



Postharvest UV-C treatment increases bioactive, ester volatile compounds and a putative allergenic protein in strawberry

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ABSTRACT

UV-C treatment applied during postharvest to control fruit decay has been shown to interfere with several aspects of fruit quality. In this study, strawberry fruit (*Fragaria × ananassa* Duch., cv 'Aromas') harvested at the 75% red stage of ripening were treated with UV-C (4.35 kJ m⁻²) and postharvest compounds indicative of quality, as well as related gene transcripts, were measured. UV-C effect in two allergenic proteins was also evaluated. After treatment, fruit was stored for six days at 20 ± 2 °C and 80 ± 5% relative humidity (RH). UV-C treated fruit maintained flesh firmness and showed lower pectate lyase transcript accumulation than control fruit. UV-C treatment increased total anthocyanin and phenolic compounds, including gallic acid, hydroxybenzoic acid, p-coumaric acid, quercetin and (+)-catechin. UV-C treatment also increased L-galactose dehydrogenase, L-galactono-1,4-lactone dehydrogenase, alcohol dehydrogenase and alcohol acetyl transferase transcript accumulation, and resulted in strawberries with higher L-ascorbic acid content and higher volatile compound production. In conclusion, UV-C treatment induces a variety of changes in the strawberry primarily via gene activation, resulting in firmer fruit with higher levels of bioactive molecules, as well as a stronger aroma. However, UV-C also induced accumulation of the putatively allergenic protein Fra a1.

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1. Introduction

The strawberry (*Fragaria × ananassa*, Duch.) is a widely consumed fruit valued for its flavor and nutritional properties. It is also highly perishable, due to an elevated metabolic rate and structure fragility, resulting in increased susceptibility to pathogen attack. Undesirable changes observed during postharvest include loss of flesh firmness and *Botrytis cinerea* induced decay (Charles, Tano, Asselin, & Arul, 2009; Pombo, Rosli, Martínez, & Civello, 2011).

The main strategies employed to reduce postharvest losses include selective breeding and genetic transformation,

refrigeration, modified and controlled atmospheres, application of edible coatings, heat and UV radiation treatments (Cordenunsi et al., 2005; Jiménez-Bermúdez et al., 2002; Wang, and Gao., 2013). UV-C radiation is a low cost and less environmentally destructive alternative, which reduces the need for fungicide application (Pombo et al., 2011; Vicente et al., 2005). The mechanisms of UV-C may controls decay include an antimicrobial effect, an impact on fruit metabolism, particularly on cell wall metabolism (Bu, Yu, Aisikaer, & Ying, 2013; Charles et al. 2009; Pombo, Dotto, Martínez, & Civello, 2009), activation of antioxidant enzymatic systems, production of pathogenesis-related (PR) proteins, and biosynthesis of antioxidant and antifungal compounds such as terpenoids, phenolics, ascorbic acid and folates, and polyamines (Charles et al., 2009; El Ghaouth, Wilson & Callahan, 2003; Erkan, Wang, & Wang, 2008; Maharaj, Arul, & Nadeau, 2014; Pombo et al., 2011). UV-C exposure has been tested in different crops such as tomato (Bu et al., 2013; Charles et al., 2009; Maharaj et al., 2014; Severo et al., 2015; Tiecher, Paula, de Chaves, & Rombaldi,

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2013), pepper (Vicente et al., 2005), peach (El Ghaouth et al., 2003; Yang, Cao, Su, & Jiang, 2014) and strawberry (Erkan et al., 2008; Pombo et al., 2009, 2011). Severo et al. (2015) proposed the action of ERF (factors of response to ethylene) in gene regulation of several metabolic biosynthesis compounds in UV-C treated tomatoes. Up-regulation of genes coding for PR proteins such as β -1,3-glucanases, chitinases, γ -thionins, osmotins, and Fra a1, have been reported in strawberry leaves infected by *Colletotrichum acutatum* (Casado-Diaz et al., 2006). The putative role of β -1,3-glucanases in disease resistance may be related to their capacity to degrade the fungal cell wall (Pombo et al., 2011). The Fra a1 family of proteins in strawberries also participates in defense against pathogens and appears to be involved in anthocyanin metabolism (Muñoz et al., 2010). While these proteins are important for plant protection, they are also primarily responsible for food allergies in humans (Karlsson et al., 2004).

Fruit softening in strawberry, similar to most fruit, largely results from the depolymerization and solubilization of cell wall polysaccharides involving proteins with or without the activity of enzymes such as expansins, pectin methylesterases, polygalacturonases, pectate lyases, β -galactosidases, α -L-arabinofuranosidases, endo-(1,4)- β -D-glucanases, β -xyloxydases, xyloglucanases, endo-transglucosylases and endo-mannanases (Jiménez-Bermúdez et al., 2002; Pombo et al., 2009; Severo, Tiecher, Chaves, Silva, & Rombaldi, 2011; Trainotti, Spinello, Piovan, Spolaore, & Casadoro, 2001). In addition, cell wall disassembly also influences vitamin C synthesis by the release of ascorbic acid precursors such as L-galactose (Cruz-Rus, Amaya, Sánchez-Sevilla, Botella, & Valpuesta, 2011). L-ascorbic acid (AA) and its oxidation product, dehydroascorbic acid (Cordenunsi et al., 2005), are both synthesized through L-galactose oxidation catalyzed by L-galactose dehydrogenase (LGalDH) which supplies a substrate for L-galactose-1,4-lactone dehydrogenase (GLDH), the last enzyme involved in the L-ascorbic acid biosynthetic pathway (Cruz-Rus et al., 2011).

In general, abiotic stress activates the synthesis of phenylpropanoids (Vicente et al., 2005) through activation of phenylalanine ammonia lyase (PAL) (El Ghaouth et al., 2003). Anthocyanins are the main compounds responsible for the red color of strawberries. Anthocyanins, along with other phenolic compounds, are synthesized in the cytosol and are present in significant amounts in vacuoles (Vicente et al., 2005). Anthocyanin synthase (ANS) acts at a latter step in this biosynthetic route, and its activity is directly correlated with anthocyanin accumulation (Severo et al., 2011).

Strawberry aroma is one of the most important quality attributes. The aroma mainly results from ester biosynthesis, and is influenced by ripening stage and postharvest treatment (Pérez, Sanz, Olías, Ríos, & Olías, 1996; Severo et al., 2011). Ultimately, these factors affect alcohol dehydrogenase (ADH) reduction of alcohols, and their esterification with acyl-CoA by alcohol acetyl transferases (AAT) (Aharoni et al., 2000).

Despite the previously identified advantages of increased fruit shelf-life, the means by which UV-C treatment affects fruit quality compounds and genes is largely unknown. We attempted to elucidate how UV-C can impact the transcriptional profile of genes related to cell wall structure, phenolic and ascorbic acid production, and volatile compound generation, as well as levels of respective metabolites in strawberry fruit. In addition, the allergenic proteins β -1,3-glucanases and Fra a1 were studied.

2. Material and methods

2.1. Plant material

Strawberry fruit (*Fragaria x ananassa* Duch., cv 'Aromas') from a

commercial plantation located in Pelotas (RS, Brazil) was harvested at the ripening stage characterized by having 75% of the fruit surface red. Fruit was immediately transferred to the laboratory for further treatment and analysis.

2.2. UV-C treatment

Harvested strawberries were packed in trays and placed at a 30 cm distance under UV-C bulbs (TUV G30T8, 30 W, Philips) providing a 4.35 kJ m⁻² UV-C dose as measured by an RS-232 digital radiometer (Model MRUR-203, Instrutherm®). This hermetic dose was chosen based on preliminary tests with doses ranging from 0 to 5 kJ m⁻². For provided this dose fruit were exposed to UV-C treatment for a total of 20 min and turned every five min to assure even exposure of the entire fruit surface. Each replicate contained fifteen fruit. After UV-C treatment, fruit was kept at room temperature (RT, 20 $\pm$ 2 °C and 80% RH). Controls fruit were directly placed at RT. Fruit were stored and monitored for six days.

2.3. RNA isolation, RT-PCR and real-time PCR

Total RNA was extracted according to the manufacturer's instructions (Pure Link, Invitrogen®). For RT-PCR, DNase-treated RNA (2 μ g) was reverse transcribed in a total volume of 20 μ L using Omniscript Reverse Transcription Kit (Qiagen, Valencia, CA, USA) and then PCR was performed using 2 μ L of cDNA in a 25 μ L reaction volume using SYBR Green PCR Master Mix™ (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 7500 sequence-detection system. Primer Express software (Applied Biosystems) was used to design gene-specific primers (Severo et al., 2011). Fourteen genes were chosen based on putative roles in strawberry quality traits, such as, cell wall disassembling (*Exp2*; *Exp5*; *PLa*, *PLb* and *PLc*; *PME*; *PG*; and β -*Gal*), anthocyanin and phenolic compound synthesis (*PAL* and *ANS*), ascorbic acid synthesis (*LGalDH* and *GLDH*) and ester volatile synthesis (*ADH* and *AAT*). Optimal primer concentration was 50 nmol/L. Real time-PCR conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min, and one cycle at 72 °C for 5 min. Samples were run in triplicate in a 96-well plate. For each sample, a Ct (threshold cycle) value was calculated from the amplification curves by selecting the optimal ΔR_n (emission of reporter dye over starting background fluorescence) in the exponential portion of the amplification plot. Relative quantitation (RQ) was calculated based on the comparative Ct method, using $RQ = 2^{-\Delta\Delta C_T}$ (Livak & Schmittgen, 2001) and using β -actin as an internal standard.

2.4. Firmness

Firmness was measured using a texture analyzer (Texture Analyzer, TA.XT plus, Stable Micro Systems Texture Technologies) fitted with a 2 mm (diameter) probe. Each fruit was penetrated 50% at a speed of 1.0 mm s⁻¹, and the maximum force developed during the test was recorded. Three measurements were taken per fruit at different points on the equatorial zone, and 30 berries per treatment were assayed. Results were expressed in Newtons (N).

2.5. Total and individual phenolic compounds, total anthocyanin, L-ascorbic acid content and antioxidant capacity

Total phenolic were determined using the Folin-Ciocalteu reagent. One gram of ground fruit flesh was suspended in 60 mL of DI water and 5 mL of Folin-Ciocalteu reagent. After eight min the solution was neutralized with 20 mL of a saturated sodium carbonate solution and kept in the dark for two hours (Singleton & Rossi, 1965). Absorbance was measured 725 nm and results were

expressed as mg of gallic acid equivalents 100 g of fruit fresh weight⁻¹ (mg GAE 100 g⁻¹ ffw).

Identification and quantification of gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, caffeic acid, (+)-catechin, (–)-epicatechin, quercetin and kaempferol were performed according to Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1998. Phenolic compounds were extracted and hydrolyzed from five grams of ground fruit flesh using acidified methanol (0.1% HCl v/v, 35 mL). The extract was stirred in the dark at 35 °C for 24 h, then filtered (Millipore membrane 0.22 µm) and analyzed by reverse-phase HPLC, using a Shimadzu HPLC system, with a Shim-Pak CLC-ODS column (3.9 cm × 150 mm × 4 µm), coupled to a UV SPD-10AV detector. The mobile phase was composed of A – acidified water (1 mL/100 mL acetic acid) and B – pure methanol. The elution gradient began with 0% B; then increased linearly to 40% B over 25 min; held for two min at 40% B; then reduced to 5% B, and held for five min; and then returning to initial conditions. The flow rate was 0.9 mL min⁻¹, and column temperature was kept at 25 °C. Quantification was based on external standard calibration curves for gallic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, quercetin and kaempferol (Sigma–Aldrich, St. Louis, MO) and results were expressed as mg 100 g⁻¹ of ffw.

Total anthocyanin content was determined according to the method described by Lees and Francis (1972). One gram of strawberry ground to powder in liquid nitrogen was suspended in 25 mL of acidified-HCl ethanol (1 mL/100 mL), for 1 h in the dark. Absorbance readings were performed in a spectrophotometer at 520 nm. Anthocyanin content was expressed as mg of cyanidin-3-glucoside 100 g⁻¹ of ffw.

L-ascorbic acid (AA) was measured using a reverse-phase HPLC method (Vinci, Rot & Mele, 1995). Total AA was extracted with metaphosphoric acid in water (1 g/100 mL) and analyzed using an HPLC system, the same system described above (for the individual phenolic compounds). The mobile phase consisted of A – 0.1% acetic acid in water, and B – methanol. The elution gradient began at 0% B, then increased linearly to 2% B over five min; then held for two min at 2% B, and then returned to initial conditions after 3 additional min. The flow rate was 0.8 mL min⁻¹ and the detector was set to 254 nm. Quantification was based on an external standard calibration curve using L-(+)-ascorbic acid (Sigma–Aldrich). Results were expressed as mg of ascorbic acid 100 g⁻¹ of ffw.

Strawberry antioxidant capacity was determined using the ABTS assay based on the method described by Re et al. (1999). ABTS radical cation (ABTS⁺) was produced by reacting 7 mmol/L ABTS solution with 2.45 mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h. The ABTS⁺ solution was then diluted with ethanol until absorbance measured at 734 nm was 0.70 ± 0.02. After the addition of 10 µL of sample or Trolox (0–1.5 mmol/L) standard to 990 µL of diluted ABTS⁺ solution, absorbance at 734 nm was measured at exactly 7 min. Results were expressed as trolox equivalent antioxidant capacity (TEAC) (µg g⁻¹ ffw).

2.6. Ester volatiles

Six major ester volatiles typical of strawberry flavor were identified by GC–MS (Shimadzu QP-5000) and quantified by CG (Varian 3800; Palo Alto, CA, USA) equipped with a flame ionization detector (FID). All extractions were performed manually using 0.75 µm carboxen-PDMS SPME fibers (Supelco, Bellefonte, PA, USA). Two gram of fruit sample, spiked with 2 µL of an internal standard solution, was placed in a 16 mL vial and volatiles were collected using a headspace collection mode (with a distance from the liquid surface of 20 mm) at 30 °C for 15 min (equilibrium) and 45 min under stirring. After extraction, the SPME device was introduced in

a splitless mode and was thermally desorbed for one min. The capillary column was a DB-5 (30 m × 0.25 mm i.d. × 0.25 µm; J&W Scientific, Folsom California, USA). Helium was used as a carrier gas at a flow rate of 1 mL min⁻¹. The injector and detector temperature were set at 280 °C. The temperature program began at 35 °C (held for 10 min) and ramped up to 210 °C at 1 °C min⁻¹, then held for 10 min. Volatile compounds were identified using a single quadrupole detector. The mass spectrometer was operated in electron ionization mode at a voltage of 70 eV. Volatile components were identified by comparison to chemical standards and the spectral library (NIST 98/EPA/MSDC 49K Mass Spectral Database, Hewlett–Packard Co., Palo Alto, CA, USA).

2.7. Antibodies and western blot

Immunodetection of proteins was performed by Western blotting, using mouse polyclonal antibodies produced against the recombinant proteins anti-β-1,3-glucanases (anti-β-1,3-Gluc, AY170376) and Fra a1 (anti-Fra a1, Q5ULZ4). Fruit samples were collected 36 h after treatment. Total strawberry protein was extracted using 1 g of fruit flesh and 1 mL of Laemmli (2x) buffer (250 mmol/L Tris–HCl pH 6.8, 4% SDS, 20% glycerol, 5% β-mercaptoethanol). After 10 min of homogenization, the material was heated to 90 °C for 5 min and then centrifuged at 14,000 × g for 30 min at 4 °C. Protein quantification was performed using Bradford reagent following precipitation with trichloroacetic acid and solubilization with 0.1 mol/L sodium hydroxide. Equal amounts of total protein (40 µg) were loaded per slot onto a denaturing polyacrylamide gel. Once migration was complete, proteins were transferred to a nitrocellulose membrane. The transfer was monitored by coloration with Ponceau S solution. The membrane was blocked with tris-tween buffer saline (TTBS) containing 2g/100 mL skim milk powder. The membrane was then incubated for 2 h with a mouse polyclonal anti-β-1,3-glucanase (diluted 1:2500) and anti-Fra a1 (diluted 1:1500) in TTBS. After three washes with TTBS, the membrane was incubated with goat anti-mouse antibody that was diluted by 1:50,000 in TTBS. Staining was performed with the ECL advanced Kit[®].

2.8. Experimental design and statistical analysis

The experiment was carried out using a completely randomized design with UV-C treated fruits and control fruits each with three biological replicates. Results were analyzed using ANOVA, with a *P* ≤ 0.05 considered significant. Post-hoc analysis was performed using Tukey's test (*p* ≤ 0.05). SAS software was used for all statistical analysis (SAS Institute, 2002).

3. Results and discussion

3.1. UV-C treatment effects on fruit flesh firmness and transcript accumulation of cell wall-associated genes

Transcript accumulation of *Exp2* and *Exp5*, and of other genes encoding enzymes acting in cell wall disassembly (*PME*, *PG*, *β-gal*, *PLa*, *PLb* and *PLc*) was monitored in order to understand the role of these putative genes in response to UV-C treatment. The expansins have a known initial role in cell wall disassembly, providing substrate for pectin methyl esterase (*PME*) de-methylation of pectin and polygalacturonase (*PG*) hydrolysis of galacturonic acid α-1-4 pectin chains (Civello, Powell, Sabehat, & Bennett, 1999). The current investigation was based on earlier studies (Civello et al., 1999; Pombo et al., 2009; Severo et al., 2011) that validated the close association between the selected cell wall genes during strawberry ripening. A decrease in fruit firmness was observed during storage

with significant retention of flesh firmness in UV-C treated fruit at days 4 and 6 of storage (Fig. 1I). The expression profile of cell wall associated genes followed two patterns during to storage. While transcript accumulation of cell wall associated genes *Exp2*, *Exp5*, *PME* and *PG*, β -Gal were higher in UV-C treated fruit (Fig. 1A, B, C, D, E), pectate lyases gene transcripts *PLa*, *PLb* and *PLc* were higher in untreated fruit (Fig. 1F, G, H). It is possible that among the selected genes, some have an important action in the first hours after treatment. Pombo et al. (2009) related delays in strawberry softening to reduced expression of a set of genes, including *Exp2*, *Exp5*, *PG* and *PME*, which are involved in cell wall disassembly during the first 24 h after treatment. In contrast, in tomatoes, Bu et al. (2013) reported a decrease in transcriptional expression of *Exp1*, *PG*, *PME* and *Cel1* in the fifth day of UV-C treated tomato storage, followed by an increase in transcript accumulation in these genes between the fifth and twentieth days of storage. These differences between strawberries and tomatoes are expected since tomatoes exhibit climacteric ripening behavior, while strawberries are non-climacteric. In despite of strawberries are known as a non-climacteric fruit, some postharvest responses had been usually associated with a climacteric organ (Iannetta et al., 2006). Increased β -Gal transcript accumulation was observed in UV-C treated strawberries until the fourth day. It is known that β -Gal promotes flesh softening by removing galactose from cell wall components (Trainotti et al., 2001). β -Gal activity could be also associated with the increase in ascorbic acid by the release of galactose, a precursor

of ascorbic acid (Cruz-Rus et al., 2011) (Fig. 3B). Between the flesh firmness-associated genes evaluated here, the three *PL* genes showed low transcript accumulation (Fig. 1F, G, H), which indicates that these genes could be associated with the prevention of flesh softening with UV-C treatment. These results are in agreement with reports by Jiménez-Bermúdez et al. (2002) who showed that low *PL* activity contributed to maintaining flesh firmness in a transgenic strawberry model. In addition, flesh firmness maintenance in UV-C treated strawberries could also be associated with the expression of other cell wall associated genes not studied here such as β -1,4 endoglucanase, cellulases, cinnamoyl-CoA reductase, and cinnamyl alcohol dehydrogenases (Bianco et al., 2009; Pombo et al., 2009). It is also possible firmness maintenance is related to the induction of phenolic compounds and cell wall lignification and suberization (Charles, Goulet, & Arul, 2008).

3.2. UV-C radiation effects on fruit bioactive compound content and transcriptional expression of associated genes

Total phenolic content was higher in UV-C treated fruit and increased until four days into storage (Fig. 2A). Individual phenolics evaluated here followed the same trend and increased upon UV-C treatment, peaking at four days of storage, except for quercetin which showed the highest content at day six (Fig. 2B, C, D, E, F). Total anthocyanin content was higher in UV-C treated fruit and also increased during storage (Fig. 3A). The increase in total

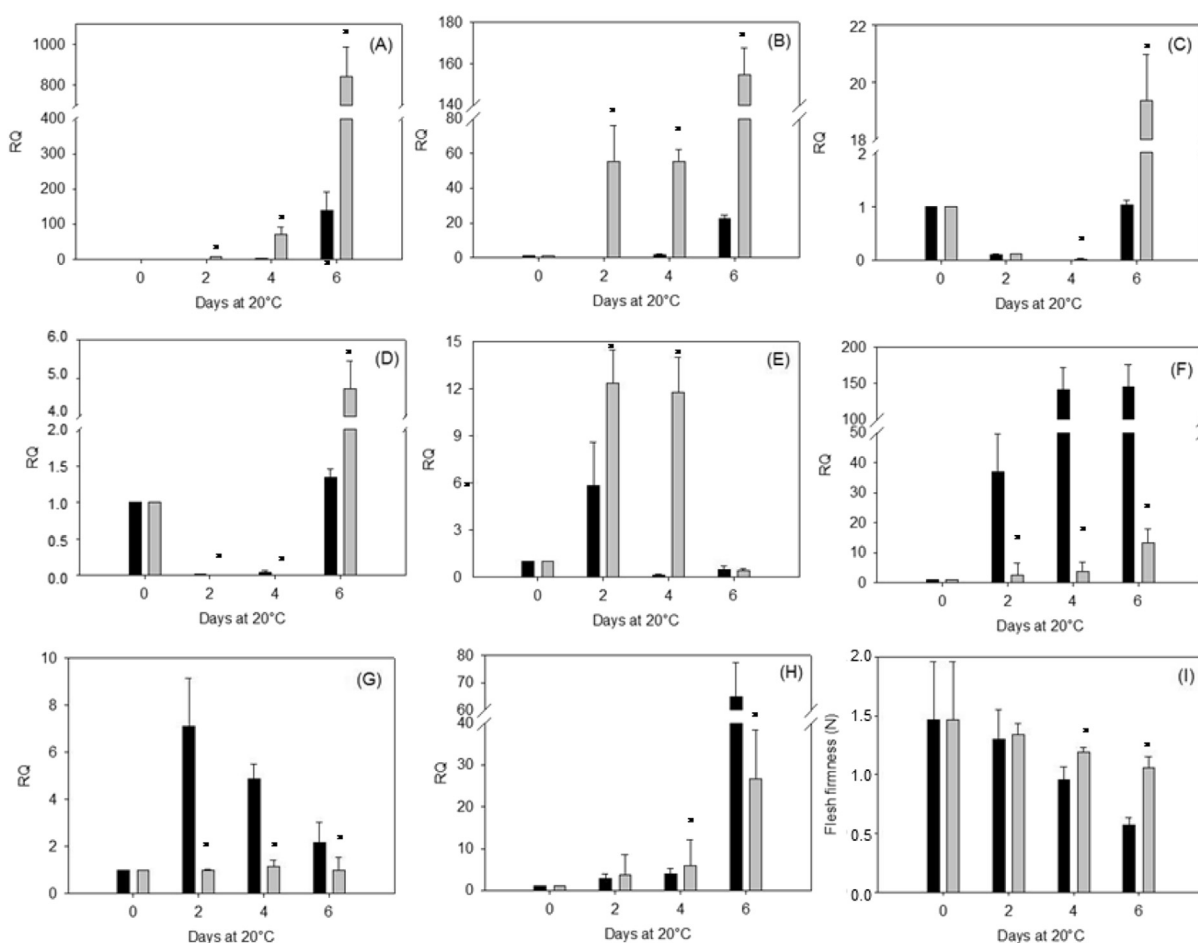


Fig. 1. Transcript accumulation of cell wall associated genes (*Exp* – A, *Exp5* – B, *PME* – C, *PG* – D, β -Gal – E, *PLa* – F, *PLb* – G, *PLc* – H) and flesh firmness (I) of strawberry treated with UV-C (■) vs. Control (■) stored at 20 ± 2°C. The relative quantification (RQ) of transcripts is relative to control fruit at harvest (0) and normalized with β -actin transcripts. The asterisks indicates that the value is statistically different from that of the corresponding control ($P \leq 0.05$). Vertical bars indicate standard deviation.

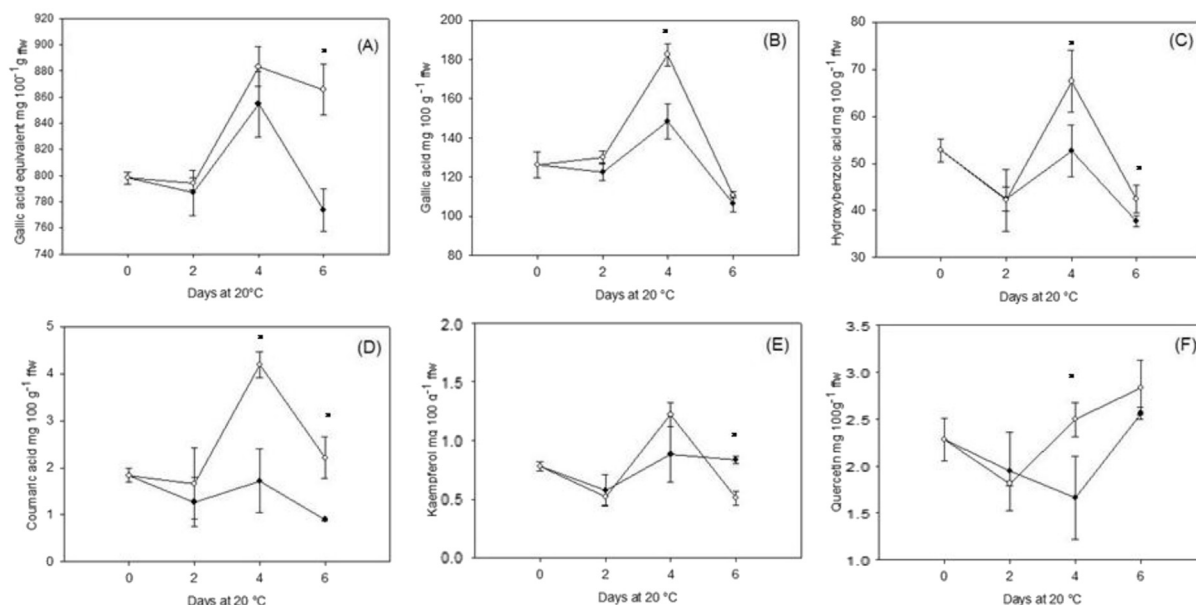


Fig. 2. Total phenolics (A), gallic acid (B), *p*-hydroxybenzoic acid (C), *p*-coumaric acid (D), kaempferol (E) and quercetin (F) in strawberry treated with UV-C (—○—) vs. control (—●—) stored at 20 ± 2 °C. The asterisks indicate that the value is statistically different from that of the corresponding control ($P \leq 0.05$). Vertical bars indicate standard deviation.

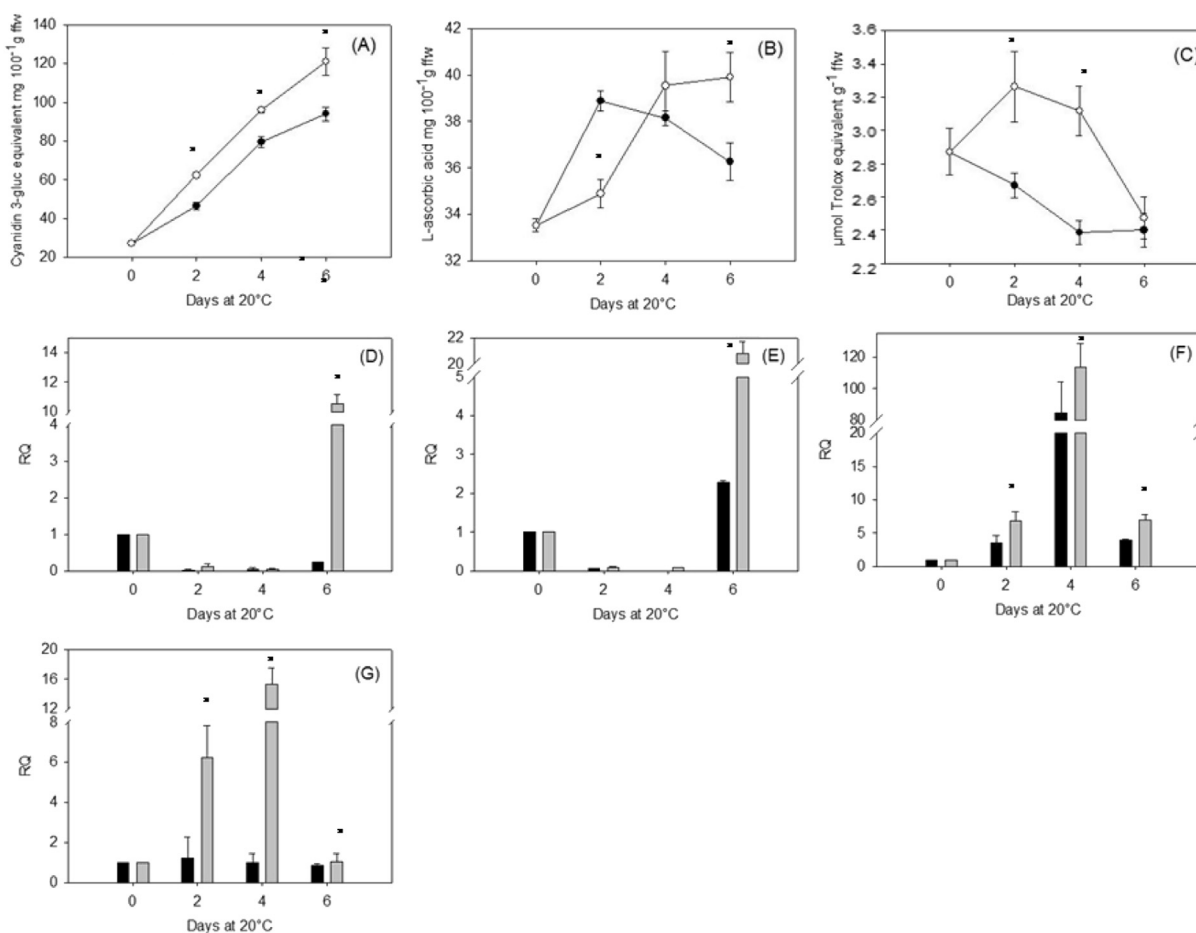


Fig. 3. Total anthocyanin content (A), L-ascorbic acid (B) antioxidant capacity (C) and transcript accumulation of PAL (D), ANS (E), LGalDH (F), and GLDH (G) in strawberry treated with UV-C (—○—) vs. Control (—●—) stored at 20 ± 2 °C. The relative quantification of transcripts (RQ) is relative to control fruit harvest (0) and normalized with β -actin. The asterisks indicate that the value is statistically different from that of the corresponding control ($P \leq 0.05$). Vertical bars indicate standard deviation.

anthocyanin contents during storage of UV-C treated strawberries agrees with a previous report by Erkan et al. (2008). However, it should also be noted that higher UV-C doses could result in the reduction of anthocyanin content in strawberry and potential surface burning Erkan et al. (2008).

PAL is one of the first committed enzymes in the anthocyanidin biosynthesis pathway. This pathway also includes anthocyanidin synthase (ANS) in the final steps, responsible for converting leucoanthocyanidins in anthocyanidins. El Ghaouth et al. (2003) and Pombo et al. (2011) reported increases in PAL activity during peach storage and in strawberries treated with UV-C, respectively. Although PAL and ANS were confirmed as being involved in strawberry ripening, they responded late to UV-C treatment (Severo et al., 2011). Highest transcript accumulation of PAL and ANS occurred in the sixth day of storage in UV-C treated fruits (Fig. 3 D, E). Thus, a direct correlation between the increase in phenolic compounds and anthocyanin compounds obtained in the first days of storage is not apparent.

The highest AA content in UV-C treated fruit was observed in the fourth day of storage, with a subsequent decrease measured on day six (Fig. 3 B). *LGaldH* and *GLDH* transcript accumulation was higher in UV-C treated fruit, particularly on the fourth day of storage (Fig. 3 F, G). Coincidentally, UV-C treated fruit had increased acid ascorbic levels after the fourth day (Fig. 3 B). It is known that biotic and abiotic stresses can interfere with ascorbic acid biosynthesis (Cordenunsi et al., 2005). Since this compound is a natural

antioxidant involved in many metabolic processes occurring during fruit ripening and senescence. The low ascorbic acid content in the second day of storage could be associated with the strong oxidative stress produced by UV-C treatment (Aharoni et al., 2000; Cordenunsi et al., 2005).

The highest antioxidant activity was found in UV-C treated strawberries (Fig. 3C), which is in agreement with findings by Erkan et al. (2008). This activity could be positively associated with phenolic, anthocyanin, and ascorbic acid content. Tabart, Kevers, Pincemail, Defraigne, and Dommes (2009) reported higher antioxidant capacity from catechins such as galocatechin and epigallocatechin, as compared to other phenolic acids and ascorbic acid. Accordingly, the reduction in the antioxidant activity on the sixth day of storage was accompanied by a reduction in phenolic (Fig. 2 A) and ascorbic acid content (Fig. 3 B).

3.3. Ester volatiles and transcript accumulation of associated genes

UV-C treated fruit showed higher production of ester volatiles throughout storage relative to controls (Fig. 4 A, B, C, D, E, F). It is well known that postharvest treatments may influence the biosynthesis of these compounds, but is the first time that an experiment proves the beneficial influence of UV-C radiation in the six more important esters aromatics in strawberry. *ADH* transcript accumulation showed higher in UV-C treated fruits after the second day of storage, while *AAT* was significantly higher after the fourth

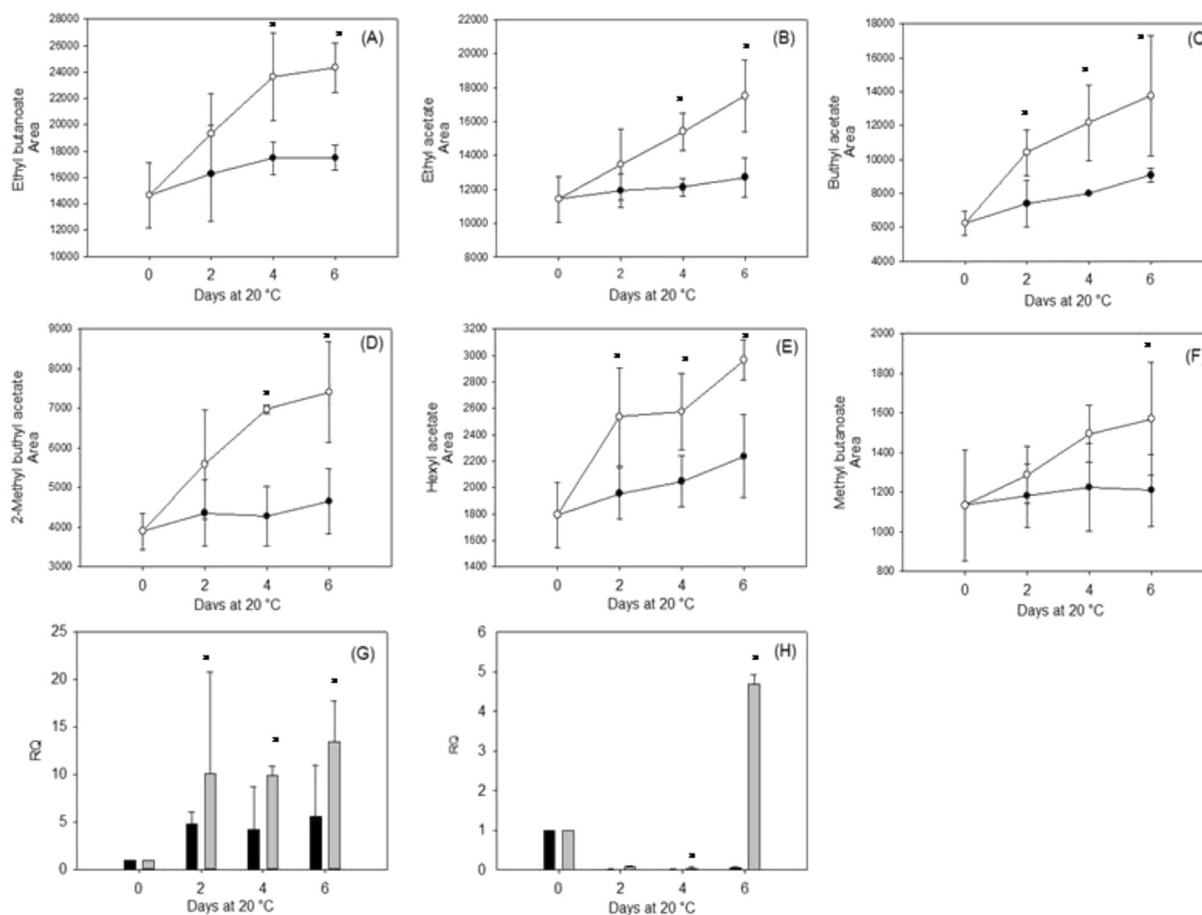


Fig. 4. Relative content of esters volatiles in strawberry treated with UV-C. Ethyl butanoate (A), ethyl acetate (B), butyl acetate (C), 2-methyl butyl acetate (D), hexyl acetate (E), methyl butanoate (F) and transcript accumulation of *ADH* (G) and *AAT* (H) of strawberry treated with UV-C (—○—; —□—) vs. control (—●—; —■—) stored at 20 ± 2 °C. The relative quantification of transcripts (RQ) is relative to control fruit harvest (0) and normalized with β -actin transcripts. The asterisks indicate that the value is statistically different from that of the corresponding control ($P \leq 0.05$). Vertical bars indicate standard derivation.

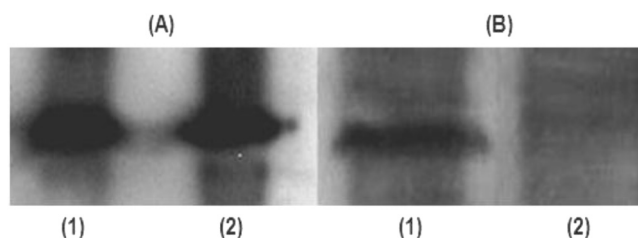


Fig. 5. Immunodetection of β -1,3-glucanases (A) and Fra a1 proteins (B) in strawberry. UV-C (1) vs. Control (2) stored at $20 \pm 2^\circ\text{C}$ for 36 h.

day of storage (Fig. 4 G, H). Pérez et al. (1996) reported the increase in AAT activity in strawberries stored under modified atmosphere, in which anoxic conditions favored alcohol production, providing substrates for ADH and AAT. Alcohol dehydrogenase (ADH) is an important enzyme in ester volatile synthesis providing substrate for alcohol acyl transferase enzyme (AAT) that participates in the final steps of volatile production in strawberry (Aharoni et al., 2000; Pérez et al., 1996; Severo et al., 2011).

3.4. Immunodetection of putatively allergenic proteins

β -1,3-glucanase activity and corresponding gene transcript accumulation have been shown to increase after UV-C application in tomato and strawberry (Charles et al., 2009; Pombo et al., 2011). However, in this study western blotting detection of β -1,3-glucanase protein did not show differences between UV-C treated and control (Fig. 5A). A variety of β -1,3-glucanases genes has been identified in several fruits and the proteins encoded by these genes differ in size, cellular localization and regulation pattern (Casado-Díaz et al., 2006; Charles et al., 2009). The isoform of β -1,3-glucanase identified in this study has a molecular weight of 14.4 kDa, whereas the only isoform of β -1,3-glucanase induced by UV-C in tomato is 33.1 kDa (Charles et al., 2009). Pombo et al. (2011) also reported different expression patterns between *FaBG2-1* and *FaBG2-3* evaluated during storage. In this study, in contrast, Fra a1 protein content was higher in UV-C treated fruit (Fig. 5 B2). Fra a1 is a PR-10 protein which is known to act in defense against pathogens in a hypersensitivity reaction (HR), followed by increased rigidity of the cell wall, synthesis of phytoalexins, phenolic compounds, and defense peptides (Van Loon & Van Strien, 1999). Fra a1 proteins are homologous to Bet V proteins known major food allergens (Karlsson et al., 2004). Furthermore, Muñoz et al. (2010) observed a decrease in gene transcripts of Fra a1 and concomitant loss of red color in strawberries after silencing chalcone synthase (*CHS*) and *PAL* genes. We speculate that the higher anthocyanin content in fruit treated with UV-C could be in part a consequence of higher levels of these Fra a1 proteins. Von Loetzen, Schweimer, Schwab, Rosch, and Hartl-Spiegelhauer (2012) related that Fra a1 proteins have a large cavity in the hydrophobic pocket of the structure, which enables amino acid residues present there to establish interaction with ligands. This interaction may then facilitate the translocation of a substance, as may be the case for the transport of flavonoid molecules responsible for the red color of strawberries (Hjerno et al., 2006; Muñoz et al., 2010). However, further work needs to be done to validate this hypothesis.

4. Conclusions

Strawberry flesh firmness maintenance in UV-C treated fruit was strongly associated with a decrease in pectate lyases transcript accumulation. UV-C exposure also induced a significant increase in phenylpropanoid-derived compounds. However, *PAL* and *ANS*

transcript accumulation profile did not match the early rise in phenolic content. In contrast, the data herein suggests that L-ascorbic acid content may be associated to the up-regulation of *LGaDH* and *GLDH* in UV-C treated fruit. UV-C also stimulated the biosynthesis of ester volatiles responsible for strawberry aroma. This increased volatile production was associated with an up-regulation of *ADH* and *ATT* transcript accumulation. Finally, UV-C also induced the accumulation of the putatively allergenic protein Fra a1 known to participate in plant defense responses.

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