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

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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 kJ m^{-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-*O*-glucoside, 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

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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 UV-B 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 20 W – 12RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands) which provided 1.69 W m^{-2} at fruit height. The apples were placed with their peduncle facing up (approximately 0.40 m 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 kJ m^{-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 8 mm 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 analysis 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: