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Preharvest UV-C radiation impacts strawberry metabolite content and volatile organic compound production

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A B S T R A C T

UV-C radiation, widely known for preventing decay and increasing fruit quality, was applied to 'Camarosa' strawberries during cultivation (preharvest) and harvested fruits were divided into external and internal tissue portions. UV-C promoted increase in total polyphenolic and volatile organic content, mostly in proanthocyanidins, anthocyanins and esters in external tissues. UV-C treatment increased vitamin C, but did not affect significantly SS, AT and, firmness. In contrast, furane and mesifurane, important volatile compounds for the aroma character of strawberry, decreased upon UV-C treatment.

Keywords:

Abiotic stress
 Furanes
 Polyphenols
Fragaria × *ananassa*

1. Introduction

Strawberry (*Fragaria x. ananassa* Duch.) pseudofruit, henceforth called fruit, is rich in bioactive compounds such as ascorbic acid, folates, and phenolic compounds that play an important role in crop quality and contribute to human health (Giampieri et al., 2015). In spite of its phytochemical richness, strawberry is susceptible to grey mold disease caused by *Botrytis cinerea* (Charles, Goulet, & Arul, 2008). Postharvest UV-C treatment has been shown to activate fruit defense mechanisms, induce the accumulation of antioxidant compounds (vitamin C) and specialized metabolites (carotenoids and anthocyanins), and limit decay incidence (Charles et al., 2008; Severo, Oliveira, Tiecher, Chaves, & Rombaldi, 2015). Additionally, recent studies have suggested positive responses to preharvest UV-C radiation in fruit quality (Oliveira et al., 2016; Xie et al., 2015). The present study investigated the effects of preharvest UV-C

application on vitamin C, phenolic contents and, volatile organic compound production of external and internal strawberry tissues.

2. Materials and methods

2.1. Plant material

Strawberry cv. Camarosa plants were grown in two greenhouses (8 × 12 m) according to Oliveira et al. (2016) in a completely random design with two treatments (UV-C treated and control plants) and three replicates (130 plants per replicate). Fruit were harvested during the highest productivity period, between the 45th and 85th days after treatment initiation. Sixty strawberries from each replicate were harvested at the full red ripening stage. Fruits after physico-chemical characterization were manually separated into internal and external tissues (Fig. 1), immediately frozen with liquid nitrogen, and stored at –80 °C until chemical analyses were performed.

2.2. UV-C treatment

UV-C "Phillips®" 30 W bulbs (24 bulbs) were placed 2 m above plants. The intensity of the applied radiation (0.5 kJ m⁻² per

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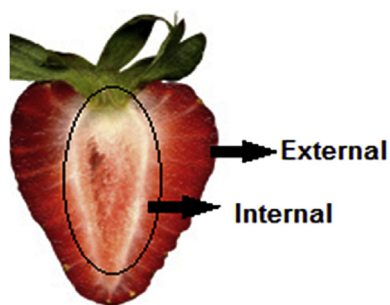


Fig. 1. Strawberry fruit tissues separated for analysis. Each representing half of fruit weight.

application) was quantified with a UV light meter (Model 232-RS-203-MRUR, Instrutherm®, São Paulo, SP, Brazil). UV-C dose and application intervals were established from exploratory tests using 0 kJ m^{-2} to 1.5 kJ m^{-2} . UV application occurred from flowering to harvest, at 7 p.m. every four days, approximately 10 min every time, totaling 28 applications. Half the plants received no UV-C treatment (control group).

2.3. Analyses

2.3.1. Physicochemical characterization

Soluble solids (SS) content was determined by refractometry, and expressed as °Brix. Total acidity (TA) was determined by titration and expressed as mg citric acid per kg^{-1} of fresh fruit. Hue angle was recorded using a colorimeter (Minolta, CR-300 TM, Osaka, Japan), at two points on the equatorial line of each fruit. Firmness was evaluated using a texture analyzer (TA.XT plus, Stable Micro Systems Texture Technologies, Godalming, United Kingdom), fitted with a 2 mm (diameter) flat probe and each fruit was penetrated 50% at a speed of 1.0 mm s^{-1} as described by Severo et al. (2015).

2.3.2. Vitamin C

Vitamin C (ascorbic acid + dehydroascorbic acid) was quantified using the spectrophotometric method described by Stevens, Buret, Garchery, Carretero, and Causse (2006).

2.3.3. Phenolic compounds

Extraction, identification, and quantitation by LC-MS were performed as describing Le Bourvellec et al. (2011). Proanthocyanidins were characterized by thioacidolysis to determine subunit composition and the average degree of polymerization (DP). Individual compounds were quantified by comparisons with external standards at 280 nm, 320 nm, at 350 nm, and 515 nm and identified by mass spectrometry (MS) (Supplementary material - Table 1).

2.3.4. Volatile organic compounds (VOC)

Solid phase micro extraction (SPME) of VOC was carried out using $75 \mu\text{m}$ carboxen-PDMS SPME fibers (Supelco, Bellefonte, PA, USA). Approximately two grams of fruit, spiked with of 4-nonanol ($100 \mu\text{L}$ of 0.8 g L^{-1} concentration in methanol) was placed in a 20 mL vial and frozen at $-80 \text{ }^\circ\text{C}$ for 30 min before analysis.

Volatiles were collected automatically with Combi PAL (Tri PlusRSH, Thermo, Zwingen, Switzerland). For extraction in oven fiber was placed at 20 mm to sample at $40 \text{ }^\circ\text{C}$ for 30 min under stirring at 250 rpm. SPME fiber was then inserted in a gas chromatograph with a mass spectrometry detector (GC-MS; Thermo Trace GC1300-ISQ LT, Waltham, MA, USA) in the split/splitless mode and thermally desorbed at $250 \text{ }^\circ\text{C}$ for 5 min. GC-MS

conditions were $5 \text{ }^\circ\text{C min}^{-1}$ ramp until $230 \text{ }^\circ\text{C}$ and then held at $230 \text{ }^\circ\text{C}$ for 5 min. The capillary column was a TG-WAXMS ($30 \text{ m} \times 0.25 \text{ mm i.d.} \times 0.5 \mu\text{m}$, Thermo Fisher Scientific, Waltham, MA, USA). Helium was used as carrier gas at a flow rate of 1 mL min^{-1} at constant pressure 150 kPa. Volatile compounds were identified by comparison to standards, using the retention index (a homologous series of C7-C30 n-alkanes) and the spectral library (NIST 2014/Hewlett-Packard Co., Palo Alto, CA, USA) and semi-quantified by comparison to the internal standard (Renard, Ginies, Gouble, Bureau, & Causse, 2013).

For accelerated solvent extraction (ASE), 10 g of frozen fruit powder were mixed with 15 g of Hydromatrix™ and 4-nonanol ($100 \mu\text{L}$ of 0.8 g L^{-1} concentration in methanol). The mixture was rapidly transferred to a pressurized extraction cell (ASE 200 system, Dionex, Sunnyvale, CA), extracted, concentrated, and a $1 \mu\text{L}$ aliquot was injected into the GC-MS (Renard et al., 2013) (Supplementary material - Table 2).

2.4. Statistical analysis

Chemical analyses of each of the three replicates were carried out in triplicate. Analysis of variance was conducted using the MIXED procedure of SAS (version 9.1). Plots of standardized residuals were examined for violation of assumptions. Mean separations were based on Fisher's test (F) and were considered different at $p \leq 0.05$. Means and pooled standard deviation are presented in supplementary data.

3. Results and discussion

Strawberry fruit in advanced stages of maturity tend to be softer, with higher soluble solids content, lower acidity and redder. In this study, flesh firmness (Control 3.4 ± 0.1 ; UV-C $3.5 \pm 0.2 \text{ N}$), soluble solids content (Control 8.0 ± 0.1 ; UV-C $7.8 \pm 0.3 \text{ }^\circ\text{Brix}$), and acidity (Control 8.0 ± 0.2 ; UV-C $7.5 \pm 0.3 \text{ mg citric acid equivalent kg}^{-1}$) were not significantly affected by UV-C treatment, however fruit showed lower °Hue (Control 32.3 ± 0.1 to UV-C $30.2 \pm 0.05 \text{ }^\circ\text{Hue}$), which indicates that UV-C treated fruit were redder in color.

No significant interaction ($p > 0.05$) between treatment and tissue was observed for L-ascorbic acid (AA), dehydroascorbic acid (DHA), and vitamin C contents, and the phenolic compounds (+)-catechin, ellagic acid deoxyhexoside, ellagic acid, kaempferol-3-O-glucoside, and the volatiles organic compounds methyl acetate, isobutyl acetate, hexyl acetate, 1-butanol-2-methylacetate, methyl butanoate, and ethyl hexanoate, mesifurane, as well as total volatile content. Mean metabolite content and volatile organic compounds (VOC) production in internal tissues was either lower than or not different from that of external tissues with the exception of the volatile production of mesifurane, which was higher in internal tissues averaged across treatments (Table 1). UV-C treatment did not affect dehydroascorbic acid, ellagic acid deoxyhexoside, ellagic acid, kaempferol-3-O-glucoside contents, degree of polymerization of proanthocyanidins, or the production of the volatiles methyl acetate, isobutyl acetate, 1-butanol-2-methyl acetate, and methyl butanoate averaged across tissue types (Table 1). On the other hand, (+)-catechin, kaempferol-3-O-acetyl glucoside, and mesifurane were reduced upon UV-C treatment, while ascorbic acid, vitamin C, quercetin glucuronide, ethyl acetate, hexyl acetate, 2-hexen-1-ol acetate, and ethyl hexanoate increased upon UV-C treatment averaged across tissue types (Table 1).

Despite the response are not tissue specific, the increase in vitamin C by UV-C treatment (Table 1) were expected because it is largely known that L-ascorbic acid (AA) and its oxidation product, dehydroascorbic acid (DHA), are activated in fruit during abiotic stress conditions (Oliveira et al., 2016; Severo et al., 2015).

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Table 1

Mean metabolite accumulation and volatile production (mg kg^{-1}) from strawberry tissues treated with UV-C during cultivation averaged across tissue type for control and UV-C and across treatment for external and internal tissues.

	Control		UV-C		External		Internal	
Ascorbic acid	494.5	B	550.4	A	654.0	A	390.9	B
Dehydroascorbic acid	2.4	A	1.2	A	2.2	A	1.4	A
Vitamin C	496.9	B	551.6	A	656.2	A	392.3	B
(+)-Catechin	51.4	A	48.1	B	52.7	A	46.8	B
Ellagic acid deoxyhexoside	7.1	A	6.6	A	11.1	A	2.6	B
Ellagic acid	15.2	A	16.8	A	28.6	A	3.4	B
Quercetin glucuronide	14.8	B	18.2	A	16.5	t	t	t
Kaempferol-3-O-glucoside	5.3	A	5.2	A	5.3	t	t	t
Kaempferol-3-O-acetyl glucoside	3.2	A	2.3	B	2.7	t	t	t
Degree of polymerization of proanthocyanidin	6.4	A	6.4	A	6.5	A	6.3	A
Methyl acetate	2.1	A	1.9	A	2.8	A	1.2	B
Ethyl acetate	0.9	B	2.3	A	2.4	A	0.8	B
Isobutyl acetate	0.1	A	0.2	A	0.2	A	0.1	A
Hexyl acetate	0.8	B	1.3	A	1.1	A	0.9	A
1-Butanol-2-methyl acetate	0.3	A	0.3	A	0.4	A	0.2	B
2-Hexen-1-ol acetate	2.4	B	3.7	A	3.7	A	2.4	B
Methyl butanoate	6.5	A	6.2	A	7.9	A	4.8	B
Ethyl hexanoate	0.6	B	1.0	A	1.4	A	0.1	B
Mesifurane	1.1	A	0.5	B	0.7	B	0.9	A
Total VOC ^a	19.1	B	22.5	A	26.4	A	15.2	B

Means followed by the same capital letter within a column are not significantly different ($p \leq 0.05$).

t – traces (below detection limit).

^a Total volatile organic compounds.

For proanthocyanidins and its monomeric subunits, coumaric acid hexoside, cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, total anthocyanin and total phenolic contents, as well as volatiles ethyl butanoate, methyl hexanoate, furaneol, and linalool, a significant interaction ($p \leq 0.05$) between treatment and tissue type was seen and therefore the effect of UV-C on these compounds was tissue specific (Table 2).

Differently than in untreated fruits, UV-C treated fruit external

tissues had higher proanthocyanidin content than internal tissues, while the other monomeric subunits did not vary between tissue types, except for (epi)afzelechin extension subunits (Table 2). UV-C treatment promoted an increase in proanthocyanidin content of 72 mg g^{-1} (+13%) in external tissue and a reduction of 126 mg g^{-1} (-21%) in internal tissue (Table 2). The changes were proximate and may indicate a potential translocation of these compounds from internal to external tissues. Charles et al. (2008) speculate

Table 2

Metabolite accumulation and volatile production (mg kg^{-1}) from strawberry tissues treated with UV-C during cultivation (significant treatment \times tissue interaction).

		Control		UV-C	
Proanthocyanidin	External	493.8	Bb	566.1	Aa
	Internal	614.3	Aa	488.0	Bb
(+)catechin terminal units of proanthocyanidins	External	74.7	Bb	89.8	Aa
	Internal	96.1	Aa	75.3	Ab
(+)catechin extension units of proanthocyanidins	External	119.9	Ba	125.0	Aa
	Internal	150.9	Aa	115.7	Ab
(–)epicatechin extension units of proanthocyanidins	External	240.0	Bb	281.7	Aa
	Internal	342.2	Aa	274.7	Ab
(epi)afzelechin extension units of proanthocyanidins	External	59.2	Ab	69.6	Aa
	Internal	25.1	Ba	22.3	Ba
Coumaric acid hexoside	External	26.7	Bb	31.7	Aa
	Internal	32.0	Aa	28.1	Bb
Cyanidin-3-O-glucoside	External	88.3	Ab	112.1	Aa
	Internal	7.2	Ba	10.5	Ba
Pelargonidin-3-O-glucoside	External	876.5	Ab	1267.3	Aa
	Internal	272.0	Ba	278.5	Ba
Pelargonidin-3-O-rutinoside	External	136.6	Ab	208.5	Aa
	Internal	58.7	Ba	51.9	Ba
Total anthocyanin content	External	1101.4	Ab	1587.9	Aa
	Internal	338.0	Ba	340.9	Ba
Total phenolic content	External	1736.8	Ab	2304.6	Aa
	Internal	1040.0	Ba	906.8	Ba
Ethyl butanoate	External	0.7	Ab	3.1	Aa
	Internal	0.2	Aa	1.1	Ba
Methyl hexanoate	External	2.4	Aa	2.0	Aa
	Internal	1.3	Ba	1.6	Ba
Total ester volatiles	External	20.8	Ab	27.3	Aa
	Internal	11.2	Ba	14.3	Ba
Furaneol	External	2.1	Aa	0.9	Ab
	Internal	1.7	Ba	1.2	Ab
Linalool	External	0.2	Aa	0.1	Bb
	Internal	0.1	Aa	0.2	Aa

Means followed by the same capital letter within a column and the same lower case letter within a row are not significantly different ($p \leq 0.05$).

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biosynthesis of phenolic compounds on the cell wall, mostly in epiderme of tomato, in response to UV-C. Further, Petrusa et al. (2013) suggest that stress conditions trigger not only the biosynthetic pathways, but also the translocation of flavonoids through expression of proteins involved in transport and accumulation. It is the first time that a study suggests a translocation of compounds in response to UV-C treatment. However, this hypothesis needs to be better tested. No free epicatechin and epiafzelechin were found in the samples although epicatechin was the major procyanidin unit. UV-C treatment promoted a reduction in coumaric acid hexoside in internal tissues and an increase in external tissues (Table 2). These changes were proportional [3.9 mg g⁻¹ (12.2%) and 5 mg g⁻¹ (15.8%)] and also suggest translocation of coumaric acid hexoside from internal to external tissues upon UV-C treatment.

Despite Xie et al. (2015) related no significant effect in anthocyanins content of strawberry fruit from plants treated by UV-C at the preharvest stage, in this study the main impact of UV-C treatment seemed to be in the increase of the three main anthocyanins quantified (cyanidin-2-O-glucoside, pelargonidin-3-O-glucoside and pelargonidin-3-O-rutinoside). UV-C treatment promoted an increase of 44% in their content in the external tissue, suggesting biosynthesis of anthocyanins in the external tissues upon UV-C treatment. However it did not influence the content of the anthocyanins in the internal tissues (Table 2). UV-C-induced biosynthesis of anthocyanins, may occur as a mechanism of radiation protection (Oliveira et al., 2016; Severo et al., 2015).

In this study, volatile esters were identified in most diversity with better reproducibility by SPME, while furane, mesifurane and linalool were better quantified after ASE extraction. UV-C treatment affected quantitatively the VOC production (Tables 1 and 2). Previous studies also reported that biotic and abiotic stressors can interfere with fruit VOC production (Neri et al., 2015; Severo et al., 2015). The UV-C impact on total ester content was tissue specific and higher in external tissues. Ethyl butanoate content was higher in external tissues of UV-C treated fruit when compared to control, however, it did not vary upon UV-C treatment in internal tissues. Methyl hexanoate production was not affected by UV-C in either tissue and was higher in external than in internal tissues in both control and UV-C treated strawberry (Table 2). However, UV-C treatment decreased furaneol production from external and internal tissues and linalool production only from external tissues (Table 2). Furanes are quantitatively a minor constituents of the fruit flavor but, because it has such a low threshold value, it is highly influential on the overall flavor (Bood & Zabetakis, 2002). Certainly fruit aroma was affected since esters are known for the particular fruity notes, while furanes by their sweet caramel-like notes.

4. Conclusions

UV-C preharvest radiation in hormetic doses affected differently external and internal strawberry fruit tissues. Fruit ripening

indicators such as firmness, soluble solids, and acidity were not affected by UV-C, however, color, anthocyanin content, and volatile production were. Tissue specific phenolic changes, in particular of proanthocyanidin and coumaric hexoside contents, decreased in internal and increased in external tissues, meanwhile the remarkable increase in anthocyanin content occurred only in external tissues upon UV-C treatments. Furthermore, UV-C increased total volatile organic production, mostly total volatile esters, however decreased furane and mesifurane production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.lwt.2016.10.032>.

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