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Post-harvest UV-C and PL Irradiation of Fruits and Vegetables

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The exposure to UV-C and Pulsed Light (PL) light causes stress in plant tissues, which stimulates the biosynthesis of defensive secondary metabolites with antimicrobial and antioxidant activity. For this reason, recent studies have examined the capability of UV-C and PL as effective methods to prolong the fresh status as well as preserve or even improve the content and activity of antioxidant compounds of fresh produce through post-harvest handling and processing.
In this work, the effect of PL and UV-C treatments on quality and antioxidant properties of tomatoes and Annurca apples intended for fresh consumption was investigated.
Fruits harvested at the green stage were exposed to both light treatments at energy dosages of 2 and 4 J/cm^2. Treated and untreated samples were allowed to ripen under day/night cycles illumination conditions at room temperature for up to 21 days for tomatoes and 28 days for apples. The effects of light treatments on the colour, pH, titratable acidity and °Brix as well as on the levels of lycopene, total carotenoids, total phenolic compounds and antioxidant activity, were evaluated through storage and compared with those of untreated samples.
Results indicate that pH, titratable acidity and °Brix of all fruit samples were not significantly affected by light treatments and remained almost the same throughout storage. The skin colour of untreated and exposed fruits changed during storage period, with no appreciable influence of the light treatments. However, the exposure of apples and tomatoes to light treatments enhanced the antioxidant properties of these fruits during storage.
These results suggest that post-harvest PL and UV-C irradiation can be utilized to the health value of tomatoes and Annurca apples by increasing the level of certain bioactive compounds without inducing significant changes to their physical properties during storage.

1. Introduction

Fruits and vegetables play a significant role in human nutrition for their richness in health-related food components with antioxidant activity.
Among vegetables typically consumed by humans, tomatoes represent an important source of many traditional nutrients and are a particularly rich source of several carotenoids (Jegadeesh et al., 2011). Carotenoids, are largely accumulated in tomato during fruit ripening by the degradation of green pigment chlorophyll and the transformation of chloroplast into chromoplast during the lag phase that precedes maturation. Within the class of carotenoids, lycopene is the most abundant and largely influence the quality perception of fresh tomatoes being it is responsible for the characteristic red colour of ripe tomatoes. This compound has notable antioxidant activity and several studies have been done to evaluate its anticancer activity, in particular against prostate cancer (Giovannucci 1999). In addition to carotenoids, tomatoes are also an effective way to supply other natural antioxidants such as ascorbic acid and phenolic compounds, namely flavonoids and phenolic acids (Jegadeesh et al., 2011), which, in turn, are characterized by health beneficial properties such as antiinflammatory, antihistaminic, and antitumor activities.

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Apple fruit also contain several health and sensory related constituents including dietary fibre, sugars, vitamins and phenolic compounds at which is mostly attributed the antioxidant capacity of this fruit. Particularly, phloridzin, a phytocemical which belongs to a class of polyphenols, has been described as capable of reducing the glucose uptake by human intestinal and liver cells (Tenore et al., 2013).
‘‘Annurca” apple fruit is one of the most important cultivars of southern Italy (Lo Scalzo et al., 2001), which, recently, has obtained the official designation of Protected Geographical Indication (PGI) from the European Council (Commission Regulation (EC) No. 417/2006). Differently from other apples, Annurca is picked still green and it undergoes a typical reddening treatment in stores called ‘‘Melai” that allows the final product to reach its typical taste and antioxidant content in about 1 month (Tenore et al., 2013).
The synthesis of all the abovementioned bioactive compounds in fruit and vegetables, may vary greatly depending not only on the cultivar, farming methods and environmental factors, such as nutrient availability, temperature and in particular, light (Jegadeesh et al., 2011). To this purpose, post-harvest exposure of horticultural crops, including many fruits and vegetables, to UV-C and Pulsed Light (PL) may cause stress in plant tissues, which stimulates the biosynthesis of defensive secondary metabolites with antimicrobial and antioxidant activity. These compounds are highly desirable as they can contribute to prolong the life and maintain the quality of vegetable and fruits as well as increase the nutritional value of UV-treated products (Ribeiro et al., 2012).
However, literature data on the use of PL and UV light to enhance the nutraceutical properties of fresh produce are relatively recent and in progress, so that no definite conclusions can yet be drawn. Nevertheless, it has been reported that the exposure (from minutes to hours) of fresh produce such as mangoes, edible mushrooms, strawberries, blueberries and grape to the short wavelength UV-C (200-280 nm) light irradiation at low (hormetic) doses (<1 J/cm^2), resulted in the enhancement of phenolic content, anthocyanin, vitamin D2, and antioxidant capacity (Ribeiro et al., 2012).
Recently, a role for post-harvest UV-C treatment at both hormetic or slightly higher energy dosages in enhancing the content of lycopene (Bravo et al., 2012), ascorbic acid (Jagadeesh et al., 2009), carotenoids (Liu et al., 2009), phenolic compounds (Jagadeesh et al., 2009) and antioxidant activity (Bravo et al., 2012). has been also demonstrated in tomatoes. Moreover, it has been also proved that the post-harvest exposure of apples to a combination of visible light and UV-B radiation can be used to improve their health benefits and colour appearance without changing important taste-related parameters or causing damage to the fruit (Hagen et al., 2007). In spite of this, further research is needed since, in some cases, investigations have resulted in different conclusions regarding the appropriate energy dosages, the optimal ripening stage of fruit, and the storage conditions.
On the other hand, with respect to continuous wave UV light irradiation, PL has higher penetration depth and emission power, which might stimulate higher production of defensive and bioactive compounds in plant tissues (Rodov et al., 2012). In spite of this, only recent studies have reported that PL treatment (0.2-10 J/cm^2) may provide a highly effective way for increasing Vitamin D2 levels in mushrooms (Koyyalamudi et al., 2011) and total anthocyanin and phenolic content in fig fruit (Rodov et al., 2012). However, to the best of our knowledge, no paper deals with the effects of post-harvest PL treatment on tomato and apple fruit.
In the present study, the effects of PL and UV-C treatments at different energy doses on the quality (colour, pH, titratable acidity, and total soluble refractive solids) and functional properties (lycopene content, total carotenoids, total phenolics, and antioxidant activity) of tomato and Aannurca apple fruit during storage were investigated.

2. Materials and Methods

2.1 Plant material

Tomatoes of “San Marzano” variety (*Solanum lycopersicum*) and Annurca apple fruits were field-grown in Camapnia Region (Southern Italy), harvested at the green stage and treated with PL and UV-C light within two days.

2.2 PL and UV-C apparatus

PL treatments were carried out using a bench-top RS-3000C SteriPulse-XL system (Xenon Corp., Wilmington, Mass., USA) which included a power/control module, a treatment chamber and lamp housing with a linear 16” xenon flash lamp mounted on top of the chamber. The system generates 3 pulse/s (360 μs width) of a polychromatic light in the wavelength range between 200 and 1,100 nm. An adjustable 15.75 x 40.64 cm stainless steel tray in the treatment chamber allowed changing the vertical distance from the quartz window surface from 1.93 to 16.63 cm. Consequently, the intensity of the flashes of light that reach the target can be changed from 1.21 (in correspondence of the smaller distance) to 0.22 J/cm^2/pulse (in correspondence of the

higher distance). A forced air system with filter was used to remove ozone and heat from both the housing lamp and treatment zone.
UV-C treatments were carried out using a laboratory scale cabinet CYTOSAFE-N 2000 (SARIN sas di Leo Temin & C., Florence, Italy) containing a 20 W germicidal UV-C lamp (G20T10 Sankyo Denki, Nagano, Japan) with a peak emission at wavelength of 254 nm.

2.3 Irradiation and storage

Before treatment, samples of tomatoes (of almost cylindrical shape of about 4 cm in diameter, 7 cm in length) and apples (of almost spherical shape of about 4 cm in diameter) were selected to form different uniform lots. Table 1 summarizes, for each fruit, the name of each lot and the corresponding process conditions.
During PL treatment, the samples of two lots of tomatoes (T-PL2 and T-PL4) and two lots of apples (A-PL2 and A-PL4) were placed in the centre of the tray and aligned with their main axis parallel to the lamp tube at the maximum vertical distance allowed. At this distance the average energy dose per pulse delivered on the upper surface of the samples was 0.35 J/cm^2 for tomatoes and 0.45 J/cm^2 for apples. During the treatment, the samples were rotated in order to expose each side of the fruit to the same dose.
Two lots of tomatoes, namely T-UV2 and T-UV4, were irradiated with continuous wave UV-C light under two different conditions. During the treatment the fruits of each lot were placed at a distance of 30 cm from the UV-C lamp source and irradiated at an average intensity of 5.50 W/m^2. Uniformity was ensured by aligning the fruits with their axis parallel to that of the lamp tube and making them to fall within the length of the lamp. UV-C irradiation was continuously applied to each of the two sides of the fruit for 1 and 2 h that provide the desired dose.
One lot of both non-irradiated tomatos and apples was used as control.
All the PL and UV-C light treatments were carried out starting with the samples at room temperature (20 ±2°C). The maximum temperature increase on the samples surface was about 2 °C for both light treatments.
Treated and untreated samples of each lot were evenly placed onto plastic trays one layer thick and then allowed to ripen under day/night cycles illumination conditions at room temperature for up to 21 days for tomatoes and 28 days for apples. All the tests and analyses were carried out in duplicate.

Table 1: Processing conditions

Food	Lot	Distance* (cm)	Exposure time (s)	Fluence (J/cm^2)
Tomato	Control	-	0	0
	T-PL2	12.6	1.9	2
	T-PL4	12.6	3.8	4
	T-UV2	30	3600	2
	T-UV4	30	7200	4
Apple	Control	-	0	0
	A-PL2	10.6	1.5	2
	A-PL4	16.6	3.0	4

*Distance between the upper surface of the fruit and either the quartz window or the UV-C lamp source
**Energy dose applied per each side of the fruit

2.4 Chemical-physical and chemical analyses

Periodically, at each of the specified period (0, 7, 14, 21, and 28 days), six fruits from each lot were randomly chosen and analyzed for color (CIE L*a*b* scale), pH, titratable acidity, and °Brix. In addition tomato samples were also analyzed for evaluating by spectrometric analysis the total lycopene, carotenoid, and phenolic content as well as antioxidant activity (DPPH assay). Similarly, total phenolic content and antioxidant activity (DPPH assay) in the peel and flesh of apple were also evaluated.

3. Results and discussion

pH plays an important role in foods, since it affects the growth and type of microbial flora spoiling the product as well as its sensory and organoleptic properties. The initial pH value of the untreated tomatoes was

3.88±0.06, which remained roughly the same after any light treatment and during all the storage period. On the other hand, the initial titratable acidity measured in apple samples containing peel and flesh was 12.2±0.89 meq/100 g FW and showed a slight decrease with storage up to a value ranging between 8.7-9.8 meq/100 g FW, depending on the treatment conditions.

The solid soluble content of fresh produce, in °Brix, is used as an indicator of quality since it may suggest how "sweet" a fruit may taste. At the harvest day, °Brix values of untreated tomatoes and apples were 4.90±0.08 and 11.2±0.48, respectively. These values were not significantly influenced by any of the light treatments studied. In addition, no significant changes of the values of this parameter were detected in both untreated and light-treated samples during storage, which remained within the range 4.9–5.4 % for tomato and showed only a slight increase up to 13.5±0.73 for apple.

Fruit colour is the most important external characteristic to assess ripeness and post-harvest life and is a major factor in the consumer's purchase decision. Overall, our results showed that the variation of the parameters L*, a* and b* for both tomato and apple was significantly affected by storage time, but no appreciable differences were observed in colour evolution and fruit ripening between untreated and light-treated samples. In addition, no signs of fruit damage resulted from the light treated fruits. In particular, it was noted a tendency of tomato and apple fruits of changing their colour during storage from light-green towards, respectively, light-red and yellow-red colour (data not shown).

Table 2 summarizes the total content of carotenoids, lycopene and phenolic compounds as well as the antioxidant activity of untreated and light treated tomato lots with UV-C and PL at different energy doses at day 0 and after 21 days of storage.

Table 2: Total content of Carotenoid, Lycopene, phenolic compounds and antioxidant activity of untreated (Control), PL and UV-C treated tomato samples at harvest and after 21 days of storage

Lot	Total Carotenoids[a] mg/kg FW		Total Lycopene[b] mg/kg FW		Total phenolics[c] mg GAE/kg FW		Antioxidant Activity[d] (% DPPH inhibition)	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Control	4.2±1.8	61.5±6.8	0.54±0.15	12.9±0.2	266±38	310.0±20.2	25.8±4. 9	40.3±2.6
T-PL2	4.1±1.1	106.0±17.4	0.58±0.21	15.0±0.8	271±7	350.1±2.1	25.1±2. 6	44.1±2.0
T-PL4	4.5±0.9	156.9±6.7	0.63±0.15	33.7±4.9	265±10	390.1±36.2	24.6±3.8	47.6±1.1
T-UV2	4.1±1.0	90.1±0.8	0.59±0.14	67.75±4.4	251±18	408.6±11.0	25.3±4.0	48.5±0.5
T-UV4	4.2±1.4	68.4±4.9	0.56±0.21	50.1±0.2	274±12	393.9±20.1	26.2±2.3	56.7±0.9

[a]Determined according to the method described by Kotikova et al. (2011)
[b]Determined according to the method described by Fish et al. (2002)
[c]Measured by the Folin–Ciocalteu assay. GAE: gallic acid equivalents
[d]Measured by the DPPH assay

The exposure of green tomatoes to light treatments did not have a significant impact on the initial content of these compounds. During the storage period, the content of total carotenoids, lycopene and phenolic compounds increased significantly in all samples (light-treated and untreated). However, the increase was greater in samples of the lots exposed to light treatments and changed in a way that was dependent on both the light treatment and energy dose (data not shown).

For each lot, the maximum content of antioxidant compounds was detected after 21 days of storage. In particular, the total carotenoids content of the lots T-PL2, T-PL4, T-UV2, and T-UV4 at day 21 of storage was, respectively, up to 1.7, 2.5, 1.5, and 1.1-fold higher than in the control lot. These differences were statistically significant only for tomatoes exposed to PL treatments. In addition, among the light treatments delivering the same energy dose, UV-C light irradiation appeared to be less effective than PL treatment in stimulating the synthesis of total carotenoids.

The lycopene content at day 21 was greater for the lots exposed to PL treatment at the higher the energy dose, increasing by 1.16 and 2.61-fold for the lots T-PL2 and T-PL4, respectively, when compared to that of untreated tomatoes. In contrast, for the samples exposed to UV-C light, the maximum lycopene content detected at end of storage was lower at the higher energy dose. In particular, the lycopene content of the lots T-UV2 and T-UV4 increased, respectively, by 5.23 and 3.88-fold, when compared to that of the untreated tomatoes. This result suggests that long exposure time to UV-C treatment might negatively affect the enzymes responsible for the synthesis of lycopene (Bravo et al., 2012).

Similarly to lycopene, at the end of storage, the highest amount of total phenolic compounds (408 mg GAE/kg FW), was detected in the fruits of the lot T-UV2, which was about 1.31-fold higher than that of control fruit,

while increments of 1.13, 1.25 and 1.27-fold were detected, respectively, for the fruits of the lots T-PL2, T-PL4 and T-UV4. However, there was no significant difference between samples of the lots PL4, UV2 and UV4.
A comparison of our results with previous reports is hard to achieve due to the differences in the tomato fruit, light treatment, characteristic of the equipment and storage conditions employed in different studies. Nevertheless, in agreement with our findings, also other studies reported a positive effect of UV-C irradiation on the synthesis of lycopene (Bravo et al., 2012) and total phenolics (Jagadeesh et al., 2009) in tomato fruits.
The main components contributing to the antioxidant activity in tomato fruit are carotenoids, ascorbic acid, and phenolic compounds (Giovanelli et al. 1999). Experimental data of overall antioxidant activity of untreated and light-treated tomato fruits evaluated by DPPH scavenging ability are reported in Table 2. As expected, it was found that the application of either PL or UV-C light, did not affect the antioxidant activity of tomatoes on the harvest day. During storage the potential antioxidant of all lots (untreated and light-treated) tended initially to increase, being the increase larger in fruits of the lots exposed to light treatments. However, during the last week of storage, the antioxidant potential tended to decrease showing an increasing trend only for the samples of the lot UV4 (data non shown). At the end of storage period, UV4 showed the highest increment in antioxidant activity (+40 %), as compared to the control sample, followed by UV2 (+20 %), PL4 (+18 %), and PL2 (+10 %) (Table 2). Enhancing effect of PL and UV-C light treatments on the antioxidant activity may be based on the increase in phenolic compounds and carotenoids observed in this study. However, in agreement with previous results (Tommonaro et al., 2008), total antioxidant activity cannot be univocally correlated to the concentrations of lycopene, carotenoids and phenolic compounds, being its value probably due to synergistic action of different bioactive compounds.
Table 3 summarizes the results of TP content and antioxidant activity measured in the peel of untreated and PL treated Annurca apple fruits at day 0 and after 28 days of storage at room temperature.
Similarly to the findings of tomatoes, also for apples the initial content of phenolic compounds was not affected by any light treatment. However, during storage, while the total phenolic content of untreated samples remained almost the same, showing a slight decrease only during the last week, the total phenolic content of PL treated samples showed a tendency to gradually increase (data not shown). After reddening (day 28), the amount of total phenolics detected in the peel of the fruits of the lots A-PL2 and A-PL4 was, respectively, 1.17 and 1.14-fold higher than the control fruits (Table 3). Similarly, Hagen et al. (2007) found that postharvest irradiation with Vis+UV-B enhanced the sum of flavonoids, sum of phenols and total phenols in the peel of shade-grown apples.
However, data not shown, revealed that no appreciable difference were found in the flesh of the untreated and PL treated fruits. This seems to confirm that effect of PL treatments is limited to the thin surface layer of the fruits.

Table 3: Total phenolic content and antioxidant activity of untreated (Control) and PL treated Annurca apple samples at harvest and after reddening

Lot	Total phenolics[a] mg GAE/kg FW		Antioxidant Activity[b] (% DPPH inhibition)	
	Day 0	Day 28	Day 0	Day 28
Control	7343±145	7245±222	27.5±3.6	33.9±2.9
A-PL2	7289±207	8442±185	27.1±4.2	38.5±1.8
A-PL4	7318±232	8283±241	28.3±3.9	37.7±2.7

[a]Measured by the Folin–Ciocalteu assay. GAE: gallic acid equivalents
[b]Measured by the DPPH assay

4. Conclusions

In conclusion, post-harvest PL and UV-C treatment of green tomatoes and Annurca apples are effective in activating biosynthesis pathways of compounds with high antioxidant potential (total carotenoid compounds, lycopene, total phenolic compounds), increasing the nutritional value of the fruits, without affecting their physicochemical and organoleptic properties (pH, acidity, °Brix and colour).
However, further experimental investigations with different tomato and apple fruits (cultivar, maturity stage), mode of light energy delivery and post-treatment storage conditions are needed in order to optimize these technologies in terms of increasing the content of health-related food components, without negatively affecting the quality and safety of products.

Finally, from our results it appears that although UV-C and PL are both effective in stimulating the synthesis of bioactive compounds, PL requires shorter exposure time (seconds rather than hours), which could foster the commercial feasibility of this of this new approach.

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