# Food and Bioprocess Technology: An International Journal


## Effect of Postharvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits

--Manuscript Draft--

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| **Abstract:** | In the present study the possibility of enhancing phenolic and flavonoid concentration in tomato (Solanum lycopersicum L.) fruits by post-harvest irradiation with UV-B light was assessed. Fruits of the commercial cv Money Maker (MM) and the mutant genotype high pigment-1 (hp-1), constitutively rich in these compounds, were harvested at mature green and turning stages and left to ripen within climatic chambers where they were daily treated with UV-B radiation (1 h, 6.08 kJ/m2 d). In control chambers UV-B radiation was screened by benzophenone-treated polyethylene film. The treatment was generally effective in increasing phenolic, flavonoid and flavonol concentration in both peel and flesh of MM and hp-1 fruits, although in this latter the positive response to UV-B treatment was mainly evident in fruits harvested at mature green stage. Following UV-B treatment antioxidant activity increased in the peel of both genotypes independently from the harvesting stage and in the flesh of hp-1 fruits harvested at mature green stage. Hydroxycinnamic acids of both genotypes reacted to UV-B treatment differently depending on harvesting stage and tissue localization, generally showing an increase in the peel of fruits harvested at mature green stage. With few exceptions, UV-B irradiation also induced a higher accumulation of individual flavonoids both in the peel and in the flesh of MM and hp-1 fruits independently from harvesting stage. Based on these results UV-B irradiation can be considered a promising technique to increase the nutraceutical potential of tomato fruits by non-molecular tools. |


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**Reviewer 1:**

*Dataset is original and it is worthy to be accepted as an original paper. One question remaining for me is how to select the appropriate wavelength band. The authors selected UV-B (280-315nm) for their experiments. On the other hand, the effect of post-harvest UV-C (less than 280nm) irradiation on phenolic compound content and antioxidant activity of tomato fruit during storage has been already reported (Liu et al., 2012 - Journal of Integrative Agriculture, 11(1), 159-165). After all, which is the better choice, UV-B or UV-C? If you can give your comments on this issue, please add them somewhere in the main text.*

**We thank the reviewer for her/his positive evaluation of the manuscript.**
**This work is part of a wider project (COST Action FA0906: 'UV-B radiation: A specific regulator of plant growth and food quality in a changing climate) aimed to evaluate the role of UV-B radiation in plant life by investigating many aspects of plant metabolism, starting from UV-B perception and signal transduction to bio-molecular responses which ultimately determine plant growth, food quality, and plant-environment interactions.**
**UV-B, differently from UV-C, reaches the Earth surface and it is known to modulate phenylpropanoid metabolism. During evolution plants have evolved the ability to perceive and respond to this environmental factor. Accordingly, the use of UV-B radiation can be considered a natural approach.**
**We previously found that tomato fruits ripened under UV-B depleted conditions were less rich in carotenoids and polyphenols, depending on genotype. The biosynthetic pathways were also affected in UVB-shielded fruits. Therefore we wondered if fruit nutraceutical quality could be improved by applying proper doses of UV-B radiation to detached fruits.**
**Regarding your specific question on the choice of the better wavelengths (UV-B or UV-C), it is really difficult to answer it.**
**In the revised manuscript we added the following two sentences (Introduction section) describing some interesting results obtained by using UV-C on tomato fruits:**
**"The effectiveness of UV-C radiation in improving nutritional quality of tomato fruits has been recently reviewed (Bravo et al. 2013)."**
**"Moreover, interesting reports on the effects of post-harvest irradiation with UV-B or UV-C on tomato phenolics and flavonoids derives from the works of Liu et al (2011, 2012) who exposed the same tomato cv (Zhenfen 202) to different doses of UV-B or UV-C irradiation, followed in both experiments by dark storage at 14°C for up to 37 d. Both UV-B and UV-C treatments were found to be effective, at proper doses, to promote the accumulation of phenolics and flavonoids."**

**Reviewer2:**

*Review of 'Effect of postharvest UV-B irradiation on polyphenol profile and antioxidant activity in flesh and peel of tomato fruits' by Castagna et al. for Food and Bioprocess Technology.*

*This study examines two cultivars of tomatoes grown in plastic tunnels, harvested at mature green or turning stages of development and given UV-B irradiation until they are red ripe. The authors concentrate on the effect of phenols in peel and fruit and do not examine other aspects of fruit taste and firmness.*

*There are sections where some English improvement is required.*

*The authors begin giving UV-B to tomatoes at two stages - mature green and turning - and assay the fruit once they have reached the red ripe stage. Understandably, the mature green fruit have less phenols than the turning fruit, and are more positively affected by UV-B irradiation. It appears that other studies have examined this effect in some detail, but the authors use a high pigment cultivar along with its non-mutant progenitor and show in some detail the changes in individual phenols. I would like more discussion of the levels of irradiation (or intensity plus time of irradiation) given in other studies and their relevance to this work. However, with proper revision the paper is suitable for publication.*

**We thank the reviewer for her/his interesting suggestions.**

**This work was addressed to study whether and how the nutraceutical quality of tomato fruits could be improved by applying proper doses of UV-B radiation. We reported data on hardness in a recently published paper (Castagna et al. 2013 Food Chemistry 137 (2013) 151–158) and we planned to repeat this kind of UV-B treatment to test the effects on organoleptic and technological traits to understand how quality (in a wider sense) can be affected by UV-B radiation.**

**Following the reviewer's suggestions we added the following sentence to compare the UV-B doses used in our and other experiments.**

**"The similar findings observed despite the different approach and UV-B doses used in the present experiment, where tomato fruits were irradiated one hour a day with until full ripening, reaching a final dose of 112 and 140 kJ m-2 (fruits harvested at MG stage) and 60 and 90 kJ m-2 (fruits harvested at TU stage) in MM and hp-1 fruits respectively, confirms that post-harvest UV-B irradiation can be effectively used to stimulate phenylpropanoid metabolism".**

**Please find below our responses to specific comments**

Corrections:

*Abstract - p. 3 Ln 9. Ripen*

*p. 3 Ln 22. Peel*

*Introduction - p. 4, ln 4. such as*

*p. 4, ln 33. thereby minimising the negative effects of heat treatments on phytochemicals (Barret & Lloyd 2012).*

*Say these treatments - not heat treatments, since most techniques mentioned are not heat treatments.*

*p. 4, ln 44. Such as tomato fruit*

*p. 5 ln 24. In this context*

*Materials and Methods - p. 6, ln 52. Not berries - fruit.*

**All the above-reported corrections have been inserted in the revised manuscript**

*Results - p. 10, ln 6 & Fig. 2. In MM fruits, all the three parameters were significantly influenced by UV-B radiation and harvesting stage in the peel, while in the flesh phenolic concentration is influenced only by the harvesting stage.*
*This is not seen according to the statistical evaluation in Fig. 2. The letters above the columns should be checked.*
**This sentence refers to the results of the statistical analysis reported under each graph. To make this more understandable, in the revised manuscript we re-wrote the sentence as follows: "In MM fruits, all the three parameters were significantly influenced by UV-B radiation and harvesting stage in the peel (*P* values ≤ 0.001), while in the flesh, phenolic concentration is influenced only by the harvesting stage (*P* values < 0.001 for phenolics and flavonoids, = 0.002 for flavonols)."**
**As far as letters of significance are concerned, according to the statistical rules, we used different letters only when interaction between the two factors is significant.**

*p. 10, ln 13. More in details, use a different phrase here - perhaps In detail or Specifically*
*p. 10, ln 20. while flavonoid and flavonol concentration increased following UVB treatment independently from harvesting stage - say instead following UVB treatment in both harvesting stages.*
**The above-reported corrections have been inserted in the revised manuscript**

*p. 11, ln 18. Phenylpropenoid*
**We did not find this word throughout the text**
*p. 11, ln 22. In figure 4 almost all columns are 'a'. How is this significant?*
**The use of the same letters is due to the fact that the interaction between the two factors (harvesting stage and UV-B treatment) is not significant and accordingly we cannot say that, for example, MG control fruit are different from TU control fruit or UV-treated fruit (and we cannot write different letters). But, in the peel of both genotypes UV-B factor alone (independently from the stage of fruit harvesting) is significant, as indicated by the P value reported in the tables, indicating that UV-B irradiation increased antioxidant potential of treated fruits.**

*Figures 2 - 4 have tables of significance underneath each figure. These are not referred to in the text. If they are important they should be discussed.*
**The tables report the results of the two-way ANOVA (*P* values). We did not specifically referred to them in the text, considering it unnecessary. However, in accordance with the reviewer's suggestion, in the revised manuscript we added references to the *P* values reported in the tables, to better describe the statistical results.**

*p. 13, ln 33. More in details, say instead - More specifically,*
**The above-reported correction has been inserted in the revised manuscript**

**Reviewer 3:**

*The present study is technically sound, the experiments well designed and the results discussed in a comprehensive way.*

*Personally, I suggest publishing the manuscript in its present version.*

*I have only a few suggestions (the lines in the PDF version of the paper, unfortunately, were incorrect and not corresponding to the text rows):*

*Page 2:*

*Line 10, change tretment into treatment;*

*Line 11, change pee into peel;*

*Page 4:*

*Line 6, change 40 kj/m2 with 40 kJ/m2;*

*Line 10, change Money maker with Money Maker;*

*Page 10:*

*Line 7, change responsivness into responsiveness*

*And the same at page 11, line 4;*

*Page 13:*

*Last line, change hydroxyvìcinnamic into hydroxycinnamic*

**We thank the reviewer for her/his positive evaluation of the manuscript. All the suggested modifications were inserted in the revised text**

*Manuscript
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**Effect of Postharvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits**

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Short running title: **Tomato postharvest irradiation with UV-B**

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**Abstract**

In the present study the possibility of enhancing phenolic and flavonoid concentration in tomato (*Solanum lycopersicum* L.) fruits by post-harvest irradiation with UV-B light was assessed. Fruits of the commercial cv Money Maker (MM) and the mutant genotype *high pigment-1* (*hp-1*), constitutively rich in these compounds, were harvested at mature green and turning stages and left to ripen within climatic chambers where they were daily treated with UV-B radiation (1 h, 6.08 kJ/$m^2$ d). In control chambers UV-B radiation was screened by benzophenone-treated polyethylene film.

The treatment was generally effective in increasing phenolic, flavonoid and flavonol concentration in both peel and flesh of MM and *hp-1* fruits, although in this latter the positive response to UV-B treatment was mainly evident in fruits harvested at mature green stage. Following UV-B treatment antioxidant activity increased in the peel of both genotypes independently from the harvesting stage and in the flesh of *hp-1* fruits harvested at mature green stage. Hydroxycinnamic acids of both genotypes reacted to UV-B treatment differently depending on harvesting stage and tissue localization, generally showing an increase in the peel of fruits harvested at mature green stage. With few exceptions, UV-B irradiation also induced a higher accumulation of individual flavonoids both in the peel and in the flesh of MM and *hp-1* fruits independently from harvesting stage. Based on these results UV-B irradiation can be considered a promising technique to increase the nutraceutical potential of tomato fruits by non-molecular tools.

**Keywords:** tomato**;** UV-B; post-harvest; phenolic compounds; antioxidant activity

## Introduction

Several evidences are recently linked to the important role offered by fruit and vegetable consumption to the protection against many cardiovascular and age-related diseases such as type II diabetes, dementia, macular degeneration (Slavin & Lloyd, 2012). This bioactive potential is mainly correlated with the high vitamin and mineral content, the good source of dietary fibre, and the diverse array of nonessential nutrients known as phytochemicals, associated with various classes of compounds with antioxidant properties. Despite the undisputable health advantages in consuming at least 400 g of fruit and vegetables per day, as recommended by World Health Organization (WHO 1990), several factors affect the nutritional value of these products during post-harvest when they are transported from cultivation area to retail store to finally reach consumer purchase and consumption (Paliyath et al., 2008).

Food industry uses a variety of preservation or processing methods to extend the shelf life of fruits and vegetables, mainly based on the application of conventional techniques as thermal and freezing processes, dehydration, or preserving agents such as acids, sugar, salt, etc. However, reported losses of phytochemicals and consumer demand for minimally processed nutritious fruit and vegetable products has resulted in the need for alternative non thermal preservation techniques, thereby minimising the negative effects of these treatments on phytochemicals (Barret & Lloyd 2012).

Among these techniques, the application of ultraviolet radiation (UV, 100-400 nm wavelengths) has been attracting attention as a promising and environmentally friendly technology for the preservation of liquid food (Koutchma 2009) and fresh horticultural crops (Guerrero-Beltrán & Barbosa-Cánovas 2004) such as tomato fruit (Jagadeesh et al. 2011). The effectiveness of UV-C radiation in improving nutritional quality of tomato fruits has been recently reviewed (Bravo et al. 2013). In addition, UV-B (280–320 nm) has been shown to enhance the nutraceutical content in plant food favouring the accumulation of antioxidant and UV-protective molecules (Jansen et al. 2008). Interesting information on the effects of post-harvest irradiation with UV-B or UV-C on tomato phenolics and flavonoids derives from the works of Liu et al (2011, 2012) who exposed the same tomato cv (Zhenfen 202) to different doses of UV-B or UV-C irradiation, followed in both experiments by dark storage at 14°C

for up to 37 d. Both UV-B and UV-C treatments were found to be effective, at proper doses, to promote the accumulation of phenolics and flavonoids.

Tomato is among the most popularly consumed fresh vegetables in the world and it could be considered as an important source of dietary antioxidants being a ‘‘functional food’’, capable of providing desirable physiological or health benefits, in addition to the supply of basic nutrients (Canene-Adams et al., 2005). Several recently published studies have explored the effect of UV-B radiation during “in planta” ripening of tomato fruits showing how the treatment can significantly affect ascorbate, carotenoid, flavonoid and hydroxycinnamic acid content: this effect is genotype-dependent and is different in the flesh and the peel (Bacci et al. 1999; Giuntini et al. 2005; Giuntini et al. 2008; Calvenzani et al. 2010, Lazzeri et al. 2012). Conversely, less literature information is available on the effect of post-harvest UV-B irradiation on tomato fruit. Liu and co-authors (2011) found that UV-B promoted the accumulation of total phenols and flavonoids also enhancing antioxidant capacity when doses of 20 or 40 $kJ/m^2$ of irradiation were applied at the beginning of fruit storage.

More recently, Castagna et al (2013) reported that post-harvest UV-B treatment at different ripening stages (mature green and turning) affected the quality of tomato fruits increasing the concentration of ascorbic acid and carotenoids in flesh and peel of the wild type Money Maker but also causing a loss of firmness.

In this context, the aim of the present work is to assess the effectiveness of post-harvest UV-B treatment in improving the nutraceutical quality of tomato fruits linked to phenolic compounds. To this aim total phenols and flavonoids content, flavonoid and hydroxycinnamic acid profiles and antioxidant activity were measured in flesh and peel of tomato fruits collected at two different ripening stages (mature green and turning) and subjected to daily irradiation with UV-B radiation until complete ripening.  A commercial cv (Money Maker, MM) and a mutant genotype (*high pigment-1*; *hp-1*) constitutively rich in these compounds were tested.

## Materials and Methods

*Tomato samples*

The tomato (*Solanum lycopersicum* L.) *high pigment-1* (*hp-1*) mutant and the corresponding wild-type (cv Money Make) seeds, obtained from the Tomato Genetics Resource Center (http://tgrc.ucdavis.edu/) were used. *High pigment-1* is a photomorphogenic mutant characterised by enhanced light responsiveness and elevated flavonoid and carotenoid content in mature ripe fruits (Liu et al., 2004).

*Treatment and storage conditions*

Plants were cultivated as reported by Castagna et al. (2013). Healthy and sun exposed fruits were harvested at two different ripening stages: mature green (MG, 35–40 DPA) and turning (TU, breaker + 3), according to the procedure reported by Grierson and Kader (1986) and randomly distributed into different climatic chambers (18 °C; R.H. 80%). Each chamber was equipped with three UV-B lamp tubes (Philips Ultraviolet B, TL 20W-12RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands) providing 1.69 $W/m^2$ at fruit height (about 45 cm under the lamps). Irradiation was carried out daily (1 h, 6.08 $kJ/m^2$ d) until RR stage, which was achieved after 10 and 18 days for TU and MG fruits of cv MM, and after 13 and 22 days for TU and MG fruits of hp-1 mutant, respectively. Control fruits received the same treatment but UV-B lamps were screened by benzophenone-treated polyethylene film, known to block UV-B radiation (Figure 1).

Flesh and peel of RR fruits were separated and freeze-dried with a lyophilizer (model 1700, Edwards Alto Vuoto, Milano, Italy).

*Flavonoid and hydroxycinnamic acid extraction*

Flavonoids and hydroxycinnamic acids were extracted from flesh and peel of freeze-dried fruits as previously described (Giuntini et al. 2008).

Freeze-dried flesh or peel (1 g) were extracted with 20 mL of a 20% methanol aqueous solution with morin as internal standard (1 $\mu$g/ml) and BHT (0.1% w/w) to prevent oxidation using an Ultra-Turrax homogenizer for 5 min at 3000 rpm. After centrifugation at 4000 rpm for 5 min, the supernatant was

filtered on HPLC filters (0.45 $\mu$m) and used for HPLC analysis (injection volume, 10 $\mu$L). Quantitative analysis of hydroxycinnamic acids was performed after alkaline hydrolysis in order to obtain the free forms, using *o*-coumaroyl methyl ester as internal standard (Giuntini et al. 2008). In particular, alkaline hydrolysis was performed at 25 °C in the dark for 4 h, by adding 5 mL of 1 M NaOH to the supernatant (10 mL). After neutralization with 1 M HCl, the hydrolyzed fraction was extracted twice with ethyl acetate (10 mL), the organic layers were pooled and evaporated under vacuum, and the residue was dissolved in methanol (1 mL) and injected in HPLC.

*Total phenolic and flavonoid analysis*

Total phenols were determined using the Folin-Ciocalteu method, modified as described by Barbolan et al. (2003). The reaction mix was composed by 1.85 mL of distilled water, 0.125 mL of Folin-Ciocalteu reagent, 0.5 mL of a 20% $Na_2CO_3$ solution and 25 µL of extract. After 30 min of incubation at room temperature the absorbance was recorded at 750 nm. Total phenols were expressed as mg of gallic acid equivalents 100 $g^{-1}$ FW.

Total flavonoids were determined as described by Kim et al. (2003), by mixing 60 µL of 5% $NaNO_2$, 40 µL of 10% $AlCl_3$, 400 µL of 1 M NaOH and 100 µL of extract. The solution was diluted with 200 µL of distilled water and the absorbance was recorded at 510 nm. The flavonoid concentration was expressed as mg of catechin equivalents 100 $g^{-1}$ FW.

Flavonol concentration was determined by mixing 25 µL of sample extract with 225 µL of 10% ethanol, 250 µL of 0.1% HCl in 95% ethanol and 1 mL of 2% HCl. The absorbance was recorded at 360 nm and data were expressed as milligrams of quercetin 100 $g^{-1}$ FW (Romani et al. 1996).

*Antioxidant activity analysis*

Antioxidant activity of phenolic extracts was assessed by the method reported by Re et al. (1999) based on the ability of antioxidants to reduce pre-formed radical cations of 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) to ABTS. Briefly, a stable, dark blue-green solution of $ABTS^{\bullet+}$ radicals was generated following 12–16 h of incubation in the dark of 2.45 mM (final concentration) potassium persulfate and 7 mM ABTS. The solution was then diluted to an absorbance

of 0.700±0.02 at 734 nm (30°C) and 1 ml $ABTS^{\bullet+}$ solution was mixed with 10 μl of adequately diluted sample. The absorbance was read exactly after 4 min and the percentage inhibition was calculated against a blank control. The results were expressed as μmol Trolox (a water-soluble vitamin E analogue) equivalents 100 $g^{-1}$ FW.

*Flavonoid and hydroxycinnamic acid quantification*

Quantification of flavonoids and hydroxycinnamic acids was performed by LC-DAD-ESI–MS/MS, by using a 2695 Alliance separation module equipped with a Phenomenex Hydro C18 column (100 x 4.6 mm, 5 μm particles), a 996 photodiode array detector and a Quattro triple–quadrupole mass spectrometer with an Electrospray ionisation (ESI) source (all from Waters, Milford, MA, USA). Gradient elution was performed with solvent A (HPLC grade water acidified with 0.2% formic acid) and solvent B (acetonitrile with 0.2% formic acid). Flow rate was 1 ml/min and a linear gradient was used; after an isocratic step at 100% A for the first 15 min, eluent B was increased at 40% in 20 min, then kept constant for 10 min, finally a re-equilibration step at 100% A was performed for 5 min (total run time, 50 min). The electrospray probe was operated in the positive ion mode with cone voltage 30 V, capillary voltage 3 kV, total ion current scan 100–1,500 m/z, scan duration 2 s, interscan delay 0.2 s and nitrogen flow 100 and 450 l/h in spray and desolvation phases, respectively.
Data were acquired by the software Masslynx 4.1 (Waters). PDA chromatograms (scan range, 200–600 nm) and mass chromatograms (scan range, 100–1500 m/z) were compared for polyphenol identification according to UV and mass spectra. Quantification was based on UV chromatograms recorded at 320 nm and 360 nm for hydroxycinnamic acids and flavonoids, respectively. Calibration experiments were obtained for naringenin chalcone, rutin and quercetin (Extrasynthese, Genay, France) in the range of 1–200 mg/kg, showing a very good linearity (r2 > 0.999). Since no standard was available for the pentosyl-rutinoside, it was quantified by using the rutin calibration curve, with the assumption of the same UV response.

*Statistical analysis*

Means and standard deviations were calculated with NCSS 2000 (NCSS Statistical Software, Kaysville, Utah, USA) statistical software. All analyses were performed in triplicate. NCSS was also used to perform two-way-analysis of variance (ANOVA) and Tukey-Kramer post-hoc test at a 95% confidence level to evaluate the effect of harvesting stage and the presence/absence of UV-B irradiation at a significance level of 0.05 ($P < 0.05$). Tomato harvesting stages (MG and TU) and UV-B treatment were considered as variables.

## Results and Discussion

*Effect of UV-B radiation on the amount of total phenolics, flavonoids and flavonols*

The effect of UV-B radiation and of the harvesting stage on the concentration of total phenolics, flavonoids and flavonols was studied both in the peel and in the flesh of tomato fruits. In MM fruits, all the three parameters were significantly influenced by UV-B radiation and harvesting stage in the peel ($P$ value $\leq 0.001$), while in the flesh, phenolic concentration is influenced only by the harvesting stage ($P$ value $< 0.001$ for phenolics and flavonoids, $= 0.002$ for flavonols) (Figure 2).

In detail, fruits harvested at TU stage always showed a higher concentration of these compounds in both tissues in comparison to MG fruits (Figure 2). Post-harvest treatment with UV-B radiation induced a significant increase of total phenolics in the peel only when fruits were harvested at MG stage (+32%, Figure 2 A), while flavonoid and flavonol concentration increased following UV-B treatment in both harvesting stage (mean increment of 21% for both flavonoids and flavonols) (Figure 2 C, E). In the flesh, UV-B irradiation was effective in increasing flavonoid and flavonol concentration only in MG fruits, leading to 27% and 48% increase in comparison to untreated fruits, respectively (Figure 2 D, F).

According to the two-way ANOVA, UV-B treatment influenced the concentration of total phenolics, flavonoids ($P$ value = 0.024 both peel and flesh) and flavonols ($P$ value = 0.014, peel; < 0.001, flesh) of the tomato mutant *hp-1* both in peel ($P$ value = 0.024 for phenolics and flavonoids, 0.014 for flavonols), and flesh ($P$ value = 0.024 for flavonoids, < 0.001 for phenolics and flavonols). The positive response to UV-B treatment occurred only in fruits harvested at MG stage, both in peel and flesh tissues, with the exception of flesh flavonols which accumulated at greater concentrations in UV-B treated fruits independently from the harvesting stage (mean increase of 20%, Figure 3). In particular, in MG-harvested fruits, post-harvest irradiation with UV-B light led to 11%, 19% and 21% increase in peel phenolic, flavonoid and flavonol concentration, respectively, and to 17% and 24% increase in flesh phenolic and flavonoid concentrations, respectively, in comparison to non-irradiated control samples (Figure 3 A, C, E).

The positive effect of UV-B irradiation on phenols and flavonoids is in accordance with the findings reported by Liu et al. (2011), who observed a significant increase of these compounds in tomato fruits treated with 20 e 40 kJ/m$^2$ UV-B at the beginning of fruit storage (MG stage) and analysed after 14, 21, 28 and 37 days of ripening in the dark. The similar findings observed despite the different approach and UV-B doses used in the present experiment, where tomato fruits were irradiated one hour a day with until full ripening, reaching a final dose of 112 and 140 kJ m$^{-2}$ (fruits harvested at MG stage) and 60 and 90 kJ m$^{-2}$ (fruits harvested at TU stage) in MM and *hp-1* fruits respectively, confirms that post-harvest UV-B irradiation can be effectively used to stimulate phenylpropanoid metabolism.

Increased accumulation of stilbenes, hydroxycinnamic acids and some anthocyanins also occurred in the skin of table grapes following a short irradiation with UV-B light (30 min, 17,8-23 W/m$^2$) (Cantos et al. 2000).

Similarly, Hagen et al. (2007) reported an increase in flavonoid and phenol concentration in the peel of shade-grown apples following irradiation with 0.20 W/m$^2$ UV-B for 12 h per day during a period of 10 days, while no response was observed in the flesh.

A stimulation of anthocyanin biosynthetic gene expression, accompanied by an increased anthocyanin accumulation in Chinese sand pears (*Pyrus pyrifolia* Nakai), but not in European pears (*Pyrus communis* L.), under bagging treatment and postharvest UV-B/visible irradiation was recently reported by Qian et al. (2013), underlying the importance of the genetic background in the responsiveness to UV-B radiation.

UV-B-mediated stimulation of phenilpropanoid accumulation occuring also in the flesh of both genotypes is a particularly positive result because flesh is constitutively less rich in bioprotective compounds than peel, and also because peel is often discarded during tomato processing or cooking. In a previous research, we found that UV-B post-harvest tratment was effective in increasing lycopene concentration in the flesh of MM tomato fruits as well (Castagna et al. 2013), making this approach extremely interesting in the view of achieving tomato-based foods enriched in healthy-protecting molecules. Indeed, it should be remembered that the bioprotective effect of plant food seems to

depend on the combined action of different metabolites (matrix effect) rather than on a single component (Jacobs et al. 2009; Liu 2004).

*Effect of UV-B radiation on antioxidant activity*

Post-harvest UV-B irradiation significantly influenced antioxidant activity in the peel of both genotypes, independently from the fruit harvesting stage (*P* value = 0.018 and 0.025 for MM and *hp-1*, respectively), leading to a mean increment in TEAC values of 20% and 8% in MM and *hp-1* peel, respectively (Figure 4 A, C). In the flesh, no significant effect of UV-B treatment was observed in MM fruits (Figure 4 B), while *hp-1* showed a light-dependent increase of antioxidant activity in fruits harvested at MG stage (+18%, Figure 4 D).

Similarly to what observed for phenylpropanoid compounds, antioxidant activity was higher in the peel than in the flesh, and showed higher values in *hp-1* fruits as compared to MM ones. In both genotypes, the increase of peel TEAC following UV-B irradiation was generally in good agreement with the UV-B-induced accumulation of phenolics, flavonoids and flavonols.

Liu et al. (2011) found increased antioxidant activity in tomato fruits after 7 or 14 days from irradiation with 20, 40 o 80 kJ/m$^2$, even if in fully ripened fruits (37 days after UV-B treatment) antioxidant activity dropped down to control values. This partly different result could be attributed not only to genotype-dependent responsiveness to the treatment but also to the different kind of UV-B irradiation. Indeed, Liu et al. (2011) performed a sort of acute treatment (high UV-B dose for a short period) followed by ripening in the dark, while in the present research fruits were daily irradiated with lower doses until fruit ripening.

*Effect of UV-B radiation on hydroxycinnamic acids and flavonoids profile*

In MM fruits, with the exception of flesh p-coumaric acid, UV-B treatment always influenced hydroxycinnamic acids concentration (*P* value ranging from <0.001 to 0.035). However, UV-B radiation played a different role on the accumulation of hydroxycinnamic acids in the two tissues and as a function of harvesting stage. In UV-B treated peel, p-coumaric acid showed a mean concentration about twice that of control, independently of harvesting stage. A marked stimulating effect of UV-B

radiation on the other hydroxycinnamic acids was observed only in MG-harvested fruits, leading to about 196- and 3.4- fold increase of caffeic and ferulic acid concentration, respectively and to the accumulation of sinapic acid, which was not detectable in control peel (Table 1). Instead, MM fruits harvested at TU stage showed a decrease of caffeic and ferulic acid concentration in the peel following UV-B irradiation (-30 and -19%, respectively), while sinapic acid did not change (Table 1).

In MM flesh, higher amount of caffeic and sinapic acids was measured following UV-B treatment, independently from harvesting stage (mean increase of 48 and 27%, respectively), while ferulic acid underwent a slight but significant increase (13%) in MG fruits and a decrease (-39%) in TU ones, and p-coumaric acid was unaffected by the treatment (Table 1).

UV-B treatment increased the concentration of caffeic (99%), ferulic (absent in control sample) and p-coumaric acid (35%) of *hp-1* peel only when fruits were collected at MG stage, being ineffective (ferulic and p-coumaric acid) or leading to a decrease (caffeic acid, -44%) in the peel of TU-harvested fruits (Table 1). Independently from harvesting stage, sinapic acid concentration always underwent a significant increase following UV-B irradiation (mean increase of 42%; Table 1).

At the flesh level, only caffeic and ferulic acids of *hp-1* fruits were influenced by UV-B irradiation, but only which led to a significant increase of their concentration only at MG harvesting stage (108% and 144%, respectively, Table 1).

The effect of UV-B radiation on hydroxycinnamic acids of tomato fruits was previously checked in two commercial tomato cultivars grown under plastic tunnels transparent or not to UV-B rays (Giuntini et al. 2005), finding a genotype-dependent effect of UV-B screening on these compounds, which underwent a decrease or an increase in the peel of DRW 5981 or Esperanza RR fruits, respectively.

The generalised increment of hydroxycinnamic acids concentration in the peel, following UV-B treatment, observed in both MM and *hp-1* fruits harvested at MG stage, suggests an influence of UV-B radiation on the early biosynthetic steps of the phenylpropanoid biosynthetic pathway. Indeed, gene transcription as well as activity of phenylalanine ammonia lyase (PAL), one of the key enzymes in the synthesis of phenolic compounds, is often stimulated by UV-B radiation (Liu and McClure 1995; Avena-Bustillos et al. 2012; Huang et al. 2010; Morales et al. 2010; Eichholz et al. 2012).

In both MM and *hp-1* fruits, naringenin was present only at peel level. In general, flavonoids underwent an increase following UV-B irradiation, both in the peel and in the flesh (*P* value ranging from <0.001 to 0.028), the only exception being 3-quercetin-pentosyl-rutinoside of *hp-1* peel.

More specifically, in MM peel, naringenin and rutin were significantly higher in UV-B-treated fruits independently of harvesting stage (mean increase of 93% and 53%, respectively), while 3-quercetin-pentosyl-rutinoside accumulation was stimulated only in MG-harvested fruits, reaching a concentration more than 3-fold higher than in control (Table 2). UV-B treatment, independently of the stage of fruit harvesting, led to a significant increase in flesh concentration of both 3-quercetin-pentosyl-rutinoside and rutin (mean increase of 50% and 136%, respectively) (Table 2).

The positive effect of UV-B radiation on flavonoid accumulation was also evident in *hp-1* fruits, with the exception of 3-quercetin-pentosyl-rutinoside at the peel level (Table 2). In this tissue naringenin increased in MG-harvested fruits (158%), and rutin showed a mean increment of 98% independently of harvesting stage (Table 2). Similarly to what detected in MM flesh, also in *hp-1* flesh UV-B radiation induced a stage-independent increase in 3-quercetin-pentosyl-rutinoside (127%), while the effect of UV-B treatment on rutin was significant only in MG-harvested fruits, leading to about 12-fold higher concentration (Table 2).

An indirect confirmation of the results obtained in the present research derives from the study carried out by Calvenzani et al. (2010) on MM and *hp-1* tomato fruits ripened *in planta* under UV-B screened plastic tunnels. In this paper, UV-B-deprived MM fruits showed a noteworthy reduction in the peel content of naringenin chalcone, rutin, quercetin and a quercetin-glycoside in comparison to control fruits ripened under UV-B transparent film. These authors also showed that activation of flavonoid biosynthetic genes was UV-B-dependent at MG ripening stage. Similarly, Giuntini et al. (2008) observed a negative impact of UV-B shielding on flavonod accumulation in the peel of one commercial tomato cultivar, although the effect was opposite in the other investigated cultivar.

In the present research the detection of a higher concentration of flavonoids also in the flesh, a tissue not directly exposed to UV-B irradiation, is particularly interesting because this tissue is constitutively poorer in bioprotective molecules than the peel which, moreover, is often discarded in processed tomato products. Whether UV-B radiation is able to pass the peel barrier of tomato fruits is still

unclear at present. UV-B was shown to reach leaf mesophyll of *Brassica napus* stimulating phenolic and flavonoid production also in the inner cell layers (Alenius et al. 1995). Similar data are not available in fruits, but the detection of higher flavonoid levels in UV-B treated tomato flesh suggests that UV-B, directly or indirectly (through signaling cascade), is involved in modulation of phenylpropanoid metabolism also in this tissue.

## Conclusions

Considerable efforts are being made to increase nutraceutical levels in fruits and vegetables, also because the overall consumption of plant food is still often below the recommended five serves per day. In addition to molecular tools, often seen with suspicion by consumers and many national governments, alternative approaches, as modulation of the environmental parameters in post-harvest, may represent valid techniques to stimulate the biosynthetic pathways ultimately leading to nutraceutical production. The results presented in this work indicateas that UV-B irradiation was indeed effective for obtaining flavonoid- and hydroxycinnamic-enriched tomato fruits. This observation, together with previously reported positive effects played by UV-B treatment on tomato carotenoid and ascorbic acid content, suggests that post-harvest irradiation with UV-B may be a promising tool to improve the healthy-nutritional properties of tomato fruits.

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## References

Alenius CM, Vogelmann TC & Bornman JF (1995). A three-dimensional representation of the

relationship between penetration of UV-B radiation and UV screening pigments in leaves of *Brassica napus*. *New Phytologist*, *131,* 297–302.

Avena-Bustillos RJ, Du W-X, Woods R, Olson D, Breksa AP III & McHugh TH (2012). Ultraviolet-B light treatment increases antioxidant capacity of carrot products. *Journal of the Science of Food and Agriculture, 92,* 2341–2348.

Bacci L, Grifoni D, Sabatini F & Zipoli G (1999). UV-B radiation causes early ripening and reduction in size of fruits in two lines of tomato (*Lycopersicon esculentum* Mill.). *Global Change Biology*, *5*, 635-646.

Barbolan AMA, Zorro L, Guillen DA & Barroso CG (2003). Study of the polyphenol content of red and white grape varieties by liquid chromatography-mass spectrometry and its relationship to antioxidant power. *Journal of Chromatography A, 1012,* 31-38.

Barret DM & Lloyd B (2012). Advanced preservation methods and nutrient retention in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *92,* 7–22.

Bravo S, García-Alonso J, Martín-Pozuelo G, Gómez V, García-Valverde V, Navarro-González I, Periago MJ (2013). Effects of postharvest UV-C treatment on carotenoids and phenolic compounds of vine-ripe tomatoes. *International Journal of Food Science and Technology, 48*, 1744–1749.

Calvenzani V, Martinelli M, Lazzeri V, Giuntini D, Dall'Asta C, Galaverna G, Tonelli C, Ranieri A & Petroni 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.

Canene-Adams K, Campbell JK, Zaripheh S, Jeffery EH & Erdman JW (2005). The tomato as a functional food. *The Journal of Nutrition*, *135*, 1226–1230.

Cantos E, Garcia-Viguera C, Pascual-Tersa S & Tomas-Barberan FA (2000). Effect on post-harvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleon table grapes. *Journal of Agricultural and Food Chemistry,* 48, 4606-4612.

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 Chemistry*, *137*, 151-158.

Eichholz I, Rohn S, Gamm A, Beesk N, Herppich WB,. Kroh LW, Ulrichs C & Huyskens-Keil S

(2012). UV-B-mediated flavonoid synthesis in white asparagus (*Asparagus officinalis* L.). *Food Research International, 48,* 196–201.

Giuntini D, Graziani G, Lercari B, Fogliano V, Soldatini GF & Ranieri A (2005). Changes in carotenoid and ascorbic acid contents in fruits of different tomato genotypes related to the depletion of UV-B radiation. *Journal of Agricultural and Food Chemistry*, *53*, 3174-3181.

Giuntini D, Lazzeri V, Calvenzani V, Dall'Asta C, Galaverna G, Tonelli C, Petroni K & Ranieri A (2008). Flavonoid profiling and biosynthetic gene expression in flesh and peel of two tomato genotypes grown under UV-B-depleted conditions during ripening. *Journal of Agricultural and Food Chemistry*, *56*, 5905-5915.

Guerrero-Beltrán JA & Barbosa-Cánovas GV (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *10*, 137-147.

Hagen SF, Borge GI, Bengtsson GB, Bilger W, Berge A, Haffner K & Solhaug KA (2007). Phenolic contents and other health and sensory related properties of apple fruit (*Malus Domestica* Borkh., cv. Aroma.): Effect on post-harvest UV-B irradiation. *Postharvest Biology and Technology*, 45, 1-10.

Huang J, Gu M, Lai Z, Fan B, Shi K, Zhou YH., Yu J-Q & Chen Z (2010). Functional analysis of the *Arabidopsis* PAL gene family in plant growth, development, and response to environmental stress. *Plant Physiology*, *153(4)*, 1526–1538.

Jacobs DR Jr, Gross MD & Tapsell L (2009). C Food synergy: an operational concept for understanding nutrition. *The American Journal of Clinical Nutrition, 89*:1543S–1548S.

Jansen MAK, Hectors K, O'Brien NM, Guisez Y & Potters G (2008). Plant stress and human health: Do human consumers benefit from UV-B acclimated crops? *Plant Science*, *175*, 449–458.

Jagadeesh SL, Charles MT, Gariepy Y, Goyette B, Raghavan GRV & Vigneault C (2011). Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment handling. *Food and Bioprocess Technology, 4*:1463-1472.

Kim D-O, Chun OK, Kim YJ, Moon HY & Lee CY (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry, 51*, 6509–6515.

Koutchma T (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology*, *2*, 138–155.

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 Biology and Technology*, *59*, 232-237.

Liu C, Cai L, Lu X, Han X, Ying T (2012). Effect of postharvest UV-C irradiation on phenolic compound content and antioxidant activity of tomato fruit during storage. *Journal of Integrative Agriculture, 11(1),* 159-165.

Liu L & McClure JW (1995). Effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. *Physiologia Plantarum*, *93,* 734-739.

Liu RH (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of Nutrition 134,* 3479S–3485S.

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C & Giovannoni J (2004). Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 9897–9902.

Morales LO, Tegelberg R, Brosché M, Keinänen M, Lindfors A & Aphalo PJ (2010) Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in *Betula pendula* leaves. *Tree Physiology*, *30(7)*, 923–934.

Paliyath G, Murr D P, Handa A K & Lurie S (ed) (2008) Postharvest Biology and Technology of Fruits, Vegetables, and Flowers. New York, USA: Wiley-Blackwell.

Qian M, Zhang D, Yue X, Wang S, Li X & Teng Y (2013). Analysis of different pigmentation patterns in 'Mantianhong' (*Pyrus pyrifolia* Nakai) and 'Cascade' (*Pyrus communis* L.) under bagging treatment and postharvest UV-B/visible irradiation conditions. *Scientia Horticulturae,* 151, 75–82

Romani A, Mancini P, Tatti S & Vincieri FF (1996). Polyphenols and polysaccharides in Tuscan grapes and wines. *Italian Journal of Food Science 1*, 13–24.

Slavin JL & Lloyd B (2012). Health benefits of fruits and vegetables. *Advances in nutrition*, *3*, 506-516.

World Health Organization (1990). Diet, Nutrition, and the Prevention of Chronic Diseases. Technical report series 797. WHO, Geneva, CH.

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## Captions for figures

**Figure 1**. Schematic representation of the experimental design. Fruits were harvested at mature green (MG) and turning (TU) stages from plants cultivated under UV-B transparent tunnels and left to ripe until red ripe (RR) stage within climatic chambers. One group of tomatoes was irradiated (1 h a day) with UV-B light (+UVB), while the second group (-UVB) was maintained under the same conditions but UV-B radiation was screened.

**Figure 2.** Concentration (mg/100 g) of total phenols (A, B), flavonoids (C, D) and flavonols (E, F) in tomato peel (A, C, E) and flesh (B, D, F) of Money Maker (MM) fruits harvested at mature green (MG) and turning green (TU) stages and left to ripe in the presence (+UVB) or absence (-UVB) of UV-B radiation. Data represent the mean of 4 replicates ± SE. The two-way ANOVA P values are shown and different letters indicate significantly different values according to Tukey-Kramer test.

**Figure 3.** Concentration (mg/100 g) of total phenols (A, B), flavonoids (C, D) and flavonols (E, F) in tomato peel (A, C, E) and flesh (B, D, F) of *high pigment 1* (*hp-1*) fruits harvested at mature green (MG) and turning green (TU) stages and left to ripe in the presence (+UVB) or absence (-UVB) of UV-B radiation. Data represent the mean of 4 replicates ± SE. The two-way ANOVA P values are shown and different letters indicate significantly different values according to Tukey-Kramer test.

**Figure 4.** Antioxidant activity (μmol Trolox 100 $g^{-1}$ FW) of phenolic extracts of tomato peel (A, C) and flesh (B, D) of Money Maker (MM, A, B) and *high pigment 1* (*hp-1*, C, D) fruits harvested at mature green (MG) and turning green (TU) stages and left to ripe in the presence (+UVB) or absence (-UVB) of UV-B radiation. Data represent the mean of 4 replicates ± SE. The two-way ANOVA P values are shown and different letters indicate significantly different values according to Tukey-Kramer test.

**Table 1.** Hydroxycinnamic acids (µg/100 g) contents in tomato peel and flesh of Money Maker (MM) and *high pigment 1* (*hp-1*) fruits harvested at mature green (MG) and turning green (TU) stages and left to ripe in the presence (+UVB) or absence (-UVB) of UV-B radiation. Data represent the mean of 4 replicates ± SE. The two-way ANOVA P values are shown and different letters indicate significantly different values according to Tukey-Kramer test.

| **MM** | MG | | TU | | *P* | | |
|---|---|---|---|---|---|---|---|
| | -UVB | +UVB | -UVB | +UVB | stage | UVB | stage x UVB |
| ***peel*** | | | | | | | |
| caffeic acid | 6.5±1.5 c | 1380±144 b | 2124±10 a | 1486±61 b | <0.001* | 0.009* | <0.001* |
| ferulic acid | 10.5±0.5 d | 47.5±0.5 c | 72.5±0.5 a | 58.5±2.5 b | <0.001* | <0.001* | <0.001* |
| p-coumaric acid | 6.0±1.0 a | 224±10 a | 515±59 a | 810±65 a | <0.001* | 0.004* | 0.431 |
| sinapic acid | nd c | 12.5±0.5 b | 20.0±1.0 a | 22.0±0.1 a | <0.001* | <0.001* | <0.001* |
| ***flesh*** | | | | | | | |
| caffeic acid | 937±38 a | 1793±134 a | 1822±269 a | 2237±245 a | 0.025* | 0.028* | 0.286 |
| ferulic acid | 16.4±0.3 c | 18.3±3.3 c | 70.0±5.8 a | 43.3±3.7 b | <0.001* | 0.035* | 0.022* |
| p-coumaric acid | 518±15 a | 797±89 a | 992±20 a | 982±100 a | 0.008* | 0.119 | 0.100 |
| sinapic acid | 83.6±0.5 a | 101.0±12.9 a | 92.2±7.3 a | 122.2±3.5 a | 0.119 | 0.034* | 0.440 |
| ***hp-1*** | MG | | TU | | *P* | | |
| | -UVB | +UVB | -UVB | +UVB | stage | UVB | stage x UVB |
| ***peel*** | | | | | | | |
| caffeic acid | 1055±57 b | 2101±112 a | 1885±10 a | 1055±68 b | 0.205 | 0.205 | <0.001* |
| ferulic acid | nd c | 10.5±1.6 b | 22.0±1.0 a | 23.0±2.0 a | <0.001* | 0.013* | 0.024* |
| p-coumaric acid | 601±53 b | 812±68 a | 695±6 b | 643±11 b | 0.439 | 0.142 | 0.039* |
| sinapic acid | 101±10 a | 147±5 a | 134±2 a | 188±1 a | 0.003* | <0.001* | 0.550 |
| ***flesh*** | | | | | | | |
| caffeic acid | 1553±244 b | 3237±463 a | 2279±419 ab | 1565 ±734 b | 0.327 | 0.317 | 0.047* |
| ferulic acid | 18.4±3.3 b | 44.5±0.2 a | 31.6±6.4 ab | 13.4±3.4 b | 0.093 | 0.384 | 0.006* |
| p-coumaric acid | 704±100 a | 957±141 a | 649±117 a | 605±104 a | 0.154 | 0.420 | 0270 |
| sinapic acid | 177±13 a | 201±27 a | 136±24 a | 204±28 a | 0.472 | 0.122 | 0.399 |

Abbreviations: : nd not detectable; MM Money Maker; *hp-1 high pigment 1*; MG mature green; TU turning green

**Table 2.** Flavonoid (mg/100 g) contents in tomato peel and flesh of Money Maker (MM) and *high pigment 1* (*hp-1*) fruits harvested at mature green (MG) and turning green (TU) stages and left to ripe in the presence (+UVB) or absence (-UVB) of UV-B radiation. Data represent the mean of 4 replicates ± SE. The two-way ANOVA P values are shown and different letters indicate significantly different values according to Tukey-Kramer test.

| **MM** | MG | | TU | | *P* | | |
|---|---|---|---|---|---|---|---|
| | -UVB | +UVB | -UVB | +UVB | stage | UVB | stage x UVB |
| ***Peel*** | | | | | | | |
| narigenin | 423±39 a | 634±130 a | 288±7 a | 748±48 a | 0.842 | 0.010* | 0.148 |
| 3-quercetin-pentosyl-rutinoside | 19.2±2.1 b | 83.9±8.6 a | 23.2±0.5 b | 34.6±0.8 b | 0.007* | 0.001* | 0.004* |
| rutin | 9.0±0.4 a | 17.9±1.3 a | 17.7±2.1 a | 22.9±1.9 a | 0.012* | 0.011* | 0.304 |
| ***Flesh*** | | | | | | | |
| narigenin | nd | nd | nd | nd | - | - | - |
| 3-quercetin-pentosyl-rutinoside | 17.1±0.9 a | 31.0±5.2 a | 19.2±0.4 a | 23.5±0.9 a | 0.373 | 0.028* | 0.150 |
| rutin | 8.7±0.7 a | 20.2±2.0 a | 10.7±0.7 a | 25.4±3.4 a | 0.152 | 0.003* | 0.471 |
| ***hp-1*** | MG | | TU | | *P* | | |
| | -UVB | +UVB | -UVB | +UVB | stage | UVB | stage x UVB |
| ***Peel*** | | | | | | | |
| narigenin | 438±40 b | 1133±121 a | 464±6 b | 756±2 b | 0.051 | 0.002* | 0.034* |
| 3-quercetin-pentosyl-rutinoside | 59.3±8.5 a | 88.6±9.3 a | 88.0±1.7 a | 100.5±10.4 a | 0.069 | 0.063 | 0.364 |
| rutin | 15.4±2.4 a | 32.4±4.6 a | 13.5±4.1 a | 24.9±2.1 a | 0.247 | 0.015* | 0.465 |
| ***Flesh*** | | | | | | | |
| narigenin | nd | nd | nd | nd | - | - | - |
| 3-quercetin-pentosyl-rutinoside | 11.9±0.4 a | 32.5±2.5 a | 15.2±0.2 a | 29.0±1.8 a | 0.951 | <0.001* | 0.096 |
| rutin | 5.6±0.3 c | 75.0±3.2 a | 15.2±0.2 bc | 29.0±1.8 b | <0.001* | <0.001* | <0.001* |

Figure
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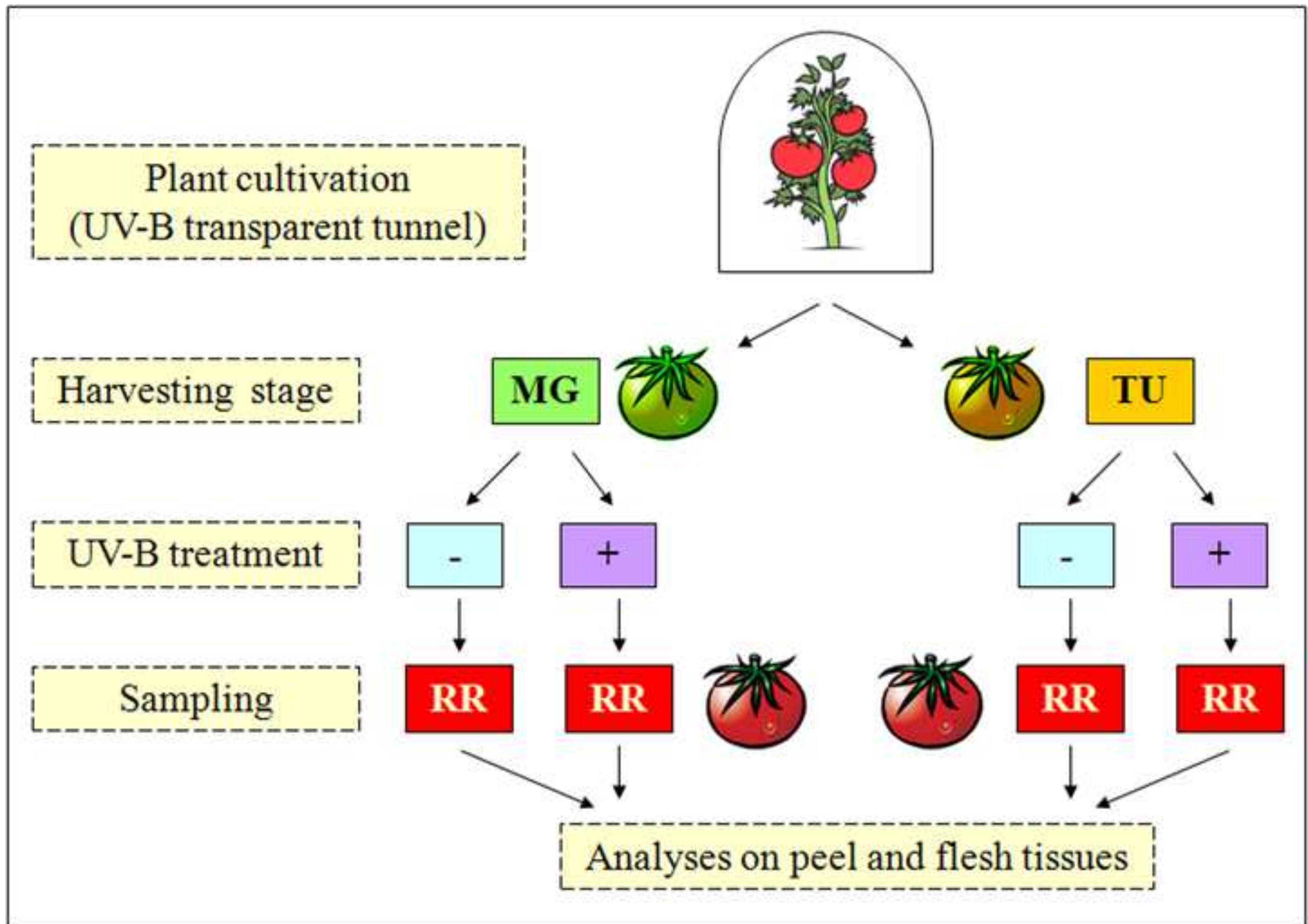

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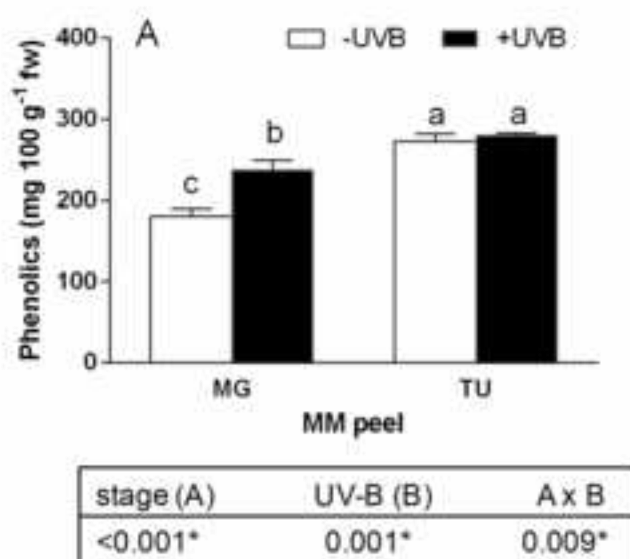


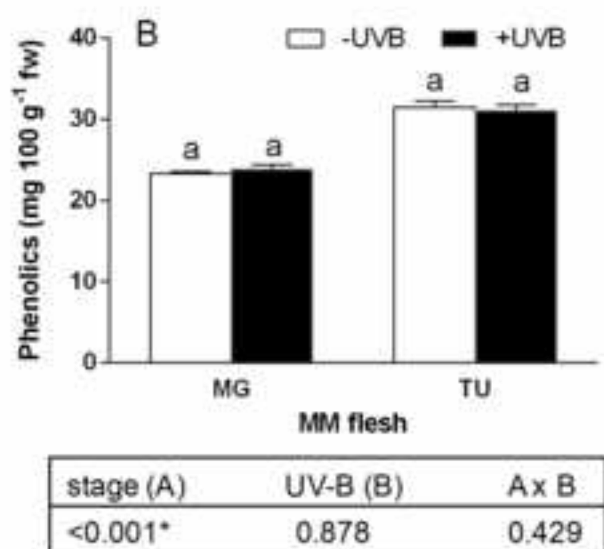


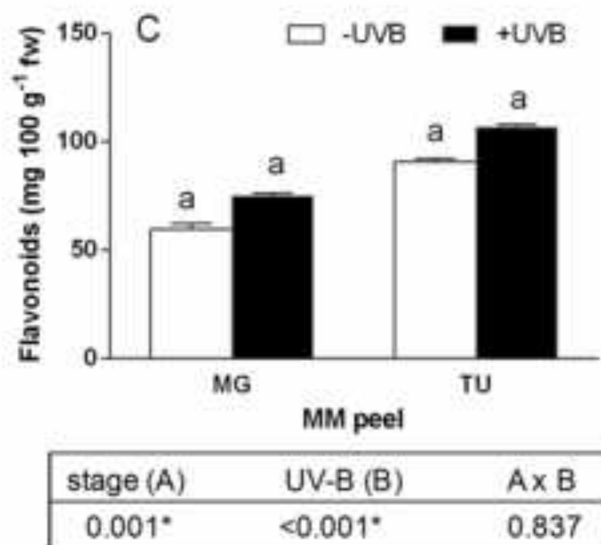


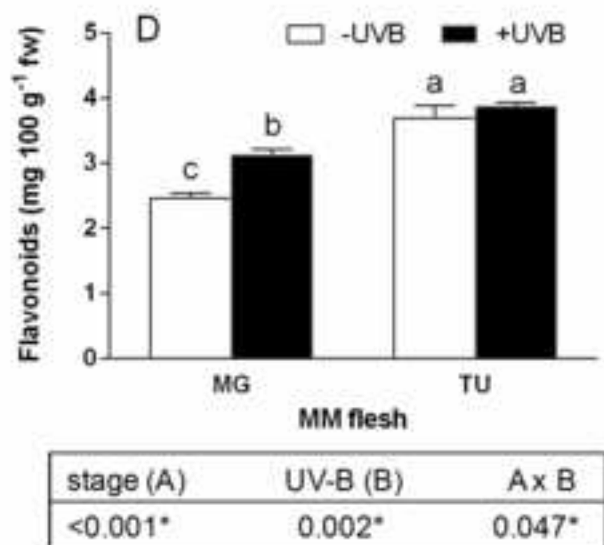


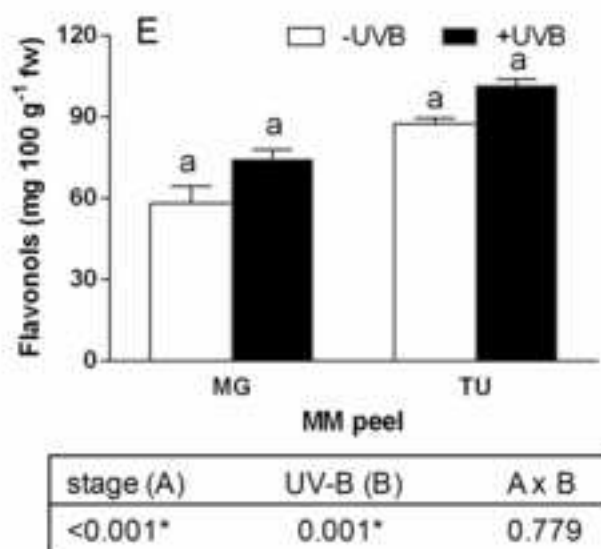


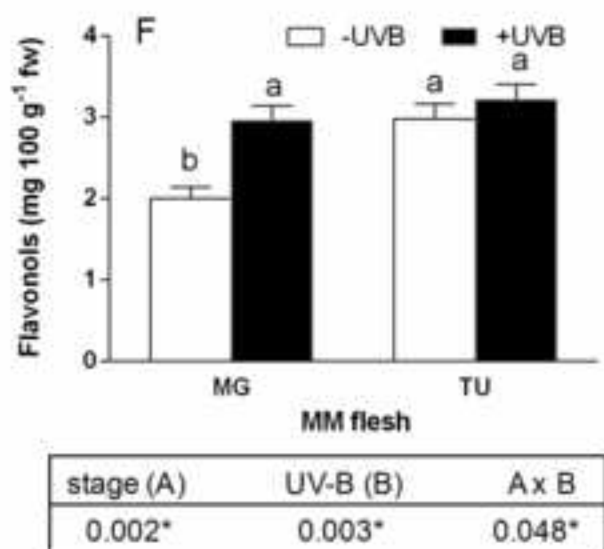

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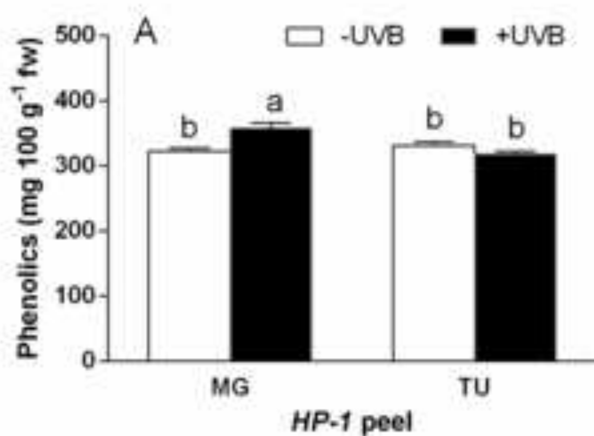
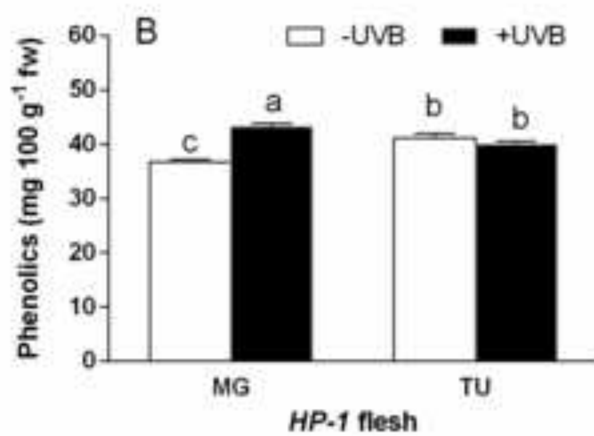
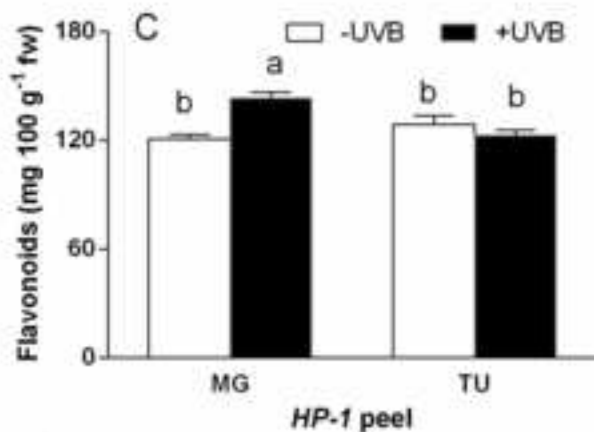


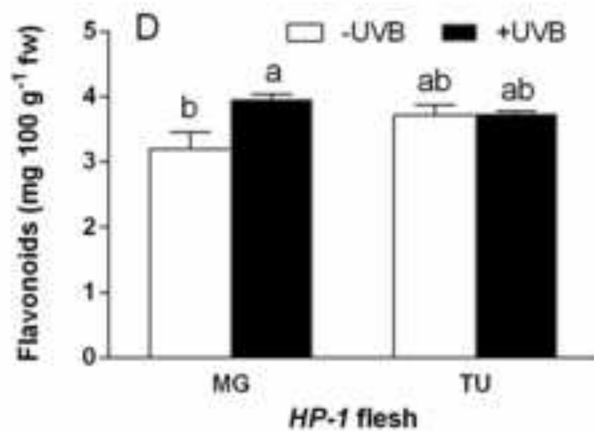

| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.006* | 0.024* | 0.005* |



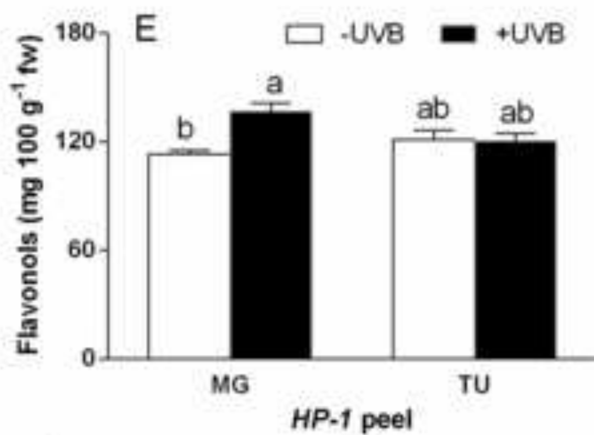

| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.607 | <0.001* | <0.001* |



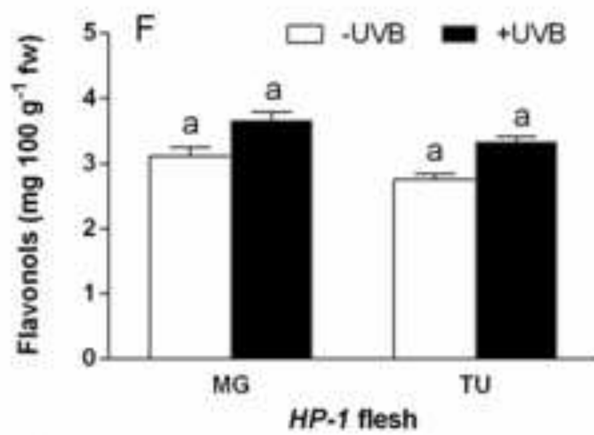

| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.072 | 0.024* | <0.001* |




| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.354 | 0.024* | 0.029* |

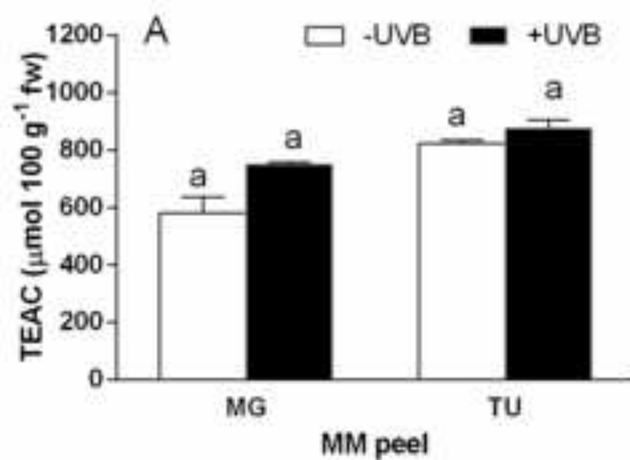



| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.324 | 0.014* | 0.008* |

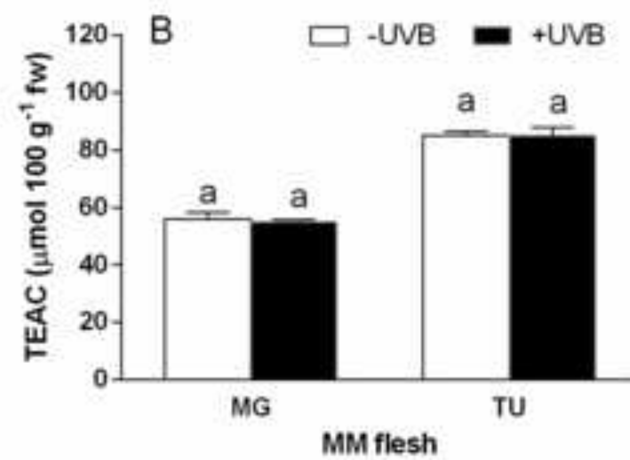



| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.007* | <0.001* | 0.874 |

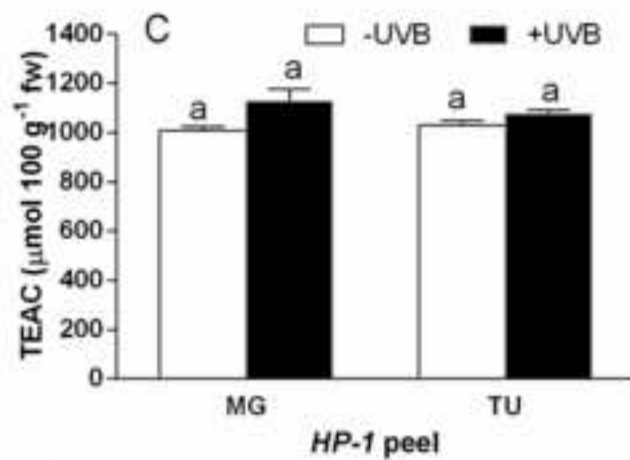
Figure
Click here to download high resolution image



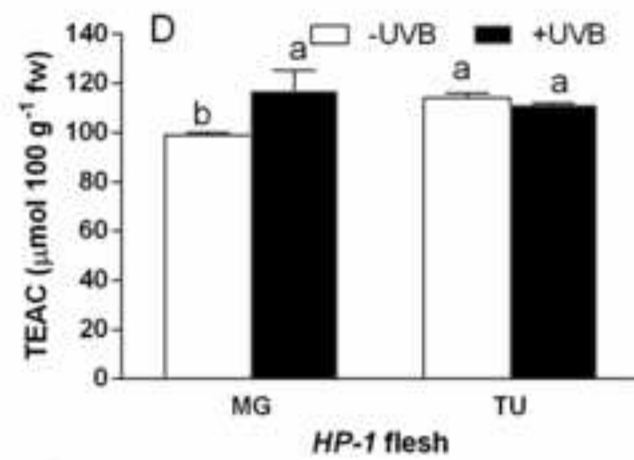

| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.001* | 0.018* | 0,162 |




| stage (A) | UV-B (B) | A x B |
|---|---|---|
| <0.001* | 0,982 | 0,760 |




| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.624 | 0.025* | 0.281 |




| stage (A) | UV-B (B) | A x B |
|---|---|---|
| 0.288 | 0.119 | 0.041* |