



## Research article

## Study of UV-C treatments on postharvest life of blueberries ‘O’Neal’ and correlation between structure and quality parameters



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## ABSTRACT

The effect of different doses of UV-C light (5.3, 8.3 and 11.4 kJ/m<sup>2</sup>) on native mycobiota and *Botrytis cinerea* incidence, micro and ultrastructure, biomechanical properties and weight loss of blueberry fruit cv. O’Neal during 20 days of storage at 8 ± 1 °C was evaluated. Decay incidence was significantly reduced by all UV-C light doses for both, native mycobiota and inoculated *B. cinerea*. The highest UV-C dose studied (11.4 kJ/m<sup>2</sup>) was the most effective in delaying the onset of fungal and *B. cinerea* infection (6 and 4 days, respectively). UV-C irradiation caused some distinctive changes in fruit structure characterized by redistribution, alteration and partial removal of epicuticular waxes, reinforcement of epicarp cell walls, and modifications in the cuticle. Biomechanical parameters were not affected by UV-C treatments excepting at day 15 where irradiated samples showed higher values of rupture force (F<sub>R</sub>) and deformation (D). Structure changes partially explained the significant increase in weight loss, F<sub>R</sub> and D values in irradiated fruit after 15 days of storage. UV-C irradiation could be an alternative for delaying and reducing fungal infection. However, postharvest shelf-life of irradiated blueberries could be limited by the negative effect on weight loss.

## 1. Introduction

Highbush blueberries have become the second most popular soft fruit species after strawberries. Fresh blueberries are relatively perishable and prone to postharvest pathological and physiological disorders. One of the most important field and post harvest pathogen of this fruit is *Botrytis cinerea* Pers., being the stem scar the predominant site of infection (Tournas and Katsoudas, 2005). Stem end infections can develop and spread to the entire fruit during cold storage, inducing gray mould and producing several cell wall degrading enzymes (Romanazzi et al., 2016). Restrictions on use of synthetic fungicides in the field as well as limitations in the use of hard thermal methods once harvested promoted the use of “non thermal” stressors as a “kill step” for increasing longevity of blueberry crops (Barkai-Golan, 2001; Umagiliyage and Choudhary, 2018; Alzamora et al., 2000; Romanazzi et al., 2016).

UV-C light (wavelength range from 200 to 280 nm in the electromagnetic spectrum) is a simple, environmentally friendly and low cost non thermal alternative. Direct germicidal effect of UV-C is due its ability to damage nucleic material, cytoplasmic membrane integrity and some enzyme activities (Fan et al., 2017; Schenk et al., 2011). Indirect UV-C action is due to the elicitation of defence mechanisms in plants and in harvested organs when it is applied at hormetic doses (Romanazzi et al., 2016; Shama and Alderson, 2005). Low UV-C doses have been demonstrated to induce the production of antimicrobial compounds, slow down ripening and senescence processes, and activate the accumulation of secondary metabolites, mainly phenolic compounds (Umagiliyage and Choudhary, 2018).

The efficacy of UV-C light in inactivating pathogenic and deteriorative microorganisms has been demonstrated in different fruit including berries, naturally or artificially contaminated with *Salmonella enterica*,

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*Escherichia coli* O 157:H7, *B. cinerea*, and *B. dothidea*, (Janisiewicz et al., 2015; Nigro et al., 1998; Pinheiro et al., 2015; Sari et al., 2016; Terao et al., 2015). However limited studies on blueberries have been performed. An inhibitory effect of UV-C on native mycobiota of treated blueberries was reported by Perkins-Veazie et al. (2008); Nguyen et al. (2014); Xu et al. (2016) and Xu and Liu (2017). Nevertheless, their findings about the impact of UV-C exposure on shelf-life extension, weight loss, firmness, bioactive compounds content and other quality traits are not conclusive, and sometimes contradictory. These discrepancies could be associated to many factors affecting UV-C light effectiveness, such as UV-C dose, fruit type and cultivar, maturity stage and storage conditions, species and serotype of microorganisms, initial level and location of contamination/inoculation, and structure characteristics of the fruit (Fan et al., 2017). To the best of our knowledge, there are no reports dealing with the response of *B. cinerea* to UV-C treatments. In addition, changes in the micro and ultrastructure of blueberry fruit have been studied after UV-C light exposure but not during storage, when most changes were reported to occur (Gómez et al., 2011).

In this context, the aim of this work was to assess the effect of UV-C light applied at different doses on native mycobiota and *B. cinerea* incidence, micro and ultrastructure, biomechanical characteristics and weight loss of blueberry fruit (cv. O'Neal) throughout storage at  $8 \pm 1$  °C.

For facilitating a commercial adoption of this technology and increasing the antimicrobial efficacy, the design of the UV-C light device used in this study assured the exposure of the entire blueberry surface.

## 2. Materials and methods

### 2.1. Plant material

Fully-ripe stage organic highbush blueberries (*V. corymbosum* L.) cultivar O'Neal (pH =  $3.4 \pm 0.1$ ;  $12.1 \pm 0.5$  °Brix) with 100 % blue colour in their surface were hand-picked in the early morning in Suipacha, province of Buenos Aires, Argentina. Blueberries that presented fungal development or mechanical injuries were discarded. Fruit with uniform size ( $1.51 \pm 0.05$  cm in diameter;  $1.64 \pm 0.2$  g in weight) were randomly distributed in plastic boxes (35 cm long, 20 cm wide and 15 cm high, filled with ~2 kg of blueberries) and kept under refrigeration at  $4 \pm 1$  °C and 95 % of relative humidity until processed, within a day. Special care was taken to avoid epicuticular wax removal or damage.

### 2.2. UV-C treatment

The UV-C radiation device consisted of four germicidal emitting lamps (maximal emission at 253.7 nm) inside a hermetically closed wooden cabinet covered with aluminium foil. Two lamps were placed at the top of the cabinet (TUV-15W/G13 T8 55V, rated power 15 W, Philips, Holland) and the other two at each side (one left and one right) (TUV-6 W/G6 T5, rated power 6 W, Philips, Holland). A ventilation system

(Ecoclima, Argentina) was used to avoid fruit overheating during UV-C radiation. Initially the fruit was at room temperature  $20 \pm 1$  °C. The maximum temperature reached after UV-C treatments was  $30 \pm 1$  °C. To ensure a homogeneous irradiation of the fruit surface, sixty blueberries were shaken at 250 rpm on a M-23 Vicking orbital shaker (Decalab S.R.L, Argentina) covered with aluminium foil. Samples were located within a uniform area of the radiation field, at 8 cm from the upper lamps and at 30 cm from the lateral lamps. The Figure 1 clarifies the arrangement of all the elements mentioned. The intensity of UV-C irradiation at the fruit surface was measured with a radiometer (Broadband Power/energy Meter 13 PEM 001, Melles Griot, USA). UV-C lamps were turned on 15 min before use to allow its stabilization.

Exposure times of 7, 11 and 15 min were evaluated (fluences: 5.3, 8.3 and 11.4 kJ/m<sup>2</sup>, respectively). Irradiated fruit were compared against two non-irradiated controls: untreated fresh fruit (FF) and blueberries subjected to a rotational movement for 15 min inside the UV-C cabin with the lamps turned off (control 0 kJ/m<sup>2</sup>). UV-C treated fruit and non-irradiated controls were packed in closed air permeable polypropylene boxes (26 cm × 19 cm × 6 cm, 30 blueberries per box) and stored for up to 15 days at  $8 \pm 1$  °C and 90–95 % RH. The storage temperature selected in this work is commonly used in commercial retail sale.

### 2.3. Fungal decay

#### 2.3.1. Native mycobiota

Fungal decay was evaluated daily during storage by visual inspection. Samples that showed fungal development were considered as decayed, regardless of the severity of the infection. Three replicates of 30 blueberries (n = 90) were analysed for each condition. Results were expressed as percentage of decayed fruit.

#### 2.3.2. *Botrytis cinerea*

*B. cinerea* BAFC 3003 strain was provided by BAFC Culture Collection (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina) (<http://www.inmibo.exactas.uba.ar>). Isolates were incubated on malt extract agar (MEA) (Merck, Germany) during 14 days at  $25 \pm 1$  °C; then, conidia were harvested by washing the culture in a Tween 80 (Biopack, Argentina) detergent solution (0.05 % v/v) in peptone water (0.1 % w/v) and gently shaken on a vortex mixer. The final conidia concentration (approximately  $10^7$  conidia/mL) was determined with a Neubauer counting chamber (Exacta, Germany).

Blueberry surface was decontaminated by immersing the fruit in a sodium hypochlorite solution ( $200 \text{ mg.L}^{-1}$ ) for 2 min and then, were rinsed three times with sterile water and dried with towel paper. A spot-inoculation method was used; 10 µl of the conidia suspension were inoculated in a small injury made at the picking scar. Finally, inoculated blueberries were kept at  $21 \pm 2$  °C overnight in a laminar air flow cabinet to promote mould adhesion (Contigiani et al., 2020a).

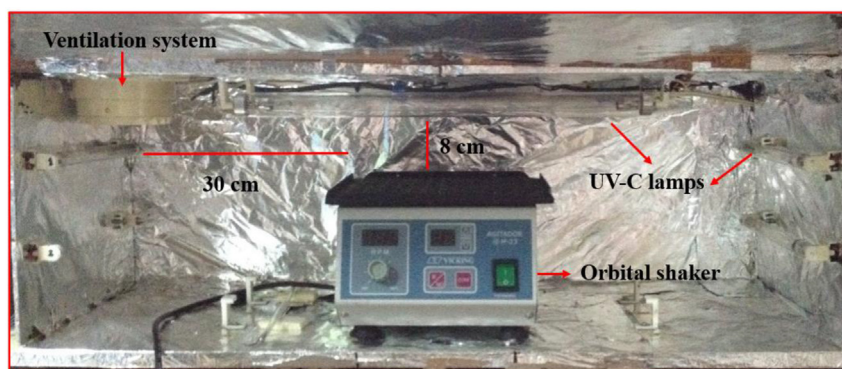


Figure 1. Photograph of the UV-C chamber.

*B. cinerea* incidence was evaluated daily by visual inspection, as previously described for native mycobiota. Untreated inoculated fresh fruit (FF<sub>(BC)</sub>) was considered as control. Three replicates of 20 blueberries (n = 60) were analysed for each condition.

#### 2.4. Light microscopy (LM) observations

Preparation of fruit tissue was performed as previously described by Fava et al. (2006). Briefly, sections (≈3 mm) of FF and irradiated blueberries were fixed in glutaraldehyde solution (3 % w/w) and then in 0.1 M potassium phosphate buffer (pH = 7.4) during 48 h at room temperature. After rinsing three times with distilled water, samples were treated with OsO<sub>4</sub> solution (1.5 % w/w) at room temperature and dehydrated in a graded acetone series prior to be embedded in low viscosity Spurr resin. Sections (1–2 μm thick) of the Spurr-embedded tissue were cut on a Sorvall MT2-B Ultracut microtome and stained with toluidine blue (1 % w/w) and basic fuchsin (1 % w/w) solutions. Samples were observed under a Zeiss AxiosKop 2 light microscope (Carl Zeiss AG, Jena, Germany). Images were captured with a Cannon EOS 1000D camera (Cannon, Tokyo, Japan) and analysed with the Axio Vision 4.8.2 Software package (Carl Zeiss AG, Jena, Germany). Laboratory reagents were purchased from Merck Química Argentina S.A. (Buenos Aires, Argentina).

#### 2.5. Environmental scanning electron microscopy (ESEM) observations

ESEM observation were carried out according to Fava et al. (2006) with slight modification. Whole blueberries (FF, control 0 kJ/m<sup>2</sup> and irradiated samples) without previous preparation were placed in the sample holder of an Environmental Scanning Electron Microscope (Philips XL 30, Holland) and observed at a pressure of 0.9 Torr and a voltage acceleration of 20.0 kV.

#### 2.6. Mechanical test

Biomechanical properties were evaluated at 0, 5, 10 and 15 days of storage by a puncture test, using an Instron Testing Machine model 3345 (Instron, Canton, Massachusetts, USA) with a flat-end cylindrical probe (diameter: 0.003 m), at a penetration speed of 0.05 m.min<sup>-1</sup>, and a 5 N-load cell. Puncture measurements were performed on the equatorial side of the fruit (perpendicular to the abscission zone) at 25 ± 1 °C. From the force-displacement curve four mechanical parameters were obtained: F<sub>R</sub>, D<sub>R</sub>, W and Stif. F<sub>R</sub> is the maximum force required to puncture the blueberry epidermis; D<sub>R</sub> represents the probe displacement at the epidermis rupture point; W corresponds to the mechanical work needed to puncture the epidermis, calculated by the area under the curve up to the epidermis rupture point; and Stif represents the stiffness, calculated as the slope of the initial linear portion of the force/displacement curve (Rolle et al., 2012). Thirty berries were analysed for each condition and storage time.

#### 2.7. Weight loss

Weight loss of irradiated and control blueberries was determined at 5, 10, and 15 days of storage. Results were expressed as the percentage of weight loss compared to the weight of blueberries at day 0, according to Eq. (1) (Contigiani et al., 2020b).

$$WL(\%) = \frac{W_0 - W_t}{W_0} \times 100 \quad (1)$$

where WL (%) is the percentage of weight loss, W<sub>0</sub> is the initial weight of each blueberry and W<sub>t</sub> is the weight at time t. Thirty fruits were evaluated for each condition.

#### 2.8. Statistical analysis

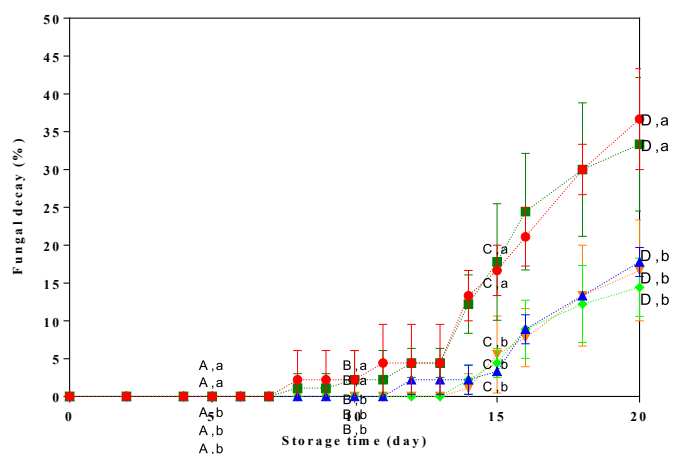
Statistical analyses were carried out using SPSS software v.19 (SPSS Inc, Chicago, USA) and InfoStat v. 2008 (InfoStat Group, FCA, UNC, Córdoba, Argentina). Two-way analysis of variance (ANOVA) was used to compare the effect of “UV-C treatment” and “storage time” on decay incidence data. Mean values were compared by Tukey's test. Prior to conducting analyses, assumptions of normal distribution and homogeneous variance among groups were verified. Weight losses of blueberries were analysed by a general linear mixed model (GLMM) followed by LSD Fisher post hoc tests. The Akaike Information Criterion (AIC) was used for choosing the best-fitting model as a minimal adequate one. The GLMM analysis was conducted by using the lme function (lme4 package, R Core Team, 2014). When variances were not homogeneous, the variance structure of the residual was corrected using VarExp, VarIdent or VarPower option (nlme package). Multivariate analysis of variance (MANOVA) followed by Hotelling test with the Bonferroni correction were used to detect differences in the mechanical properties of the samples. Multivariate outliers were detected by the Mahalanobis distance and removed from the dataset. Significance level was set at 0.05.

### 3. Results

#### 3.1. Fungal incidence

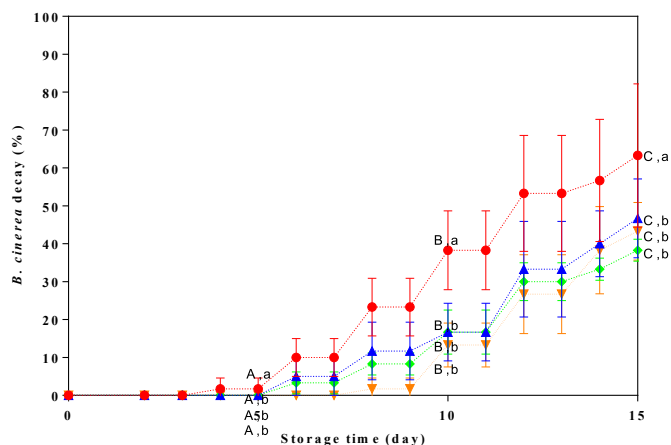
The effect of UV-C treatments on native mycobiota incidence during storage is shown in Figure 2. The ANOVA indicated that main effects were significant (p < 0.0001 and; p < 0.0001, respectively) but their interaction was not (p = 0.680). Therefore, refrigerated storage affected both irradiated and control samples in the same way, showing an increase in the percentage of infected fruit during storage. Irradiated samples presented a delay on the onset of infection of 4 (5.3 kJ/m<sup>2</sup>) and 6 (8.3 and 11.4 kJ/m<sup>2</sup>) days when compared to FF and control 0 kJ/m<sup>2</sup>. In addition, the percentage of infected fruit in irradiated blueberries after 20 days of storage was significantly lower (~50 %) than both controls. However, differences among the UV-C doses assayed were not significant.

*B. cinerea* infection in inoculated fresh (FF<sub>(BC)</sub>) and irradiated fruit during storage is shown in Figure 3. No significant interaction between



**Figure 2.** Mycobiota decay incidence in fresh fruit (FF), control 0 kJ/m<sup>2</sup> and irradiated blueberries stored during 20 days at 8 ± 1 °C. (red circle) FF (untreated fruit); (dark green square) 0 kJ/m<sup>2</sup>; (blue triangle) 5.3 kJ/m<sup>2</sup>; (light green diamond) 8.3 kJ/m<sup>2</sup>; (orange triangle) 11.4 kJ/m<sup>2</sup>. Values are the mean of samples, and vertical bars represent standard deviation (n = 90). Different uppercase letters indicate significant differences throughout storage time. Different lowercase letters indicate significant differences between treatments (p < 0.05). To avoid excessive overlapping of text boxes, statistical results were only shown on days 5, 10, 15 and 20 of storage.





**Figure 3.** *B. cinerea* decay incidence in inoculated fresh fruit (FF<sub>(BC)</sub>) and irradiated blueberries stored during 15 days at  $8 \pm 1$  °C. (red circle) FF<sub>(BC)</sub>; (blue triangle) 5.3 kJ/m<sup>2</sup>; (light green diamond) 8.3 kJ/m<sup>2</sup>; (orange triangle) 11.4 kJ/m<sup>2</sup>. Values are the mean of samples, and vertical bars represent standard deviation (n= 60). Different uppercase letters indicate significant differences throughout storage time. Different lowercase letters indicate significant differences between treatments ( $p < 0.05$ ). To avoid excessive overlapping of text boxes, statistical results were only shown on days 5, 10 and 15 of storage.

“treatment” and “storage time” was observed ( $p = 0.2519$ ) as observed for native mycobiota results. The percentages of infected fruit by *B. cinerea* in irradiated blueberries were significantly lower ( $p < 0.0001$ ) than in FF<sub>(BC)</sub>; and similarly to the native mycobiota incidence, there were no significant differences among the UV-C doses assayed. There was at least a 2-day delay on the onset of *B. cinerea* infection in irradiated blueberries when compared to FF<sub>(BC)</sub>, but the highest dose (11.4 kJ/m<sup>2</sup>) delayed the infection in 4 days. *B. cinerea* decay in

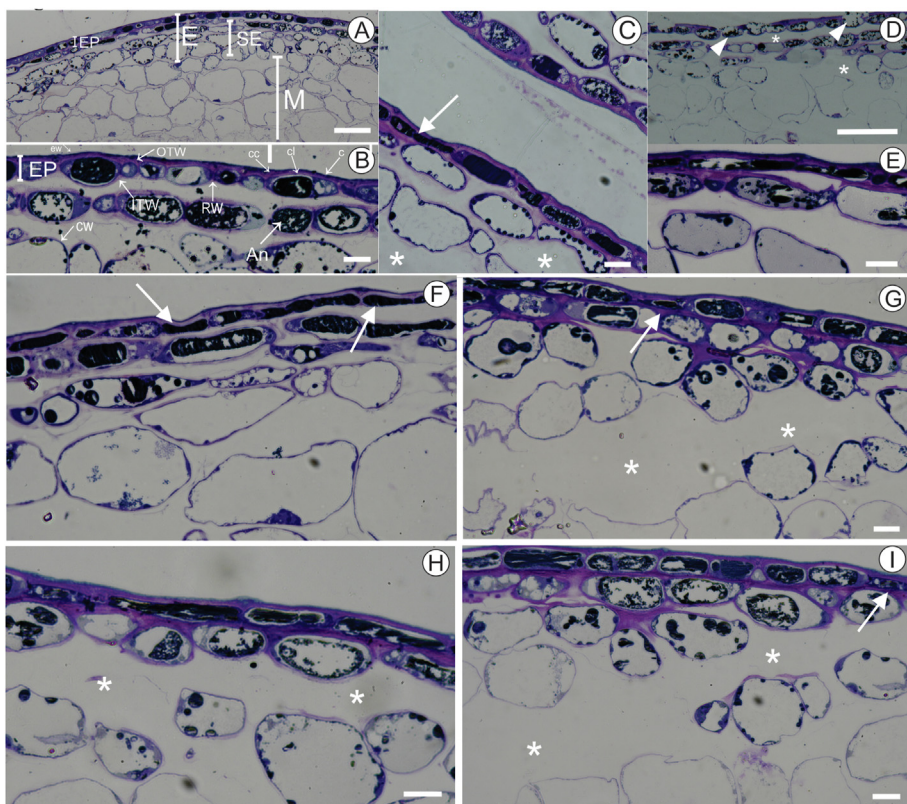
irradiated berries was reduced by 32–40 % when compared to inoculated untreated fruit at day 15.

### 3.2. Microstructure features

LM images of transversal sections of FF, control (0 kJ/m<sup>2</sup>) and irradiated (5.3 and 11.4 kJ/m<sup>2</sup>) blueberry tissues at 0, 10 and 15 days of storage were evaluated. The microstructure of blueberry fruit was described in previous studies (Jaramillo et al., 2019; Fava et al., 2006) therefore, only some distinctive aspects of the epicarp (E) and the mesocarp (M) of FF will be mentioned forward (Figure 4A and B). Briefly, the epicarp was composed by a layer of quadrangular to rectangular epidermal cells (EP), followed by two or three layers of more rounded and more loosely attached subepidermal collenchymatous cells (SE) (hypoderm). Outer and inner tangential walls (OTW and ITW, respectively) showed intense staining. In the OTW, the cuticle(c), the cutinized layer (cc) and the cellulose layer (cl) could be distinguished by different staining density. In some parts, epicuticular waxes (ew) could be detected covering the cuticle. ITW appeared thinner and less stained than OTW and radial epidermal walls (RW). Anthocyanins (An), in the form of small and large globoids, were located in the epidermal and hypodermal cells. The mesocarp (M) exhibited turgent cells, irregular or rounded in shape, with parietal cytoplasm and separated by conspicuous intercellular spaces.

Control 0 kJ/m<sup>2</sup> and FF tissues exhibited similar microstructure traits, although rotation of the berries in the tray provoked flattening of epidermal cells in some regions (white arrow) (Figure 4C). Most of epidermal cells showed parietal cytoplasm and turgid central vacuoles with visible anthocyanins clumps and no alteration of OTW.

Immediately after 5.3 and 11.4 kJ/m<sup>2</sup> UV-C irradiation (Figure 4F and H respectively) tissues microstructure showed slight differences in comparison with FF. In some regions, the whole epicarp exhibited slight tangential flattening and plasmolysis, not only seen in epidermal cells but



**Figure 4.** LM images of transverse sections of irradiated and control blueberry tissues. A, B, C, F, H: day 0; D, E, G, I: day 15. A, B, D, E: FF; C: control 0 kJ/m<sup>2</sup>; F–H: irradiated at 5.3 kJ/m<sup>2</sup>; G–I: irradiated at 11.4 kJ/m<sup>2</sup>. E: epicarp; M: mesocarp; An: anthocyanin deposits; EP: epidermis; OTW: outer tangential wall; ITW: inner tangential wall; SE: subepidermis; c: cuticle proper layer; cc: cutinized layer; cl: cellulose layer; cw: cell wall; ew: epicuticular waxes; →: tangential compaction of epidermal cells; \* : intercellular spaces; +: tannins granularities. Scale: A and E: 100 μm; B–J: 20 μm.

also in sub-epidermal ones. However, in other areas, irradiation did not modify tissue structure.

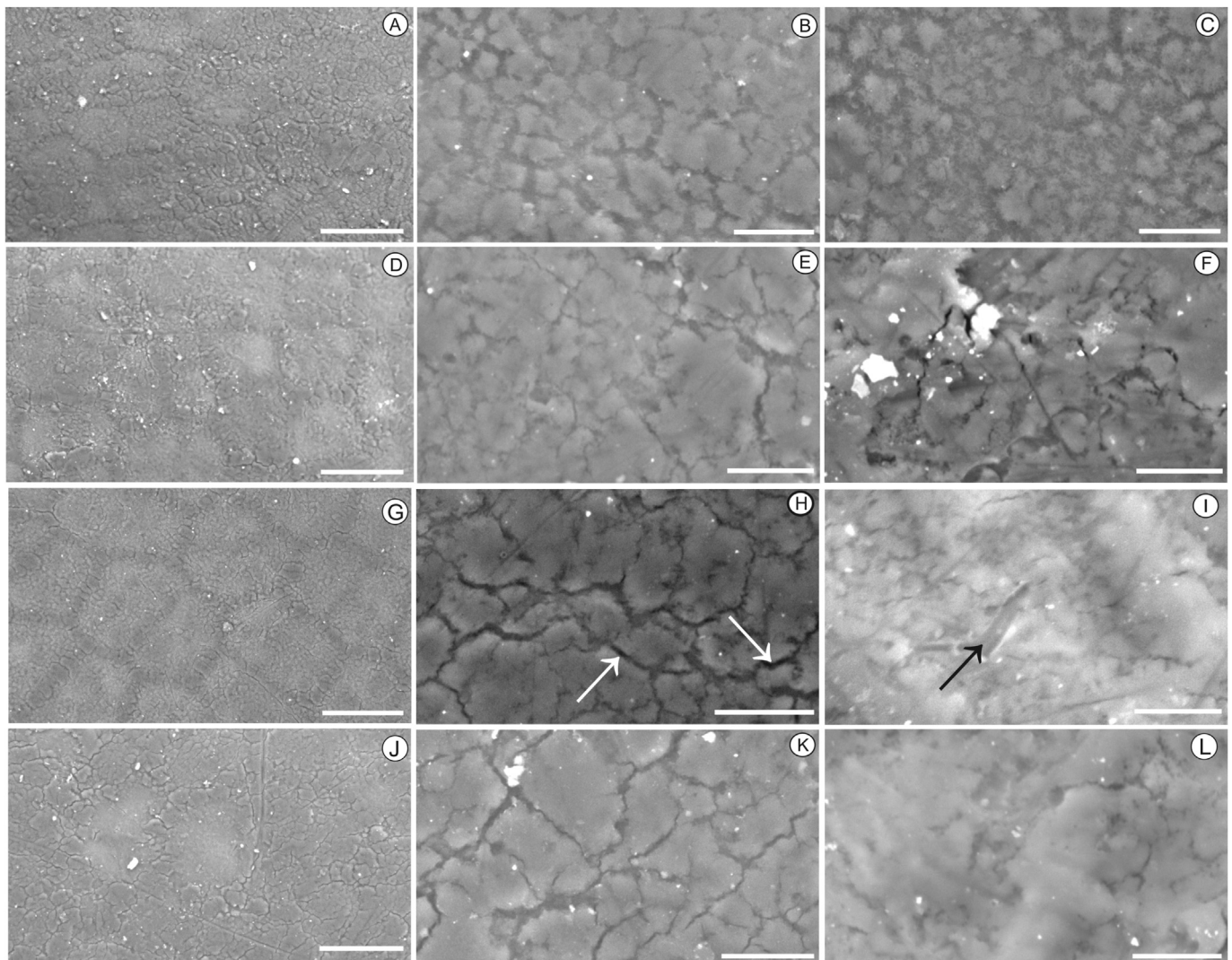
Microstructure of FF and irradiated fruit after 10 days under cold storage showed slight differences with respect to day 0 (data not shown). Cold storage induced in both, FF and irradiated samples, a strengthening of epicarp walls. OTW and ITW maintained the staining density but the thickness of ITW was much wider than at day 0. A much reinforced cell-to-cell adhesion was observed between epidermal cells and the cells of the first layer of the hypodermis. Skin cells showed a very dense cytoplasm, with some tannins granularities. As light tangential cell contraction and some alterations in the cuticle coloration were detected in irradiated blueberry tissues. Intercellular spaces in the epidermis of samples exposed to  $5.3 \text{ kJ/m}^2$  were similar to those observed in FF tissues; however, irradiated tissue at  $11.4 \text{ kJ/m}^2$  exhibited larger spaces among hypodermal cell layers.

After 15 days of storage, epicarp and mesocarp of FF showed compaction, and epidermal cells looked tangentially flattened with signs of plasmolysis (Figure 4D,E). A slight increase in intercellular spaces was detected between subepidermal cells. In some regions, content of epidermal and subepidermal cells appeared conserved, although contracted (Figure 4E). In others, rupture episodes of OTW

(extended from the cuticle to the walls of epidermal cells), as well as no contact between epidermal and hypodermal cells were observed (Figure 4D). Walls in the mesocarp cells appeared with low staining. Irradiated tissues showed a notorious reinforcement of junction areas between epidermis and subepidermis (first layer and in some parts second layer of cells) (Figure 4G,I). Alterations in the cuticle were evidenced by different colour staining. Detachment of the skin (epidermal and hypodermal cells) from the fruit flesh was also observed (Figure 4G,I).

### 3.3. Ultrastructure features

ESEM images of irradiated and control berries at day 0 and day 15 are shown in Figure 5. Figure 5A,B showed a general aspect and a detail of the surface of FF epidermis at day 0, respectively. The whole surface was covered by a discontinuous and reticulated wax layer which exhibited a heterogeneous aspect. Partially amorphous waxes appeared densely arranged in form of patches, surrounded by bands or channels with lower dense crystalline wax structures in the form of rods, rodlets and platelets (Figure 5B). Below the surface covered by abundant wax coating, the contour of epidermal cells could be detected (Figure 5A). General aspects



**Figure 5.** ESEM images of superficial sections of irradiated and control blueberry tissues. A, B, D, E, G, H, J, K: day 0; C, F, I, L: day 15. A–C: FF; D–F: control  $0 \text{ kJ/m}^2$ ; G–I: irradiated at  $5.3 \text{ kJ/m}^2$ ; J–L: irradiated at  $11.4 \text{ kJ/m}^2$ . Scale: A, D, G, J:  $100 \mu\text{m}$ ; B, C, E, F, H, I, K, L:  $20 \mu\text{m}$ . White arrows: absence of waxes; black arrow: rupture in epidermis.



of wax layer in control 0 kJ/m<sup>2</sup> samples were observed similar to those of FF (Figure 5D). However, a detailed image showed a slight redistribution of waxes, which resulted in a reduction of band or channel areas and an increase in patches size (Figure 5E). UV-C treatment, regardless of the dose, induced not only slightly larger amorphous patches but a reduction in the content of crystalline waxes, detected in the channels around (Figure 5G,H,J,K). Moreover, absence of waxes in some sites (white arrow) allowed seeing the cuticle (Figure 5H).

After 15 days of storage, wax layer in FF covered the whole surface of the fruit and presented a similar arrangement than at day 0, although it appeared thinner, less dense, more translucent, and loosely attached (Figure 5C). In control 0 kJ/m<sup>2</sup> fruit, waxes characteristics did not show severe changes respect to day 0; however, in some sites the presence of crystalline waxes was minor (Figure 5F). Epicuticular waxes in stored irradiated samples showed important alterations. They looked disorganized and more amorphous, mainly in tissues irradiated at the highest dose (Figure 5I,L). Some rupture episodes in the epidermis of irradiated fruit (black arrow) were also detected (Figure 5I).

Waxes redistribution could be due to fruit movement in the orbital shaker: berries rubbed each other and with the shaker platform. However, structure differences among irradiated and control 0 kJ/m<sup>2</sup> fruit would indicate a specific effect of UV-C treatment on waxes.

### 3.4. Biomechanical properties

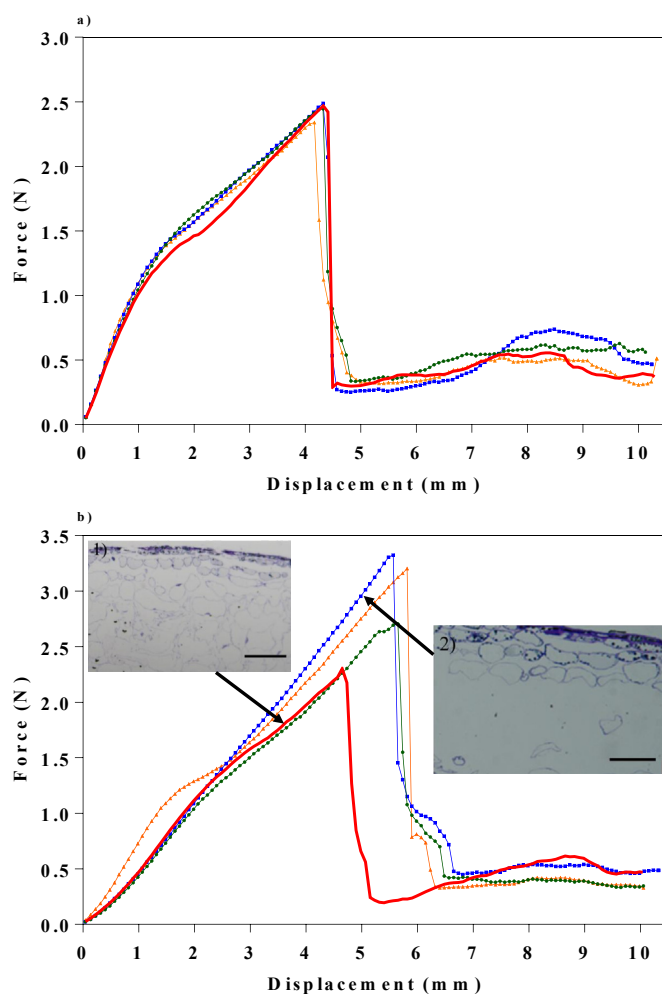
Force-displacement curves from puncture test were registered since the moment the probe touched the sample (epicarp) until it penetrated the whole fruit (mesocarp) (Figure 6). In irradiated, FF and control 0 kJ/m<sup>2</sup> blueberry fruit, with or without storage, skin (outer surface layers plus epidermal and subepidermal tissues) contributed between 78 % and 85 % of the firmness before the rupture point. Mean values of biomechanical parameters ( $F_R$ ,  $D_R$ ,  $W$  and  $Stif$ ) are shown in Table 1. Mechanical work was excluded from multivariate analyses since this parameter showed a strong and positive correlation with force and displacement (Pearson coefficient = 0.87). Significant interaction between “treatment” and “storage time” was observed ( $p < 0.0001$ ). Namely, the refrigerated storage did not cause the same changes in the mechanical parameters of the irradiated fruits compared to the control. Irradiated, FF and control 0 kJ/m<sup>2</sup> fruit exhibited similar mechanical behaviour except at day 15, when significant although small differences were detected.  $F_R$  values showed a slight increase in all samples up to 10 days of storage, but at day 15,  $F_R$  of irradiated samples were significantly larger than those of FF at day 0 and day 15.  $D_R$  values increased (mainly in irradiated berries), while  $Stif$  values decreased throughout storage.  $W$  values of irradiated fruit at day 15 were greater in comparison with FF, indicating UV-C induced skin resistance to rupture.

### 3.5. Weight loss

Weight loss of FF, control 0 kJ/m<sup>2</sup> and irradiated blueberries throughout cold storage is shown in Figure 7. No significant interaction between “treatment” and “storage time” was observed ( $p = 0.064$ ) but main effects of each factor were statistically significant. Refrigerated storage caused a significant increase in weight loss ( $p < 0.0001$ ) in all fruits (irradiated, FF and control 0 kJ/m<sup>2</sup>). Differences among all the UV-C doses tested were not significant and all of them increased the loss of weight ( $p < 0.0001$ ) when compared to FF and control 0 kJ/m<sup>2</sup>. On the other hand, the 0 kJ/m<sup>2</sup> control exhibited greater weight losses than the FF, which could be attributed to the effect shaking had on the epicuticular wax layer.

## 4. Discussion

Postharvest treatment with UV-C light has been previously investigated in an attempt to inhibit native mycobiota in blueberry fruit. Xu et al. (2016) and Xu and Liu (2017) evaluated the UV-C light effect (4



**Figure 6.** Typical penetrometric force-displacement curves for irradiated, control 0 kJ/m<sup>2</sup> and fresh blueberry fruit: a) 0 day and b) 15 day. (red line) FF; (dark green circle) control 0 kJ/m<sup>2</sup>. Irradiated fruit: (blue square) 5.3 kJ/m<sup>2</sup>, (orange triangle) 11.4 kJ/m<sup>2</sup>. Major contribution to firmness before rupture point in irradiated and control fruit was giving by the skin (outer surface layers plus epidermal and subepidermal tissues). LM images showed a general aspect of FF (1) and 5.30 kJ/m<sup>2</sup> irradiated (2) tissues at day 15 (scale 100  $\mu$ m). The notorious detachment of the skin from the mesocarp in irradiated fruit would be responsible for the greater  $D_R$  value observed in irradiated fruit in comparison with untreated one.

kJ/m<sup>2</sup>) on blueberries cv. Berkeley at two harvest years (2016 and 2017) and, after 8 days of storage ( $4 \pm 1$  °C) irradiated blueberries showed a significant reduction of fungal infection (15 and 28 %, respectively) when compared to untreated fruit (22 and 32 %, respectively), although a delay on the onset of the infection was not detected. Similar results were reported by Nguyen et al. (2014) in blueberries (cv. Duke) irradiated with 6 kJ/m<sup>2</sup> and stored for 28 days at 0 °C and 95 % RH. These authors observed a delay (7 days) and a reduction of the infection of 7.2 % in UV-C treated samples respect to untreated fruit. Perkins-Veazie et al. (2008) reported a reduction of fungal incidence (10 %) in UV-C treated (1–4 kJ/m<sup>2</sup>) blueberries 'Collins' and 'Bluecrop' after 7 days of storage at 5 °C and 2 days at 20 °C (to simulate retail conditions).

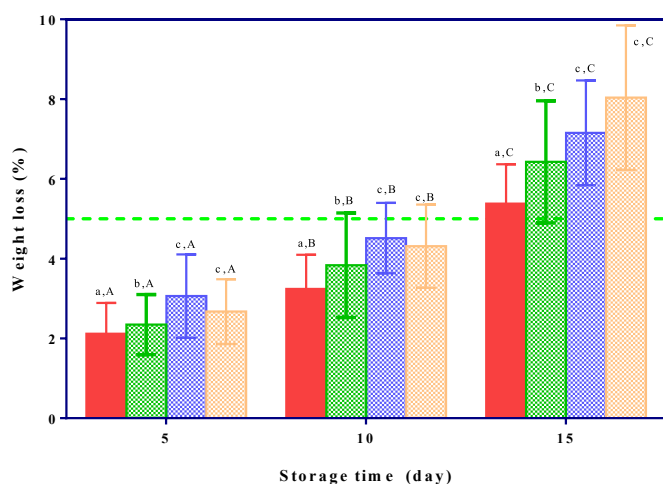
In comparison with the literature, native mycobiota incidence was more effectively reduced by the UV-C treatments applied in this study. The observed differences could be associated to the higher UV-C doses applied and the more homogeneous irradiation due to shaking as well as to the different cultivar and epiphytic fungal community. However, it is remarkable that for both native mycobiota and *Botrytis cinerea* incidence,

**Table 1.** Biomechanical parameters of irradiated and control blueberries stored at  $8 \pm 1$  °C during 15 days.

Day	Treatment	$F_R$ (N) $\pm$ SD	$D_R$ (mm) $\pm$ SD	W (mJ) $\pm$ SD	Stif (N/mm) $\pm$ SD	
0	FF	$2.4 \pm 0.4$	$4.2 \pm 0.7$	$6.0 \pm 1.4$	$0.93 \pm 0.3$	a
	0 kJ/m <sup>2</sup>	$2.4 \pm 0.4$	$4.2 \pm 0.9$	$5.9 \pm 2.0$	$0.94 \pm 0.3$	a
	5.3 kJ/m <sup>2</sup>	$2.4 \pm 0.4$	$4.2 \pm 0.6$	$6.0 \pm 1.5$	$0.90 \pm 0.2$	a,b
	11.4 kJ/m <sup>2</sup>	$2.3 \pm 0.4$	$4.3 \pm 0.6$	$5.9 \pm 1.6$	$0.85 \pm 0.2$	a
5	FF	$2.4 \pm 0.6$	$4.4 \pm 0.9$	$5.7 \pm 1.8$	$0.80 \pm 0.4$	a,b, f,g
	0 kJ/m <sup>2</sup>	$2.6 \pm 0.5$	$4.5 \pm 0.7$	$6.4 \pm 1.7$	$0.82 \pm 0.3$	a,b, d, f,g
	5.3 kJ/m <sup>2</sup>	$2.8 \pm 0.5$	$4.8 \pm 0.7$	$7.3 \pm 1.8$	$0.82 \pm 0.3$	d,e,f,g
	11.4 kJ/m <sup>2</sup>	$2.7 \pm 0.6$	$4.8 \pm 0.7$	$7.3 \pm 2.1$	$0.83 \pm 0.2$	a,b, d,e,f,g
10	FF	$2.8 \pm 0.5$	$4.3 \pm 0.5$	$6.8 \pm 1.4$	$0.87 \pm 0.2$	b, e,f
	0 kJ/m <sup>2</sup>	$3.0 \pm 0.6$	$4.8 \pm 0.6$	$7.7 \pm 1.6$	$0.87 \pm 0.2$	d,e,f,g
	5.3 kJ/m <sup>2</sup>	$2.8 \pm 0.6$	$4.9 \pm 0.8$	$7.5 \pm 2.0$	$0.78 \pm 0.3$	c,d,e,f,g
	11.4 kJ/m <sup>2</sup>	$3.0 \pm 0.6$	$5.2 \pm 0.9$	$8.6 \pm 2.2$	$0.85 \pm 0.3$	d,e
15	FF	$2.4 \pm 0.5$	$4.9 \pm 1.1$	$6.5 \pm 2.0$	$0.66 \pm 0.2$	g
	0 kJ/m <sup>2</sup>	$2.6 \pm 0.7$	$5.5 \pm 1.0$	$7.6 \pm 2.5$	$0.61 \pm 0.2$	e
	5.3 kJ/m <sup>2</sup>	$3.2 \pm 0.5$	$5.5 \pm 0.9$	$8.7 \pm 1.9$	$0.66 \pm 0.2$	h
	11.4 kJ/m <sup>2</sup>	$3.2 \pm 0.7$	$5.4 \pm 1.0$	$8.6 \pm 2.4$	$0.69 \pm 0.2$	h

Mean values  $\pm$  standard deviation (SD) of mechanical parameters of blueberries ( $n = 30$ ).

Different letters indicate significant differences ( $p < 0.05$ ) between treatments.



**Figure 7.** Weight loss (%) for irradiated, control 0 kJ/m<sup>2</sup> and fresh blueberry fruit throughout storage at  $8 \pm 1$  °C. (red square) FF; (green square) control 0 kJ/m<sup>2</sup>. Irradiated fruit: (blue square) 5.3 kJ/m<sup>2</sup>; (orange square) 11.4 kJ/m<sup>2</sup>. At the same storage day, means followed by different lowercase letter indicate significant differences between treatments. Means followed by different uppercase letter indicate significance differences throughout storage time ( $n = 30$ ) ( $p < 0.05$ ). Dotted line indicates the maximum acceptable weight loss according to Salunkhe et al. (1991).

the UV-C doses evaluated in this study showed similar effects. The change observed in wall architecture of irradiated fruit could contribute to a lower decay incidence and could be attributed to an hormetic effect.

Reports about the effect of UV-C light on blueberry softening were only focused in the instrumental measurement of firmness, without taking into account other important parameters that help understanding the whole mechanical behaviour, such as those related to probe displacement after the rupture of the epidermis. According to Xu et al. (2016), UV-C irradiation ( $4 \text{ kJ/m}^2$ ) delayed softening of ‘Berkeley’ blueberries when compared with untreated samples after 8 days of storage at  $4 \pm 1$  °C. Contrary, Xu & Liu (2017) reported no effect of the same UV-C dose and storage conditions. Nguyen et al. (2014) found that ‘Duke’ blueberries subjected to UV-C at  $6 \text{ kJ/m}^2$  showed higher firmness values ( $\sim 25\%$ ) than untreated fruit after 28 days at  $0$  °C. Our results are in partial agreement with existing literature:  $F_R$  values of UV-C treated

fruit and controls resulted similar during cold storage, except at day 15 when rupture force of irradiated fruit showed slightly greater values.

Biomechanical behaviour of fruit depends on the structure features of the cellular conglomerate that compose the tissues (Aguilera and Stanley, 1999). In soft fruit with a thick epicarp, such as blueberries, the reaction to compression and probe penetration is almost exclusively exerted by the skin, which serves as a mechanical support to the pulp. Thus, key structure factors determinant of mechanical properties include the integrity and rigidity of OTW, ITW, RW and others cell walls, turgor (the forced exerted on cell membrane by intracellular fluid), cell – cell adhesion (determined by middle lamella and plasmodesmata) and volume of intercellular spaces in the epicarp (Chen et al., 2015). Based on fine structure studies, OTW can be distinguished from all other cell walls of fruit by the presence of the following layers, from outer to inner: (1) epicuticular waxes (amorphous layer, crystalline or semi-crystalline); (2) cuticle (constituted only by cutin); (3) cutinised or fibrillar layer (mainly composed by a polysaccharide matrix, cutin and intracuticular waxes); (4) pectic layer (that links cutinised layer to the epidermis; and (5) cellulosic layer (composed mainly of cellulose, hemicelluloses, pectic polysaccharides, and additional minor components such as phenolic substances and proteins) (Esau, 1953; Fava et al., 2006).

Present results could be partially related to the changes observed in epicarp microstructure (Figure 4). After treatment and up to 10 days of storage, minor structure changes observed in the epicarp of irradiated fruit in comparison with untreated berries were not traduced in significant differences in biomechanical parameters among different samples. This could be partially explained by the high biological variability within and between berries, either fresh or treated, and the consequent high deviation of the mechanical parameters that may mask the effect of slight structure modifications. Slight decreases in Stif values during storage in all berries could be attributed to plasmolysis of the epidermis and sometimes subepidermal cells, associated with loss of turgor; and to the lower cell-to-cell contact with the formation of empty spaces between the skin and the parenchyma, or between epicarp layers.

At day 15, the maintenance of  $F_R$  values in FF could be partially ascribed to opposing effects: compression of epidermis (dense packed cellulose microfibrils) versus degradation and micro cracks occurrence in the OTW. Cell wall disassembly in untreated fruit could be associated with increased water soluble pectin content and decreased levels of sodium carbonate soluble pectin, hemicellulose and cellulose and the reduced activities of cell wall degrading enzymes (Chen et al., 2015). On contrary, the notorious reinforcement of OTW, ITW and RW structure,

extended in some sites to the walls of the cells in deeper layers of sub-epidermis, were reflected in higher  $F_R$  values of irradiated fruit. The increase in intercellular spaces, more notorious in fruit irradiated at the highest dose, could be also responsible for the increase in D values, due to a lower resistance to deformation in the tissues beneath the epidermis (Figure 6).

Disease resistance against a wide range of pathogens, delayed physiological processes and inhibition of the expression of cell wall degrading enzyme activities elicited by UV-C in berries are well documented (Umagiliyage and Choudhary, 2018). However, information about cell wall strengthening mechanisms induced by UV-C in fruit is scarcer. An hormetic dose (3.7 kJ/m<sup>2</sup>) of UV-C caused ultrastructure modifications of the pericarp in tomato fruit (Charles et al., 2008, 2009). Cell wall stacking zones caused by plasmolysis and collapse of epicarp cells and accumulation of protective phenolic compounds such as lignin and suberin deposited on cell walls could serve as reinforced barriers against *B. cinerea* development. An increase in cellulose, hemicellulose, neutral sugars and pectins were found in UV-C treated strawberry (Langer et al., 2018). In cotyledons and grape leaves, changes in cell wall architecture and composition under UV-B light provided mechanical barriers through lignin deposition (Le Gall et al., 2015). Other biotic and abiotic elicitors induced oxidative cross-linking of cell wall structural proteins in peripheral tissues of soybean cells, and further strengthening by oxidative crosslinking of pectin (Bradley et al., 1992). It is important to highlight the need of future studies focus on the evaluation of the accumulation of bioactive compounds and cell wall degrading enzymes to get a better description of the defence response triggered by the UV-C treatments evaluated in this study.

Transpiration rate varies greatly among cultivars, maturity stage within a singular cultivar, storage temperature, composition and ultrastructure of epicarp, and radiation dose (Duarte-Molina et al., 2016; Moggia et al., 2016). Our findings on the influence of UV-C light on weight loss of blueberries are not in agreement with the literature. Nguyen et al. (2014) reported that the percentage of weight loss was comparable for UV-A, -B and -C exposed and untreated 'Berkeley' blueberries until 14 days of storage, but from 14 to 21 days control fruit showed higher values when compared to irradiated samples. Xu et al. (2016) found a decrease in the weight loss of 'Duke' blueberries treated with 4 kJ/m<sup>2</sup> from day 2–8 during storage at 4 ± 1 °C. Considering the dynamic of water loss and firmness, our results also differ from the findings of Paniagua et al. (2013), who suggested that moisture loss has been the major cause of firmness changes during storage of blueberries.

Nevertheless, microscopic observations could contribute to understand fruit weight loss during storage. Peripheral layers of the fruit control mechanical integrity and provide protection not only against biotic and abiotic factors, but also against water loss and withering (Konarska, 2015). A number of studies mentioned the close relationship among water permeability, cuticle composition, and epicuticular waxes (Heredia, 2003; Järvinen et al., 2010; Lara et al., 2014). It was demonstrated that these last play a vital role in limiting the non-stomatal water loss in blueberry fruit (Chu et al., 2018). Water diffusion is considered to occur mostly in the amorphous fraction of the waxes, while the crystalline cover would prevent water transport (Vogg et al., 2004). Weight loss values observed in this work could be strongly associated with modifications in epicuticular waxes and cuticle occasioned by UV-C radiation and also to the partial removal and redistribution of blueberry waxes by mechanical abrasion during fruit rotation. Accordingly, control 0 kJ/m<sup>2</sup> that was not exposed to UV/C, presented higher weight loss than FF, but weight loss in irradiated berries was higher than in FF and control 0 kJ/m<sup>2</sup>, evidencing a *per se* UV-C action on epicuticular waxes (Figure 5 H,I,K,L) and in the cuticle (Figure 4 H,I). Interestingly, previous studies mentioned that a water loss greater than 5 % (discontinuous line in Figure 7) resulted in unattractive appearance in blueberries affecting their commercial value (Dinamarca et al., 1986; Salunkhe et al., 1991). This would indicate that water loss levels reached after 15 days of storage would be unacceptable, limiting blueberry shelf-life.

## 5. Conclusions

All UV-C doses assayed resulted effective in delaying and reducing the native mycobiota and *B. cinerea* infection in blueberries cv. O'Neal. The highest dose assayed (11.4 kJ/m<sup>2</sup>) resulted the most effective in delaying mycobiota (6 days) and *B. cinerea* (4 days) infection.

The higher values of FR and DR observed in irradiated fruit at day 15 of storage were associated with strengthened epicarp walls and skin detachment from the mesocarp, which could be probably due to an hormetic effect. A *per se* effect of UV-C light in waxes and cuticle could be identified by the micro and ultrastructural features and could explain the higher weight loss observed in irradiated fruit. UV-C light treatment mainly at the highest doses assayed could be an effective alternative for prolonging the storage life of blueberries cv. O'Neal delaying and inhibiting fungal infection. Postharvest shelf-life of irradiated blueberries would be mainly determined by weight loss and *B. cinerea* decay rather than by mechanical properties modification and native mycobiota incidence. The low impact of rotational movement on blueberry quality would suggest that UV-C device used in this work would be suitable for scaling up for a commercial adoption.

## Declarations

### Author contribution statement

Gabriela Jaramillo-Sánchez, Eunice V. Contigiani: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

María Bernarda Coronel: Performed the experiments; Wrote the paper.

Stella M. Alzamora, Andrea B. Nieto: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Anaía García Loredó: Analyzed and interpreted the data; Wrote the paper.

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### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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