenolic compounds were determined in apple skin extracts by UPLC–MS analysis and they are present as free or glycoside forms (Table 2). The identification of the molecules is reported in Table 3. Most phenolic compounds are naturally present in food as conjugated forms. In fact, in higher plants, low molecular weight phenols occur as glycosides or esters with sugars or related compounds (Imeh and Khokhar, 2002).

The compounds that mostly contributed to the increase in hydroxycinnamic acids after 36 h of UV-B treatment was feruloyl glucoside, while cryptochlorogenic and chlorogenic acids showed an increment during storage, this latter phenol exhibiting values from 4.5 to 6.7-fold higher than the controls (Table 2). An enhancement in chlorogenic acid amounts was also found by Lancaster et al. (2000) in UV-B exposed apples. Since this phenolic compound is known to have a high in vitro antioxidant activity, more than vitamin C and E (Rice-Evans et al., 1997), its behavior could explain,

in part, the higher value of the antioxidant activity of treated samples during post-harvest (Table 2, Fig. 1). It is worth noting that a positive correlation between DPPH• scavenging ability and the presence of chlorogenic acid has been demonstrated in lettuce (Złotek et al., 2014). For this reason, phenolic acids have attracted considerable interest in the past few years due to their potential health benefits (Mattila and Hellström, 2007), the antioxidant nature of phenolic acids being related to the number and kind of substituents on their aromatic rings (Rice-Evans et al., 1996).

The significant increases in chlorogenic, protocatechuic and cryptochlorogenic acid, observed in treated samples at the end of the storage period are in accordance with the accumulation of total phenolic compounds (Table 2, Fig. 1). Conversely, in the earlier storage periods, these compounds exhibited a different trend of response to UV-B treatment in respect to total phenols, being importantly increased by the treatment despite decreased or unchanged phenolic levels. It should be noted, however, that, due to their low content in comparison to other phenolic classes (ranging from 0.1% to 1.2%), their contribution to changes in total phenols is low. It was not possible to establish a trend in relation to the response of neochlorogenic acid to UV-B radiation. This may be because of the small amount present in the samples.

Within the flavan-3-ols, catechin, epicatechin, and procyanidin B1–B4 were identified in apple skin (Table 2), the most representative compounds being epicatechin and procyanidin B3 (Table 2). None of these compounds were affected by the UV-B-treatment while a reduction by 29 and 33% was observed at the end of the storage period in the levels of catechin and procyanidin B1, respectively (Table 2). In agreement with these results, both Lancaster et al. (2000) and Hagen et al. (2007) observed little or no effect of UV-B radiation on skin procyanidins of different apple cultivars. Moreover, the group of flavan-3-ols in apple skin of Red Delicious includes main phenolic compounds in the free state (Table 2), which could mostly contribute to the antioxidant capacity (Fig. 1). In fact, aglycones have been demonstrated to be more potent antioxidants than their corresponding glycosides (Heim et al., 2002; Vinson et al., 2001) and this presence could have a particular biological significance in fruit (Vinson et al., 2001).

The lower content of quercetins after the treatment for 36 h is in agreement with the decrease of total flavonoids (Fig. 1B). This could be related to the consumption of these antioxidants by the cell to counteract the possible ROS generation due to increased oxidative metabolism (Hideg et al., 2013). In fact, the appearance of stable carbon-centered free radicals occurred after UV-B treatment (Figs. 3 and 4) most likely as the result of Fenton-type reactions responsible for tissue damage including lipid peroxidation (Sgherri et al., 2015).

Besides quercetin, different conjugated forms of flavonols were identified in apple skin (Table 2), similarly with what was reported by (Boyer and Liu, 2004). Glycosylated forms of quercetin tended

to decrease as the conservation time increased. The UV-B radiation negatively affected the concentration of these compounds immediately after treatment (36 h) and at day 7. However, at the end of the experimental time, there was a significant increase in quercetin 3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-arabfuranoside, and quercetin-3-O-arabpiranoside, respectively by 87, 103, 40 and 34% (Table 2).

However, the increases of the most quercetin conjugated forms, at the end of storage, indicated that UV-B radiation induced the synthesis of this class of flavonoids, improving health properties of the fruit and contributing to extending their shelf-life. In agreement with these results, also Hagen et al. (2007) found increases in quercetins in the peel of shade-grown apples even if a different dose and duration of the UV-B treatment was applied.

Anthocyanins in apple skin are mainly represented by cyanidin-3-Ogalactoside (Table 2), but peonidin-3-O-galactoside was also identified (Table 3). In contrast to the latter, the former was not affected by the treatment of UV-B for 36 h (Table 2). Similarly to flavonols during storage times, cyanidin-3-O-galactoside showed values about 1.5–2.7 higher in the treated samples compared to their controls (Table 2), which influenced the antioxidant activity of apple skin extracts (Fig. 1). The importance of accumulation of this kind of molecules following UVB treatment was previously demonstrated in peach skin where the capacity of cyanidin-3-O-glucoside radicalization was related to protection of other cell organic molecules from oxidation (Sgherri et al., 2015). This could represent a mechanism by which shelf life of UV-B irradiated fruit can be prolonged in the presence of anthocyanins.

The typical dihydrochalcones found in apple skin were phloretin 2′-O-xyloside-glucoside and phloridzin (Table 2), the former being a product of phloridzin oxidation by polyphenoloxidase in the presence of ascorbic acid (Treutter, 2001). No differences were shown between the treated and untreated samples, only phloretin glucoside exhibiting a reduction by 20% in the UV-B treated apples during 7 d of storage (Table 2).

The differences observed in our study for each phenolic compound could be ascribed to the fact that in apple skin the different genes involved in the phenylpropanoid pathway respond to UV-B uncoordinatedly (Hagen et al., 2007). This uncoordinated synthesis has also been demonstrated regarding flavonoid accumulation in response to high sunlight (Solovchenko and Schmitz-Eiberger, 2003) as well as in the case of phloridzin, catechin and chlorogenic acid, which did not change between shade condition or exposure to sunlight (Awad et al., 2000). Moreover, some authors pointed out that different genotypes have a strong influence on the sensitivity of apple skin to UV-B radiation (Bakhshi and Arakawa, 2006; Glenn and Yuri, 2013).

3.5. Electron paramagnetic resonance (EPR) measurements

Apples exposed to UV-B for 36 h, presented in treated skin the occurrence of stable free radicals as shown in Figs. 3 and 4. The addition of the spin trap DEPMPO to the sample skin extract borught to the generation of the spectrum reported in Fig. 4. Following spectra simulation, two DEPMPO carbon-centered adducts can be identified. Similarly to what was previously reported for the peach skin (Sgherri et al., 2015) values for EPR hyperfine splitting constants of the first radical (74.5%) were: aP: 46.57 G, aN, 14.39 G; aH: 20.85 G whereas those for the second one (25.5%) were: aP: 45.39 G, aN, 14.24 G; aH: 22.53 G.

Carbon-centered free radicals occurred in the tissues after 36 h of UV-B treatment as the result of oxidative reactions induced by ROS (Fig. 4), in particular, hydroxyl radical, the most harmful species (Sgherri et al., 2015). In the presence of an efficient antioxidative system, tissue damage can be avoided, and apple skin is particularly enriched with antioxidants, mainly phenolic compounds such as quercetins, which are consumed following UV-B treatment (Table 2).

Following storage (7, 14 and 21 d) also control samples of apple skin showed the presence of stable radicals probably due to aging (Fig. 4). A relationship between aging and free radical production has been well established in sunflower seeds and pigeonpea orthodox seeds (Bailly et al., 1996; Kalpana and Rao, 1994) as well as in Araucaria seeds (Francini et al., 2006), but evidence on fruits has not been shown yet.

The sample treated with UV-B for 36 h maintained all over the storage period values of carbon-centered radicals always higher than the relative controls. Moreover, the difference in concentration between treated and control fruit increased during the storage (+128%, +169% and 382% after 7, 14 and 21 d respectively) indicating increasing oxidative conditions (Fig. 4). However, free radicals are known to act also as signal molecules (Schieber and Chandel, 2014), and the increase in some phenolic compounds in the skin of the apple following storage (Table 2) can be the result of the induction of some biosynthetic pathways by some radical species. In particular, synthesis appeared to be induced as regards hydroxycinnamic acids and anthocyanins, which responded positively to the dose of UV-B applied (Table 2). This could explain why, notwithstanding the free radical increase, tissue of apple skin was able to counteract oxidative damages, such as lipid peroxidation (Sgherri et al., 2017), with the result that main quality parameters of fruits were unaffected.

4. Conclusions

Post-harvest UV-B radiation is a promising tool to modulate the concentration of bioactive compounds in apple fruit. A UV-B for 36 h treatment induced the generation of carbon-centered radicals in the skin, the tissue more exposed to radiation, but quality parameters of the fruit were not

affected. Even if firmness progressively decreased and an increasing weight loss occurred during storage, differences between treated and control fruit were not observed. Moreover, in the treated-apple skin, an increase in some important nutraceutical compounds, among which anthocyanins, was observed together with the enhancement in total phenolic compounds at the end of the storage period. Phenols in fruit and vegetables may have a diverse range of properties, and a higher presence of these compounds increases the marketability of the products. In fact, the fruit skin showed a higher antioxidant activity increasing the healthy properties of the fruit, thus demonstrating that UV-B radiation is a valid eco-friendly approach to obtain phenolic-enriched apple fruit.

Acknowledgement

The authors are grateful for the financial support from Capes (071/2013 PVE).

References

Allan, A.C., Hellens, R.P., Laing, W.A., 2008. MYB transcription factors that colour our fruit. Trends Plant Sci. 13, 99–102.

Awad, M.A., de Jager, A., van Westing, L.M., 2000. Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci. Hortic. 83, 249–263.

Bailly, C., Benamar, A., Corbineau, F., Come, D., 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant 97, 104–110.

Bakhshi, D., Arakawa, O., 2006. Effects of UV-B irradiation on phenolic compound accumulation and antioxidant activity in ‘Jonathan’ apple influenced by bagging, temperature and maturation. J. Food Agric. Environ. 4, 75–79.

Becatti, E., Chkaiban, L., Tonutti, P., Forcato, C., Bonghi, C., Ranieri, A.M., 2010. Short-term postharvest carbon dioxide treatments induce selective molecular and metabolic changes in grape berries. J. Agric. Food Chem. 58, 8012–8020.

Boyer, J., Liu, R.H., 2004. Apple phytochemicals and their health benefits. Nutr. J. 3, 5.

Calvenzani, V., Martinelli, M., Lazzeri, V., Giuntini, D., Dall'Asta, C., Galaverna, G., Tonelli, C., Ranieri, A.P.K., 2010. Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. Planta 231, 755–765.

Castagna, A., Chiavaro, E., Dall’Asta, C., Rinaldi, M., Galaverna, G., Ranieri, A., 2013. Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. Food Chem. 137, 151–158.

Castagna, A., Dall'Asta, C., Chiavaro, E., Galaverna, G., Ranieri, A., 2014. Effect of postharvest UV-B irradiation on polyphenol profile and antioxidant activity in flesh and peel of tomato fruits. Food Bioprocess Technol. 7, 2241–2250.

Chitarra, M.I.F., Chitarra, A.B., 2005. Pós colheita de frutos e hortaliças: fisiologia e manuseio, 2nd ed. FAEPE, Lavras.

Du, W., Avena-Bustillos, R.J., Breksa III, A.P., McHugh, T.H., 2014. UV-B light as a factor affecting total soluble phenolic contents of various whole and fresh-cut specialty crops. Postharvest Biol. Technol. 93, 72–82.

Duling, D.R., 1994. Simulation of multiple isotropic spin trap EPR spectra. J. Magn. Reson. 104, 105–110.

Francini, A., Galleschi, L., Saviozzi, F., Pinzino, C., Izzo, R., Sgherri, C., Navari-Izzo, F., 2006. Enzymatic and non-enzymatic protective mechanisms in recalcitrant seeds of Araucaria bidwillii subjected to desiccation. Plant Physiol. Biochem. 44, 556–563.

Glenn, D.M., Yuri, J.A., 2013. Photosynthetically active radiation (PAR)×ultraviolet radiation (UV) interact to initiate solar injury in apple. Sci. Hortic. 162, 117–124.

Hagen, S.F., Borge, G.I.A., Bengtsson, G.B., Bilger, W., Berge, A., Haffner, K., Solhaug, K.A., 2007. Phenolic contents and other health and sensory related properties of apple fruit (Malus domestica Borkh, cv. Aroma): effect of postharvest UV-B irradiation. Postharvest Biol. Technol. 45, 1–10.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584.

Hernández-Herrero, J.A., Frutos, M.J., 2014. Colour and antioxidant capacity stability in grape, strawberry and plum peel model juices at different pHs and temperatures. Food Chem. 154, 199–204.

Hideg, É., Jansen, M.A.K., Strid, Å., 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci. 18, 107–115.

Imeh, U., Khokhar, S., 2002. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. J. Agric. Food Chem. 50, 6301–6306.

Kalpana, R., Rao, K.V.M., 1994. Absence of the role of lipid peroxidation during accelerated ageing of seeds of pigeonpea Cajanus cajan (L.) Millsp. Seed Sci. Technol. 22, 253–260.

Kim, D.-O., Chun, O.K., Kim, Y.J., Moon, H.-Y., Lee, C.Y., 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51, 6509–6515.

Lancaster, J.E., Dougall, D.K., 1992. Regulation of skin color in apples. CRC Crit. Rev. Plant. Sci. 10, 487–502.

Lancaster, J.E., Reay, P.F., Norris, J., Butler, R.C., 2000. Induction of flavonoids and phenolic acids in apple by UV-B and temperature. J. Hortic. Sci. Biotechnol. 75, 142–148.

Lin-Wang, K., Micheletti, D., Palmer, J., Volz, R., Lozano, L., Espley, R., Hellens, R.P., Chagnè, D., Rowan, D.D., Troggio, M., Iglesias, I., Allan, A.C., 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ. 34, 1176–1190.

Liu, C., Han, X., Cai, L., Lu, X., Ying, T., Jiang, Z., 2011. Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. Postharvest Biol. Technol. 59, 232–237.

Lotito, S.B., Frei, B., 2004. Relevance of apple polyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. Free Radic. Biol. Med. 36, 201–211.

Majidi, H., Minaei, S., Almasi, M., Mostofi, Y., 2011. Total soluble solids, titratable acidity and repining index of tomato in various storage conditions. Aust. J. Basic Appl. Sci. 5, 1723–1726.

Mattila, P., Hellström, J., 2007. Phenolic acids in potatoes, vegetables, and some of their products. J. Food Compos. Anal. 20, 152–160.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C., 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. Methods Enzymol. 299, 379–389.

Peng, T., Moriguchi, T., 2013. The molecular network regulating the coloration in apple. Sci. Hortic. 163, 1–9.

Pinzino, C., Forte, C., 1992. Esr-endor. Istituto di Chimica Quantistica ed Energetica Molecolare-CNR, Pisa, Italy.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Rice-Evans, C., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152–159.

Ryan, K.G., Swinny, E.E., Markham, K.R., Winefield, C., 2002. Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. Phytochemistry 59, 23–32.

Saure, M.C., 1990. External control of anthocyanin formation in apple. Sci. Hortic. 42, 181–218.

Scattino, C., Castagna, A., Neugart, S., Chan, H.M., Schreiner, M., Crisosto, C.H., Tonutti, P., Ranieri, A., 2014. Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines. Food Chem. 163, 51–60.

Schieber, M., Chandel, Navdeep S., 2014. ROS function in redox signaling and oxidative stress. Curr. Biol. 24, 453–462.

Sgherri, C., Scattino, C., Pinzino, C., Tonutti, P., Ranieri, A.M., 2015. Ultraviolet-B radiation applied to detached peach fruit: a study of free radical generation by EPR spin trapping. Plant Physiol. Biochem. 96, 124–131.

Sgherri, C., Pinzino, C., Quartacci, M.F., 2017. Reactive oxygen species and photosynthetic functioning: past and present. In: Singh, V.P., Singh, S., Tripathi, D.K.,

Prasad, S.M., Chauhan, D.K. (Eds.), Reactive Oxygen Species in Plants: Boon or Bane? Revisiting the Role of ROS. J. Wiley & Sons, New York (USA), pp. 137–155.

Solovchenko, A., Schmitz-Eiberger, M., 2003. Significance of skin flavonoids for UV-B protection in apple fruits. J. Exp. Bot. 54, 1977–1984.

Suesslin, C., Frohnmeyer, H., 2003. An Arabidopsis mutant defective in UV-B light-mediated responses. Plant J. 33, 591–601.

Sun, S., Xin, L., Gao, H., Wang, J., Li, P., 2014. Response of phenolic compounds in 'Golden Delicious' and 'Red Delicious' apples peel to fruit bagging and subsequent sunlight re-exposure. Sci. Hortic. 168, 161–167.

Takshak, S., Agrawal, S.B., 2014. Effect of ultraviolet-B radiation on biomass production, lipid peroxidation, reactive oxygen species, and antioxidants in *Withania somnifera*. Biol Plant. 58, 328–334.

Treutter, D., 2001. Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regul. 34, 71–89.

Ubi, B.E., Honda, C., Bessho, H., Kondo, S., Wada, M., Kobayashi, S., Moriguchi, T., 2006. Expression analysis of anthocyanin biosynthetic genes in apple skin: effect of UV-B and temperature. Plant Sci. 170, 571–578.

Vinson, J.A., Su, X., Zubik, L., Bose, P., 2001. Phenol antioxidant quantity and quality in foods: fruits. J. Agric. Food Chem. 49, 5315–5321.

Wildman, R.E.C., 2006. Handbook of Nutraceuticals and Functional Foods, 2nd ed. CRC Press, Boca Raton.

Złotek, U., Świeca, M., Jakubczyk, A., 2014. Effect of abiotic elicitation on main health-promoting compounds, antioxidant activity and commercial quality of butter lettuce (*Lactuca sativa* L.). Food Chem. 148, 253–260.

Zhao, C., Mao, K., You, C.-X., Zhao, X.-Y., Wang, S.-H., Li, Y.-Y., Hao, Y.-J., 2016. Molecular cloning and functional analysis of a UV-B photoreceptor gene MdUVR8 (UV Resistance Locus 8), from apple. Plant Sci. 247, 115–126.

Figure legends

Fig. 1. Total phenols, flavonoids (g kg^{-1} DW) and antioxidant activity (gmol kg^{-1} DW) of apple skin irradiated with UV-B for 36 h and left to stand in the dark until 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

Fig. 2. Total hydroxycinnamic acids (A), flavan-3-ols (B), flavonols (C), anthocyanins (D) and dihydrochalcones (E) (g kg^{-1} DW) of apple skin irradiated with UV-B for 36 h and left to stand in the dark untill 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

Fig. 3. EPR spectra relative to the generation of carbon-centered free radicals following irradiation of apple skin with UV-B (A, before and B after the addition of DEPMPO); a, c experimental; b, d simulated. Two carbon-radicals can be recognized. The first (74.5%) presents the following hyperfine splitting constants: aP: 46.57 G, aN, 14.39 G; aH: 20.85 G. The second one (25.5%) presents the following hyperfine splitting constants: aP: 45.39 G, aN, 14.24 G; aH: 22.53 G.

Fig. 4. Stable carbon radicals (Spin×1013 g−1 DW) generated in skin of apples irradiated for 36 h with UV-B and left to stand in the dark until 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B- treated apples; UV-B means UV-B-treated apples.

Table 1. Quality parameters of apple skin irradiated with UV-B for 36 h and left to stand in the dark untill 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UVB means UV-B-treated apples.

		Treatment	Storage		
		36 h	7 days	14 days	21 days
Titratable Acidity	Control	0.1518 ± 0.01^{a}	0.1281 ± 0.01^{a}	0.1532 ± 0.01^{a}	0.1560 ± 0.01^{a}
	UV-B	0.1776 ± 0.01^{a}	0.1382 ± 0.01^{a}	0.1437 ± 0.01^{a}	0.1664 ± 0.01^{a}
TSS	Control	13.33 ± 0.04^{a}	14.14 ± 0.25^{a}	13.60 ± 0.35^{a}	12.90 ± 0.21^{a}
	UV-B	12.51 ± 0.07^{b}	13.29 ± 0.13^{b}	12.79 ± 0.20^{a}	12.90 ± 0.19^{a}
pH	Control	3.64 ± 0.04^{a}	3.55 ± 0.07^{a}	3.75 ± 0.06^{a}	3.53 ± 0.04^{a}
	UV-B	3.51 ± 0.02b	3.61 ± 0.05^{a}	3.73 ± 0.05^{a}	3.63 ± 0.06^{a}
Firmness	Control	68.20 ± 3.62^{a}	59.07 ± 2.12^{a}	53.33 ± 3.12^{a}	47.62 ± 1.90^{a}
	UV-B	71.44 ± 4.17^{a}	63.62 ± 2.24^{a}	44.28 ± 4.60^{a}	38.16 ± 6.73^{a}
Weight loss	Control	0.63 ± 0.06^{a}	2.39 ± 0.22^{a}	4.05 ± 0.25^{a}	5.08 ± 0.74^{a}
	UV-B	0.72 ± 0.02^{a}	2.76 ± 0.22^{a}	3.24 ± 0.10^{b}	5.66 ± 0.25^{a}

Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples. Acidity as % of malic acid; TSS (Total Soluble Solids) as %; Firmness as Newton and Weight loss as %.

Table 2. Phenolic compounds (g kg^{-1} DW) determined after UPLC‑MS analysis of methanolic extracts from apple skin irradiated with UV-B for 36 h and left to stand in the dark until 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukeys test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples. irradiated with UV-B for 36 h and left to stand in the dark until 21 days.

Group of compounds	Identified compounds		Treatment	Storage		
			36 h	7 days	14 days	21 days
Hydroxycinnamic acids	Caffeoyl glucoside	Control	0.0018 ± 0.01[a]	0.0020 ± 0.01[a]	0.0010 ± 0.01b	0.0011 ± 0.01[a]
		UV-B	0.0015 ± 0.01[a]	0.0017 ± 0.01[a]	0.001.32 ± 0.01a	0.0008 ± 0.01[a]
	Chlorogenic acid	Control	0.0031 ± 0.01[a]	0.0071 ± 0.01[b]	0.006.49 ± 0.01[b]	0.0046 ± 0.01[b]
		UV-B	0.0045 ± 0.01[a]	0.0326 ± 0.01[a]	0.034.76 ± 0.01[a]	0.0315 ± 0.01[a]
	Neochlorogenic acid	Control	0.0006 ± 0.01[a]	0.0011 ± 0.01[a]	0.0009 ± 0.01[a]	0.0011 ± 0.01[a]
		UV-B	0.0004 ± 0.01[a]	0.0011 ± 0.01[a]	0.0007 ± 0.01 [b]	0.0006 ± 0.01[b]
	p-Coumaroyl glucose	Control	0.0017 ± 0.01a	0.0028 ± 0.01[a]	0.0013 ± 0.01[b]	0.0021 ± 0.01[a]
		UV-B	0.0013 ± 0.01[b]	0.0025 ± 0.01[a]	0.0025 ± 0.01[a]	0.0016 ± 0.01[a]
	p-Coumaroyl quinic acid	Control	0.0006 ± 0.01[a]	0.0015 ± 0.01[a]	0.0007 ± 0.01[a]	0.0013 ± 0.01[a]
		UV-B	0.0005 ± 0.01[a]	0.0013 ± 0.01[a]	0.0007 ± 0.01[a]	0.0010 ± 0.01[a]
	Protocatechuic acid	Control	0.0003 ± 0.01[a]	0.0004 ± 0.01[a]	0.0006 ± 0.01[a]	0.0002 ± 0.01[b]
		UV-B	0.0004 ± 0.01[a]	0.0004 ± 0.01[a]	0.0005 ± 0.01[a]	0.0005 ± 0.01[a]
	Cryptochlorogenic acid	Control	0.0002 ± 0.01[a]	0.0004 ± 0.01[b]	0.0004 ± 0.01[b]	0.0004 ± 0.01[b]
		UV-B	0.0004 ± 0.01[a]	0.0016 ± 0.01[a]	0.0013 ± 0.01[a]	0.0017 ± 0.01[a]
	Feruloyl glucoside	Control	0.0257 ± 0.01[b]	0.0541 ± 0.01[a]	0.0335 ± 0.01[b]	0.0527 ± 0.01[a]
		UV-B	0.0378 ± 0.01[a]	0.0598 ± 0.01[a]	0.0478 ± 0.01[a]	0.0452 ± 0.017[a]
Flavan-3-ols	(+)Catechin	Control	0.0340 ± 0.01[a]	0.0445 ± 0.01[a]	0.0229 ± 0.01[a]	0.0283 ± 0.01[a]
		UV-B	0.0317 ± 0.01[a]	0.0405 ± 0.01[a]	0.0281 ± 0.01[a]	0.0200 ± 0.01[b]
	(−)Epicatechin	Control	0.8431 ± 0.02[a]	1.4051 ± 0.02[a]	0.8007 ± 0.06[a]	0.7645 ± 0.08
		UV-B	0.7244 ± 0.07[a]	1.2534 ± 0.05[a]	0.9028 ± 0.04[a]	0.6807 ± 0.02[a]
	Procyanidin B1	Control	0.0091 ± 0.01[a]	0.0153 ± 0.01[a]	0.0082 ± 0.01[a]	0.0107 ± 0.01[a]
		UV-B	0.0079 ± 0.01[a]	0.0141 ± 0.01[a]	0.0101 ± 0.01[a]	0.0071 ± 0.01[b]
	Procyanidin B2	Control	0.0828 ± 0.01[a]	0.1456 ± 0.01[a]	0.0798 ± 0.01[a]	0.0810 ± 0.01[a]
		UV-B	0.0737 ± 0.01[a]	0.1251 ± 0.01[b]	0.0876 ± 0.01[a]	0.0725 ± 0.01[a]
	Procyanidin B3	Control	0.3046 ± 0.01[a]	0.4877 ± 0.01[a]	0.2579 ± 0.01[a]	0.2556 ± 0.03[a]
		UV-B	0.2474 ± 0.02[a]	0.4169 ± 0.01[b]	0.2825 ± 0.01[a]	0.2327 ± 0.01[a]
	Procyanidin B4	Control	0.0039 ± 0.01[a]	0.0072 ± 0.01[a]	0.0059 ± 0.01[a]	0.0051 ± 0.01[a]
		UV-B	00032 ± 0.01[a]	0.0108 ± 0.01[a]	0.0055 ± 0.01[a]	0.0045 ± 0.01[a]
Flavonols	Quercetin 3-O-glucoside	Control	0.2967 ± 0.02[a]	0.2869 ± 0.01[a]	0.1891 ± 0.01[b]	0.0815 ± 0.01[b]
		UV-B	0.1201 ± 0.01[b]	0.2137 ± 0.01[b]	0.2540 ± 0.01[a]	0.1533 ± 0.01[a]
	Quercetin 3-O-galactoside	Control	0.7664 ± 0.05[a]	0.9774 ± 0.02[a]	0.6825 ± 0.03[b]	0.2161 ± 0.01[b]
		UV-B	0.4199 ± 0.01[b]	0.6174 ± 0.01[b]	0.8481 ± 0.04[a]	0.4386 ± 0.01[a]
	Quercetin 3-O-rhamnoside	Control	0.1585 ± 0.01[a]	0.1575 ± 0.01[a]	0.1335 ± 0.01 [a]	0.0622 ± 0.01[a]
		UV-B	0.0755 ± 0.01[b]	0.0921 ± 0.01[b]	0.1397 ± 0.01 [a]	0.0636 ± 0.01[a]
	Quercetin 3-O-arabfuranoside	Control	0.3091 ± 0.02[a]	0.3226 ± 0.01[a]	0.2578 ± 0.01[a]	0.1058 ± 0.01[b]
		UV-B	0.1681 ± 0.01[b]	0.2363 ± 0.01[b]	0.2833 ± 0.01[a]	0.1478 ± 0.01[a]
	Quercetin 3-O-arabpiranoside	Control	0.2255 ± 0.01[a]	0.2442 ± 0.01[a]	0.1823 ± 0.01[a]	0.0728 ± 0.01[b]
		UV-B	0.1097 ± 0.01 [b]	0.1504 ± 0.01[b]	0.1941 ± 0.01[a]	0.0979 ± 0.01[a]
	Quercetin	Control	0.1244 ± 0.11[a]	0.1593 ± 0.01[a]	0.1105 ± 0.01[a]	0.0444 ± 0.01[a]
		UV-B	0.0545 ± 0.01[b]	0.0841 ± 0.01[b]	0.1096 ± 0.01[a]	0.0586 ± 0.01[a]
Anthocyanins	Cyanidin 3-O-galactoside	Control	0.1843 ± 0.01[a]	0.2042 ± 0.01[b]	0.1366 ± 0.01[b]	0.0784 ± 0.01[b]
		UV-B	0.1826 ± 0.01[a]	0.3050 ± 0.01 [a]	0.3072 ± 0.01 [a]	0.2091 ± 0.01 [a]
	Peonidin 3-O-galactoside	Control	0.0026 ± 0.01 [a]	0.00242 ± 0.01 [a]	0.0031 ± 0.01 [a]	0.0003 ± 0.01[b]
		UV-B	0.0007 ± 0.01[b]	0.00130 ± 0.01[b]	0.0012 ± 0.01[b]	0.0008 ± 0.01 [a]
Dihydrochalcones	Phloridzin	Control	0.3291 ± 0.01[a]	0.6532 ± 0.01[a]	0.5677 ± 0.03[a]	0.43267 ± 0.03[a]
		UV-B	0.3149 ± 0.01[a]	0.5979 ± 0.02[a]	0.6538 ± 0.03[a]	0.4502 ± 0.01[a]
	Phloretin 2′-O-xyloside-glucoside	Control	0.0777 ± 0.01[a]	0.2002 ± 0.01 [a]	0.1492 ± 0.01[a]	0.1388 ± 0.01[a]
		UV-B	0.0862 ± 0.01[a]	0.1587 ± 0.01b	0.1829 ± 0.01[a]	0.1369 ± 0.01[a]

Table 3. Polyphenolic compounds identified in apple skin by m/z, adduct and MRM transition in the HPLC–MS and MS/MS analysis. The details on the chromatographic conditions and mass spectrometric detection are described in Section 2.6.

Polyphenol group	Compound	Abbreviation	m/z	adduct	MRM transition
Anthocyanins	Cyanidin 3-O-galactoside	Cy-3gal	449.01	[M+H]+	449.01→286.7
	Peonidin 3-O-galactoside	Pn-3gal	463.01	[M+H]+	463.01→301.1
Dihydrochalcones	Phloritzin (Phloretin 2′-O-glucoside)*	Phlor	481.0	[M+HCOO−]−	481.0→434.9
	Phloretin 2′-O-xyloside-glucoside	Phlo-xyl	567.1	[M−H]−	567.1→273.0
Flavan-3-ols	(+)Catechin*	Cat	289.0	[M−H]−	289.0→203.1
	(−)Epicatechin*	Epi	289.0	[M−H]−	289.0→245.0
	Procyanidin B1	ProB1	577.0	[M−H]−	577.0→289.0
	Procyanidin B2	ProB2	577.0	[M−H]−	577.0→289.0
	Procyanidin B3	ProB3	577.0	[M−H]−	577.0→406.9
	Procyanidin B4	ProB4	577.0	[M−H]−	577.0→289.0
Flavonols	Quercetin 3-O-glucoside*	Q-glu	463.0	[M−H]−	463.0→301.1
	Quercetin 3-O-galactoside	Q-gal	463.0	[M−H]−	463.0→301.1
	Quercetin 3-O-rhamnoside	Q-rha	447.1	[M−H]−	447.1→301.1
	Quercetin 3-O-arabfuranoside	Q-arab-fur	433.0	[M−H]−	433.0→301.1
	Quercetin 3-O-arabpiranoside	Q-arab-pyr	433.0	[M−H]−	433.0→301.1
	Quercetin	Q	301.0	[M−H]−	301.0→179.0
Hydroxycinnamic acids	Caffeoyl glucoside	Caf-glu	341.2	[M−H]−	341.2→178.9
	Chlorogenic acid*	Clor	353.0	[M−H]−	353.0→191.1
	Neochlorogenic acid	Neoclor	353.0	[M−H]−	353.0→172.8
	p-Coumaroyl glucose	p-Cou-glu	325.1	[M−H]−	325.1→264.8
	p-Coumaroyl quinic acid	p-Cou-qui	337.0	[M−H]−	337.0→172.9
	Protocatechuic Acid	Procat	153.0	[M−H]−	153.0→108.9
	Cryptochlorogenic Acid	CryptoClor	353.0	[M−H]−	353.0→191.1
	Feruloyl glucoside	Fer-glu	355.3	[M−H]−	355.3→193.1

Figure 1

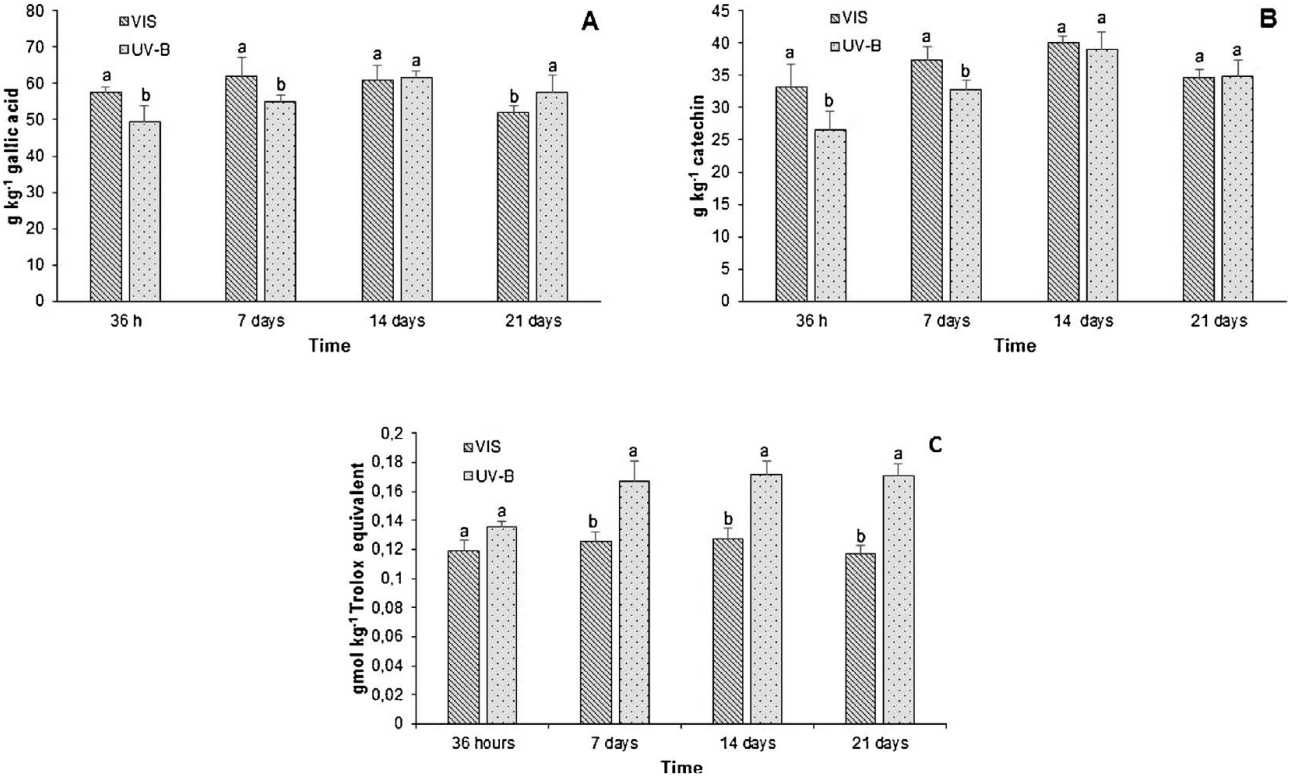

A
VIS
UV-B
g kg-1 gallic acid
36 h
7 days
14 days
21 days
Time
B
g kg-1 catechin
C
gmol kg-1 Trolox equivalent
36 hours

Figure 2

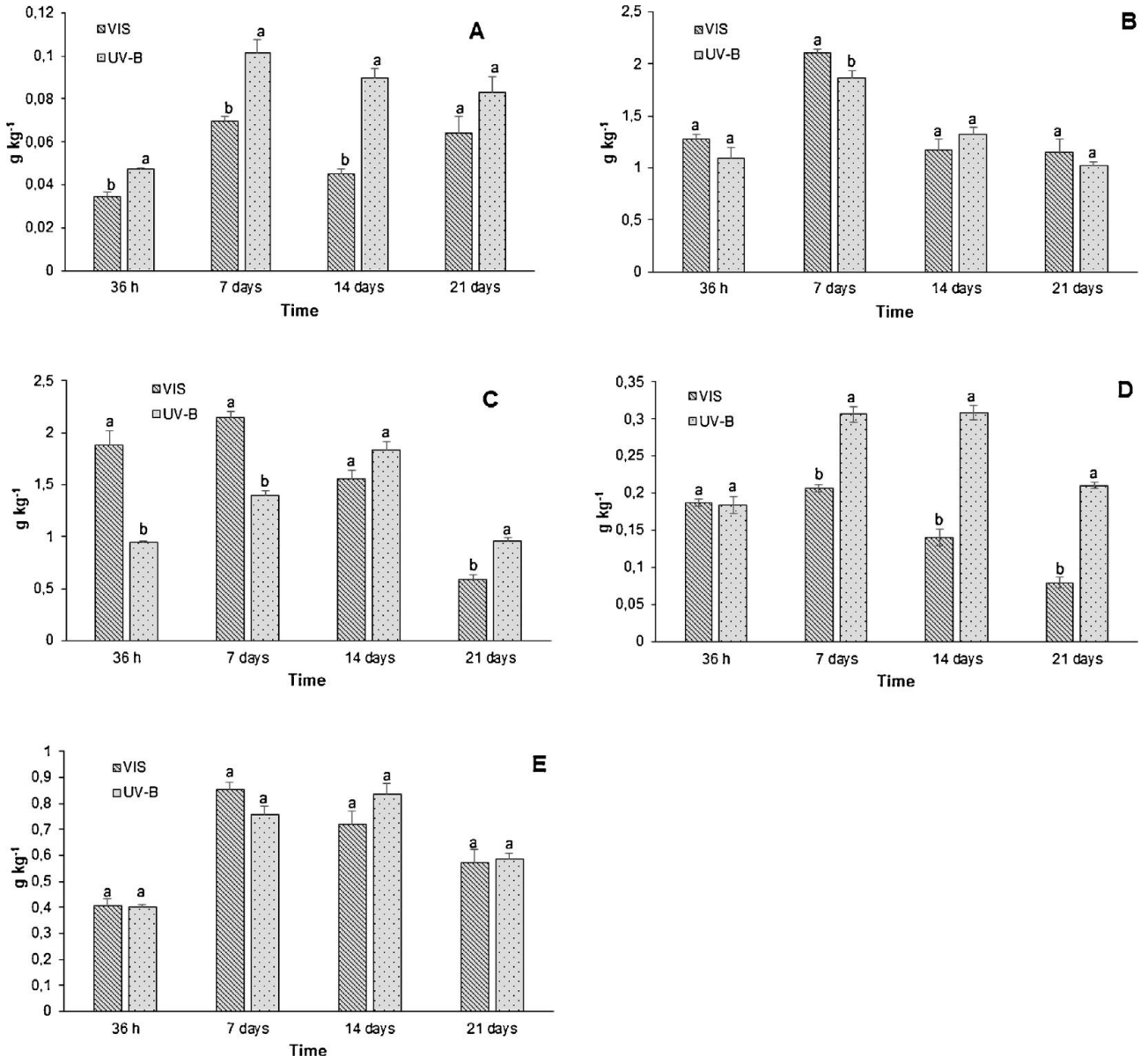


Figure 3

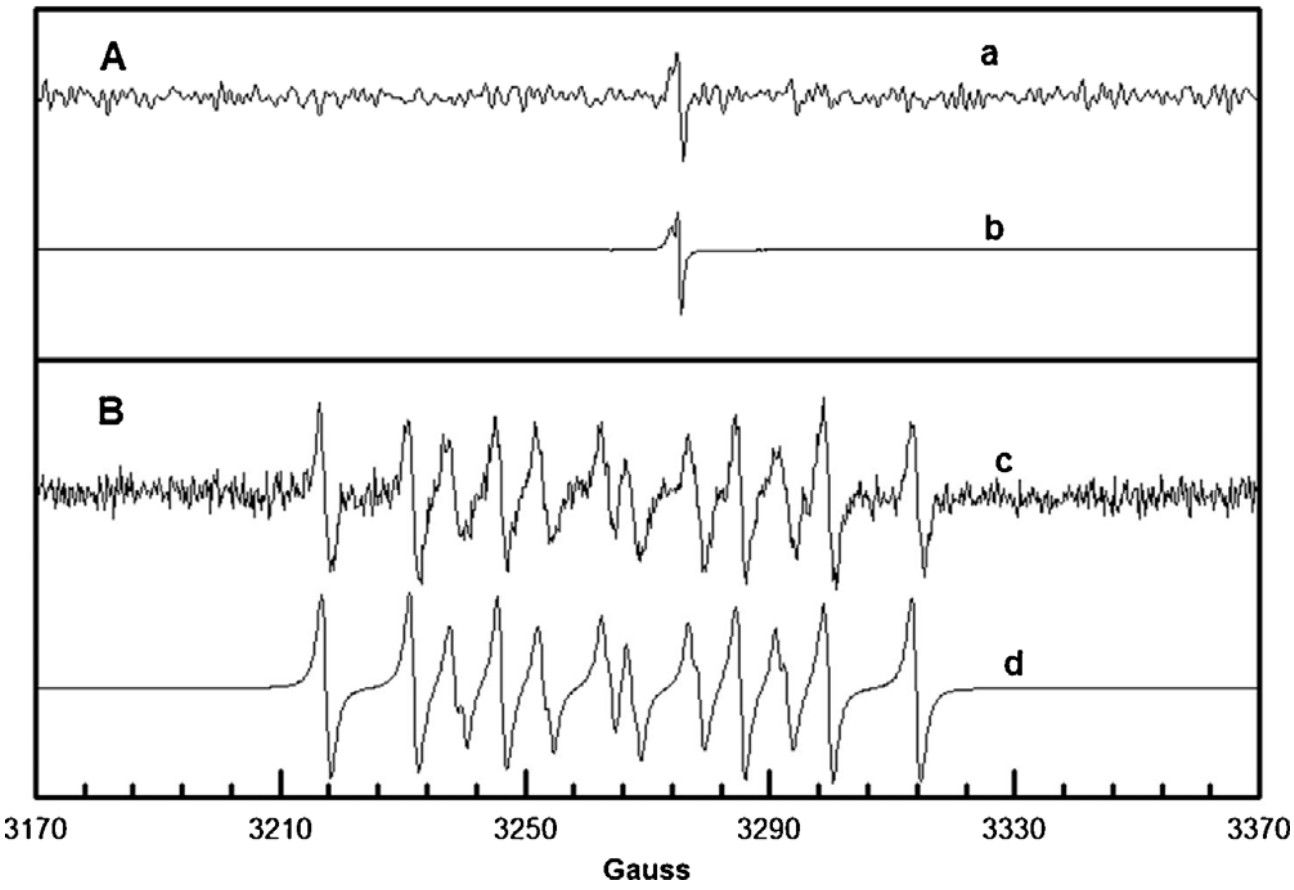
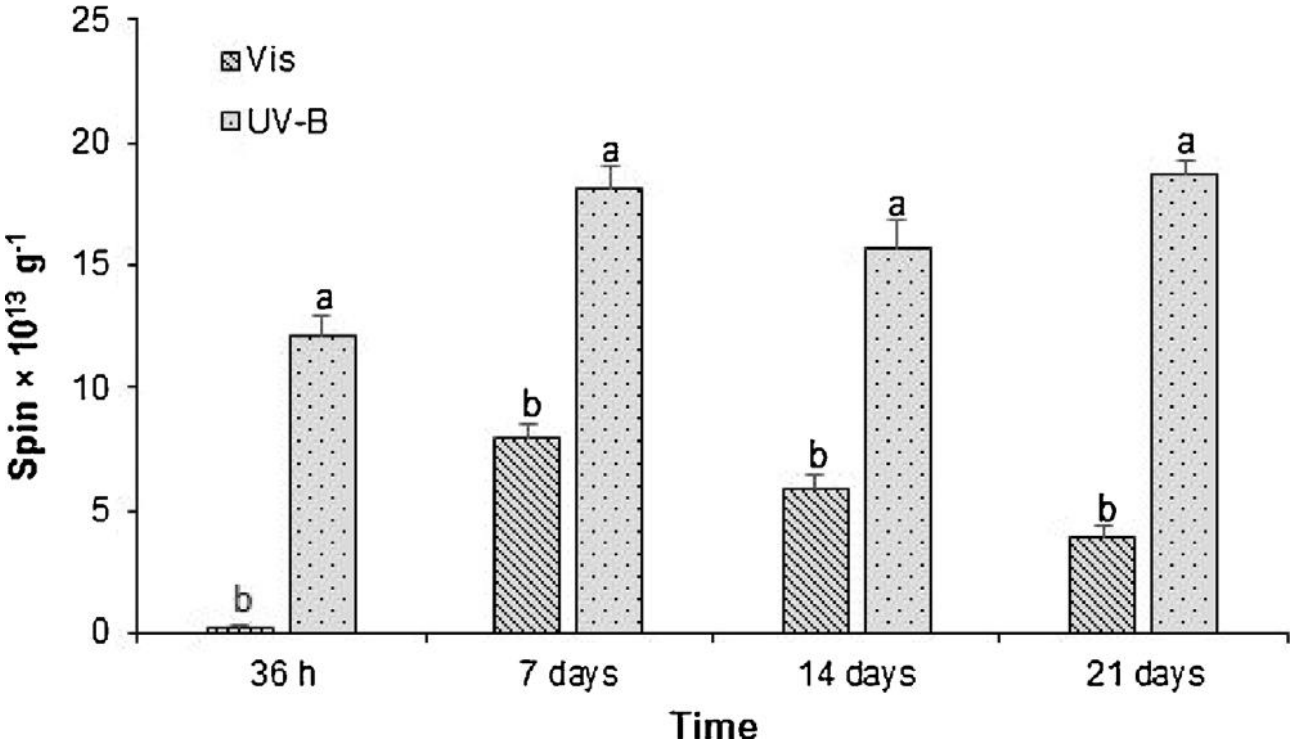


Figure 4


25
20
15
10
5
0
Vis
UV-B
Spin × 10^13 g^-1
a
b
a
b
a
b
a
b
36 h
7 days
14 days
21 days
Time