Blueberry (*Vaccinium corymbosum* L.) postharvest UV-B treatments induce changes in bioactive compounds and reduce weight losses during cold storage

A. Varaldo[a], F. Alchera, N.R. Giuggioli and G. Giacalone

Department of Agricultural, Food and Forest Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy.

Abstract

Postharvest management of blueberry is challenging. Complications are related to the monitoring of ripeness, which is manifested by the colouring of the peduncle area. This results in premature harvesting, with possible retrogradation during storage as consequence of a decrease in bioactive compounds and reduction in quality characteristics. Aiming to mitigate these complications, the effect of increased artificial UV-B radiation on the nutraceutical compounds of 'Cargo' blueberries (*Vaccinium corymbosum* L.) was evaluated. Blueberry samples, from pot-based production, were homogeneously harvested partially unripe (peduncle area still green/pink) and immediately processed under UV-B action. UV treatments were performed with a total peak emission, at 310 nm, of 18.58 W m^{-2}. Two exposure times (5-20 min) were performed in triplicate, then the samples were stored for 1, 2, and 24 h at 20°C (adaptation time) in a perforated plastic box, thereafter cold stored at 2°C for 6 days (storage time) and finally processed for qualitative analyses. The findings show that shorter treatments and longer adaptation times resulted in significantly greater accumulation of anthocyanins than the control. Short irradiation (5 min) caused some marked changes in fruit pigmentation, characterized by a colour change towards darker shades than the control. It was observed that the treatment improved in samples kept in the chamber at 20°C for 2 h. In addition, the results for weight loss during cold storage remain lower than the control. On the other hand, the samples seem to be negatively affected by the UV dose: the firmness of the berries decreases significantly with increasing exposure to lamps. Thus, anthocyanin accumulation between adaptation times is quite similar and the mechanical properties are better preserved with short UV treatments. Therefore, low doses would be promising to improve postharvest management. These latter results would provide consumers with fully ripened, defect-free products rich in nutraceutical compounds.

Keywords: UV radiation, blueberry, batch standardized, shelf-life, physical elicitor

INTRODUCTION

Recently ultraviolet (UV) radiation has been positively evaluated for several purposes, including the control of postharvest diseases and human pathogens, the modulation of ripening and senescence, the induction of cross-stress tolerance, and the synthesis of nutraceutical compounds (Darré et al., 2022). Numerous studies claim a wide range of positive effects in plant physiology through the induction of secondary antioxidant pathways and natural defenses. Studies have shown their usefulness in enhancing the fruits nutraceutical and mechanic properties. For example, postharvest treatments of strawberries (Li et al., 2019), combined with cold storage, have shown promising results in reducing microbial growth. In addition, UV irradiation allowed a greater accumulation of bioactive components, such as phenolic compounds. Furthermore, postharvest UV-B treatments increased the levels of non-volatile phenolic compounds in blueberries (Eichholz et al., 2011). Delayed loss of resistance to skin penetration and reduction of pitting were demonstrated in

[a]E-mail: alice.varaldo@unito.it

sweet cherries pretreated with UV-C after 10 days of cold storage (Michailidis et al., 2019).

On the basis of the above considerations, UV-B pre-treatments could be of particular interest in an application to blueberries prior to cold storage. The main complexities of storage are the great weight losses and the bioactive compounds decay. In addition, it is economically important to improve harvesting and storage, given the high purchasing demand. In fact, blueberries reveal some challenges in the management of the postharvest chain that frequently results in low-quality products sold to consumers. These include complications related to the monitoring of full ripeness, which is only visible in the peduncle area. Maturity is essentially expressed by the development of colour from the basal area to the peduncular zone, which is barely visible due to the fruiting mode typical of the *Vaccinium corymbosum* L. plant, which produces cluster of berries very close together. This phenomenon leads to premature picking with possible colour retrogradation during storage, as an effect of decrease in bioactive compounds and a reduction in quality characteristics.

In view of the above, a new postharvest treatment was investigated in the present study. In particular, UV-B radiation (280-315 nm) was applied to fresh blueberries, in order to promote an increase in bioactive components. The aim is therefore to market a product with uniform appearance and superior organoleptic qualities, based on the improvement of fruit nutraceutical properties.

MATERIALS AND METHODS

Plant material and UV treatments

Highbush blueberries 'Cargo', pot cultivated, were used in this study. The test was carried out on six even-aged plants (5 years) at DISAFA - University of Turin (Grugliasco, TO) during the summer (July-August) 2022. Harvesting started on July 20 and samples were collected weekly for 3 times. Blueberries were harvested partially unripe (peduncle insertion point still green/pink) in order to simulate a retrogradation of the fruit or an improper premature harvest. After each collection, defect-free fruits were randomly divided into 9 rPet baskets of 125 g each and immediately processed under UV-B action.

UV treatments were performed using a prototype UV test box; a closed cabinet containing 20 LED strips (composed of 6 LEDs each) on the top, including 10 UV-B (PU35BM2 V0 - C3, LEXTAR, Taiwan) and 10 UV-C (PU35CM1 V3 B4, LEXTAR, Taiwan). The total peak emission of all UV-B LEDs at 100 mA, 25°C and 310 nm is 8.37 mW m^{-2}, while the peak emission of UV-C at 100 mA, 25°C and 278 nm is 8.15 mW m^{-2}. Treatments were carried out by placing the blueberries contained in the rPet boxes on a grid at 78.5 mm (first level) from the radiation source by carefully exposing the fruit insertion stem point, which is the last to develop the pigmentation, to UV lamps. In order to identify the most promising method, 2 exposure times (ET) were performed in triplicate (Table 1). One set of unirradiated fruits was used as a control. Samples were placed in a dark climate chamber at 20°C, in a perforated plastic box for an adaptation time (AT) of 1, 2, and 24 h, and finally cold stored (CS) at 2°C for 6 days until analysis.

Quality analysis

Quality analyses were performed at the DISAFA laboratory - University of Turin (Grugliasco, TO) before the UV treatments (D0) and immediately after the storage period (CS). For each of the three set of tests the following data were collected:

1. Weight loss (WL%).

At the beginning of the storage period and after 6 days of storage samples were weighted with an analytical balance (Acculab Vicon, Germany). The weight loss was calculated using Equation 1. Results were expressed as percentage.

$$WL = (W_{D0} - W_{CS})/W_{D0} \quad (1)$$

Table 1. Set of tests performed in triplicate. Exposure time (ET), target dose, adaptation time at 20°C (AT) and duration of cold storage at 2°C (CS).

Set	Class	UV-B treatment		AT (h)	CS (days)
		ET (min)	Target dose (kJ m^{-2})		
1	Control	0	0.0	1 – 2 – 24	6
	Low-dose	5	5.6	1 – 2 – 24	6
	High-dose	20	23.0	1 – 2 – 24	6
2	Control	0	0.0	1 – 2 – 24	6
	Low-dose	5	5.6	1 – 2 – 24	6
	High-dose	20	23.0	1 – 2 – 24	6
3	Control	0	0.0	1 – 2 – 24	6
	Low-dose	5	5.6	1 – 2 – 24	6
	High-dose	20	23.0	1 – 2 – 24	6

2. Colour development (Δh° %).

Berries surface colour was determined using a colourimeter (CR400, Minolta, Japan) under similar lighting conditions. The results were expressed in the CIE colour space with C as the standard illuminant and an observation angle of 2°.

For the present study was considered the hue angle (h°) which is calculated from the parameters a* and b* using the equation $h° = tan^{-1}{}^{b*}/_{a*}$ (Clydesdale and Ahmed, 1978). The h° parameter is an angular measure that identifies the colour according to its similarity to red, yellow, green, or blue, or a combination of two of these attributes in sequence. The colour of the samples was measured at the point of insertion of the peduncle of 30 fruits per sample, the last to develop colour. In the present study colour development was considered, than Δh° was calculated using Equation 2 and the result was expressed as a percentage.

$$\Delta h° = (h°_{CS} - h°_{D0})/h°_{CS} \quad (2)$$

3. Softening (S%).

Mechanical properties were evaluated using a digital durometer tester (53215 TP-Turoni, Forlì, Italy) that returns firmness (F) values on a Shore scale (10-90). The analyses were carried out on the equatorial zone of the berries at room temperature on 30 fruits. In order to understand the change in firmness of the berries, softening was calculated as consistency loss using Equation 3. The result was expressed as a percentage.

$$S = (S_{CS} - S_{D0})/S_{CS} \quad (3)$$

4. Total anthocyanin content variation (ΔTAC %).

TAC analysis followed the pH differential protocol (Roidoung et al., 2017). From blueberry samples, clear juice was obtained by adding 10 mL of solvent extraction (MeOH 500 mL + HCl 1.4 mL + H_2O 14 mL) to 4 g of fruit, put in the dark for 2 h, and homogenizing at 24,000 rpm for 1 min using an Ultra-Turrax T18 basic (Janke and Kunkel, IKA®-Labortechnik, Germany). Solutions were then centrifuged at 4,000 rpm for 10 min using the AVANTIM J-25 centrifuge (Beckamn Instruments Inc.). The clear juice (supernatant) was collected and stored in vials at - 26°C until analysis. The juice (20 µL) was diluted separately with 2 mL each of pH 1 (potassium chloride 0.025 mol L^{-1}) and pH 4.5 (sodium acetate 0.4 mol L^{-1}) buffer solution. The absorbance values of the solution were determined spectrophotometrically at both λ 520 nm and λ 700 nm (U-5100, Hitachi, Japan). The total anthocyanin content was determined using the following equation:

$$TAC = A \times MW \times DF \times \frac{10^3}{\varepsilon} \times L \quad (4)$$

where TAC = total anthocyanin content as mg pelargonidin-3-glucosidine L^{-1}; A = difference of absorbances ($A = (A520_{nm}\text{-}A700_{nm})_{pH1} - (A520_{nm}\text{-}A700_{nm})_{pH4.5}$); MW = molecular weight of pelargonidin (433.2 g mol^{-1}); DF = dilution coefficient (10); L = optical path in cm; E = extinction coefficient (48,340 L mol^{-1} cm^{-1})

Three replicates per treatment were performed and the results were calculated in ΔTAC using Equation 5 to study the increase in anthocyanin as affected by the UV treatments and the storages. The results were expressed as a percentage.

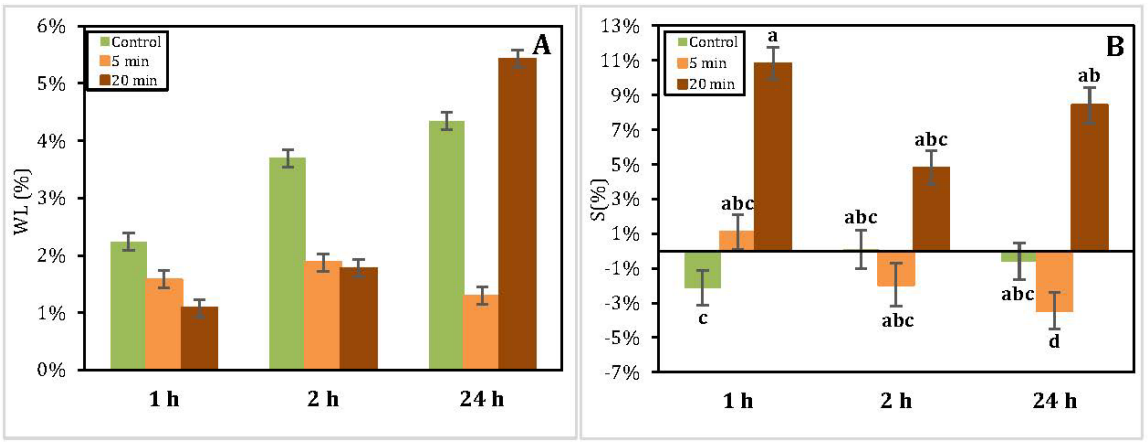
$$\Delta TAC = (TAC_{CS} - TAC_{D0})/TAC_{CS} \quad (5)$$

Statistical analysis

The statistical processing was conducted using R Studio software version 4.1.2 (Integrated Development for R. RStudio, PBC, Boston, MA, USA). Factor analysis of variance (two-way ANOVA) was used to study the quality data collected. Factors considered were ET, AT, and their interaction. Least significant differences (LSD) at 0.05 significance level ($p \leq 0.05$) were used to compare means with Tukey's test.

RESULTS AND DISCUSSION

The weight loss (WL) and the softening (S) after 6 days of cold storage are shown in Figure 1. The results showed distinctive changing among the ETs, that need to be well discussed as these parameters play a key role in the postharvest supply chain.



Factor	F value	Pr (>F)
ET	25.7564	2.01E-10 ***
AT	0.1975	0.8243

Figure 1. Effects of pre-storage UV-B treatment and adaptation time (1, 2, and 24 h) at 20°C on 'Cargo' blueberries quality attributes after cold storage at 2°C. A: weight loss (WL), data are the mean ± SD. B: consistency loss (S), interaction between factors (**), data are the mean ± SE of 30 replications, same letter indicates no statistical differences among data; $p \leq 0.05$.

The main difference lies in the treated samples, where the UV treatments have a positive effect on the WL, especially with 5 min irradiation and 24 h of AT (Figure 1A). On the contrary the greatest losses are those recorded in correspondence of fruits treated 20 min and with 24 h of AT. Furthermore, the 20 min treated and the control show the same downward trend with an increase in decay with the passage of AT. This may be due to the blueberries being treated for too long under UV lamps and kept at 20°C for too long. These conditions can actually cause excessive moisture loss, which is the main cause of firmness and weight changes during postharvest storage of blueberries (Paniagua et al., 2013).

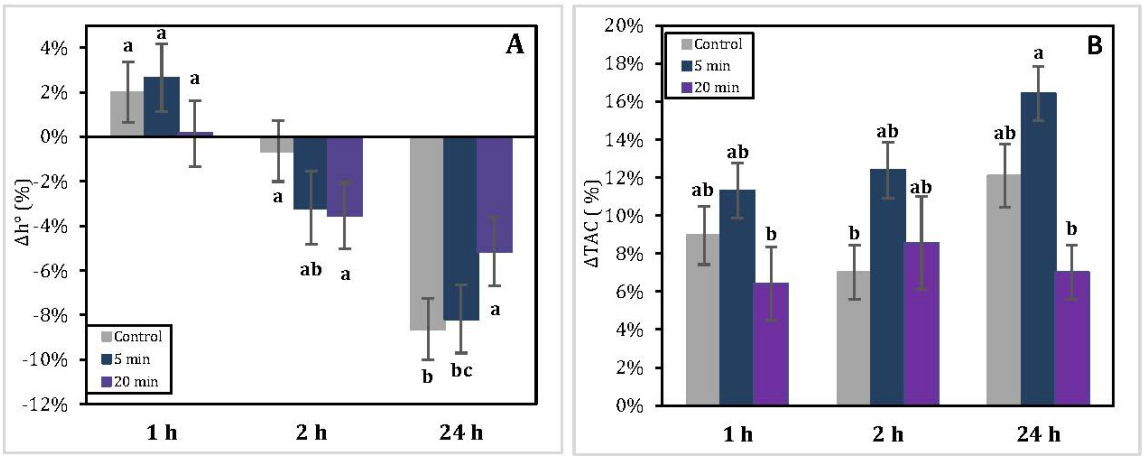
Low doses of UV light effectively inhibited weight loss, but softening does not show the

same tendency (Figure 1B). The statistically lowest value of S is observed with 5 min ET and 24 h of AT, which results in a firmness decrease. An opposite change seems to develop between the 5 min sample and the control in the first 2 h of AT. While S first increased and then decreased in the control, the opposite was found for the treated sample. Similar findings have been observed in several works (Chen et al., 2015, 2017), where it was observed that, as in the case of the low-dose irradiation, the firmness of blueberry fruits increased in the early stages of storage and then decreased with prolonged storage.

Fruits treated for 20 min showed the greatest firmness variation, with the 1 h of AT showing the statistically highest value (11%). This latter result is probably the consequence of excessive exposure to UV lamps which causes the decadence.

In general, the high-dose irradiation, as was for the WL, provided a negative effect on the S of berries, which showed a clear increase in firmness with 1 and 24 h of AT, consequence of the high WL. It is also clear how the low-dose UV exposure mitigated fruit decay compared to the control and the high-dose irradiation. Finally, even though the control showed a slight S variation in correspondence of 2 and 24 h of AT, the WL is too high to be considered as a successful process.

The evolution of berry pigmentation and anthocyanin accumulation compared to day 0, after six days of cold storage, is represented in Figure 2. It was observed, in all treatments, a decreasing of Δh° which means a variation from red to blue pigmentation (Figure 2A). However, after 1 h of AT, there were no significant differences among the treatments. In this case, there is only a slight increase in the h° values, which is evident for the 5 min treatment and the control samples. The Δh° suggests a shift towards more red hues. This is due to the colour regression that could occur in the early stages of fruit storage. The interaction between the two factors of variation (ET and AT) showed that the statistically highest change in blue colour occurred in both the control and the 5 min UV treated samples after 24 h of AT. Conversely, the lowest pigmentations are those found for all the other treatments. This means less variation into blue hues and therefore less influence from the combined effect of UV irradiation and the AT.



Factor	F value	Pr (>F)
ET	31.2657	1.01E-11 ***
AT	0.2107	0.8103

Factor	F value	Pr (>F)
ET	6.5764	0.0089 **
AT	8.1425	0.0040 **

Figure 2. Effects of pre-storage UV-B treatment and adaptation time (1, 2, and 24) at 20°C on 'Cargo' blueberries quality characteristics after cold storage at 2°C. A: colour development (Δh°), data are the mean of the interaction between factors (**) ± SE of 30 replications. B: variation of total anthocyanin content (ΔTAC), data are the mean of the interaction between factors (***) ± SE of three replications. Same letter indicates no statistical difference among data; p≤0.05.

The colourimetric results are consistent with the anthocyanin accumulation (Figure 2B). Low-dose ET promotes TAC synthesis, and accordingly the pigmentation evolves toward blue tones, with ΔTAC showing the statistically higher increase. The anthocyanins accumulation, mainly in the skin, is closely correlated with the pigmentation of the fruit (Lancaster et al., 1997). Thus, the trend observed is due to anthocyanins, which, as the main pigments in blueberry (*Vaccinium* spp.) fruits, determine the typical purple-blue colouring (Cesa et al., 2017). On the contrary, the 20 min treatments seem to have a pejorative effect on the fruits, with a lower TAC accumulation than the control.

More precisely, studying the adaptation time, it emerges that the blueberries treated with UV-B for 5 min, constantly increase the TAC during the AT. It emerged that 24 h in a climate chamber at 20°C, allowed the greatest accumulation of anthocyanins, with a significantly higher increase of 17%. A similar evolution was also observed for the control, so it seems that the accumulation of bioactive compounds could be due to the AT, as well as to the ET. However, short irradiation times seem to enhance this trend, while longer ETs, appear to overturn the tendency.

The benefit of short-duration UV irradiation on fresh blueberries has been found in other studies especially with UV-B action. Low-dose pre-treatments have been confirmed to delay weight loss and softening after a period of cold storage (Abdipour et al., 2019; Nguyen et al., 2014; Perkins-Veazie et al., 2008).

Concerning bioactive compounds, the results of the present study are in agreement with those of (Reyes-Díaz et al., 2016), who observed that the UV-B action improved the synthesis of anthocyanins during storage. As a confirmation of these results Yang et al. (2018) found that UV-B irradiation significantly promoted anthocyanin biosynthesis and the transcripts of genes involved in the activities of the anthocyanin pathway. Similar outcomes have also been demonstrated in other fruits, such as peach (Abdipour et al., 2019), apple (Assumpção et al., 2018), lemon (Ruiz et al., 2017) and tomato (Castagna et al., 2013) where the UV-B pre-treatments have improved anthocyanin production and nutraceutical properties.

CONCLUSIONS

The present study aimed to identify a novel-chemical-free strategy to mitigate the quality deterioration of blueberries during cold storage as a consequence of incorrect harvesting. Berry samples were treated with UV-B radiation after harvest, placed in a climate chamber at 20°C for 1, 2, and 24 h as adaptation time and finally stored at 2°C for 6 days until analysis.

The main results showed that low doses of UV-B can provide better quality characteristics after post-harvest storage. In fact, 5 min of UV radiation minimized weight loss and softening rates, and allowed higher anthocyanin concentrations than non-irradiated and high-dose treated fruits. While the better efficiency was obtained by changing the exposure time at the minimum, no significant differences with the control were observed when the berries were placed in the climate chamber for 1 and 2 h concerning the ΔTAC and S development. Conversely, statistically better performances were discovered with 5 min treatments and 24 h of adaptation time. In the latter case, the berries showed the least weight loss and softening and the greater increase in anthocyanins accumulation.

Although the research has allowed the introduction of a new technique to improve the blueberries postharvest quality, future studies should focus on lower UV-B doses to make the treatment even more energy efficient and to find out if exposure times of less than 5 min provide better post-storage performance. In addition, longer cold storage periods should be tested to verify if UV-B radiation improves blueberry shelf-life and reduces the problems due to incorrect picking. In this way, it would be possible to implement the limits of research that are lacking in surveys of cold storage times and methods.

In conclusion, the application of UV-B pre-treatment seems to be a promising approach to the supply chain strategy for improving the storage and shipping quality of harvested blueberries. This could open up new opportunities for fruit that would otherwise become food waste due to its low quality characteristics.

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