Effect of LED lights on the antioxidant properties of baby spinach leaves (*Spinacia oleracea* L.)

Efecto de las luces LED sobre las propiedades antioxidantes de hojas de espinaca tipo "baby" (*Spinacia oleracea* L.)

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Abstract

The present study employed white (W), blue (B: 468 nm), red (R: 629 nm) and green (G: 524 nm) monochromatic LED lights for 26 days, from 11:00 to 18:00 h (7 h per day), with an average photosynthetic photon flux density (PPFD) of 26.0 m^{-2} s^{-1} on baby spinach leaves (*Spinacia oleracea* L.), cvs. Falcon F1 and Viroflay, grown in a hydroponic system. Regardless of the cultivar, the fresh and dry weights were positively influenced when the plants were irradiated by R-light in comparison to W-light. Independent of the cultivar, the leaves treated with B-light reached a significantly higher phenolic compound concentration and antioxidant capacity than plants irradiated with W-light. In addition, the green light increased total phenolic compound concentration. According to the results, the use of LED lights is a promising technique for the production of antioxidant compound-enriched leafy vegetables.

Keywords
LED light • biomass • spectrum • antioxidant capacity • hydroponic system • spinach

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Resumen

El presente estudio empleó luces LED monocromáticas blanca, azul (468 nm), roja (629 nm) y verde (524 nm) durante 26 días, desde las 11:00 hasta las 18:00 h (7 horas por día), con una densidad media de flujo fotosintético de 26,00 µmol m^{-2} s^{-1} sobre dos cultivares de espinaca (*Spinacia oleracea* L.) de hoja baby (Falcon F1 y Viroflay) cultivadas en un sistema hidropónico. Respecto del cultivar, los pesos fresco y seco fueron influenciados positivamente cuando las plantas fueron irradiadas con luz roja en comparación con las irradiadas con luz blanca. Independientemente del cultivar, las hojas tratadas con luz azul alcanzaron una concentración de compuestos fenólicos y capacidad antioxidante superiores a la de las plantas irradiadas con luz blanca. Además, la luz verde incrementó la concentración de compuestos fenólicos. De acuerdo con los resultados, el uso de luces LED es una técnica prometedora para la producción de hortalizas de hoja enriquecidas en compuestos antioxidantes.

Palabras clave

luces LED • biomasa • espectros • capacidad antioxidante • sistema hidropónico • espinaca

Introduction

Spinach (*Spinacia oleracea* L.) is a leafy vegetable belonging to the family *Amaranthaceae*. This species is recognized for its high concentration of iron, calcium, potassium and vitamins such as A, B and C. Moreover, it has several phenolic compounds that promote good health beyond their basic nutrition, which are also attributed to its antioxidant characteristics. These compounds help avoid diseases and cell damage caused by reactive oxygen species (ROS) produced in the respiration process (1, 3, 15).

To increase the quality of leafy vegetables, light supplements are being studied. Nowadays, light-emitting diode (LED) lights are being used, given their advantage of a long lifespan, low energy cost and low temperature production. Its operation is based on the adjustment to different light spectrum, which can also be adjusted for different light quality, mainly red (R), green (G) and blue (B)-lights, stimulating germination, promoting vegetative growth and synthesizing antioxidant compounds in plants grown in greenhouses (30). Weston and Barth (1997) Rouphael *et al.* (2012) indicated that the factors that most influenced the antioxidants content of vegetables were temperature and light intensity. These climatic factors can be modified by the use of greenhouses, which offers a productive advantage by reducing the high climatic variability present in an outdoor crop (16).

Colonna *et al.* (2016) reported a propensity to increase the nutritive value and phenolic compounds of different species of leafy vegetables grown in a greenhouse when were harvested at 8:00 h (with a low photosynthetic photon flux density (PPFD): 200 – 400 µmol m^{-2} s^{-1}) in comparison to harvesting at 14:00 h (with a high PPFD: 800 – 1200 µmol m^{-2} s^{-1}).

On the other hand, Samuolienè *et al.* (2012) determined a positive trend of B and G LED light supplementation (30 µmol m^{-2} s^{-1}) for 16 hours per day on vitamin C and tocopherol concentration in lettuce (*Lactuca sativa* L.) cultivated in a greenhouse compared to natural light intensity conditions. Moreover, Son and Oh (2015) showed that the use of R (600 – 700 nm), G (500 – 600 nm) and B (400 – 500 nm) LED lights with a PPFD of 173 µmol m^{-2} s^{-1} for 12 hours per day for 18 days increased the production of biomass and secondary metabolites on lettuce treated with a greater proportion of R and B-lights, respectively. Johka *et al.* (2010) indicated that blue LED improved seedling quality and growth after transplanting red leaf lettuces. Son and Oh (2015) observed that total phenolic concentration, total flavonoid concentration, and antioxidant capacity of lettuces grown under high ratios of blue LED were significantly higher compared with red LED or control conditions. Among the secondary metabolites, Ávalos and Pérez-Urria (2009) indicated that changes in quality and light intensity increased phenolic compounds and the antioxidant capacity of vegetables.

On the other hand, spinach (*Spinacia oleracea* L.) is widely regarded as a functional food due to its diverse nutritional composition, which includes vitamins and minerals, and to its phytochemicals and bioactives that promote health beyond basic nutrition (33). Spinach leaves showed biological activities that contribute to the anti-cancer, anti-obesity, hypogly-

cemic, and hypolipidemic properties (33). Viroflay baby spinach grown under red photoselective filter reached higher phenolic compound content, foliar area, width, length and yield than control plants (25). In this same study, Viroflay baby leaves cultivated under blue filter showed significantly higher dry weight than those in red and gray filters (25).

The aim of this study was to evaluate light supplementation with B, G and R- lights on the physical aspects and synthesis of antioxidant compounds in two baby spinach leaves (*Spinacia oleracea* L.) cultivars grown in a floating hydroponic system in a plastic greenhouse.

Materials and methods

Plant material and experiment

The experiment and analysis were conducted in the greenhouse and laboratory of the Postharvest Study Center (CEPOC) at the Faculty of Agricultural Sciences, University of Chile, located in La Pintana, Santiago, Chile. The experiment lasted from the end of September to November 2017 in a closed hydroponic floating system located inside a plastic greenhouse.

The plant material used was spinach belonging to two cultivars: Falcon F1, which is a vigorous plant, with a smooth, large, dark green leaf (World South Seeds Ltda., Chile) and Viroflay characterized by a very fast growing, very productive, with dark green semi-smooth leaves (World South Seeds Ltda., Chile).

A germination test was performed according to ISTA standards (20) and the results of which showed 96% for both cultivars. Inert substrate was mixed in a 1:1 proportion with granulated rock wool (Agrolan®, El Volcán S. A., Chile) and expanded perlite A6 (Harbolite Chile Ltda., Chile) and used in the sowing. Irrigation was done with tap water after sowing and when the plants had the first true leaf completely expanded, modified 50% Hoagland II nutritive solution was added (18). The pH of the solution was kept between 5.5 and 5.8 to maximize nutrient absorption by the crop (19, 21). The transplant was done when the plants had 1 to 2 completely expanded true leaves (18 days after sowing) in a floating root closed hydroponic system, making sure that the roots were in contact with the nutrient solution. The hydroponic system was 7.0 x 1.5 m with a maximum capacity of approximately 950 L. The plants were placed on high-density expanded polystyrene plates located in the system, which were previously perforated with a 3 bobbin arrangement, with a density of 63 plants m^{-2}. One day after transplant, the same nutrient solution was added keeping the range of pH between 5.5 to 5.8. The experimental unit (EU) was a quadrant of 0.9 x 0.5 m polystyrene floating plate containing the root system, with 15 plants located in the central area to avoid interaction with other treatments. An empty space of 14 cm without plants was kept between each EU. Treatments were distributed randomly in a margin of 7.0 x 0.5 m of the floating system.

The plants were grown in the greenhouse with natural sunlight. During this period, the natural sunlight was between 11.85 to 13.82 h per day (from sunrise to sunset time).

The treatments were applied with LED tapes (DEMASLED, TEXMCCW, Chile) located in each EU divided into 9 sections. The light spectrum of the LED lights were measured in each EU in 9 equidistant sectors with a smart spectrometer sensor (Lighting Passport, Asensetek, Taiwan). Additionally, the PPFD of each LED light used to enrich the natural light of the greenhouse was measured with the point sensor of a radiometer (DELTA OHM, HD9021, Italy) in the same 9 equidistant sectors per EU as previously described. The natural sunlight was enriched with white (W), blue (B), green (G) or red (R) LED lights for 26 days from transplanting to the harvest. LED light supplementation was performed daily from 11:00 to 18:00 h (to take advantage of the period of maximum photosynthetic activity). The average PPFD was 26 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR): white (W) 25.85 ± 0.55; blue (B) 25.59 ± 0.60; green (G) 26,60 ± 1.50; and red (R) 25.93 ± 0.60 µmol m^{-2} s^{-1}. The light spectrum were 468, 534 and 629 nm for B, G and R- lights, respectively.

The harvest took place when the plants had 5 to 6 fully expanded true leaves (45 days after sowing) and they had a maximum length of 10 cm (commercial size for baby leaves). At this time, each EU was assessed independently. All the leaves in each EU were harvested and packed into low-density polyethylene sealed bags. Five or six leaves per plant from three plants were packed per bag. After harvest, early in the morning, five randomly selected bags

from each EU were stored at 5 °C for two hours till afternoon for the physical measurements of the leaves. For the chemical analysis, another eight bags were frozen at -80 °C in an ultra-low temperature freezer (SANYO, MDF – U33V, Japan).

Physical parameters

At harvest, a visual assessment was made of leaf damage per plant. The damage intensity on the leaves and the number of affected leaves per repetition were recorded. The damage intensity was obtained according to the affected leaf area, dividing the leaf into four quadrants. According to the affected quadrants, a damage coefficient was calculated per leaf considering the expression $Damage = (C1 * n) + (C2 * n) + (C3 * n) + (C4 * n)$

Where:

C = the damage coefficient value (Dc) based on the number of affected quadrants

n = the number of damaged leaves per EU (figure 1).

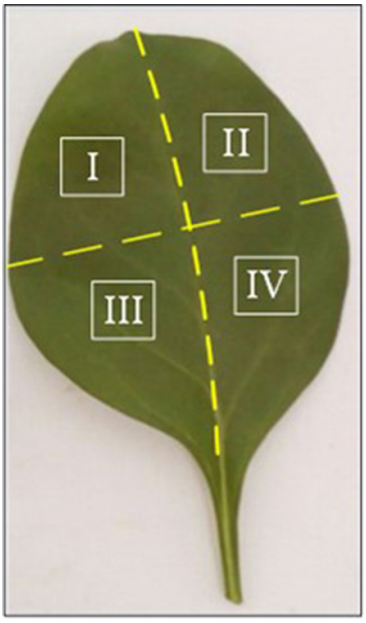


Figure 1. Quadrants used for the damage scale of spinach leaves under light supplementation with LED lights.

Figura 1. Cuadrantes utilizados para la escala de daño de las hojas de espinaca bajo suplementación lumínica con luces LED.

The color was measured in six random leaves selected from three random plants. The measurements were taken in the axial part of the distal sector of the leaf using a tristimulus compact colorimeter (Minolta Chroma meter, CM – 2500d, Japan). The results were expressed as hue, chroma (C) and lightness (L) (25). The longest three leaves were measured from another five random plants per repetition (to limit the length). The measurements were made with a metric rule, where the maximum length and width were represented for the distance obtained from the base to the apex of the leaf above the midrib and the greatest distance perpendicular to the central rib of the leaf, respectively (25). The results were expressed in cm. The fresh weight (FW), dry weight (DW) and dry weight percentage (%DW) were measured with a precision balance (Radwag, AS 100/C/2, Poland) (25). For the DW, the samples were dried in a freeze dryer (ilShinBioBase, FD5508, Korea) until reaching a constant weight. The FW and DW were expressed in g per plant. The %DW was calculated as a quotient between DW and FW, expressed as g of DW in 100 g of FW (25).

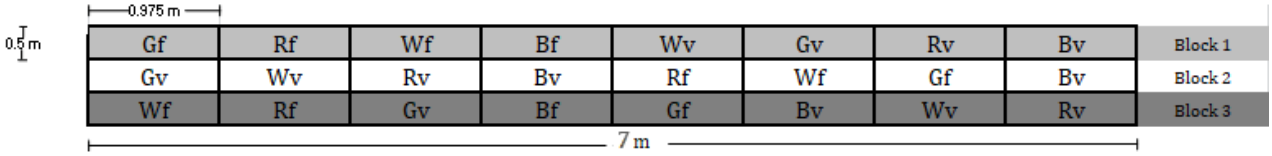
Antioxidant parameters

Initially, 1 g of frozen leaves obtained from a sample of 5 random plants per repetition were extracted with methanol 70% (v/v) following the adapted method proposed by Swain and Hills (1059). The sample was crushed in Ultraturrax (IKA, T18 basic, USA) until a uniform consistency (approximately 30 s) and centrifuged (HERMLE Labortechnik, Z326K, Germany) for 15 min at 6037 gN. The supernatant liquid was filtered through a 0.45 µm PVDF filters and stored at -20 ° C in a horizontal freezer (Electrolux, EC305ZBGW / 220/50, Sweden) for 1 week until the analysis. Total phenolic content (TPC) were measured using the method proposed by Singleton and Rossi (1965), with the Folin-Ciocalteu method. The results were expressed as µg of gallic acid equivalent (GAE) g^{-1} of fresh weight (FW).

The antioxidant capacity was measured using the DPPH (1, 1 – diphenyl 2 – picrylhydrazyl) and FRAP (ferric reducing antioxidant power) measurement protocols. The DPPH assay was performed using the method proposed by Brand-Williams *et al*. (1995), while the FRAP assay followed the method proposed by Benzie and Strain (1996). The results were expressed as µg Trolox equivalent (TE) g^{-1} of fresh weight (FW).

Statistical analysis

A completely random block design was produced with a factorial structure of 4 x 2 with three repetitions, where the first factor was the spectrum of LED light employed in the light supplementation, which had 4 levels: W, B, G and R. The second factor corresponded to the spinach cultivar: Falcon F1 and Viroflay (figure 2).



B: blue, W: white, R: red, G: green, f: Falcon F1, and v: Viroflay.

B: azul, W: blanco, R: rojo, G: verde, f: Falcon F1, v: Viroflay.

Figure 2. Scheme of the experimental design used for light supplementation with blue (468 nm), red (629 nm), green (524 nm) and white LED lights, with a light intensity of 26 µmol m^{-2} s^{-1} of PAR, on two cultivars of spinach for 26 days.

Figura 2. Esquema del diseño experimental usado para la suplementación lumínica con luces LED de espectro azul (468 nm), rojo (629 nm), verde (524 nm) y blanco, con una intensidad lumínica de 26 µmol m^{-2} s^{-1} de PAR, sobre dos cultivares de espinaca durante 26 días.

An analysis of variance (ANOVA) was carried out and Tukey's multiple comparison test was done in order to find differences among treatments. All the analyses were done with a 5% significance level. The statistical analyses were performed with the InfoStat statistics software (9).

Results

Physical parameters

The leaves of two spinach cultivars treated by W, B, G and R- lights showed no visible damage and did not affect the hue, C or L color parameters as described in table 1.

Table 1. Hue, chroma (C) and lightness (L) of the leaves from two spinach cultivars (Falcon F1 and Viroflay) cultivated for baby leaves under white, blue (468 nm), green (524 nm) and red (629 nm) LED light supplementation for 26 days.

Tabla 1. Tono, croma (C) y luminosidad (L) de las hojas de los dos cultivares de espinaca (Falcon F1 y Viroflay) cultivadas para hojas baby bajo suplemento de luces LED blanca, azul (468 nm), verde (524 nm) y roja (629 nm) durante 26 días.

LED light spectrum	Hue		C		L	
	Falcon F1	Viroflay	Falcon F1	Viroflay	Falcon F1	Viroflay
W	109.59±1.78aA	110.01±1.32aA	40.66±3.04aA	38.77±1.99aA	25.14±3.69aA	22.23±1.29aA
B	109.85±1.72aA	109.57±1.40aA	40.17±2.34aA	40.66±3.04aA	24.84±3.05aA	23.38±2.65aA
G	111.34±0.57aA	108.31±0,99aA	37.74±0.38aA	41.86±2.49aA	21.82±0,70aA	23.09±2.73aA
R	109.84±1.75aA	110.09±1.56aA	40.32±2.82aA	39.82±1.46aA	24.79±3.39aA	22.82±1.50aA
Significance						
LED	NS	NS	NS	NS	NS	NS
Cv	NS	NS	NS	NS	NS	NS
LED x Cv	NS	NS	NS	NS	NS	NS

Values indicate means ± standard deviation (S.D.). W: White; B: blue; G: green; R: red. LED: LED light spectrum. Cv: Cultivar. Lowercase letters compared in the column and uppercase letters compared in the rows indicate significant differences between LED light spectrum and cultivars, respectively (p – value < 0.05). NS: not significant (p – value > 0.05).

Los valores indican las medias ± la desviación estándar (S.D.). W: blanco; B: azul; G: verde; R: rojo. LED: Luz LED. Cv: Cultivar. Letras minúsculas comparan en columnas y letras mayúsculas comparan en líneas indicando diferencias significativas entre espectros de luces LED y cultivares, respectivamente (valor p < 0,05). NS: no significativo (valor de p > 0,05).

According to the results obtained, cv. Viroflay had significantly longer and narrower leaves than cv. Falcon F1. Regardless of the cultivar, significant differences were observed in the length of the leaves irradiated with different spectrum of LED light. Thereby, plants irradiated with R-light obtained the longest leaves compared to the other treatments. Falcon F1 leaves grown under R-light had a length of 6.91 cm and those treated with W-light reached 6.33 cm. In the case of Viroflay, the leaves grown under R and W-lights had lengths of 7.68 and 7.03 cm, respectively. Regarding the maximum width, regardless of the cultivar, no significant differences were observed among leaves treated with different LED light spectrum (table 2).

Table 2. Length and width of the leaves from two spinach cultivars (Falcon F1 and Viroflay) cultivated for baby leaves, under white, blue (468 nm), green (524 nm) and red (629 nm) LED light supplementation for 26 days.

Tabla 2. Longitud y anchura de las hojas de espinaca de los dos cultivares (Falcon F1 y Viroflay) de hojas baby, bajo un suplemento de luces blanca, azul (468 nm), verde (524 nm) y roja (629 nm) durante 26 días.

LED light spectrum	Length (cm)		Width (cm)	
	Falcon F1	Viroflay	Falcon F1	Viroflay
W	6.33±0.15aA	7.03±0.15aB	3.55±0.28aB	2.69±0.03aA
B	6.42±0.22aA	6.81±0.58aB	3.44±0.18aB	2.64±0.37aA
G	6.21±0.39aA	7.38±0.34aB	3.35±0.16aB	2.68±0.01aA
R	6.91 ± 0.07bA	7.68 ± 0.31bB	3.54 ± 0.26aB	2.83 ± 0.37aA
Significance				
LED	*	*	NS	NS
Cv	*	*	*	*
LED x Cv	NS	NS	NS	NS

The values show means ± standard deviation (S.D.). W: White; B: blue; G: green; R: red. LED: LED light spectrum. Cv: Cultivar. Lowercase letters compared in the column and uppercase letters compared in the rows and indicate significant differences among LED light spectrum and cultivars, respectively. * p – value < 0.05 NS: not significant (p – value > 0.05).

Los valores muestran las medias ± la desviación estándar (S.D.). W: blanco; B: azul; G: verde; R: rojo. LED: Luz LED. Cv: Cultivar. Letras minúsculas comparan en columnas y letras mayúsculas comparan en líneas indicando diferencias significativas entre espectros de luces LED y cultivares, respectivamente. *valor p < 0,05. NS: no significativo (valor de p > 0,05).

Falcon F1 had significantly higher values of FW, DW and DW% than Viroflay. With respect to the LED light spectrum, regardless of the cultivar, plants irradiated with R-light reached significantly higher values of FW and DW than the other treatments, where FW was 2.59 g (Falcon F1) and 2.24 g (Viroflay) and DW was 0.25 g (Falcon F1) and 0.21 g (Viroflay) respect W-light, what FW was 2.04 g (Falcon F1) and 1.95 g (Viroflay) and DW was 0.19 g (Falcon F1) and 0.18 g (Viroflay). %DW showed no significant differences among the plants irradiated with different LED light spectrum (table 3, page 104).

Antioxidant parameters

A significantly higher total phenolic content (TPC) was found in Viroflay than Falcon F1. Regardless of the LED lights, the highest TPC was obtained in leaves treated by B and G- lights compared to the W treatments (table 4, page 104).

The highest antioxidant capacity was detected in Falcon F1 leaves by both the DPPH and FRAP protocols. In Falcon F1 and Viroflay, 925.8 and 886.1 µg TE g^{-1} FW were observed using the DPPH protocol, respectively. On the other hand, Falcon F1 and Viroflay showed 1092.3 and 989.1 µg TE g^{-1} FW using the FRAP protocol, respectively. Independent of the cultivar, leaves irradiated with B-light showed the highest antioxidant capacity compared to W, G and R- lights using both the DPPH and FRAP protocols (table 4, page 104). The DPPH protocol detected 998.8 and 949.5 µg TE g^{-1} FW in Falcon F1 and Viroflay treated with B-light, respectively. Using the FRAP protocol, the leaves treated with B-light showed 1234.9 and 1115.9 µg TE g^{-1} FW in Falcon F1 and Viroflay, respectively. In Falcon F1 leaves treated with W, G and R-lights, antioxidant capacity yielded 904.7, 879.3 and 920.6 µg TE g^{-1} FW using the DPPH protocol, respectively and 1070.3, 1041.9 and 1022.1 µg TE g^{-1} FW using the FRAP protocol, respectively. On the other hand, in Viroflay leaves antioxidant capacity was 856.3, 846.4 and 892.0 µg TE g^{-1} FW using the DPPH protocol respectively, and 987.2, 916.4 and 936.9 µg TE g^{-1} FW using the FRAP protocol, respectively.

Table 3. Fresh weight (FW), dry weight (DW) and dry weight percentage (%DW) of the leaves from two spinach cultivars (Falcon F1 and Viroflay) cultivated for baby leaves, under white, blue (468 nm), green (524 nm) and red (629 nm) LED light supplementation for 26 days.

Tabla 3. Peso fresco (FW), peso seco (DW) y porcentaje de peso seco (%DW) de las hojas de dos cultivares de espinaca (Falcon F1 y Viroflay) cultivados para hojas baby, bajo suplementos de luces LED blanca, azul (468 nm), verde (524 nm) y roja (629 nm) durante 26 días.

LED light spectrum	FW (g)		DW (g)		%DW (g DW 100 g^{-1} FW)	
	Falcon F1	Viroflay	Falcon F1	Viroflay	Falcon F1	Viroflay
W	2.04±0.13 aB	1.95±0.03 aA	0.19±0.01 aB	0.18±0.02 aA	9.54±0.38 aB	9.30±0.17 aA
B	1.93±0.03 aA	1.95±0.04 aA	0.18±0.01 aA	0.18±0.01 aA	9.55±0.32 aB	9.24±0.13 aA
G	2.17±0.17 aB	2.09±0.18 aA	0.21±0.0 2 aB	0.19±0.0 2aA	9.60±0.34 aB	9.30±0.10 aA
R	2.59±0.20 bB	2.24±0.14 bA	0.25±0.01 bB	0.21±0.01 bA	9.58±0.20 aB	9.36±0.11 aA
Significance						
LED	*	*	*	*	NS	NS
Cv	*	*	*	*	*	*
LED x Cv	NS	NS	NS	NS	NS	NS

The values show means ± standard deviation (S.D.). W: White; B: blue; G: green; R: red. LED: LED light spectrum. Cv: Cultivar. Lowercase letters compared in the column and uppercase letters compared in the rows and indicate significant differences among LED light spectrum and cultivars, respectively. * p - value < 0.05. NS: not significant (p - value > 0.05).

Los valores muestran las medias ± la desviación estándar (S.D.). W: blanco; B: azul; G: verde; R: rojo. LED: Luz LED. Cv: Cultivar. Letras minúsculas comparan en columnas y letras mayúsculas comparan en líneas indicando diferencias significativas entre espectros de luces LED y cultivares, respectivamente. *valor p <0,05. NS: no significativo (valor de p > 0,05).

Table 4. Total phenolic content and antioxidant capacity using the DPPH and FRAP protocols of the leaves from two spinach cultivars (Falcon F1 and Viroflay) cultivated for baby leaves, under white, blue (468 nm), green (524 nm) and red (629 nm) LED light supplementation for 26 days.

Tabla 4. Contenido de fenoles totales y capacidad antioxidante por los métodos DPPH y FRAP de las hojas de dos cultivares de espinaca (Falcon F1 y Viroflay) cultivados para hojas baby, bajo suplementos de luces LED blanca, azul (468 nm), verde (524 nm) y roja (629 nm) durante 26 días.

LED light spectrum	Total phenolic content (µg GAE g^{-1} FW)		DPPH (µg TE g^{-1} FW)		FRAP (µg TE g^{-1} FW)	
	Falcon F1	Viroflay	Falcon F1	Viroflay	Falcon F1	Viroflay
W	1019.4±44.2 aA	1054.1±70.72 aB	904.7±34.5 aB	856.3±38.0aA	1070.3±57.1aB	987.2±33.2aA
B	1245.8±61.3 bA	1483.4±205.8 bB	998.8±25.0bB	949.5±39.0bA	1234.9±58.7bB	1115.9±65.8bA
G	1263.4±86.4 bA	1244.0±42.1bA	879.3±33.1aB	846.4±27.8aA	1041.9±39.4 aB	916.4±40.7aA
R	1036.1±113.0 aA	1143.7±41.48 aB	920.6±58.1bB	892.0±34.5bA	1022.1±46.9 aB	936.9±54.8aA
Significance						
LED	*	*	*	*	*	*
Cv	*	*	*	*	*	*
LED x Cv	NS	NS	NS	NS	NS	NS

The values show means ± standard deviation (S.D.). W: White; B: blue; G: green; R: red. LED: LED light spectrum. Cv: Cultivar. Lowercase letters compared in the column and uppercase letters compared in the rows and indicate significant differences among LED light spectrum and cultivars, respectively. * p – value < 0.05. NS: not significant (p – value > 0.05).

Los valores muestran las medias ± la desviación estándar (S.D.). W: blanco; B: azul; G: verde; R: rojo. LED: Luz LED. Cv: Cultivar. Letras minúsculas comparan en columnas y letras mayúsculas comparan en líneas indicando diferencias significativas entre espectros de luces LED y cultivares, respectivamente. *valor p <0,05. NS: no significativo (valor de p > 0,05).

Discussion

Physical parameters

According to Tadeo and Gómez-Cadenas (2008), the stress caused in plants by excessive radiation occurs when the light collector antennae of the photosystems absorb more light energy than they can use in photosynthesis. Light stress initially produces photoinhibition, which prevents oxidative damage of the photosynthetic apparatus by the generation of ROS. Should this process continue, it can trigger a lower dry matter accumulation and ultimately producing the wilting of the leaves. On the other hand, low radiation causes lengthening of the internodes, thinner stems, and wider and thinner leaves with little root development.

In case of almost no illumination or darkness, etiolation is provoked in the vegetal tissues (12). These results, along with those of Bula *et al*. (1991) and Lin *et al.* (2013) in lettuces, are consistent with our results, where spinach leaves were not damaged by this low radiation intensity supplementation. Additionally, Olle and Viršilé (2013) mentioned that LED light supplementation did not produce damage associated with high temperature.

From the results of the present study, none LED lights had a significant effect on color leaves. According to previous studies, high radiation can disrupt the photosynthetic apparatus, generating a mild yellowing coloration (44). Conversely, suboptimal levels of radiation cause losses of chlorophyll and a whitening coloration that could affect the color of the plants (12). Therefore, it seems that the increase in light intensity used in this work has not been sufficient to produce a photoinhibition of the photosynthetic apparatus and changes in the green color of the spinach leaves for both cultivars. These results are consistent with those of Lin *et al.* (2013) in lettuce, where they found similar contents of photosynthetic pigments such as chlorophyll a, b, a/b and carotenoids under different light treatments. Conversely, Son and Oh (2015) found changes in total chlorophyll concentration of lettuce cultivated under R- light with a PPFD of 173 ± 3 µmol m^{-2} s^{-1} for 4 weeks, which caused changes in leaf color due to a greater pigment synthesis. This result could confirm that LED supplementation with a PPFD of 26 µmol m^{-2} s^{-1} does not have a significant effect on the color parameters hue, C or L, because it does not induce a greater synthesis of pigments or stress capable of generating color changes in spinach leaves.

The effect of R-light on spinach leaves could be related to a high energy efficiency for a photosynthesis process from 600 to 700 nm (36). The results obtained are in agreement with those recorded by Johkan *et al.* (2010) and Borowski *et al.* (2015), who showed a significant increase in size of red and green lettuce leaves exposed to R- light compared to W and B-lights. Additionally, the same authors found no significant size differences between leaves treated with W and B-lights, following a similar trend to the one observed in our study in spinach leaves irradiated with B and W-lights. These results were contrary to those reported by Yanagi *et al.* (1996), where shorter lettuce leaves were reached under B-light (PPFD of 85 and 170 µmol m^{-2} s^{-1}) than W-light. In previous studies, plants irradiated with R- light demonstrated a greater growth of leaves in species like dill (14), potato (29) and lettuce (5, 17). R-light is considered an efficient energy source for plant photosynthesis (13, 35, 36). On the other hand, B-light causes the saturation of the photosynthetic apparatus, generating less accumulation of dry matter and less length. However, in our study a low PPFD supplementation (26 µmol m^{-2} s^{-1}) was used, and leaves were smaller than those treated with W- light. On the other hand, G- light supplementation at high intensity (300 µmol m^{-2} s^{-1}) promoted the growth and development of lettuce (23). Son and Oh (2015) also indicated that G-light generates an increase in the length of lettuce leaves compared to B and W-lights.

Previous studies have shown a significant increase in length and biomass of plants cultivated under monochromatic R- light (17, 22). This is in accordance with our work, in which R- light increased fresh and dry weights and length of the leaves. Using a PPFD of 130 ± 7 µmol m^{-2} s^{-1} for 23 days (photoperiod of 12 h), a significant increase in FW and DW of lettuce treated with R- light was observed compared to those exposed to B, G or W- lights (40). This may be because wavelengths between 600 and 700 nm (R- light) generate a higher level of photosynthesis per absorbed energy unit in plants, promoting greater vegetative growth (35, 36). On the other hand, plants exposed to wavelengths between 500 and 600 nm (G- light) reflect a large proportion of the G- light spectrum, thus becoming a spectrum of light that by itself could not sustain plant growth (38), which is also in agreement with the results obtained in this work. A combination of the G- light with another spectrum or alone as supplementary light, however, could have a positive effect on plants due to its photosynthetic efficiency per absorbed energy unit is similar to R- light (45). Additionally, PPFD of G- light higher than 100 µmol m^{-2} s^{-1} could increase FW and DW accumulation (23). The wavelengths of 400 to 500 nm had less photosynthetic efficiency per absorbed energy unit than 500 to 700 nm (36). Accordingly, higher PPFD must be used in plants treated with B-light to have the same photosynthesis values as R-light due to B-light being less efficient at increasing biomass. Son and Oh (2013) used combinations of R and B- lights with a PPFD of 173 ± 3 µmol m^{-2} s^{-1} for 4 weeks. Their results found that to achieve a greater biomass accumulation in lettuce, monochromatic B and R-lights must be used. Additionally, they found that a high R-light proportion favored higher FW and DW accumulation compared to B-light proportion. All these results are consistent with our study, and the highest biomass accumulation recorded in plants treated with R-light compared to B and W-lights.

Chemical parameters

The effect of the light treatments on spinach leaves were consistent with those described by Qian *et al.* (2016), who like us found an increase in TPC of cauliflower treated with B-light compared to those exposed to W-light. In similar conditions, Liu *et al.* (2016) found a significant increase in TPC of pea seeds under B-light compared to those treated with W-light. Lee *et al.* (2010) also found a significant effect of B-light on TPC in barley leaf compared to W-light. In this work, B and G-lights increased TPC in spinach leaves compared to those treated with W-light. In the case of B-light, probably was due to the capacity of this spectrum to stress plants due to its high energy content, which induces polyphenolic compound synthesis (10). The increase in TPC in plants is associated with the synthesis of the PAL enzyme, a key factor in the secondary metabolite biosynthesis pathway (40). In addition, other enzymes such as 3-deoxy-D-arabino-heptulosonate 7-phospate synthase (DS) and chorismate mutase (CM) can activate the synthesis of enzymes in this pathway (48). Plants produce a higher biosynthesis of the PAL enzyme in the presence of stress like B-light irradiation, which could be capable of saturating the photosynthetic apparatus due to its high energy content (2). Consequently, the PPFD of 26 µmol m^{-2} s^{-1} used in our study would enough to stimulate the phenolic synthesis without altering the visual appearance of the leaves. Regarding the G-light, there is no certainty of the role that it plays in biomass production such as antioxidant compound synthesis like phenols, because previous studies have noted that the green spectrum could be completely reflected by leaves. Nevertheless, studies carried out on lettuce have demonstrated that G-light could play an important role in phenol synthesis and vegetative growth when this spectrum is used as a supplementary light or with PPFD higher than 100 µmol m^{-2} s^{-1} (23). Furthermore, Fazal *et al.* (2016) found that G- light caused an increase in total phenolics and flavonoids production in callus cultures of *Prunella vulgaris* L. compared to the control. These conflicting results by different researchers might be due to differences in light sources, intensities and vary according to species (11). Moreover, previous studies have confirmed that R-light reduces TPC in vegetables like lettuce in comparison with those treated with W-light (22). In the present experiment, plants exposed to R-light did not present significant differences with those treated with W-light, because its energy content was not capable of saturating the photosynthetic apparatus by the PPFD used (38).

In previous studies, a significant increase in antioxidant capacity was found in plants exposed to B-light compared to R- light (24, 32, 41). In the present study, we also found a significant effect of B-light on the antioxidant capacity of spinach leaves; however, R-light had no significant impact compared to W-light, which could indicate that a PPFD of 26 µmol m^{-2} s^{-1} was not enough to cause significant changes in antioxidant capacity. Also, spinach leaves treated with G-light showed an antioxidant capacity similar to those exposed to W-light. According to other studies, there is no clarity regarding of the effect of G-light on the antioxidant capacity of plants. Broccoli stored under G-light (520 nm) with a PPFD of 12 – 13 µmol m^{-2} s^{-1} showed a significantly higher antioxidant capacity than W-light (21). It has also been reported that G-light could cause a positive effect in biomass accumulation when it was used as a supplementary light; however, there is no clear evidence that G-light can induce a high synthesis of antioxidant compounds in vegetables (45, 46).

Conclusion

R-light stimulates biomass production and B-light increases total phenolic content and antioxidant capacity in spinach leaves. On the other hand, G-light increase total phenolic compound concentration in comparison with W-light treatment. LED light supplementation could benefit both, the yield and antioxidant properties of spinach plants, without affecting their visual and color characteristics. Thus, the possibility of increasing the PPFD and exposure time remains open, making use of different spectrum to optimize yield and antioxidant compound accumulation in leafy vegetables like spinach.

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