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Postharvest UV-B and Photoperiod with Blue + Red LEDs as Strategies to Stimulate Carotenogenesis in Bell Peppers

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Featured Application: Illumination with blue + red LEDs during the nights of retail sale periods is recommended to increase carotenogenesis in bell peppers. This night-time photoperiod combined with a low postharvest UV-B dose in a logistic centre may present a synergistic effect on the carotenoid accumulation in these fruits.

Abstract: Background: Our objective was to evaluate carotenoid accumulation in bell peppers during shelf life under different light conditions. Methods: Fruit stored for 6 d at 7 °C received a 9 kJ m^{-2} UV-B treatment, while non-UV-treated were used as control (CTRL). Subsequently, all peppers were disposed for a retail sale period of 4 d at 20 °C with a photoperiod of 14 h under fluorescent light (FL) + 10 h under darkness (D), FL, or blue + red LEDs (BR LED). Results: Total antioxidant capacity (TAC) was increased by the UV-B treatment and the photoperiods supplemented with FL and BR LED, which was directly related to the carotenoid content. In fact, CTRL peppers (225 mg β-carotene kg^{-1}) under FL+BR LED showed an increase of ~33% of 13-cis-β-carotene, ~24% of all-trans-β-carotene, and ~27.5% of 9-cis-β-carotene compared to FL + D and FL + FL. Capsaicinoids showed an increase by ~22%, ~38%, and ~27% in the content of capsanthin, capsanthin laurate, and capsanthin esters, respectively, after the UV-B treatment, which was even enhanced after the LED-supplemented photoperiod by ~18% compared to FL+D. Conclusions: Illumination with BR LEDs + UV-B during the retail sale period nights is recommended to increase the bioactive content of bell peppers via carotenoid accumulation to 270 mg β-carotene kg^{-1}.

Keywords: *Capsicum annum* L.; ultraviolet; lighting emitting diode; abiotic stress; bioactive compounds; nutraceuticals; antioxidants; shelf life

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1. Introduction

Fruit and vegetables are essential in human health due to the high content in bioactive compounds [1]. Indeed, these nutraceuticals have been associated with a reduction in the risk of chronic and pro-inflammatory diseases [2]. Red peppers (*Capsicum annuum* L.) in particular are demonstrated to be an important source of carotenoids [3], flavonoids, and vitamins [4], being a powerful ingredient able to fight against ageing and prevent chronic diseases [5]. In this context, Spain is the fifth largest producer of bell peppers in the world after China, Mexico, Turkey, and Indonesia [6] and the first European exporter country [7] with 1.4 million tons.

As light is an essential factor directly related to the synthesis of primary and secondary metabolites [8], the bioaccumulation pathways of these compounds can vary with changes in the quantity and quality of lighting applied. In fact, application of abiotic stresses throughout non-visible spectral regions, such as UV-B (280–315 nm), are reported to have an hormetic effect characterized by positive stimulation of the metabolite pathways under low doses [9,10], but a negative response of the plant tissues may appear under moderate to high UV doses [11]. In fact, UV RESPONSE LOCUS 8 (UVR8), as the main UV-B receptors,

are responsible for regulating carotenoid biosynthesis [12]. Thus, UV-B has shown to improve the contents of carotenoids and flavonoids as the main antioxidant compounds in bell peppers [13]. In this way, UV can be used to induce the expression of antioxidant enzymes, which increase the biosynthesis of vitamins, flavonoids, phenolic acids, and carotenoids, among others [14–16].

From another point of view, the environmental conditions in the final steps of the fresh bell pepper retail chain in supermarkets widely apply conventional fluorescent lamps (2 W m^{-2} on the floor and 8 W m^{-2} on the shelves) for 14 h between 15 to 22 °C [17]. However, when supermarkets close, the lamps above the horticultural commodities are usually switched off for approximately the next 10 h in order to save energy, with such conditions biologically promoting the accumulation of phytochemicals. In recent years, the use of coloured LEDs in supermarkets and the industry have increased due to their low energy requirements and their possibility to customize the light intensity and spectral properties. In this sense, the use of postharvest LED lighting has already shown potential beneficial effects in broccoli florets [18–20], brussels sprouts [21], and broccoli sprouts [22].

However, the effect in quality and phytochemicals accumulation of horticultural commodities of the combination of different postharvest lighting conditions from the visible and non-visible spectrum is mostly unexplored. Pérez-Ambrocio et al. [23] combined postharvest blue LED and UV-C lighting obtaining promising results in habanero pepper (*Capsicum chinense*) by increasing chlorophyll, carotenoid, capsaicin, phenolic, and flavonoid content.

The combination of UV illumination and a photoperiod with lights from the visible spectrum during the postharvest shelf life of fruit and vegetables could improve the bioactive compound contents without compromising other quality attributes, or even improving them. Therefore, the aim of the present work was to evaluate the effect on carotenoid accumulation of UV-B radiation treatment jointly with a photoperiod under different illumination spectra during the commercialization of red bell peppers.

2. Materials and Methods

2.1. Plant Material

The selected bell pepper (*Capsicum annum* L.) variety was 'Angus' from Syngenta España. S.A. (Torre-Pacheco, Murcia, Spain) of the California type and with red ripening. Bell peppers were grown in a greenhouse in the Southeast of Spain by a local producer under integrated pest management (Tárraga y Henarejos, S.L., Murcia, Spain) and harvested on 21st July 2020, when the maturity index reached 21.8 ± 0.7. Quality characterization at harvest of the studied fruit is shown in Table 1.

Table 1. Main quality parameters at harvest of studied bell peppers (n = 25).

Quality Attribute		Value at Harvest
Weight (g)		198.9 ± 26.5
Calibre (mm)	Equatorial	87.6 ± 7.1
	Longitudinal	87.5 ± 8.6
	Thickness	5.9 ± 0.6
Colour	L*	33.3 ± 1.9
	a*	26.8 ± 5.7
	b*	19.5 ± 3.1
	Chroma	33.3 ± 5.8
	Hue angle	36.6 ± 6.0

Plant material was transported for 30 min to the Universidad Politécnica of Cartagena. After reception, the healthy and undamaged peppers were selected and washed with 5% peracetic acid (Citrocide® PC, Citrosol, Valencia, Spain) at 7 °C for 2 min and rinsed in tap water for 1 min. A total of 550 bell peppers were washed and dried with absorbent paper.

2.2. Postharvest Treatments and Light Photoperiod

Bell red peppers were stored for 6 d at 7 °C and 80% relative humidity (RH) under conditions of darkness, simulating a short postharvest storage + commercial exportation within Europe. Then, considering that the fruit arrived at a logistic platform, or even though that they could be repacked, peppers were divided into two groups: CTRL and UV-B. UV-B red peppers were exposed to 9 kJ m^{-2} UV-B (19 min 30 s) in a radiation chamber consisted of a reflective stainless-steel chamber described by Formica-Oliveira et al. [24], which was equipped with 6 UV-B unfiltered emitting lamps (TL 40W/01 RS; Philips, Eindhoven, The Netherlands). The applied UV-B intensity was calculated as the mean of 20 readings with a LP 471 UVB radiometer (Delta OHM, Padua, Italy): 8.94 ± 0.40 W m^{-2}, and this dose was chosen based on our preliminary test and findings by Formica-Oliveira et al. [10,24].

After treatment, bell peppers were kept at 20 °C and 60% RH during 2, 3, and 4 d, simulating a retail sale period in supermarkets on the destination country. In order to adapt our model to what usually happens in supermarkets, three different photoperiods were applied following the same pattern: 14 h under fluorescent lighting (**FL**) with 403 kJ m^{-2} when supermarkets are open + 10 h under the next variable lighting treatment when supermarkets are close at night)

- **D:** darkness, as it is usually performed.
- **FL:** fluorescent lighting with lower energy (280 kJ m^{-2}) to save costs provided by fluorescent lamps (OSRAM DULUX L 36W/840, Munich, Germany) with broad white spectrum and 8 W m^{-2}, simulating the conventional storage conditions measured by us for this study in several Spanish supermarkets during June–July 2020.
- **BR LED:** a combination of blue + red LEDs (LEDMurcia S.L., Murcia, Spain) with 576 kJ m^{-2}. LED lamps were applied with a simultaneous combination (1:1) of blue (peak at 450 nm) and red (peak at 660 nm). This combination was chosen due to our previous preliminary test based on previous findings by Martínez-Zamora et al. [25] and Pennisi et al. [26].

A constant photosynthetic photon flux density (PPFD) for FL and LED lights of 36.7 and 73.1 µmol m^{-2} s^{-1}, respectively, were measured using a Quantum-Photo Radiometer Data Logger DO 9721 (Delta Ohm, S.R.L., Venice, Italy). Quality sampling days throughout the studied postharvest period were at harvest (day 0), after 6 d at 7 °C (end of the simulated storage/transportation period), and after 2, 3, and 4 d at 20 °C of the simulated retail sale period. A schematic representation of this study is shown in Figure 1.

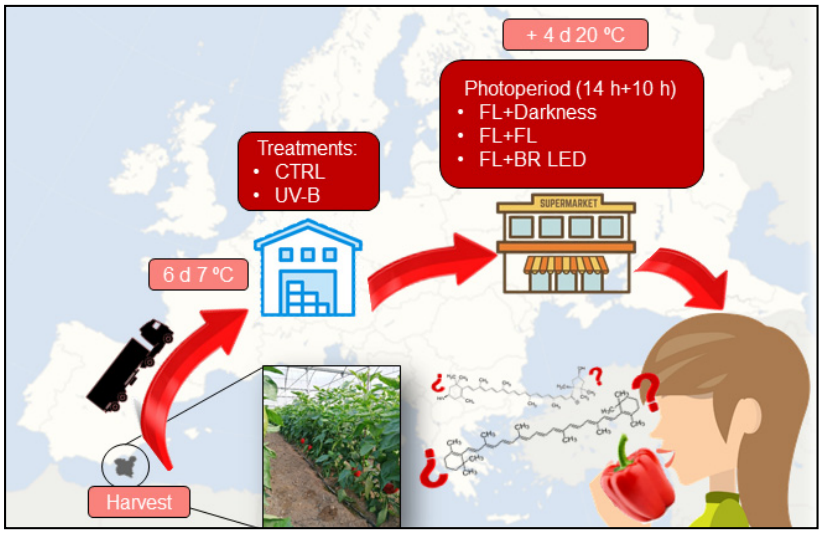

Figure 1. Schematic representation of the experimental design of the study.

2.3. Physicochemical and Sensory Quality Determination

The physicochemical quality analyses were carried out on each sampling day. Bell peppers were weighed (g) using a FH-2000 scale (GRAM, Barcelona, Spain). Weight losses (%) were calculated by using the next formula: ((Initial Weight−Final Weight)/Initial Weight) × 100. Thickness and longitudinal and equatorial calibre were initially measured using a digital calliper (Mitutoyo, Neuss, Germany), expressing the results in mm. Colour was determined using a Konica Minolta CR-400 colorimeter (Tokyo, Kanto, Japan). Measurements were recorded using the CIELab system (L*, a*, and b* coordinates). Total colour differences (ΔE) throughout the shelf life were compared to their respective initial values according to equations previously described by Torres-Sánchez et al. [27]. A texturometer (Brookfield, CT3-4500, Toronto, ON, Canada) was used to test firmness at room temperature (RT) with a cylinder of 8 mm diameter and a surface area of 2.01 cm^2 and compressing the fruit by 5 mm at a contact speed of 2 mm s^{-1}, expressing these results in newtons (N).

After that, bell peppers were cut and blended using a Robot Coupe J80 Ultra (Vincennes, Île-de-France, France). The total soluble solids content (TSS), pH, and titratable acidity (TA) of the obtained pepper juice were determined. TSS were measured by a digital handheld refractometer (Atago N1; Tokyo, Kanto, Japan) and expressed as %. A pH meter (GLP21, Crison; Alella, Cataluña, Spain) was used to determine the pH while TA was determined by titration of 5 mL of pepper juice plus 45 mL of distilled water with 0.1 M NaOH to pH 8.1 (T50, Metter Toledo; Milan, Lombardia, Italy) and expressed as mg citric acid 100 mL^{-1}.

Each sample was analysed in quintuplicate on each sampling day per treatment, and 5 bell peppers formed a replicate (n = 25). After the physicochemical analyses were performed, three replicates of 5 juiced peppers each per treatment were frozen in liquid nitrogen for every sampling time and stored at −80 °C. Frozen samples were ground to fine powder prior to analyses using liquid N_2 with a mincer (IKA, A 11 basic, Berlin, Germany) at 12,700 g for 10 s.

The characterization was completed with a sensory evaluation each sampling day. The tasting room was air-conditioned and free of disturbing factors. Bell peppers were cut into 1 × 5 mm pieces. Ten panellists were trained following the ISO guide [28]. Samples were coded with three random digits and were presented individually to the panellists. Mineral water was provided for mouth rinsing between samples. The attributes measured for the colour, odour, and taste characteristics were 'Dehydration', 'Visual appearance', 'Colour (green/mature)', 'Odour', 'Firmness (touch, when slightly compressing with the fingers)'. 'Firmness (mouth, when eating)', and 'Flavour'. Overall acceptability was measured by a panel of 30 consumers. A hedonic scale in intensity from 1 to 5 was used, being 1 'Extremely bad', 2 'Bad'; 3 'Limit of commercialization', 4 'Good', and 5 'Excellent'.

2.4. Extraction and Determination of Bioactive Compounds

One gram of bell pepper powder was placed in plastic tubes and 10 mL methanol:H_2O (80:20, v/v) was added. The extraction was carried out in an orbital shaker (Stuart, Stone, UK), where the samples were vigorously shaken for 1 h at 4 °C in darkness. The extracts were centrifuged at 3220× g for 10 min at 5 °C and the supernatant was collected and kept at −80 °C until further analysis of total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC). Extractions were carried out on each sampling day in triplicate.

The TPC was determined as previously described by Castillejo et al. [22]. For that, 19 µL sample extract were mixed with 29 µL of 1 mol L^{-1} Folin–Ciocalteu reagent. Three minutes later, 192 µL of 0.4% Na_2CO_3 and 2% NaOH were added. After 1 h incubation in darkness, the absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200, Männedorf, Switzerland). The TPC was expressed as g gallic acid equivalents (GAE) kg^{-1} fresh weight (fw). Each sample was analysed in triplicate.

The TFC was determined following the method described by Hamed et al. [5]. Briefly, 0.030 mL of extract were mixed with 80 µL of aluminium chloride (20 g L^{-1}). The samples

were shaken for 30 s and then incubated in darkness for 1 h. After that, the reaction absorbance was measured at 415 nm. TFC was expressed as g rutin equivalents (RE) kg^{-1} of fw. Each sample was analysed in triplicate on each sampling day.

The TAC was analysed following the DPPH procedure [29]. DPPH assay was performed by adding 194 µL of DPPH (0.7 mM) solution to 21 µL of bell pepper extract. After 30 min incubation in darkness, absorbance was measured at 515 nm. Obtained data were expressed as g of trolox equivalents (TE) kg^{-1} fw. Each sample was analysed in triplicate on each sampling day.

2.5. Extraction and Determination of Carotenoids

Preparation and carotenoid analysis were performed according to Martínez-Zamora et al. [25] with slight modifications. A sample of 1 g was homogenized with 5 mL of chloroform:dichloromethane (2:1, v/v) in a basic grinder (IKA A11, Staufen, Germany). The extraction was carried out in an orbital shaker (Stuart, Stone, UK) for 20 min at 200 rpm at 4 °C. After this time, 2 mL of 1 M NaCl solution were added for phase separation. The extracts were centrifuged at 5000× g for 10 min at 4 °C and the organic phase was collected. The supernatant was re-extracted by adding 2.5 mL of chloroform:dichloromethane (2:1, v/v) and centrifuging again, and the organic phase was collected. Finally, the extracted phase was dried by centrifugal evaporation and re-dissolved in 4 mL methanol:MTBE (25:75, v/v) and filtered using 0.2 µm PTFE membrane filters.

An ultrahigh-performance liquid chromatography (UHPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater, and SPDM-20A photodiode array detector was used. Chromatographic analyses were carried out into a C30 column (250 mm × 4.6 mm, 3 µm particle size; YMC Co., Kyoto, Japan). Carotenoids were quantified as equivalents of β-carotene. The results were expressed as mg kg^{-1} fw. Each sample was analysed in triplicate.

2.6. Data Analysis

The experiment was a three-factor (treatment -T- × photoperiod -P- × time -t-) design subjected to analysis of variance (ANOVA) using Statgraphics Plus software (v. 5.1. Statpoint Technologies. Inc., Warrenton, VA, USA). Statistical significance was assessed at the level $p < 0.05$, and Tukey's multiple range test was used to separate means.

3. Results

3.1. Physicochemical Quality Changes throughout Shelf Life

Bell pepper weight at harvest was 198.9 ± 26.5 g (Table 1), for which 0.5% weight losses were reported after 6 d at 7 °C (Table 2). Although there were no weight loss differences after 6 d at 7 °C after the UV-B treatment for CTRL fruit, dehydration was higher after the retail sale period of 4 d at 20 °C, especially under the FL + BR LED photoperiod. Hence, such losses are directly related to firmness, which was slightly lower under the FL + FL and FL + BR LED photoperiods and accentuated after 4 d at 20 °C by ~40%–50% reductions (Table 2).

The optimum ripening stage as a non-climacteric fruit was reached after 6 d at 7 °C, which can be observed when the ΔE achieves constant values just before the retail sale period at 20 °C (Table 2). In this way, a slight colour variation in fruit under the FL + FL photoperiod was shown regarding peppers stored under FL+D and FL+BR LED. TSS also showed a slightly increase throughout the shelf life, especially on UV-B-treated samples. Indeed, the interaction among all variables (T, P, and t) of the experimental design was highly significant, which also occurs with TA and MI (TSS/TA). While TA decreased throughout shelf life, MI increased, especially in CTRL red peppers under the FL+FL photoperiod, reaching values of 0.28 ± 0.05 mg citric acid 100 mL^{-1} and 28.7 ± 0.6 for MI (Table 2). In contrast, pH values remained quite constant during shelf life at 4.9 ± 0.0 and no interaction was found among dependent variables.

Table 2. Physicochemical quality attributes of UV-B-treated and untreated CTRL bell peppers after 6 d at 7 °C followed by a retail sale period of 2, 3, and 4 d at 20 °C under a photoperiod of 14 h in fluorescent light + 10 h in different lighting conditions.

	T	P 14 h + 10 h	t	Weight Losses (1)	Firmness (2)	ΔE	TSS (3)	TA (4)	TSS/TA
At harvest			0 d	-	19.8 ± 7.3	-	7.5 ± 0.4	0.35 ± 0.02	21.8 ± 0.7
After 6 d at 7 °C	CTRL	-	6 d	0.5 ± 0.1	19.7 ± 4.5	8.4 ± 6.1	7.6 ± 0.3	0.35 ± 0.03	21.7 ± 1.1
	UV-B	-		0.5 ± 0.1	17.2 ± 3.6	5.9 ± 3.9	7.6 ± 0.3	0.31 ± 0.01	24.7 ± 1.6
After a retail sale period at 20 °C	CTRL	FL + D	+2 d	1.1 ± 0.2	17.2 ± 2.6	6.2 ± 2.6	7.8 ± 0.2	0.30 ± 0.02	26.2 ± 1.1
			+3 d	2.5 ± 0.5	13.8 ± 3.3	6.4 ± 2.6	7.5 ± 0.1	0.28 ± 0.02	26.5 ± 1.2
			+4 d	3.7 ± 0.7	10.1 ± 2.0	6.3 ± 2.2	8.1 ± 0.3	0.32 ± 0.01	25.6 ± 0.2
		FL + FL	+2 d	1.3 ± 0.3	16.7 ± 3.0	7.4 ± 5.8	7.9 ± 0.2	0.36 ± 0.02	21.8 ± 0.7
			+3 d	2.8 ± 0.6	13.7 ± 3.0	7.7 ± 4.6	7.6 ± 0.3	0.31 ± 0.01	24.4 ± 0.6
			+4 d	4.0 ± 0.8	9.9 ± 2.2	7.4 ± 5.7	7.8 ± 0.3	0.28 ± 0.05	28.7 ± 0.6
		FL + BR LED	+2 d	1.5 ± 0.2	15.2 ± 3.2	6.3 ± 3.5	7.7 ± 0.2	0.30 ± 0.01	25.3 ± 1.2
			+3 d	3.1 ± 0.5	13.7 ± 3.4	6.0 ± 2.4	7.6 ± 0.2	0.31 ± 0.02	24.3 ± 1.4
			+4 d	4.6 ± 0.7	8.1 ± 2.1	6.2 ± 2.6	7.7 ± 0.1	0.31 ± 0.01	25.1 ± 1.1
	UV-B	FL + D	+2 d	1.2 ± 0.3	16.7 ± 4.0	6.1 ± 2.7	7.7 ± 0.2	0.32 ± 0.01	23.7 ± 0.8
			+3 d	2.5 ± 0.6	14.4 ± 3.2	6.6 ± 2.8	7.5 ± 0.1	0.30 ± 0.01	25.2 ± 1.2
			+4 d	3.7 ± 0.9	9.7 ± 2.6	6.2 ± 2.3	7.8 ± 0.2	0.30 ± 0.01	26.4 ± 0.1
		FL + FL	+2 d	1.3 ± 0.2	17.4 ± 3.3	8.5 ± 5.6	7.6 ± 0.5	0.31 ± 0.02	24.7 ± 0.7
			+3 d	2.7 ± 0.4	12.2 ± 2.9	8.1 ± 5.4	7.6 ± 0.1	0.29 ± 0.02	26.4 ± 2.1
			+4 d	4.0 ± 0.5	9.1 ± 1.8	6.8 ± 4.7	7.7 ± 0.3	0.30 ± 0.01	25.7 ± 1.0
		FL + BR LED	+2 d	1.5 ± 0.2	14.5 ± 2.5	7.1 ± 3.4	7.5 ± 0.3	0.27 ± 0.03	27.8 ± 1.7
			+3 d	3.1 ± 0.4	11.2 ± 2.5	6.8 ± 4.1	7.8 ± 0.2	0.33 ± 0.06	24.1 ± 0.5
			+4 d	4.7 ± 0.6	8.4 ± 1.8	6.5 ± 2.5	8.1 ± 0.2	0.31 ± 0.00	25.7 ± 0.9
		T		n.s.	n.s.	n.s.	(0.042) *	(0.004) **	n.s.
		P		(0.139) ***	(0.758) ***	(1.039) *	n.s.	n.s.	n.s.
		t		(0.139) ***	(0.758) ***	n.s.	(0.052) ***	(0.005) ***	(0.477) ***
		T × P		n.s.	n.s.	n.s.	(0.074) ***	(0.008) ***	(0.675) ***
		T × t		n.s.	n.s.	n.s.	(0.074) ***	(0.008) ***	(0.675) **
		P × t		(0.241) **	n.s.	n.s.	(0.090) ***	(0.009) ***	(0.827) ***
		T × P × t		n.s.	n.s.	n.s.	(0.127) ***	(0.013) ***	(1.169) ***

FL: fluorescent light; D: darkness; BR LED: blue and red LEDs. Data are vertically compared. T: treatment; P: photoperiod; t: time (days). * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; n.s.: no significant differences. (1) (%); (2) N; (3) (%); (4) mg citric acid 100 mL^{-1}.

These physicochemical quality parameters can be related to sensory perception of red peppers (Figure 2), which did not report differences among the studied treatments and photoperiods. In this way, 'Colour' increased by 2 points according to the evaluation scale after the retailing period (Table 2). 'Firmness' (both when slightly compressing with fingers and when eating) experimented an important decrease after 6 d 7 °C + 4 d 20 °C which was directly related to dehydration, justified with the objective quality attributes of weight losses and firmness shown in Table 2. As observed, these parameters also affected the acceptability of the fruit at the end of the retail sale period, being close to the limit of commercialization, giving then some extra days to consumers. In our previous unpublished shelf life experiments with this 'Angus' cv, a shelf life of 15 d was mainly reached being the first 6–8 d under refrigeration conditions simulating the storage + transportation period.

3.2. Bioactive Compound Content and Total Antioxidant Capacity

Total phenolic content of red peppers at harvest was 2.12 ± 0.11 g GAE kg^{-1}, which decreased by ~20% after 6 d at 7 °C, although such an initial amount was again reconstituted after the retailing period at 20 °C, especially under the FL + BR LED photoperiod (Table 3). In contrast, flavonoid biosynthesis was not affected by the photoperiod nor UV-B treatment, increasing its content at harvest by ~18% in CTRL fruit after 6 d at 7 °C + 4 d at 20 °C. As total phenolic and flavonoid contents have been directly related to the antioxidant capacity of these fruit, the TAC was positively affected by FL + FL and FL + BR LED photoperiods regarding FL + D, which was also highly remarkable after 6 d at 7 °C + 4 d 20 °C ($p < 0.05$).

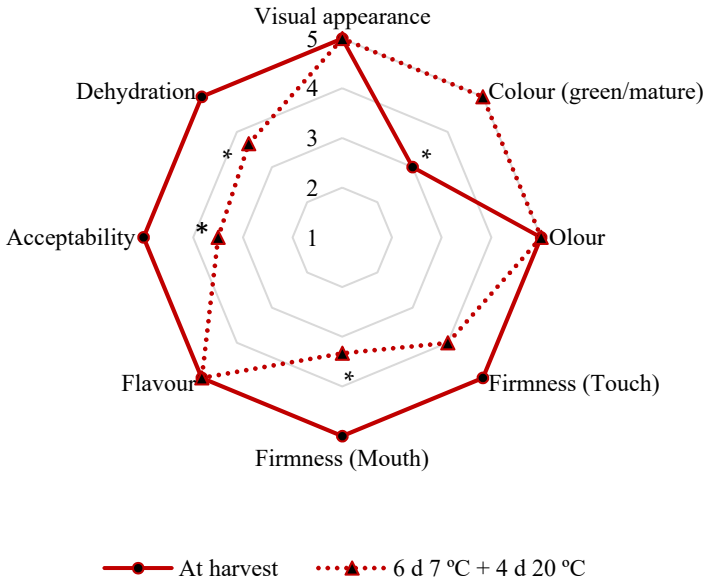

Figure 2. Sensory analysis of untreated and UV-B-treated bell peppers after 6 d at 7 °C and after a complimentary retail sale period of 4 d at 20 °C as mean values under all light conditions, which did not reported differences. * denotes statistical differences ($p < 0.05$). A 5-point hedonic scale was used, with 1 meaning 'Extremely bad', 3 'Limit of commercialization', and 5 'Excellent'.

Table 3. Total phenolic compounds (g gallic acid equivalents kg^{-1}), total flavonoid compounds (g rutin equivalents kg^{-1}), and total antioxidant capacity (g trolox equivalents kg^{-1}) of UV-B-treated and untreated (CTRL) bell peppers during a storage and transportation period of 6 d at 7 °C followed by a retail sale period of 2, 3, or 4 d at 20 °C under a photoperiod of 14 h in fluorescent light + 10 h in different light conditions.

	T	P (14 h + 10 h)	t	TPC	TFC	TAC
At h ar vest			0 d	2.12 ± 0.11	0.17 ± 0.04	1.57 ± 0.10
After 6 d at 7 °C	CTRL	-	6 d	1.81 ± 0.14	0.13 ± 0.02	1.64 ± 0.07
	UV-B	-		1.62 ± 0.14	0.16 ± 0.04	1.19 ± 0.08
After a retail sale period at 20 °C	CTRL	FL + D	+2 d	1.51 ± 0.08	0.16 ± 0.02	1.09 ± 0.12
			+3 d	1.79 ± 0.12	0.17 ± 0.02	1.26 ± 0.26
			+4 d	1.83 ± 0.17	0.21 ± 0.04	1.10 ± 0.15
		FL + FL	+2 d	1.69 ± 0.24	0.17 ± 0.04	1.17 ± 0.11
			+3 d	1.56 ± 0.14	0.18 ± 0.02	1.24 ± 0.16
			+4 d	1.80 ± 0.00	0.20 ± 0.01	1.43 ± 0.06
		FL + BR LED	+2 d	2.12 ± 0.09	0.20 ± 0.02	1.28 ± 0.13
			+3 d	1.87 ± 0.24	0.17 ± 0.04	1.43 ± 0.16
			+4 d	1.96 ± 0.10	0.19 ± 0.01	1.36 ± 0.26
	UV-B	FL + D	+2 d	1.56 ± 0.11	0.15 ± 0.02	1.10 ± 0.05
			+3 d	1.81 ± 0.17	0.17 ± 0.00	1.39 ± 0.05
			+4 d	1.76 ± 0.04	0.19 ± 0.05	1.28 ± 0.08
		FL + FL	+2 d	1.69 ± 0.22	0.17 ± 0.04	1.25 ± 0.17
			+3 d	1.78 ± 0.12	0.17 ± 0.03	1.29 ± 0.12
			+4d	1.74 ± 0.12	0.18 ± 0.03	1.62 ± 0.06
		FL + BR LED	+2 d	1.74 ± 0.02	0.17 ± 0.01	1.24 ± 0.26
			+3 d	1.90 ± 0.15	0.18 ± 0.02	1.48 ± 0.12
			+4 d	1.86 ± 0.16	0.18 ± 0.01	1.28 ± 0.11
		T		n.s.	n.s.	n.s.
		P		(0.097) ***	n.s.	(0.092) *
		t		n.s.	(0.019) *	(0.092) **
		T × P		n.s.	n.s.	n.s.
		T × t		n.s.	n.s.	n.s.
		P × t		n.s.	n.s.	(0.160) *
		T × P × t		n.s.	n.s.	n.s.

TPC: total phenolic compounds; TFC: total flavonoid compounds; TAC: total antioxidant capacity; FL: fluorescent light; D: darkness; BR LED: blue and red LEDs. Data are vertically compared. T: treatment; P: photoperiod; t: time (days). * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; n.s.: no significant differences.

3.3. Carotenoid Biosynthesis

As it was presumed with TAC values, the carotenoid biosynthesis was highly enhanced by FL + FL and FL+BR LED photoperiods, especially after a low UV-B dose (Table 4). β-Carotene and its derivatives were those reporting a higher bio stimulation with UV-B, mainly when it was combined with the lighting conditions of a supermarket (Figure 3). Capsaicinoids were the main carotenoid compounds found in red peppers. Their content was also positively affected with the synergistic effect of a postharvest UV-B treatment and a FL+BR LED photoperiod during the retail sale period (Figure 4). In this sense, a general increase of the total carotenoid content after UV-B postharvest treatment and photoperiods is shown in Figure 5.

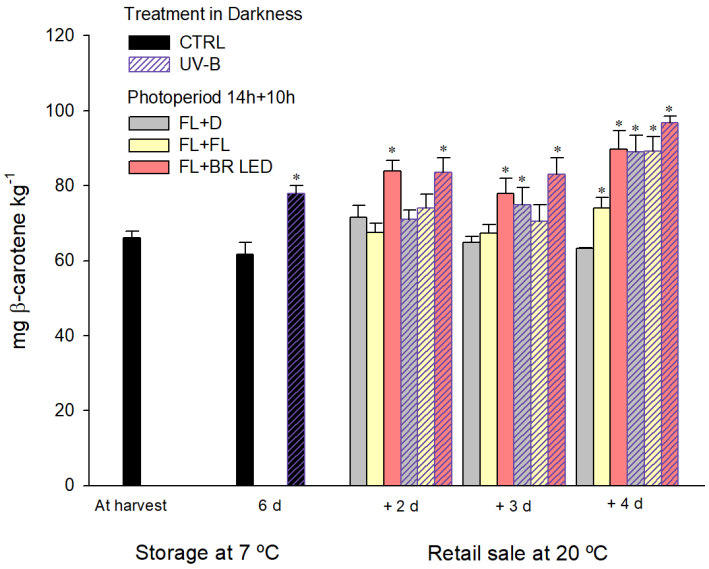

Figure 3. Total β-carotene content of UV-B-treated and untreated CTRL bell peppers after 6 d at 7 °C followed by a retail sale period of 2, 3, and 4 d at 20 °C under different lighting conditions. *: significant differences compared to CTRL. Treatment in darkness (T): CTRL (non-striped bars) and UV-B (striped bars); Photoperiod during retail sale (P): FL+D (grey bars), FL+FL (yellow bars), FL+BR LED (light red bars); and time (t). LSD_T = (1.999) †††; LSD_P = (2.449) †††; LSD_t = (2.449) †††; $LSD_{T\times P}$ = (3.463) †††; $LSD_{T\times t}$ = (3.463) †††; $LSD_{P\times t}$ = n.s.; $LSD_{T\times P\times t}$ = (5.998) ††. n.s.: no significant; ††: $p > 0.005$; †††: $p > 0.001$.

Table 4. Individual carotenoid content (mg β-carotene kg^{-1}) of UV-B-treated and untreated (CTRL) bell peppers during a storage and transportation period of 6 d at 7 °C followed by a retail sale period of 2, 3, or 4 d at 20 °C under a photoperiod of 14 h in fluorescent light + 10 h in different light conditions.

	T	P (14 h + 10 h)	t	All-Trans-Neoxanthin	All-Trans-Lutein	Capsanthin	Capsanthin Laurate	13-Cis-β-Carotene	All-Trans-β-Carotene	9-Cis-β-Carotene	Capsanthin Myristate	Capsanthin Esters
At harvest			0 d	9.9 ± 0.7	2.3 ± 0.3	54.6 ± 4.8	7.8 ± 0.3	19.0 ± 0.9	35.2 ± 0.6	6.2 ± 0.2	7.7 ± 0.1	77.5 ± 2.7
After 6 d at 7 °C	CTRL	-	6 d	9.6 ± 1.8	2.2 ± 0.3	39.5 ± 0.8	8.2 ± 0.0	15.3 ± 0.5	33.5 ± 1.6	6.8 ± 0.9	10.5 ± 1.1	80.2 ± 6.9
	UV-B	-		10.8 ± 0.2	2.2 ± 0.3	48.0 ± 4.5 ^{A}	11.3 ± 0.4 ^{A}	19.6 ± 0.8 ^{A}	36.6 ± 1.6	7.2 ± 0.6	10.4 ± 1.1	102.2 ± 7.2 ^{A}
After a retail sale period at 20 °C	CTRL	FL+D	+2 d	10.5 ± 0.2	2.4 ± 0.1	54.7 ± 0.7	9.7 ± 0.7	18.4 ± 1.3	33.9 ± 3.1	7.8 ± 0.1	8.5 ± 0.8	82.7 ± 3.3
			+3 d	14.0 ± 0.2	2.8 ± 0.0	51.9 ± 0.0	10.0 ± 0.3	16.9 ± 0.7	30.9 ± 0.7	6.6 ± 0.4	8.1 ± 0.5	76.5 ± 2.9
			+4 d	15.3 ± 1.2	3.1 ± 0.2	78.1 ± 3.4	9.9 ± 0.8	17.2 ± 0.7	28.8 ± 0.4	8.4 ± 0.2	8.4 ± 0.1	75.9 ± 7.7
		FL + FL	+2 d	10.1 ± 0.3	2.5 ± 0.2	58.5 ± 5.8	8.7 ± 0.4	17.9 ± 0.4	31.6 ± 1.7	7.5 ± 0.1	7.4 ± 0.5	80.0 ± 2.4
			+3 d	14.2 ± 1.0	2.7 ± 0.1	52.0 ± 2.3	10.4 ± 0.5	19.6 ± 1.0	31.3 ± 1.4	6.2 ± 0.1	8.0 ± 0.2	94.9 ± 2.6
			+4 d	15.8 ± 1.0	2.9 ± 0.1	84.6 ± 2.4	10.5 ± 0.7	20.0 ± 1.0	34.2 ± 1.9	8.3 ± 0.4	9.4 ± 0.3	81.7 ± 2.3
		FL + BR LED	+2 d	14.1 ± 1.3	2.8 ± 0.2	70.3 ± 4.5	13.2 ± 0.0	24.5 ± 1.8	43.8 ± 2.9	9.8 ± 0.4	10.5 ± 0.0	108.1 ± 7.2
			+3 d	14.4 ± 1.8	2.7 ± 0.2	70.5 ± 5.6	9.8 ± 0.4	24.0 ± 2.2	32.3 ± 1.7	9.4 ± 0.6	9.3 ± 0.7	90.6 ± 8.8
			+4 d	18.2 ± 0.7	3.5 ± 0.2	102.4 ± 4.3	12.7 ± 1.1	24.6 ± 1.3	42.2 ± 2.7	9.4 ± 0.5	9.8 ± 0.4	88.6 ± 2.5
	UV-B	FL + D	+2 d	9.6 ± 0.4	2.2 ± 0.0	69.2 ± 0.6	9.9 ± 0.9	18.3 ± 0.5	34.3 ± 2.8	7.1 ± 0.5	8.6 ± 0.5	83.8 ± 5.6
			+3 d	14.2 ± 0.2	2.8 ± 0.1	67.1 ± 1.0	10.7 ± 0.4	19.3 ± 0.9	35.7 ± 2.1	7.7 ± 1.0	10.4 ± 0.7	81.6 ± 12.3
			+4 d	17.7 ± 0.5	3.4 ± 0.0	78.7 ± 2.1	12.1 ± 0.5	23.7 ± 1.4	41.5 ± 2.4	9.8 ± 0.8	11.0 ± 0.9	90.7 ± 8.2
		FL + FL	+2 d	9.9 ± 0.3	2.7 ± 0.1	67.8 ± 2.1	10.3 ± 0.2	18.5 ± 0.4	36.6 ± 1.9	7.2 ± 0.5	10.8 ± 0.5	91.1 ± 6.6
			+3 d	13.9 ± 0.9	2.8 ± 0.2	68.5 ± 3.6	9.8 ± 1.1	18.0 ± 0.2	33.7 ± 3.2	7.6 ± 0.3	9.3 ± 0.8	77.2 ± 6.3
			+4 d	17.2 ± 0.9	3.3 ± 0.0	87.6 ± 6.3	12.8 ± 0.8	23.8 ± 0.9	43.2 ± 1.6	8.5 ± 1.0	9.5 ± 0.3	88.8 ± 5.9
		FL + BR LED	+2 d	14.6 ± 1.1	2.8 ± 0.1	73.6 ± 3.9	11.4 ± 1.1	20.4 ± 1.6	37.5 ± 2.6	8.4 ± 0.3	10.0 ± 0.7	87.3 ± 5.4
			+3 d	16.5 ± 0.7	3.1 ± 0.2	81.1 ± 3.9	12.7 ± 1.3	21.3 ± 0.3	38.8 ± 2.5	8.3 ± 0.7	11.1 ± 0.7	92.8 ± 9.1
			+4 d	18.1 ± 0.2	3.5 ± 0.0	98.3 ± 4.3	13.3 ± 0.5	26.1 ± 0.1	45.9 ± 1.3	10.0 ± 0.4	11.5 ± 1.2	97.1 ± 6.3
		T		(0.461) *	(0.079) ***	(2.017) ***	(0.401) ***	(0.602) *	(1.204) ***	n.s.	(0.343) ***	n.s.
		P		(0.564) ***	(0.097) ***	(2.470) ***	(0.491) ***	(0.737) ***	(1.474) ***	(0.360) ***	(0.420) ***	(4.370) ***
		t		(0.564) ***	(0.097) ***	(2.470) ***	(0.491) ***	(0.737) ***	(1.474) ***	(0.360) ***	(0.420) *	n.s.
		T × P		n.s.	n.s.	(3.493) *	n.s.	(1.042) ***	(2.085) **	(0.509) **	n.s.	n.s.
		T × t		n.s.	n.s.	(3.493) ***	(0.694) *	(1.042) ***	(2.085) ***	(0.509) ***	n.s.	(6.179) **
		P × t		(0.978) ***	(0.167) *	(4.278) **	(0.851) *	n.s.	(2.553) *	n.s.	n.s.	n.s.
		T × P × t		(1.382) *	(0.237) *	n.s.	(1.203) ***	n.s.	(3.611) **	(0.881) *	(1.030) ***	(10.703) ***

FL: fluorescent light; D: darkness; BR LED: blue and red LEDs. Data are vertically compared. T: Treatment; P: Photoperiod; t: time (days). ^{A}: denotes significant differences ($p < 0.05$) between T on 6 d at 7 °C. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; n.s.: no significant differences.

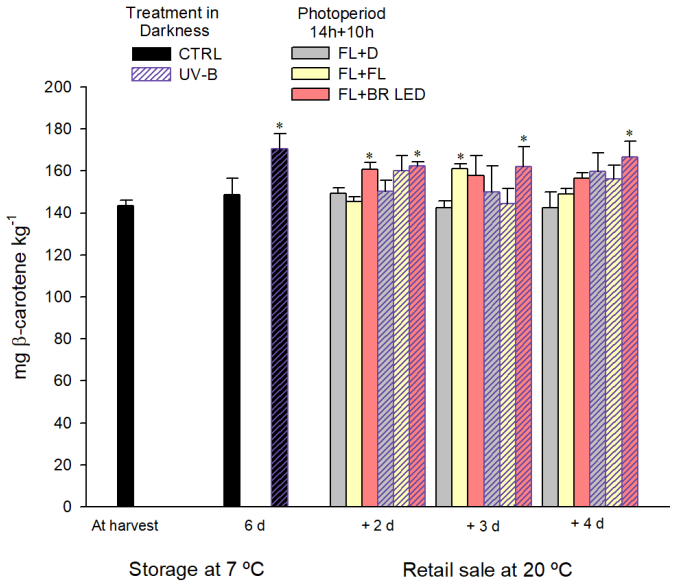

Figure 4. Total capsanthin content of UV-B-treated and untreated CTRL bell peppers after 6 d at 7 °C followed by a retail sale period of 2, 3, and 4 d at 20 °C under different lighting conditions. *: significant differences compared to CTRL. Treatment in darkness (T): CTRL (non-striped bars) and UV-B (striped bars); Photoperiod during retail sale (P): FL + D (grey bars), FL + FL (yellow bars), FL+BR LED (light red bars); and time (t). LSD_T = n.s.; LSD_P = (4.577) ††; LSD_t = n.s.; $LSD_{T \times P}$ = n.s.; $LSD_{T \times t}$ = (6.473) †; $LSD_{P \times t}$ = n.s.; $LSD_{T \times P \times t}$ = (11.211) †††. n.s.: no significant; †: $p > 0.05$; ††: $p > 0.005$; †††: $p > 0.001$.

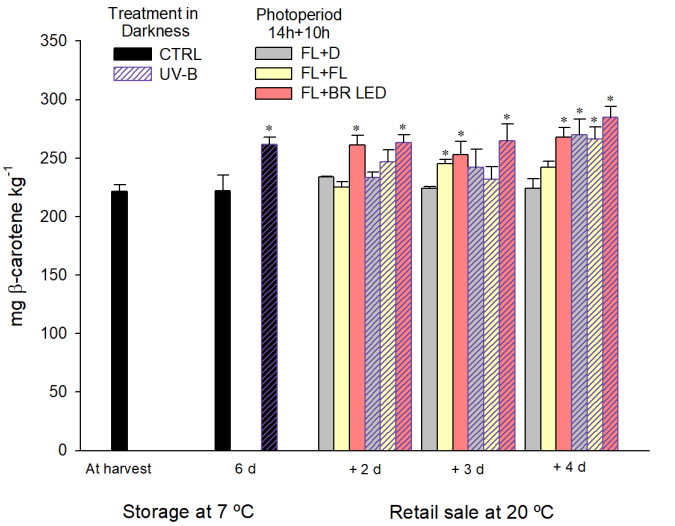

Figure 5. Total carotenoid content of untreated and UV-B-treated red peppers during 6 d at 7 °C followed by commercialization of 2, 3, and 4 d at 20 °C under different light conditions measured by UHPLC. *: significant differences compared to CTRL. Treatment in darkness (T): CTRL (non-striped bars) and UV-B (striped bars); Photoperiod during retail sale (P): FL+D (grey bars), FL+FL (yellow bars), FL+BR LED (light red bars); and time (t). LSD_T = (5.255) †††; LSD_P = (6.435) †††; LSD_t = (6.435) †††; $LSD_{T \times P}$ = (9.101) †††; $LSD_{T \times t}$ = (9.101) †††; $LSD_{P \times t}$ = n.s.; $LSD_{T \times P \times t}$ = (15.764) ††. n.s.: no significant; †: $p > 0.05$; ††: $p > 0.005$; †††: $p > 0.001$.

At harvest, red peppers reported a total of 221.7 ± 5.5 mg β-carotene kg^{-1}, which was maintained during the refrigerated period (Figure 5). Capsaicinoids represented the 64.5% of the total carotenoid content, followed by β-carotene and its derivatives (30%), all-trans-neoxanthin (4.5%), and all-trans-lutein (1%) (Table 4).

All-trans-neoxanthin and all-trans-lutein compounds showed a similar behaviour, being positively affected by UV-B treatment, the photoperiod with light combinations, and the retailing period at 20 °C. In fact, UV-B-treated and untreated red peppers after the supplementary 4 d at 20 °C under FL+BR LED photoperiod registered the same content of these compounds, but UV-B red peppers exhibited an increase of ~15% and ~10% under FL + D and FL + FL, respectively.

All-trans-β-carotene and 13-cis-β-carotene biosynthesis was slightly increased after 9 kJ m^{-2} UV-B treatment, which was especially remarkable on the same day of the treatment. In contrast, no differences were found in the accumulation of 9-cis-β-carotene after the UV-B treatment. Nevertheless, all β-carotene derivatives were positively affected by the FL + BR LED photoperiod, which was highly observed after the supplementary 4 d at 20 °C. In fact, as a mean of the retailing period, CTRL red peppers under FL+BR LED showed an increase of ~33% of 13-cis-β-carotene, ~24% of all-trans-β-carotene, and ~27.5% of 9-cis-β-carotene compared to FL + D and FL + FL. To a lesser extent, UV-B red peppers under FL + BR LED photoperiod also reported ~11.5%, ~8.5%, and ~11% more accumulation of these compounds, respectively, than FL + D and FL + FL. Moreover, no differences were found between CTRL and UV-B red peppers under the same photoperiod, alternating 14 h FL and 10 h BR LED lighting.

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Concerning the major carotenoid compounds, capsanthin and its derivatives were also bio stimulated by the UV-B treatment, photoperiod, and the time at 20 °C. Indeed, immediately after the 9 kJ m^{-2} UV-B dose applied on 6 d at 7 °C, red peppers showed an amelioration effect in the accumulation of capsanthin, capsanthin laurate, and capsanthin esters by ~22%, ~38%, and ~27%, respectively. After that, as a mean of the capsanthin content during the retailing period at 20 °C, CTRL red peppers under FL + BR LED showed an increase by ~31.5% and ~25% regarding FL + D and FL + FL, respectively. Accordingly, capsanthin laurate and capsanthin myristate were enhanced by ~20.5% and ~19.3%, respectively, compared to FL+D and FL+FL. Moreover, the amount of capsanthin esters was enhanced after the FL+BR LED photoperiod in ~22% compared to FL+D and in ~12% compared to FL+FL photoperiod.

UV-B-treated red peppers under the supplemented 10 h BR LED photoperiod showed an increase of ~18% and ~13% in the capsanthin content regarding FL + D and FL + FL, while capsanthin laurate, capsanthin myristate, and capsanthin esters were increased by ~14.7%, ~9.5%, and ~8.1%, respectively, compared to the other tested photoperiods.

From a general point of view, a 10 h photoperiod supplemented with BR LED was demonstrated to be as effective as a carotenoid biosynthesis stimulator, compared to turning off the lights at night during the retail sale period, and without compromising the quality.

An additional postharvest light stress of 9 kJ m^{-2} UV-B even increased such accumulation. In fact, a combination of both UV-B treatment and a photoperiod under FL+BR LED has shown a symbiotic effect during the retailing period concerning the accumulation of carotenes, lutein, and neoxanthin, due to their amount being similar under single and combined light conditions. Nevertheless, a synergistic behaviour was observed regarding the capsanthin accumulation and its esters (~60% of the total carotenoid content). This fact can be due to the stimulation of the carotenoid biosynthesis, as phytochemicals involved in the plant photoprotection, throughout several biochemical pathways.

4. Discussion

The studied bell peppers showed ~27% more water losses than those under the FL+D photoperiod. This fact can be related to previous results obtained by Pennisi et al. [30], who recently showed similar losses in fresh-cut red chard (26.8%–38.2%) and rocket leaves (22% and 31%) after 10 d at 5 °C in response to monochromatic red and blue LEDs (35 μmol m^{-2} s^{-1}), respectively. Such low weight losses are in agreement to previous results reported by Kasim and Kasim [31], who reported 1% dehydration after 6 d at 7 °C, whose data were not affected after UV-B treatments of 4.46 and 8.93 kJ m^{-2}. Similar results were obtained by Miranda-Molina et al. [4], who showed weight losses higher than 50% in serrano chilli peppers after 33 d at 25 °C.

Regarding colour variations (ΔE), this effect is similar to results reported by Kasim and Kasim, [31], who showed that low UV-B doses (4.46 kJ m^{-2}) did not affect the colour development during 6 d at 7 °C. As observed, TSS has been related to MI and, particularly in red peppers, these two parameters have also been associated with colour development [32], which can also be contrasted with our present results. Moreover, sensory quality values can be related to the overall marketability, which has been previously reported to be increased after applying blue and red LEDs [33] or UV low doses [31,34].

As total phenolic and flavonoid contents have been directly related to the antioxidant capacity of these fruit [5], the TAC was positively affected by FL + FL and FL + BR LED photoperiods regarding FL + D, which was also highly remarkable after 6 d at 7 °C + 4 d 20 °C. Values obtained can be comparable to previous studies in which UV-B increased the TAC according to DPPH values [31], and this effect has also been shown in UV-C-treated red peppers [35]. Moreover, an increase of the TAC measured by FRAP in fresh-cut red peppers for 11 d at 7 °C has also been reported for the application of 8 h blue and red LEDs separately [33]. Nevertheless, there are no previous studies combining both UV-B and LED photoperiod to study their synergistic effect. Indeed, this behaviour can be explained by the biosynthesis of nutraceuticals as carotenoids, which were potentiated by both postharvest strategies as it is subsequently detailed.

The carotenoid profile of the studied red peppers is in agreement with previous studies in different varieties of red peppers reported by Hassan et al. [3].

Our hypothesis to explain the basis of this positive effect on the carotenogenesis of carotenes and xanthophylls in red bell peppers, which especially occurs during fruit ripening, is that it is controlled by transcript genes which are mainly regulated by light incidence and high temperatures [36]. As the initial precursor of lycopene in the top of the carotenoid chain, phytoene synthase (PSY) plays an essential role in the conversion of geranylgeranyl diphosphate to phytoene, whose stimulation directly depends on light stimuli [37]. For instance, UVR8 as the main UV-B receptors, and cryptochromes, as receptors of blue light, are in charge to activate several transcription factors (COP1 protein, constitutive photomorphogenesis 1), which link with HY5 (elongated hypocotyl5) as the main PSY stimulator [37]. Furthermore, red and far-red wavelengths are absorbed by phytochromes, which also regulate the photomorphogenesis of carotenoids through the control of phytochrome interacting factors (PIF) [37,38].

Regarding to this behaviour, Pola et al. [39] have recently shown that the exposure to continuous red LEDs is more effective at inducing the accumulation of carotenoids than blue LEDs in mature green chilli peppers exposed to 946 kJ m^{-2} for 3 d at 30 °C

and 75% RH. This positive effect was also shown in fresh-cut red peppers for 14 d at 7 °C and 85% RH under 8 h of 946 kJ m^{-2} red LED in comparison with 630.7 kJ m^{-2} blue LED [33]. Moreover, the exposition to UV-B and UV-C radiation are also demonstrated to affect the expression of genes directly related to carotenogenesis. For instance, UV-B has shown a regulator effect of the initial steps of carotenoid synthesis, stimulating the PSY and lycopene biosynthesis in filamentous cyanobacterium *Chlorogloeopsis fritschii* PCC 6912 after 9.5 kJ m^{-2} (4 h) and 37.8 kJ m^{-2} (4 h/d during 4 d) [40]. Similarly, this positive effect of UV-B radiation on carotenoid accumulation has been reported in tobacco leaves after 9.75 kJ m^{-2} for 8 d [41]. Moreira-Rodríguez et al. [14] also demonstrated an increase in the carotenoid content in broccoli sprouts after 7.16 kJ m^{-2} UV-B.

Knowing that colour development throughout the postharvest storage is directly related to continuous carotenogenesis during the ripening process [3], the ΔE results that were previously mentioned can be linked to the higher carotenoid concentration in FL + BR LED red peppers. Hence, postharvest UV-B treatment of 9 kJ m^{-2} single or combined with a photoperiod of 14 h FL + 10 h BR LED was demonstrated to be a useful tool to increase the carotenoid content in red peppers after the retailing period. Furthermore, it is important to remark that such alternative lighting treatment is able to reduce the operational cost by ~71% compared to a FL + FL photoperiod according to a fixed price of energy.

5. Conclusions

A retail sale period of 4 d at 20 °C under 14 h FL + 10 h under blue and red LEDs reported an increase of ~33% of 13-cis-β-carotene, ~24% of all-trans-β-carotene, and ~27.5% of 9-cis-β-carotene in red bell peppers when compared to the other conventional photoperiods used in supermarkets (including darkness or FL). Biosynthesis of capsaicinoids, as the major carotenoid compound in red bell peppers, was greatly stimulated after a UV-B postharvest treatment, which was even enhanced after supplementation with the described BR LED photoperiod regarding the conventional retail sale lighting procedures. Therefore, our first proposal is to illuminate peppers with BR LEDs during the retail sale period nights, when supermarkets close, instead of switching off the lights. In this way, an increase in the bioactive content of bell peppers via carotenoid accumulation is induced without compromising quality. Moreover, this proposal can even be included in domestic refrigerators, installing BR LEDs in the fruit and vegetables compartment.

Therefore, our results suggest transferring this technology to the retail sale procedure of bell peppers, and probably other fruit and vegetables, in order to enhance healthiness while decreasing energy and operational costs up to ~70%.

Author Contributions: Conceptualization, F.A.-H. and L.M.-Z.; methodology, N.C. and L.M.-Z.; software, L.M.-Z.; validation, F.A.-H., N.C., and L.M.-Z.; formal analysis, N.C., and L.M.-Z.; investigation, F.A.-H., N.C., and L.M.-Z.; resources, F.A.-H.; data curation, N.C., and L.M.-Z.; writing—original draft preparation, F.A.-H. and L.M.-Z.; writing—review and editing, F.A.-H., N.C., and L.M.-Z.; visualization, F.A.-H.; supervision, F.A.-H.; project administration, F.A.-H.; funding acquisition, F.A.-H. All authors have read and agreed to the published version of the manuscript.

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