Influence of UV-B Light Emitting Diodes on Colour Development and Accumulation of Polyphenols and Antioxidants in Apple Skin

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Summary

UV light provoked stress can evoke antioxidant defence system and consequently induces the synthesis of several phytochemical compounds. The aim of the present study was to investigate the influence of UV-B light irradiation on surface colour and synthesis of some bioactive compounds in apples skin of cultivars 'Elstar' and 'Jonagold'. Half of each apple was shaded with aluminium foil to compare irradiated and non-irradiated parts of skin. Apple fruits were exposed to constant UV-B light at 310 nm in chamber for 13 days at 8.5 °C. The UV-B light emitting diodes were positioned *ca.* 13 cm above the apple fruit and the average irradiance fruit received was 4.6 μW/cm^2. Measurements of colour parameters (L^*, a^*, b^*) were performed on the same spots of irradiated and non-irradiated surface of each apple fruit before and after 7, 10 and 13 days of storage constant UV-B light irradiation. All apple fruits started to develop red colour on the irradiated surface. The antioxidant potential, ascorbic acid content, total phenolic compounds, six flavonols and anthocyanin cyanidin-3-galactoside were quantitatively evaluated. Apple skins exposed to UV-B light irradiation contained significantly more total phenolic compounds and had higher antioxidant potential as compared to non-irradiated. UV light treated apple skin contained more cyanidin-3-galactoside and flavonols.

Key words

antioxidant potential, ascorbic acid content, led emitting diode, total phenolic compounds, UV-B light irradiation

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Introduction

Apples (*Malus domestica*) are some of the most ancient and popular fruits grown worldwide and represent one of the major fruit for fresh consumption (Lee and Smith, 2000). Various health promoting properties of fruits, including apples, are associated with several vitamins, dietary fibre and especially secondary plant metabolites, such as polyphenols (Sgherri et al., 2015), natural effective antioxidants with the potential health benefits for humans (Balasundram et al., 2006). Content of polyphenols in fruits is affected by many agrotechnical factors, including light availability (Caballero et al., 2015).

Light presents one of the most important environmental factors which has the influence on various biological processes and can act as stressor for plants. Photo stress can evoke antioxidant defence system and consequently induce the synthesis of phytochemical compounds (Li and Kubota, 2009). The biologically active light radiation consists of the spectrum from 300 nm to 800 nm, including UV light (Zoratti et al., 2014). UV light is composed of UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (<280 nm). UV-B light has extensive photo-biological effects on fruits during postharvest life, thus its intensity can affect physiological responses such as phytochemical accumulation (Ferreyra et al., 2012).

Apple maturation is a complex interaction of intricate physiological processes (Pech et al., 2013). Differences in apple peel appearance and morphology based on the degree of exposure to light during the growth period, even on the same fruit, were observed (Rudell et al., 2008). There is nevertheless a lack of results concerning postharvest LED light applications. Apple skin colour plays an important role in consumer appeal, therefore developing of postharvest treatments to achieve desirable colour would be highly beneficial (Hamadziripi et al., 2014). UV-B irradiation is considered a promising technique to increase the health promoting potential of mature fruits and at the same time to improve shelf-life and quality characteristics (Sgherri et al., 2015).

The aim of the present study was to gain knowledge about the postharvest influence of UV-B LED light irradiation on surface colour and accumulation of selected bioactive compounds in apple skin: antioxidant potential, ascorbic acid, total phenolics, anthocyanin and flavonols. For this purpose, half of each apple fruit was shaded in order to compare irradiated and non-irradiated parts. Apple fruits were exposed to constant UV-B LED light irradiation for 13 days, and changes in apple skin were investigated.

Materials and methods

In the preliminary experiment apples (*Malus domestica*) cv. 'Elstar' and 'Jonagold' were purchased from the commercial supermarket (Ljubljana, Slovenia) in October 2015. Ripe apple fruits were of commercial size and adequate colour. The apple fruit samples were immediately prepared for the postharvest storage under controlled conditions with UV-B light irradiation. Four UV-B LEDs with the wavelength of 310 nm were installed in cardboard box. Half of each apple was shaded with aluminium foil to prevent the exposure to UV-B light, and this shaded surfaces presented control. Four apples were put into UV-B LED light irradiation cardboard box *ca.* 13 cm below UV-B LED. UV-B light irradiation was provided constantly for 13 days at 8.5 °C, with four LEDs providing a total radiant flux of 4.6 μW/cm^2 on the irradiated surface.

The surface colour of the apple skins was measured using a colour-measurement device (Minolta CR-400, Minolta, Japan). Before irradiation, and after 7, 10 and 13 days of irradiation under UV-B light colour parameters L^*, a^* and b^* were measured. L^* represents the lightness of the colour, a^* the position between red (positive value) and green (negative value), and b^* the scale between yellow (positive value) and blue (negative value). The colour measurements were performed on three marked points on UV-B light irradiated and three marked points on shaded side (non-irradiated control) of the each individual apple fruit.

Analyses of bioactive compounds included evaluation of antioxidant potential (AOP), ascorbic acid content (AA) and total phenolic compounds (TPC) in apple skin only. Strips of apple skin were obtained using ceramic knife. Each apple fruit was sampled on the irradiated and non-irradiated side. For the sample extraction, 500 mg of apple skin was homogenized in liquid nitrogen and mixed with 10 g of metaphosphoric acid dissolved in distilled water (2 % by mass). Homogenized samples were then centrifuged and filtered through a 0.45 μm filters (17 mm syringe filter CA). The extracts were further stored at −80 °C until analysed.

AA content analysis were performed on an HPLC system (Agilent 1260; Agilent Technologies) using a diode array detector, with the wavelength set at 254 nm. The separation of AA was carried out on a 100 × 2 mm i.d., 3 μm Scherzo SM-C18 column (Imtakt, Japan), at a flow rate of 0.3 ml/min. The mobile phase consisted of water (A) and acetonitrile (B), both of which contained 0.3 % (by volume) formic acid. The following elution gradient was used for solvent B: 0 – 3 min, 0 % – 10 %; 3 – 4 min, 10 % – 100 %; 4 – 6 min, 100 %. The temperature of the column was maintained at 30 °C, while the temperature of the automatic sample feeder was set at 4 °C. AA content was calculated using an external standard method and is expressed as mg/100 g fresh weight (FW).

AOP of the samples was determined spectrophotometrically, as the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma, Germany) free-radical-scavenging capacity, based on the modified method of Kokalj et al. (2016). Calibration curve was made through seven-point standard curves of Trolox (Sigma, Germany) as standard (R^2 = 0.9985). AOPs were determined in triplicate and expressed as mg Trolox equivalents (TE)/100 g FW.

TPC was determined according to Folin-Ciocalteu method described by Singleton and Rossi (1965), and modified by Kokalj et al. (2016). Calibration was made through eight-point standard curves of gallic acid (Fluka, Germany) as standard (R^2 = 0.9988). TPCs were determined in triplicate and expressed as mg gallic acid equivalents (GAE)/100 g FW.

The phenolic compounds were analysed on a HPLC system Agilent 1260 Infinity (Agilent Technologies, Palo Alto, USA) with a diode array detector (DAD), the wavelength was set at 280, 320, 370 and 520 nm. The separation of phenolic compounds was carried out on a 100 x 2.1 mm i.d., 2.7μm Poroshell 120 EC-C18 column (Agilent Technologies, USA) using a flow rate of 0,2 ml/min. The mobile phase consisted of (A) water containing

1% (v/v) formic acid and (B) methanol, the following elution gradient was used: 0-20 min, 2-23% B; 20-40 min, 23-35% B; 40-46 min: 35-38% B, 46-60 min: 60 % B, 60-65 min: 95 % B. The column temperature was kept at 30°C and the autosampler at 4°C. Concentrations of phenolic compounds were calculated using external standard calibration method. For the compounds lacking of standards, the quantification was achieved using similar compounds. Thus quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinopyranoside, quercetin-3-arabinofuranoside and quercetin-3-rhamnoside were quantified in equivalents of quercetin (Sigma-Aldrich, Germany). Cyanidin-3-galactoside was quantified using cyanidin chloride standard (Extrasynthese, France). An Agilent 6460 triple quadropole mass sprectrometer coupled to electrospray ionization (ESI) interface with Agilent Jet Stream (Agilent Technologies, Palo Alto, USA) was used for mass analysis of phenolic compounds. Mass spectra were recorded in positive and negative mode from m/z 100 to 1000. The ESI source parameters were as follows: gas temperature 300°C, drying gas flow 6 L/min, cappilary voltage 4000 V, sheath gas temperature 350°C, sheath gas flow 12 L/min and nebulizer pressure was set at 35 psi. Data were analyzed according to the method of least squares, using a general linear model procedure (SAS Software, 1999). All of the measurements were performed in triplicates (n = 3). Differences at p <0.05 were considered as statistically significant.

Results and discussion

Several studies confirmed the influence of light irradiation on plant metabolism during growth (Harbaum-Piayda et al., 2010, Samuolienė et al., 2012, Shin et al., 2012, Wu et al., 2007), while postharvest light irradiation data are still lacking. Present study deals with analyses of colour parameters and content of several bioactive compounds in the skin of UV-B irradiated and non-irradiated apples. Our UV-B radiation consisted of very narrow spectrum with a peak at 310 nm.

Measurements of colour parameters (L^*, a^*, b^*) performed on the marked points of each apple fruit skin before and after 7, 10 and 13 days of constant UV-B light emitting diode irradiation are presented in Figure 1. All irradiated apple fruit skins showed tendency for developing red colour, since colour parameter a^* increased. Non-irradiated control apple fruit skin surfaces, which were shaded and exposed to identical conditions as UV-B light irradiated surfaces, showed no significant changes in colour parameters. Similar results were also obtained by Dong et al. (1995) were irradiation with broad wavelength UV fluorescent light produced a marked increase in the development of red apple skin colour.

Data of AA content, AOP and TPC of irradiated and non-irradiated apple skin samples after 13 days postharvest storage under constant UV-B light emitting diodes irradiation are presented in Table 1. AA content was higher in UV-B light irradiated apple skin compared to non-irradiated control samples, but the differences were not significant. Similar findings are reported by Hagen et al. (2007) who found an increase of AA in 'Aroma' apples after postharvest irradiation with combination of visible and UV-B light. On the other hand, AOP was significantly higher in irradiated apple skin with value of 345 mg TE/100 g FW, as compared to non-irradiated control skin samples with value of 276 mg TE/100 g FW. Correspondingly, significant higher TPC was also observed for UV-B light exposed fruit skin (421 mg GAE/100 g FW) in comparison to non-irradiated control apple skin (297 mg GAE/100 g FW). The results are in agreement with Hagen et al. (2007) who found an increase of TPC and AOP in 'Aroma' apples after postharvest irradiation

Table 1. Bioactive compounds analysed in irradiated and non-irradiated apple skin samples after 13 days postharvest storage under constant UV-B LED light irradiation.

Parameter	UV-B irradiation	Non-irradiated
AA (mg/100 g FW)	151 ±49	142 ±44
AOP (mg TE/100 g FW)	345 ±35[a]	276 ±42[b]
TPC (mg GAE/100 g FW)	421 ±59[a]	297 ±47[b]

Data are means ±SD (n = 4). Means with different letters within each row are significantly different (p <0.05). AA, ascorbic acid; AOP, antioxidant potential; TPC, total phenolic compounds; FW, fresh weight; TE, trolox equivalents; GAE, gallic acid equivalents.

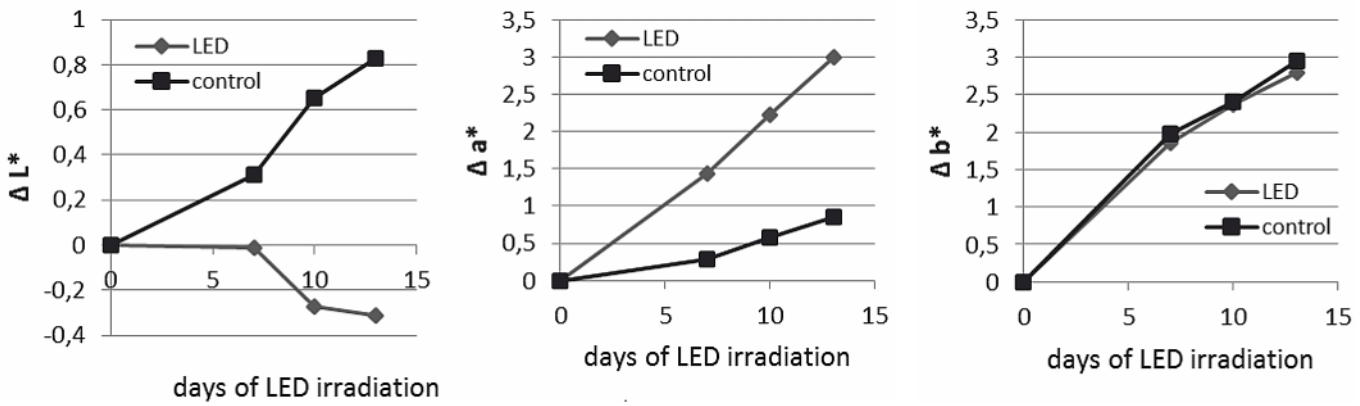

Figure 1. Change in colour parameters (L^*, a^*, b^*) of irradiated and non-irradiated apple skin after 7, 10 and 13 days postharvest storage under constant UV-B LED light irradiation.

Table 2. Content of anthocyanin and flavonols in irradiated and non-irradiated apple skin of 'Elstar' samples after 13 days under constant UV-B LED light irradiation.

Phenolic compound	UV-B irradiation	Non-irradiated
Anthocyanin (mg CE/100 g FW)		
C-3-G	3.06 ±1.06	1.86 ±0.52
Flavonols (mg QE/100 g FW)		
Q-3-Gal	7.83 ±2.80	4.59 ±1.74
Q-3-Glu	3.95 ±1.22	2.65 ±0.75
Q-3-Xyl	2.64 ±0.82	1.93 ±0.76
Q-3-Ara*p*	0.48 ±0.09	0.45 ±0.09
Q-3-Ara*f*	3.08 ±1.15	2.81 ±1.23
Q-3-Rha	2.20 ±0.72	1.68 ±0.60

Data are means ±SD (n = 3). FW, fresh weight; C-3-G, cyanidin-3-galactoside; CE, cyanidin equivalents; Q-3-Gal, quercetin-3-galactoside; Q-3-Glu, quercetin-3-glucoside; Q-3-Xyl, quercetin-3-xyloside; Q-3-Ara*p*, quercetin-3-arabinopyranoside; Q-3-Ara*f*, quercetin-3-arabinofuranoside; Q-3-Rha, quercetin-3-rhamnoside; QE, quercetin equivalents.

with visible and UV-B light. As also reported by Liu et al. (2011), postharvest UV-B irradiation of tomato promoted the accumulation of TPC and total flavonoids, resulting in higher AOP during storage. With regard to other wavelengths Kokalj et al. (2016) found an increase in TPC and AOP in apple skin as consequence of postharvest irradiation with LED light at 590 nm.

HPLC analysis were used to determine and quantitatively evaluate anthocyanin (C-3-G, cyanidin-3-galactoside) and six flavonols (Q-3-Gal, quercetin-3-galactoside; Q-3-Glu, quercetin-3-glucoside; Q-3-Xyl, quercetin-3-xyloside; Q-3-Ara*p*, quercetin-3-arabinopyranoside; Q-3-Ara*f*, quercetin-3-arabinofuranoside; Q-3-Rha, quercetin-3-rhamnoside). Content of anthocyanin and flavonols for irradiated and non-irradiated apple skin samples are presented in Table 2. Content of anthocyanin C-3-G was found higher in irradiated apple skin (3.06 mg CE/100 g FW) as compared to non-irradiated (1.86 mg CE/100 g FW). Flavonols content in irradiated apple skins were found in the following order (in mg QE/100 g FW): Q-3-Gal (7.83) > Q-3-Glu (3.95) > Q-3-Ara*f* (3.08) > Q-3-Xyl (2.64) > Q-3-Rha (2.20) > Q-3-Ara*p* (0.48). Our results are in accordance with findings presented by Josuttis et al. (2010) who found an increase of flavonols in strawberries as a consequence of solar UV-B irradiation. Scattino et al. (2014) reported an increase of flavonols in the skin of 'Suncrest' peaches after UV-B postharvest treatment. In our study the content of most represented flavonol Q-3-Gal was higher in irradiated apple skin as compared to non-irradiated.

Conclusions

Irradiation of apples stored for 13 days at 8.5 °C with UV-B light emitting diodes resulted in accumulation of phenolic compounds. Higher AOP and TPC were determined in the skin of irradiated apples in comparison to control. Accumulation of phenolic compounds was confirmed by HPLC analysis. Development of red colour of the skin was accompanied by the increase of cyanidin-3-galactoside content.

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