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Pulsed light of near-infrared and visible light wavelengths induces the accumulation of carotenoids in tomato fruits during post-treatment time

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Abstract: Pulsed light (PL) is proposed as a novel strategy for the food industry to enhance the antioxidant potential of fruits and vegetables for industrial uses. The main aim of this work is to evaluate the impact of postharvest PL treatments of different spectral ranges on the carotenoid concentration as well as quality attributes of tomatoes during post-treatment time. Doses of wide-spectrum light (180–1100 nm), full-spectrum without ultraviolet (UV)-C wavelengths (305–1100 nm), and visible (VIS) + near-infrared light (NIR) (400–1100 nm) were compared. Total carotenoids, lycopene, and chlorophyll contents were spectrophotometrically assessed just after treatments and 1, 5, and 10 days post-treatment. PL treatments accelerated the accumulation of both total carotenoids and lycopene concentrations in tomato fruits. Nevertheless, the efficacy of PL depended on the applied spectral range. Tomato subjected to VIS + NIR treatment exhibited the greatest enhancement in total carotenoids (31 %) and lycopene (35 %) content at day 5 post-treatment and quality attributes were not affected. Conversely, UV-light exposure did not enhance carotenoid concentrations. These results evidenced that VIS + NIR treatments induced a faster accumulation of carotenoids without negatively affecting tomato quality attributes.

KEYWORDS
carotenoids, physicochemical properties, pulsed light, spectral range, tomato

Practical Application: The integration of visible and near-infrared (VIS + NIR) light filters in pulsed light (PL) processing allows enhancing the accumulation of bioactive compounds in tomato tissues in a sustainable way, which can be processed to obtain derived products (e.g., juices, purees) with health-promoting properties. PL technology is characterized by a lack of residual compounds and the absence of applying chemicals potentially harmful to humans. Industries

can attract the attention of consumers through their application, which allows offering this added value.

1 | INTRODUCTION

The regular consumption of tomato, which is one of the most important vegetable crops worldwide, has been associated with a lower incidence of chronic diseases, such as cancer, atherosclerosis, and cardiovascular diseases (Tanumihardjo & Yang, 2010). These beneficial properties have been attributed to their high content of bioactive compounds, such as phenolics, vitamins, and, especially, carotenoids (Hedges & Lister, 2005). Among carotenoids, lycopene has important antioxidant and anti-inflammatory effects and is the pigment mainly responsible for the deep-red color in ripe tomatoes, which influences the quality perception of the fruits and the derived products by consumers (Shi & Maguer, 2000).

Carotenoids accumulation in tomato fruits is associated with ripening and involves various physiological, morphological, biochemical, and molecular changes including the transition from chloroplasts to chromoplasts (Ilahy et al., 2011). The carotenoid biosynthesis can be affected by both genetic and environmental factors, in which radiation intensity is included (Liu et al., 2015). The exposure of tomatoes to intense light doses either preharvest or postharvest seems to trigger the biosynthesis of different antioxidant compounds, including carotenoids (Poiroux-Gonord et al., 2010). This response has been linked to the induction of a photoprotective antioxidant defense response to oxidative stress, that eventually leads to the accumulation of carotenoids in tomato fruits (Aguiló-Aguayo et al., 2013). Colored carotenoids like β-carotene or lycopene exert a photoprotective effect by quenching excited chlorophyll molecules and singlet oxygen ($^1O_2$) to protect the photosynthetic system. In conditions of excessive radiation, uncolored carotenoids such as xanthophylls can also quench the excited chlorophyll in the photosystem II and exert their photoprotective role (Fanciullino et al., 2014; Müller et al., 2001; Robert et al., 2004). The ability of pulsed light (PL) treatments to decontaminate fresh fruit and vegetable products without causing unacceptable modifications in their sensory and nutritional characteristics has caught the interest of researchers and processors (Soliva-Fortuny & Martín-Belloso, 2016). PL consists of pulses of intense and short-time light generated by Xenon lamps (Charles et al., 2013). A PL generator system emits a radiation of broad-spectrum within the ultraviolet (UV), characterized by shorter wavelengths

and infrared (IR), corresponding to longer wavelengths range (Demirci & Krishnamurthy, 2011). Each one of these radiation bands possesses different characteristics and exhibits distinct interactions with food constituents due to their electromagnetic properties (Soliva-Fortuny & Martín-Belloso, 2016). Only 46% of radiation (400–700 nm) can be used in photosynthesis by plants, whereas UV and IR can induce stress defence responses or detrimental effects (Nwoba et al., 2021). Beyond the antimicrobial action, several research works have reported an increase in the antioxidant content of metabolically active fruit tissues after postharvest exposure to artificial light treatments. In this regard, it has been reported that postharvest broad-spectrum PL treatments significantly increase carotenoid concentrations in tomato fruits as a consequence of the activation of their biosynthetic pathway (Aguiló-Aguayo et al., 2013). In this regard, Pataro et al. (2015a) observed an increase in the concentration of total carotenoids in tomato fruits exposed to different energy dose treatments of PL in the wavelength range between 200 and 1100 nm, whereas UV-C irradiation appeared to be less effective. Some studies performed in other food matrices have also shown that PL of broad-spectrum can induce an accumulation of carotenoids during storage. A significant increase of total carotenoid content in pulp (450 %) and peel (190 %) of ripe mango treated by PL (0.6 J cm^{-2}) was reported after 7 days of storage (Lopes et al., 2016). Recently, Rybak et al. (2021) reported that the carotenoid content of fresh-cut bell pepper increased throughout storage when PL (190–1100 nm) intensities higher than 12 J cm^{-2} were applied. Other authors have observed positive effects of low-dose UV light continuous treatments on the accumulation of carotenoids and phenolic compounds in tomatoes, resveratrol in grapes, and anthocyanins in strawberries and apples (Bravo et al., 2012; Castagna et al., 2013; Liu et al., 2009; Lu et al., 2016; Soliva-Fortuny & Martín-Belloso, 2016). However, there is scarce information about the application of PL treatments with different spectral ranges, including wide-spectrum light with or without UV-C wavelengths, and VIS-NIR light, on the accumulation of carotenoids in fruits during post-treatment time. Consumers are increasingly demanding nutritive food products that are produced in a sustainable way and promote their well-being by reducing the incidence of diseases. Such population sectors are a niche market in which horticultural processing industries can

focus their innovation projects. The integration of commercially available light filters allows to optimize the accumulation of bioactive compounds (e.g., carotenoids) in plant tissues. This technology is characterized by a lack of residual compounds and the absence of applying chemicals potentially harmful to humans. Industries can attract the attention of consumers through the application of PL technology, which allows offering this added value. Therefore, this work was aimed at evaluating the effect of PL dose spectral range on the accumulation of total carotenoids and lycopene as well as on the main quality attributes of tomato fruits throughout post-treatment time.

2 | MATERIALS AND METHODS

2.1 | Reagents

Butyl hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, Spain). Acetone was acquired from Fisher Scientific Scharlau Chemie (Loughborough, UK) and hexane and ethanol were purchased from Scharlab (Sentmenat, Spain).

2.2 | Tomato fruits

Tomatoes (*Lycopersicum esculentum* cv. Raf) (220 fruits) were purchased at a wholesale distributor in Lleida (Spain) at turning stage, characterized by more than a 10% but not more than a 30% of the surface showing a definite change in color from green to red (USDA, 1991). The fruits were stored at $12 \pm 1°C$ until turning to a light red-stage (60–90 % of tomato surface was red) (USDA, 1991). At such ripening stage, tomatoes with uniform shape and size were selected and then rinsed with tap water and carefully dried with a paper cloth.

2.3 | Pulsed light treatments

Pulsed light (PL) treatments were carried out using an XeMaticA-2L system (SteriBeam Systems GmbH, SteriBeam, Kehl, Germany). The treatment chamber had two Xenon lamps separated by a gap of 17 cm. The sample holder consists of a polypropylene film (1-mm thick) supported by a metal framework, which was located at 8.5 cm between the two Xenon lamps (Figure 1). Transparency of the film was determined by measuring the amount of energy received by a photodiode coupled to an oscilloscope, and was found to be above 97 % of the total emitted energy. PL-dose was obtained by measuring the amount of energy received by a photodiode detector placed
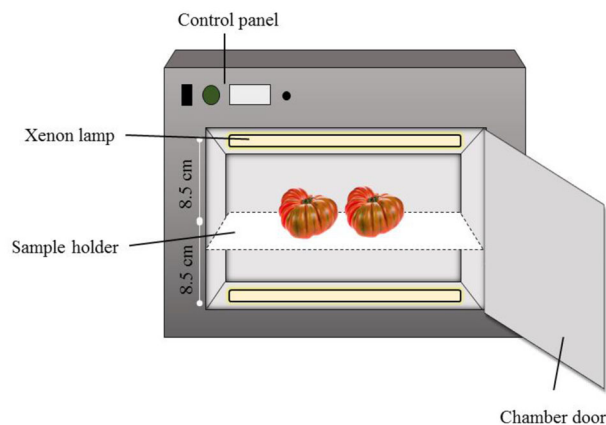

FIGURE 1 Scheme of the pulsed light treatment device

at sample height, meaning 8.5 cm away from the lamp. The photodiode was connected to the oscilloscope and the recorded signal was transformed into radiance values using a calibration with a standard light source according to the instructions of the manufacturer. The temperature was also measured at the sample height and did not go beyond 40°C. The emitted wavelengths ranged from 180 to 1100 nm, with 15–20 % of the light in the UV region. The duration of each pulse was 0.3 ms and the fluence delivered by each lamp was 0.4 J cm^{-2} per pulse. A total energy dose of 10 J cm^{-2} per side was applied and each treatment lasted 7.5 ms. This dose was selected after preliminary experiments since it was optimal for enhancing carotenoid content without affecting tomato physicochemical attributes. The application of higher energies altered texture and visual aspect of tomatoes since the reached temperature was higher than 40°C. It is worth mentioning that this energy dose (10 J cm^{-2}) is lower than the maximal cumulative treatment dose approved by FDA for treatment of food products, which is established at 12 J cm^{-2} (FDA, 2016).

To evaluate the effect of the application of light pulses of different spectrum compositions, two types of filters were used: a Makrolon® polycarbonate filter which cuts off all light below 400 nm, thus allowing only the VIS and NIR to pass through, and a 2-mm thick Pyrex® glass filter that cuts all light below 305 nm allowing to pass UV-B (280–320 nm), UV-A (320–400 nm), VIS, and NIR wavelengths. Treatments with no filter were carried out to assess the effect of broad emitted spectrum (180–1100 nm). Additionally, untreated tomato fruits served as control. Namely, three treatments were compared: wide-spectrum light (180–1100 nm) (PL1), wide-spectrum light without UV-C wavelengths (305–1100 nm) (PL2), and VIS-NIR light (400–100 nm) (PL3). Six PL treatments were applied for each treatment and post-treatment storage condition, and each replicate comprised two tomato fruits.

2.4 | Post-treatment conditions and sample preparation

Tomatoes with uniform size and shape and in the same ripening stage (60–90% of the fruit surface turned red) were divided into 4 lots of 48 fruits each. Three lots were submitted to the different assessed PL treatment conditions and the remaining lot of fruits were used as an untreated control. Each treatment was applied to batches of 2 fruits in order to ensure treatment uniformity and avoid overheating. Just after PL treatment, each replicate was labeled, randomized, and stored in darkness at $12 \pm 1°C$ until removal for analysis. Six treatment replicates were independently analyzed just after, and 1, 5, and 10 days after treatment. Color and texture analysis were determined in each individual fruit. Afterward, each treatment batch of two fruits was cut into small pieces, pooled, and ground with a laboratory blender (Solac Professional Mixter BV5722, Spain). Hence, six homogenates were obtained for each treatment and storage condition, and then subjected to pH, total soluble solids analysis, and extraction and determination of carotenoids and chlorophyll contents.

2.5 | Quality attributes of tomato fruits

2.5.1 | Color

The colorimetric CIELab values, $L*$ (lightness), $a*$ (red–green chromaticity), and $b*$ (blue–yellow chromaticity) were randomly measured over tomato fruits surface using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and an observation angle of 10°. A white standard plate ($Y = 94.00$, $x = 0.3158$, $y = 0.3322$) was used for calibration. For each assayed treatment condition and sampling time, color parameters were determined. Three readings were taken at random positions from each fruit. Twelve tomatoes were evaluated for each assayed treatment condition and post-treatment time ($n = 12$). Color changes were expressed as $L*$ and hue angle ($h°$), which were calculated following Equation (1):

$$h^0 = tan^{(-1)}b*/a* \qquad (1)$$

2.5.2 | Texture

Tomato firmness was determined with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England) by measuring the maximum force required to penetrate tomato fruits with a 4-mm diameter probe to a depth of 10 cm at a rate of 5 mm s^{-1}. The fruits were placed so that the plunger penetrated the pericarp in the equatorial region. Two readings from each fruit were taken. Twelve tomatoes were evaluated for each treatment condition and post-treatment time ($n = 12$). Results were expressed in Newtons (N).

2.5.3 | pH

The pH of tomato homogenate was determined using a Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain). Six homogenates were analyzed for each treatment and storage condition and two readings taken per homogenate ($n = 12$).

2.5.4 | Total soluble solids

TSS content was determined by the refraction index using an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25°C. Six homogenates were analyzed for each treatment and storage condition and two measurements were taken per homogenate ($n = 12$).

2.6 | Carotenoids and chlorophylls content

2.6.1 | Lycopene determination

Lycopene concentration was determined following the methodology proposed by Odriozola-Serrano et al. (2007) with slight modifications. Duplicates of 0.2 g of freeze-dried tomato samples were weighed and mixed with 20 ml of 0.05 % (w/v) BHT in ethanol:hexane (4:3). The mixture was homogenized at 6 g, for 15 min and 4°C in a Beckman Coulter centrifuge (Avanti J-26 XP, Pasadena, CA, USA). Then, 3 ml of distilled water were added and vortexed for 30 s. The mixture was kept at room temperature for 5 min to allow phase separation. The organic phase was collected and used to measure the lycopene concentration. Six homogenates were analyzed for each treatment and storage condition. All the extractions were repeated twice on each treatment homogenate ($n = 12$) and two readings were averaged per extraction. The absorbance of the extract was measured at 503 nm with a microplate spectrophotometer (Thermo Scientific Multiskan GO; Vantaa, Finland). Lycopene concentration was calculated according to the following Equation (2).

$$Lycopene\ concentration (mgkg^{-1})$$
$$= \frac{A_{503}x\mathrm{MW}x\mathrm{DF}x10^6}{\varepsilon xL} \qquad (2)$$

where A_{503} is the absorbance at 503 nm, MW is the molecular weight of lycopene (536.9 g mol^{-1}), DF is the dilution factor, ε is the molar extinction coefficient for lycopene (17.2 10^4 L mol^{-1} cm^{-1}) and L is the pathlength (cm). Lycopene concentration was expressed as mg kg^{-1} of tomato.

2.6.2 | Total carotenoids and chlorophylls determination

The determination of total carotenoids was carried out using the methodology proposed by Costache, Campeanu, & Neata (2012) with slight modifications. Freeze-dried tomato samples (0.2 g) were mixed and homogenized with 20 ml of 100% acetone in an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Then, the mixture was centrifuged at 3000 g for 10 min at 4°C (Beckman Coulter, Avanti J-26 XP,) and filtered through a Whatman no. 1 paper. The extract was transferred to a 25 ml flask and the volume was adjusted with acetone. Six homogenates were analyzed for each treatment and storage condition. All the extractions were repeated twice on each treatment homogenate ($n = 12$) and two readings were averaged per extraction. The absorbance of the extracts was measured spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 662, 645, and 470 nm, and the concentration of chlorophyll a, chlorophyll b, and carotenoids was calculated using Equations (3)–(5), respectively.

$$C_a = 11.75\,A_{662} - 2.35\,A_{645} \qquad (3)$$

$$C_b = 18.61\,A_{645} - 3.96\,A_{662} \qquad (4)$$

$$C_c = (1000\,A_{470} - 2.27\,C_a - 81.4\,C_b)\,/227 \qquad (5)$$

where C_a, C_b, and C_c stand for chlorophyll a, chlorophyll b, and total carotenoid concentrations, respectively. Total chlorophylls content was calculated as the sum of chlorophyll a and chlorophyll b. Results were expressed as mg kg^{-1}. All procedures were performed in dim lighting and using amber glassware in order to minimize carotenoid oxidation and isomerization.

2.7 | Statistical analysis

Statistical analysis was carried out using the JMP Pro v. 12.0.1 statistics software (SAS Institute, Cary, NC, USA). A two-way analysis of variance (ANOVA) was applied,
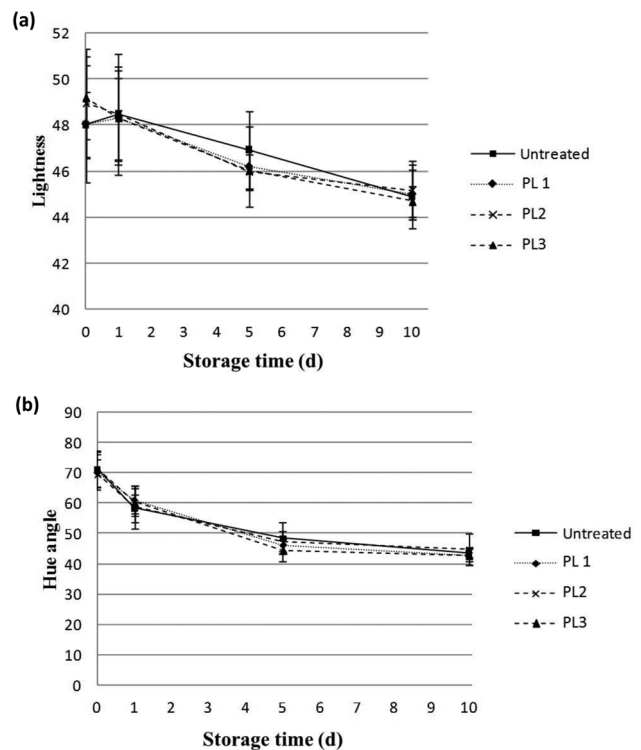

FIGURE 2 Lightness (L^*) (a) and hue angle ($h°$) (b) of tomato fruits stored at 12°C for 10 days as affected by pulsed light (PL) treatments with different spectral distributions. Different spectral ranges were assessed: wide-spectrum light ($\lambda = 180–1100$ nm) (PL1), wide-spectrum light without UV-C wavelengths ($\lambda = 305–1100$ nm) (PL2), and VIS-NIR light ($\lambda = 400–1100$ nm) (PL3). Data shown are mean $\pm$ standard deviation (n = 12). LSD$_{lightness}$ = 0.25; LSD$_{hue}$ = 0.67

considering spectral range and post-treatment time as factors. The Tukey–Kramer honestly post-hoc test was also applied. Results are expressed as mean $\pm$ standard deviation. Moreover, the relationship between variables was determined using the Pearson correlation coefficient. A confidence level of 95 % was set up in all the analyses.

3 | RESULTS AND DISCUSSION

3.1 | Quality attributes of tomato fruits

3.1.1 | Color

Color parameters (L^* and $h°$) of untreated and PL-treated tomatoes are displayed in Figure 2. Untreated tomatoes exhibited initial L^* and $h°$ values of 47.9 $\pm$ 1.4 and 71.2 $\pm$ 5.9, respectively. No significant ($p > 0.05$) differences were found in L^* and $h°$ values immediately after PL treatments, regardless the applied spectral wavelength range. L^* values of untreated and PL-treated tomatoes noticeably

decreased as time progressed. However, no significant ($p > 0.05$) differences were found between untreated and PL-treated tomatoes during the post-treatment period.

Lightness ($L*$) is the most indicative parameter associated with browning of fruits and vegetables. In this regard, the progressive decrease in $L*$ values throughout storage could be associated to the accumulation of carotenoids (Arias et al., 2000). Additionally, the mode of action of PL is related to structural changes and cell wall alterations provoked by photochemical (Gómez-López et al., 2007; Manzocco et al., 2009) and photophysical (Ramos-Villarroel et al., 2013) effects, which may lead to changes in quality attributes such as color and firmness. Ignat et al. (2014) has reported that PL can induce the breakage of cell membranes and the loss of turgor, as well as the activation of oxidative reactions (Ignat et al., 2014). Therefore, changes in $L*$ value may also be a consequence of decompartmentalization and cell membrane disruption, which is supported by observed softening (Figure 2). This fact favors the contact between oxidative enzymes, such as peroxidase (POD) and polyphenol oxidase (PPO) and their phenolic substrates, previously located in the vacuoles. No significant differences among $L*$ values of untreated and different PL-treated fruits have been reported: tomato (200–1100 nm; 2.68 and 5.36 J cm^{-2}) (Aguiló-Aguayo et al., 2013), fresh-cut cantaloupe (180–1100 nm; 0.3, 0.6, 0.9, and 1.2 J cm^{-2}) (Koh et al., 2016), or fresh-cut avocado (200–1100 nm; 3.6, 6.0 and 14 J cm^{-2}) (Aguiló-Aguayo et al., 2014). In addition, Liu et al. (2009) did not find any significant influence of short burst of UV-C light, red light, or sun light on lightness of tomatoes when they were treated daily for up to 21 days.

On the other hand, $h°$ values significantly ($p < 0.05$) decreased after treatment in both untreated and PL-treated tomatoes (Figure 2). The $h°$ values of tomato fruits were not found to be significantly ($p > 0.05$) influenced by the application of PL treatments, regardless of the applied spectral distribution. The $h°$ values decreased with time as a consequence of the increase of $a*$ values, which ranged from 8 ± 3 to 26 ± 3 at day 10 (data not shown). Pataro et al. (2015a) and Aguiló-Aguayo et al. (2013) obtained similar results since authors reported that the application of PL treatments of broad-spectrum (1–13.15 J cm^{-2}) did not affect the $h°$ value of tomato over storage (21 and 14 days, respectively). The increase in $a*$ value was also observed by Bustos et al. (2017) in avocado pulp, which was attributed to chlorophyll degradation during storage, which is supported by results shown in Table 1. Additionally, changes in $h°$ could be related to increase of redness, supported by the increase in carotenoid content (Table 2) (López-Gámez et al., 2021).
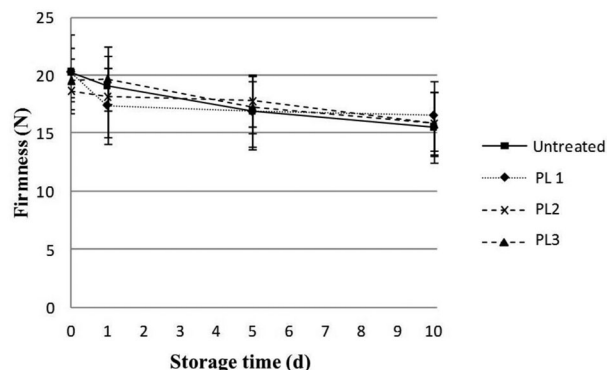

FIGURE 3 Firmness of tomato fruits stored at 12°C for 10 days as affected by pulsed light (PL) treatments with different spectral distribution. Different spectral ranges were assessed: wide-spectrum light ($\lambda = 180$–1100 nm) (PL1), wide-spectrum light without UV-C wavelengths ($\lambda = 305$–1100 nm) (PL2) and VIS-NIR light ($\lambda = 400$–1100 nm) (PL3). Data shown are mean $\pm$ standard deviation ($n = 12$). LSD = 0.51

3.1.2 | Firmness

Changes in firmness of tomato fruits after PL treatments of different spectral distribution are shown in Figure 3. A noticeable loss of firmness was observed in both untreated and PL-treated tomatoes throughout post-treatment time. However, differences in firmness of untreated and PL-treated tomatoes using different spectral distribution were not significantly noticeable.

Softening results from complex phenomena involving turgor pressure loss and the enzyme-mediated degradation of polysaccharides of the pericarp (Brashlyanova et al., 2014; Požrl et al., 2010). Many reports have described modifications in pectic polysaccharides during ripening, which contribute to cell wall disassembly (Osorio et al., 2011; Požrl et al., 2010). Therefore, tomato softening may be also a consequence of progressive changes in cell wall composition (Ait Barka et al., 2000). In accordance with the obtained results, Aguiló-Aguayo et al. (2013) did not find significant changes in tomato firmness after the application of full-spectrum PL treatments delivering a fluence of 5.36 J cm^{-2} compared to untreated tomatoes. Lopes et al. (2016) also reported that PL-treated (200–1100 nm; 0.6 J cm^{-2}) mangoes had similar firmness than those untreated at day 7 of storage. Nevertheless, authors found that the activities of the main enzymes catalyzing cell wall disintegration, pectin methyl esterase (PME), and polygalacturonase (PG) had their activity decreased. In contrast, some previous studies report that UV-C irradiation may retard fruit softening as a consequence of the down-regulated expression of genes encoding cell wall-degrading enzymes in tomato, such as PG, PME, cellulase, xylanase, β-D-galactosidase, and protease (Ait Barka et al., 2000; Bu et al., 2013). Differences from our results suggest that while UV-C

TABLE 1 Effect of pulsed light (PL) treatments with different spectral distributions on the content of total chlorophylls (mg kg^{-1}) of tomato fruits stored at 12°C

Time (days)	Untreated	PL1 (λ = 180–1100 nm)	PL2 (λ = 305–1100 nm)	PL3 (λ = 400–1100 nm)
0	3.93 ± 0.92	4.71 ± 0.54	4.62 ± 0.76	4.38 ± 1.00
1	3.87 ± 1.00	2.90 ± 0.72	3.92 ± 0.94	3.33 ± 0.79
5	3.49 ± 0.72	2.76 ± 0.31	4.42 ± 0.53	3.32 ± 0.2
10	3.14 ± 0.45	3.23 ± 0.74	3.00 ± 0.38	3.83 ± 0.74

Data shown are mean ± standard deviation (n = 12). LSD = 0.15

TABLE 2 Effect of pulsed light (PL) treatments with different spectral distributions on the content of total carotenoids (mg kg^{-1}) of tomato fruits stored at 12°C

Time (days)	Untreated	PL1 (λ = 180–1100 nm)	PL2 (λ = 305–1100 nm)	PL3 (λ = 400–1100 nm)
0	19.32 ± 2.30	23.23 ± 0.99	19.99 ± 2.00	20.65 ± 2.42
1	27.30 ± 2.35	21.94 ± 1.23	25.82 ± 2.11	22.97 ± 1.23
5	32.81 ± 4.88	35.42 ± 5.29	39.25 ± 3.13	43.10 ± 5.34
10	42.94 ± 6.09	42.11 ± 3.47	39.55 ± 4.23	45.85 ± 1.89

Data shown are mean ± standard deviation (n = 12). LSD = 0.66

continuous light may delay tomato softening, the application of PL treatments containing different spectral wavelengths within the 180–1100 nm (from UV to NIR), such as those used in this work, may not have any effect on the tomato firmness. Furthermore, the application of PL treatments instead of continuous exposure to UV light would likely cause lower photothermal effect and reduced absorption of UV range by membrane components (Koh et al., 2016). Further research about the activity of cell-wall associated enzymes should be aimed at to elucidate the influence of PL treatments with different spectral ranges on these enzymes.

3.1.3 | pH

The initial pH of untreated tomatoes was 4.17 ± 0.03 and was not affected just after the application of any of the PL treatments assessed (Figure 4). As time progressed, a marked increase in pH values was observed in both untreated and PL-treated tomatoes. The post-treatment variation of pH began to be significant ($p < 0.05$) at day 1. However, this increase was less noticeable in tomatoes subjected to PL treatments delivering wavelengths within 305–1100 nm (wide-spectrum without UV-C light), thus leading to significant lower values of pH at day 10 in comparison to untreated tomatoes. According to our results, suppression of UV-C light in PL treatments allowed maintaining low pH values in tomato fruits throughout post-treatment time. The progressive increase of pH values is usually attributed to the loss of organic acids occurring during tomato ripening (Anthon et al., 2011). These results are consistent with
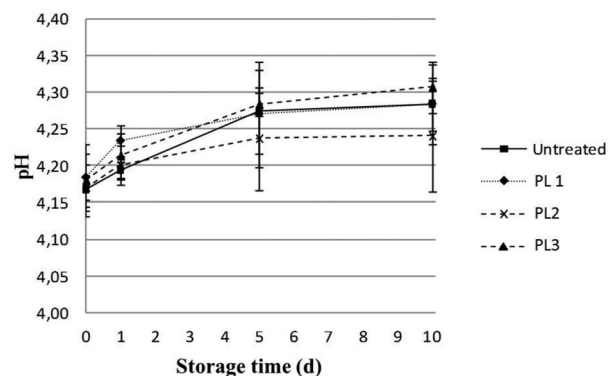
FIGURE 4 The pH of tomato fruits stored at 12°C for 10 days as affected by pulsed light (PL) treatments with different spectral distribution. Different spectral ranges were assessed: wide-spectrum light (λ = 180–1100 nm) (PL1), wide-spectrum light without UV-C wavelengths (λ = 305–1100 nm) (PL2), and VIS-NIR light (λ = 400–1100 nm) (PL3). Data shown are mean ± standard deviation (n = 12). LSD = 0.01

those reported by other authors (Pataro et al., 2015), who previously noticed that pH of tomato remained almost unchanged after light irradiation with UV-C and PL treatments of broad-spectrum. Our results seem to point out that the application of visible/NIR wavelengths could compensate the deleterious effects of UV-C treatments.

3.1.4 | Total soluble solids

The initial total soluble solids (TSS) content of untreated tomatoes was 4.50 ± 0.06°Brix and was not significantly ($p > 0.05$) affected by any of the PL treatments studied
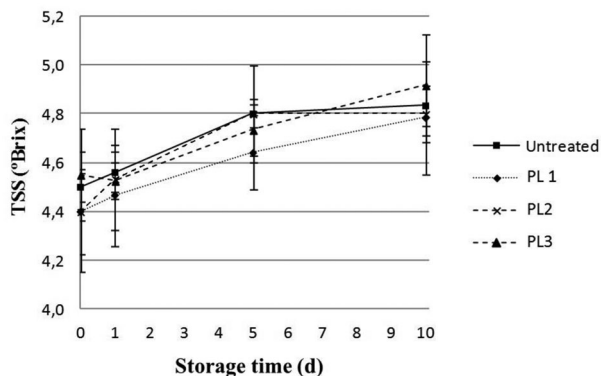
FIGURE 5 Total soluble solids (TSS) content of tomato fruits stored at 12°C for 10 days as affected by pulsed light (PL) treatments with different spectral distribution. Different spectral ranges were assessed: wide-spectrum light (λ = 180–1100 nm) (PL1), wide-spectrum light without UV-C wavelengths (λ = 305–1100 nm) (PL2), and VIS-NIR light (λ = 400–1100 nm) (PL3). Data shown are mean ± standard deviation (n = 12). LSD = 0.03

(Figure 5). TSS of both untreated and PL-treated tomatoes continuously increased during storage, which may be associated to the accumulation of soluble sugars during tomato ripening (Anthon et al., 2011; Denoya et al., 2020). However, TSS of tomato fruits were not found to be significantly (p > 0.05) affected by the application of PL treatments of different spectral wavelength range throughout the storage period. In this work, only TSS was determined, but Aguiló-Aguayo et al. (2017) also observed that the extraction of certain free sugars was enhanced in PL-treated (2.26, 4.52, 5.41, 9.38, and 13.15 J cm^{-2}) carrots as a result of hydrolysis from more complex carbohydrates by the application of PL. Although there are no previous studies regarding the influence of PL treatments of different spectral range on the TSS content of tomato, some authors have reported that the exposure of tomatoes to continuous UV-C irradiation and PL treatments of broad-spectrum did not significantly influence the TSS of tomato and other fruits (Denoya et al., 2020; Koh et al., 2016; Liu et al., 2009; Pataro et al., 2015), which is in line with our results.

3.2 | Carotenoids and chlorophylls content

3.2.1 | Chlorophyll content

Changes in chlorophyll content in tomato fruits as affected by the application of PL treatments of different spectral distributions are shown in Table 1. A significant (p < 0.05) decrease in total chlorophyll content in both untreated and PL-treated tomatoes was observed throughout storage. Initially, total chlorophyll concentration in untreated

tomatoes was 3.93 ± 0.9 mg kg^{-1}, which progressively decreased by 20.1% throughout post-treatment time. In contrast, PL-treated tomatoes exhibited a marked decrease in chlorophyll content during the first day of storage. Afterward, its concentration remained almost unchanged until the end of storage. This trend was especially evident in tomatoes subjected to PL treatments with broad-spectrum light (180–1100 nm), which exhibited a 33.4% decrease in chlorophyll concentration at day 1 in comparison to untreated tomatoes. Similar results have been reported in PL-treated fresh-cut avocado after applying the same wavelength ranges (Velderrain-Rodríguez et al., 2021). Authors attributed this reduction to photooxidative reactions, the loss of cellular compartmentalization, and the action of chlorophyllase, chlorophyll oxidase, and POD. Additionally, another alternative explanation can be proposed considering the obtained results regarding carotenoids content. Tomato ripening is affected by several environmental factors, including light irradiance (Llorente et al., 2016). During this process, chlorophylls are degraded (Table 1) and carotenoids are accumulated (Tables 2 and 3) in chromoplasts (Pataro et al., 2015). Carotenoids are photoprotectants of the photosynthetic apparatus against excess light and act as hormone precursors (Llorente et al., 2016); therefore, PL treatments could promote the tomato ripening leading to chlorophyll reduction and increase in the carotenoid biosynthesis. Contrasting results have been previously reported by other authors. In this regard, Lopes et al. (2016) observed a delay in the loss of chlorophylls in mango pulp as affected by broad-spectrum PL treatments (0.6 J cm^{-2}). On the other hand, Aguiló-Aguayo et al. (2014) determined that chlorophylls of fresh-cut avocados were better preserved during storage after PL treatments of 3.6 and 6 J cm^{-2}, whereas they were degraded after applying 14 J cm^{-2}. These results suggest that the oxidative stress response triggered by PL may differ depending on the applied dose, the wavelength range, and the type of fruit. It has been demonstrated that postharvest UV-B and UV-C irradiation significantly delay chlorophyll degradation in tomato fruits (Maharaj et al., 1999) and broccoli (Aiamla-or et al., 2010; Ribeiro et al., 2012), whereas red (660 nm) and far-red light (730 nm) are involved in chloroplast to chromoplast transition and hence, in the loss of chlorophylls (Alba et al., 2000). Therefore, the results obtained in this study could be associated to the presence of VIS + NIR wavelengths (400–1100 nm) in the PL treatments, which probably trigger carotenogenesis.

3.2.2 | Carotenoids content

The effects of PL treatments of different spectral wavelength distributions on total carotenoids and lycopene

TABLE 3 Effect of pulsed light (PL) treatments with different spectral distributions on the lycopene content (mg kg^{-1}) of tomato fruits stored at 12°C

Time (days)	Untreated	PL1 (λ = 180–1100 nm)	PL2 (λ = 305–1100 nm)	PL3 (λ = 400–1100 nm)
0	9.71 ± 1.21	11.81 ± 0.52	10.48 ± 1.22	10.65 ± 1.45
1	14.30 ± 1.79	11.08 ± 0.59	13.62 ± 1.43	11.89 ± 0.93
5	18.28 ± 2.93	20.66 ± 2.68	21.55 ± 1.39	24.75 ± 2.84
10	24.49 ± 4.00	24.40 ± 2.16	22.66 ± 2.57	25.42 ± 1.68

Data shown are mean ± standard deviation (n = 12). LSD = 0.38.

contents throughout post-treatment time are displayed in Tables 2 and 3, respectively. Total carotenoids concentration of untreated tomatoes continuously increased during storage, from 19 ± 2 mg kg^{-1} to 43 ± 6 mg kg^{-1} at day 10. Lycopene concentration followed an upward similar trend to that observed for total carotenoids. Thus, initial lycopene concentration in untreated tomatoes was 9.7 ± 1.2 mg kg^{-1} and continuously increased by 2.36-fold over the reported storage period. PL-treated tomatoes exhibited a sharp increase in total carotenoid (8–31 %) and lycopene (13–35 %) contents at day 5 after treatments and regardless of the wavelength range applied in comparison to untreated tomatoes at the same post-treatment time. Then, the contents remained constant through further storage. Nevertheless, the carotenoids concentration was differently affected depending on the spectral distribution applied. In this regard, those tomatoes subjected to PL treatments characterized by wavelength within the 400–1100 nm (VIS + NIR) exhibited the highest increase in total carotenoids concentration at day 5, 31% more than untreated tomatoes at the same post-treatment time. After such PL treatments, lycopene content also attained its maximum enhancement (1.35-fold increase) at day 5. However, treatments applying light containing UV fractions did not exert any significant (p > 0.05) impact on total carotenoids and lycopene content compared to untreated tomatoes. However, it is important to consider that the spectrophotometric method used in this study could only allow the detection of the colorful carotenoids. Nevertheless, colorless carotenoids and precursors, such as phytoene and phytofluene, which are also found in tomatoes, were not assessed. Further HPLC analysis should be carried out in order to precisely quantify the specific concentration of each individual compound.

It was reported that fruit-localized phytochromes play a fundamental role in the light-induced carotenoids biosynthesis in tomatoes (Alba et al., 2000). Phytochromes are photoreceptors involved in response-regulation by red light and far-red light (Llorente et al., 2016; Schofield & Paliyath, 2005). In this regard, the fast accumulation of carotenoids in tomatoes after the application of PL treatments was likely related to the modulation of phytoene synthase (PSY) activity, considered the main enzyme in carotenoids biosynthesis, triggered by the high ratio of

red and far-red light. Previous studies have reported similar carotenoid increment during post-treatment time of different PL-treated fruits, such as tomato (200–1100 nm; 1, 2, 4 J cm^{-2}) (Pataro et al., 2015), fresh-cut avocado (400–1100 nm; 12 J cm^{-2}) (Velderraín-Rodríguez et al., 2021), and mango (200–1100 nm; 1.2 J cm^{-2}) (Lopes et al., 2016). However, carotenoids reduction has also been reported in other vegetable products such as bell pepper (190–1100 nm; 4–8 J cm^{-2}) (Rybak et al., 2021). These differences can be explained by the applied PL fluence (Pataro et al., 2015; Rybak et al., 2021). Photooxidative stress during high light intensities may play a major role in the chlorophyll loss and accumulation of carotenoids, whereas lower intensity could likely reduce the activity of the enzymes involved in chlorophyll degradation or carotenoid biosynthesis (Aguiló-Aguayo et al., 2014).

Scarce information about the effects of PL of different wavelength ranges on carotenoid accumulation is available. High levels of light and UV radiations may induce plant defense responses. It has been reported that the UV-light exposure from minutes to hours accounts for the formation of free radicals, which lead to the initiation of photooxidation followed by photodecomposition (Demirci & Krishnamurthy, 2011). These processes could be behind the modification of both the enzymes involved in the carotenoid biosynthesis and some food constituents, leading to product quality deterioration (Bravo et al., 2012; Demirci & Krishnamurthy, 2011; Pataro et al., 2015). The differences observed in this study could be associated to the deleterious effect of UV-light (180–400 nm). In this regard, Tiecher et al. (2013) and Lu et al. (2016) noticed that the application of UV-C light delayed the carotenoids accumulation in tomato fruits. In addition, Liu et al. (2011) reported that the application of postharvest UV-B (10–40 J cm^{-2}) significantly reduced the lycopene content of tomato fruits. Therefore, these effects can probably counteract the beneficial effect of both red and far-red light on the activation of the carotenoid's biosynthesis and their accumulation.

It is known that the rapid accumulation of carotenoids during tomato ripening, particularly lycopene, is triggered by an increase in ethylene production (Liu et al., 2015). However, available literature does not always offer consistent results. On the one hand, Tiecher et al. (2013) found that UV-C irradiation promoted ethylene production but

delayed red color development in tomato fruits. On the other hand, Lu et al. (2016) observed that UV-C light postponed the ethylene production, resulting in delayed lycopene bioproduction in tomato. Similarly, Scott et al. (2018) observed a transient peak in ethylene production at 24 h after low intensity UV-C treatments and pulsed polychromatic light, followed by a lag in ethylene production. These authors also observed a significant increase in enzymes expression involved in the carotenoid's biosynthetic pathway, especially 1 day after treatment. According to these results, the fast accumulation of total carotenoids and lycopene concentration observed in this study at day 5 after treatments could be related to an increase in ethylene production promoted by PL treatments. Moreover, the inverse and significant correlation found between chlorophylls and both total carotenoids and lycopene concentration ($R = 0.709$ and $R = 0.71$, $p < 0.001$, respectively) indicates that PL treatments may accelerate the degradation of chlorophylls and the synthesis and accumulation of carotenoids, mainly lycopene, in tomato fruits. Additionally, firmness of PL-treated tomatoes was similar to those untreated, which confirms that the increase in carotenoid content is not related to their improved extractability (Figure 3). This study demonstrates that the exposure to PL can significantly stimulate the accumulation of these health-related compounds during post-treatment storage. In addition, this study confirms that the spectral wavelength range needs to be finely tuned in order to optimize the induced accumulation of carotenoids in tomato fruits by the application of PL treatments.

4 | CONCLUSION

Carotenoids concentration of tomato fruits was differently affected depending on the spectral distribution of the PL treatment. The efficiency of the emitted spectrum wavelengths increased as follows: UV + VIS + NIR < wide-spectrum light without the UV-C range < VIS + NIR. Treatments containing only VIS and NIR fractions (400–1100 nm) led to 1.31- and 1.35-fold increases in total carotenoids and lycopene concentrations, respectively, in comparison to untreated tomatoes after 5 days of post-treatment time. Quality attributes (color, firmness, pH, and TSS) of tomato fruits were not negatively affected by any of the PL treatment conditions studied. Therefore, a proper combination of dose spectral range and post-treatment storage yields positive effects on the antioxidant potential of tomato fruits. These results open new prospects regarding the application of PL technologies as an alternative to UV light continuous exposure since PL minimizes the side effects on quality attributes due to the short time application while promoting a similar effect on bioproduction of bioactive compounds. Additionally, being

a climacteric fruit, tomato metabolism continues to be active after being harvested. Some growers harvest tomato at mature green stage and let them ripen off the vine to extend the available time for transportation to the market. However, this negatively affects their color and final quality. PL treatments could be a potential solution for triggering their ripening, the accumulation of carotenoids, and maintainance of their color. Such treatments could be applied by food industries according to their appropriate time. Therefore, PL technology can be a potential way to obtain raw materials for industrial uses with improved health benefits.

AUTHOR CONTRIBUTIONS

Sandra González-Casado: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft. **Gloria López-Gámez**: Visualization; Writing – review & editing. **Olga Martín-Belloso**: Writing – review & editing. **Robert Soliva-Fortuny**: Supervision; Writing – review & editing

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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