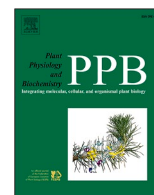
Plant Physiology and Biochemistry 165 (2021) 274–285

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy



Periodical UV-B radiation hormesis in biosynthesis of kale sprouts nutraceuticals

Noelia Castillejo, Lorena Martínez-Zamora, Francisco Artés-Hernández *

Department of Agronomical Engineering & Institute of Plant Biotechnology, Universidad Politécnica de Cartagena, Cartagena, Murcia, 30203, Spain

ARTICLE INFO

Keywords:
Brassica oleracea var. *sabellica*
Ultraviolet
Germination
Phenols
Glucosinolates
Antioxidant
Abiotic stresses

ABSTRACT

The objective of the present study was to evaluate the periodical UV-B radiation hormesis during kale seeds germination in their main content of secondary metabolite compounds (phenols; glucosinolates; total antioxidant capacity –TAC–) and their changes during a refrigerated shelf-life. The total UV-B doses received were 0, 5, 10, and 15 kJ m^{-2} (CTRL, UVB5, UVB10, and UVB15) in where the 25% was applied on the 3rd, 5th, 7th, and 10th sprouting day. UV radiation did not affect the morphological development of the sprouts. UVB10 and UVB15 treatments increased their phenolic content (>30%). Likewise, TAC was increased by UV-B lighting ~10% (DPPH) and ~20% (FRAP). The hydroxycinnamic acid content in UVB15-treated sprouts increased by 52%, while UVB5 reported an increase of 34% in the kaempferol-3,7-di-*O*-glucoside concentration, compared to CTRL. After 10 d at 4 °C of shelf-life, content of gallic acid hexoside I and gallic acid increased by 55 and 78% compared to UV-untreated kale sprouts, respectively. Glucoraphanin was the main glucosinolate found in kale sprouts and seeds, followed by 4-hydroxy-glucobrassicin, whose biosynthesis was enhanced by UVB10 (~24 and ~27%) and UVB15 (~36 and ~30%), respectively, compared to CTRL. In conclusion, periodical low UV-B illumination represents a useful tool to stimulate phytochemicals biosynthesis in kale sprouts as an important source of bioactive compounds with potential health benefits.

1. Introduction

Nowadays, society lifestyle tends to improve consumer habits introducing healthier foods rich in bioactive compounds content. In this way, the consumption of functional foods enriched in phytochemicals, most of them obtained from fruit and vegetables, has increased in last twenty years (Shahidi and Ambigaipalan, 2015). As a consequence, the consumption, and therefore its production, of ready-to-eat vegetables harvested in the early growth stages of the plant, commonly known as sprouted seeds, has exponentially increased, because of their convenience, nutritional profile, health benefits and lack of additives (Benincasa et al., 2019).

In fact, a daily consumption of more than 500 g of fruit and vegetables has been associated with a reduction of risk factors for chronic diseases, such as diabetes, obesity or cardiovascular diseases (Fernandes et al., 2018). These healthy effects are related to the presence of bioactive compounds, such as carotenoids, glucosinolates (GLs), vitamins, minerals, and phenolic compounds, which have exhibited important biological properties (Barba et al., 2017). Indeed, these phytochemical compounds have demonstrated to have an essential role in cancer prevention and oxidation or inflammatory reactions (Klug et al., 2018; Martínez-Hernández et al., 2013).

Brassica oleracea var. *Sabellica*, commonly known as kale, has been used in the present study as a model of a germinated seed. Vegetables from the *Brassica* family are rich in antioxidant compounds, especially phenolics and GLs. Besides, sprouts and young plants from Brassicas have resulted to contain 10-fold more bioactive compounds than mature plants (Šamec et al., 2018). For this reason, edible sprouts, and new technologies to improve their phytochemical compounds are a hot topic of study.

Light has the most important role in plant development, providing not only the main source of energy for photosynthesis, but also the signal for a multitude of physiological responses. Light quality (wavelength), quantity (intensity), photoperiod (duration), and uniform direction are key components of light conditions, which affect to plant morphology and secondary metabolite content (Pennisi et al., 2019). Ultraviolet (UV) and blue radiations of the electromagnetic spectrum, whose wavelength varies from 100 to 400 nm are responsible of directing the

* Corresponding author.
E-mail address: fr.artes-hdez@upct.es (F. Artés-Hernández).

https://doi.org/10.1016/j.plaphy.2021.05.022
Received 24 February 2021; Accepted 17 May 2021
Available online 26 May 2021
0981-9428/© 2021 The Author(s). Published by Elsevier Masson SAS. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

photosynthetic reaction and regulating the opening of the stomas (Taulavuori et al., 2018), as well as the photosynthetically active radiation (from 400 to 700 nm), which is also absorbed by plant pigments to regulate the plant growth and development (Chen and Blankenship, 2011; Martínez-Zamora et al., 2021).

In this way, although high UV-B radiation (280–320 nm) intensity may be harmful to plants because of its damaging effects on proteins, lipids, membranes, and DNA, the plant acclimation procedure can mitigate the damage by regulation of the secondary metabolites pathways (Klem et al., 2015). Indeed, plants detect UV-B radiation throughout the UVR8 photosystem (Jenkins, 2009, 2014), which is able to regulate the expression of key elements, which triggers enzymes of the phenylpropanoid pathway in the phenolic compounds biosynthesis, flavonoid–glycosylation (Clayton et al., 2018), and/or even UV-induced morphogenic responses (Jenkins, 2014; Robson et al., 2015). Likewise, there are many stresses that can be applied to increase the secondary metabolites of plants as elicitors including biotic stressors (carbohydrates, proteins, hormones fungus), and abiotic ones (wounding, temperature, heavy metals, light, drought, salt) (Baenas et al., 2014; Thakur et al., 2019).

Light treatments using adequate UV-B doses can act as an important regulator of secondary metabolites production in plants, such as phenolic compounds, carotenoids, and GLs (Moreira-Rodríguez et al., 2017a). In fact, the oxidative stress produced by continuous exposure to UV-B can trigger the accumulation and synthesis of several antioxidant enzymes and secondary metabolites, such as ascorbic acid, β-carotene, flavonoids, GLs, and some alkaloids that help to neutralise the impact of oxidative stress (Moreira-Rodríguez et al., 2017a). According to Moreira-Rodríguez et al. (2017b), GL accumulation was 75% increased after 24 h UV-B treatment (24.05 kJ m^{-2} dose) in 7-day broccoli sprouts. In the same study, this UV-B dose also reported an enhancement of the biosynthesis some phenolic glycosides, such as 5-sinapoylquinic acid by 121%. Nevertheless, the application of high doses of UV-B radiation results harmful to plants producing important damages on proteins, lipids, membranes and DNA (Artés et al., 2009). Therefore, an optimized dose should be found in each case to preserve from UV damages while stimulating the synthesis of bioactive compounds. From the best of our knowledge, the research of the optimal dose which could be periodically applied during kale sprouts growth to enhance the phytochemical compounds have been developed also evaluating UV-B effects on their nutritional quality. In this way, the periodical UV-B treatments can be implemented in sprout industry.

The main aim of the present study was to evaluate the effect of kale seeds germination under periodical application of low UV-B doses in the main phytochemicals content (GLs, phenols, and total antioxidant capacity) and their changes during refrigerated shelf-life as a minimally processed product.

2. Materials and methods

2.1. Plant material used and growing conditions

Black kale seeds (Brassica olearacea ssp. acephala var. Sabellica) were supplied by Intersemillas S.A. (Valencia, Spain). Two g of kale seeds were weighed and disinfected with a fungicide for 10 min (Geoxe 50 WG - Fludioxonil 50% w/w -; Syngenta). Subsequently, two 1 min rinses were carried out with autoclaved distilled water and seeds were left water submerged for 18 h in darkness. In a laminar flow hood (Telstar Bio–II–A/M, Japan), the kale seeds were disposed in polypropylene trays (170 × 120 × 50 mm), using a filter paper layer moistened with sprayed autoclaved distilled water as a support. This moment was defined as day 0, as the seeds started to sprout. A 40 μm oriented polypropylene film covered the trays to ensure a high relative humidity (RH). Kale seeds germinated in a growth chamber (Sanyo

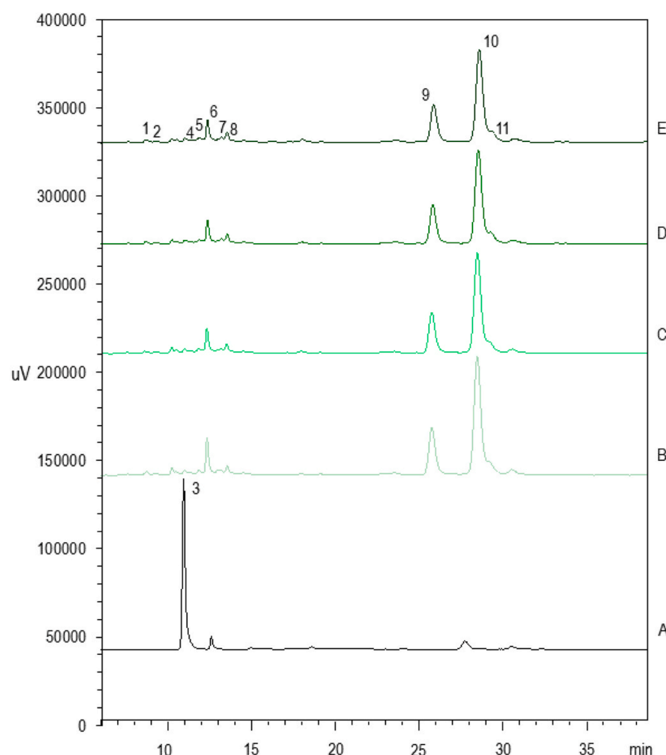


Fig. 1. UHPLC chromatograms (shown at 320 nm) of identified phenolic compounds from methanol/water (80:20, v/v) extracts of kale seed (A) and kale sprouts treated with UV-B (0, 5, 10, and 15 kJ m-2) (B, C, D, and E, respectively) during10 growing d at 20 °C in darkness and stored up to 10 d at 4 °C as a minimally processed product. Identified peaks are: (1) Gallic acid hexoside I, (2) Gallic acid, (3) 3-O-caffeoylquinic acid, (4) 4-O-caffeoylquinic acid, (5) 5-O-caffeoylquinic acid, (6) 1,2-diferuloyl-gentobiose, (7) caffeic acid, (8) caffeic acid derivative, (9) hydroxycinnamic acid, (10) Kaempferol-3-diglucoside-7-glucoside, and (11) Kaempferol-3-sinapoyl-7-diglucoside.

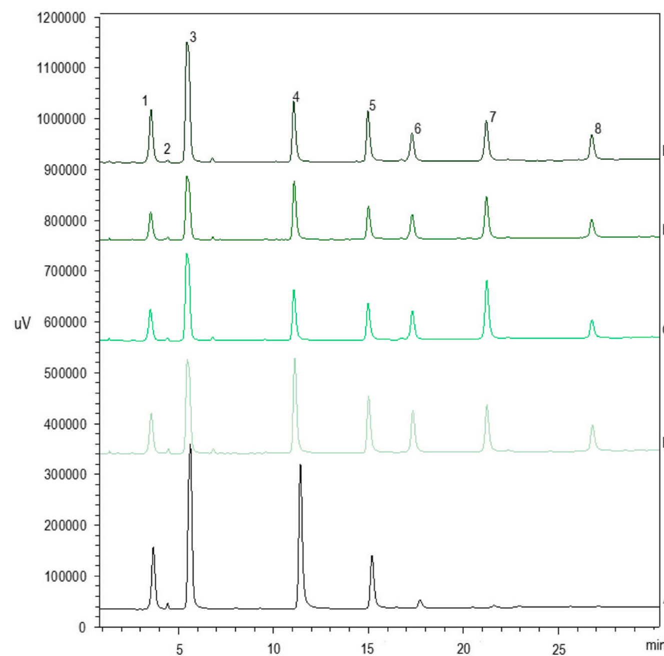


Fig. 2. UHPLC chromatograms (shown at 227 nm) of identified glucosinolates from ethanol/water (70:30, v/v) extracts of kale seed (A) and kale sprouts treated with UV-B (0, 5, 10, and 15 kJ m-2) (B, C, D, and E, respectively) during 10 growing d at 20 °C in darkness and stored up to 10 d at 4 °C as a minimally processed product. Identified peaks are: (1) Glucoiberin, (2) Progoitrin, (3) Glucoraphanin, (4) 4-hydroxy-glucobrassicin, (5) Glucoerucin, (6) Glucobrassicin, (7) 4-methoxy-glucobrassicin, and (8) Neoglucobrassicin.

MLR-350 H, Japan) for 10 d at 20 °C and 90% RH under darkness conditions.

2.2. UV-B treatments

UV-B treatments were applied during germination on days 3, 5, 7, and 10 with a 25% of the total dose received, which was based on previous unpublished experiences in sprouts and those recommended by Formica-Oliveira et al. (2017b).

The radiation chamber consisted of a reflective stainless-steel chamber with 2 lamp banks being fitted to each bank 6 UV-B unfiltered emitting lamps (TL 40 W/01 RS; Philips, Eindhoven, The Netherlands). It was also provided with a ventilator to renovate the air from inside of the chamber (Formica-Oliveira et al., 2017b). Kale sprouts grown under darkness conditions were placed between the two lines of lamps at 17.5 cm above and below on a polystyrene net. The UV-B (280–320 nm) intensity applied (7.99 ± 0.40 W m^{-2}) was calculated as the mean of 20 readings on each side of the net by using a UV radiometer (LP 471 UVB, Delta OHM, Italy). For that, kale sprouts under darkness conditions were sited under UV-B lamps. The light intensities were kept constant and the applied doses varied by altering the exposure time at the fixed distance. UV-B treatments applied were the following:

- **CTRL**: No UV radiation was our control treatment.
- **UVB5**: the sprouts received at the end of the germination process 5 kJ m^{-2} UV-B in 4 equal applications of 1.25 kJ m^{-2} d^{-1} conducted on days 3, 5, 7, and 10.
- **UVB10**: the sprouts received at the end of the germination process 10 kJ m^{-2} UV-B in 4 equal applications of 2.50 kJ m^{-2} d^{-1} conducted on days 3, 5, 7, and 10.
- **UVB15**: the sprouts received at the end of the germination process 15 kJ m^{-2} UV-B in 4 equal applications of 3.25 kJ m^{-2} d^{-1} conducted on days 3, 5, 7, and 10.

The UV-B dose was selected based on our previous experiments and the data reported by Formica-Oliveira et al. (2017b) in order to obtain maximum phytochemicals synthesis while minimizing heating and evaporation processes during treatment, which may affect the quality of the product. After 1 h from the end of the UV-B application, samples were immediately frozen at −80 °C until they were freeze-dried (Lyoquest-85, Telstar Technologies, Terrassa, Spain). Freeze-dried samples were kept at room temperature until the analytical determinations were performed.

2.3. Minimal processing unit operations

After 1 h of the last UV treatment applied on the 10th germination day at 20 °C, the sprouts were hand harvested and disinfected in a cold room at 5 °C with a sodium hypochlorite solution (1 min; 150 ppm; 5 °C; pH = 6.5) and then rinsed 1 min in water at 5 °C. Kale sprouts were placed in 250 mL polypropylene closed containers (10 × 8 × 3.5 cm) under 90–95% RH and atmospheric gas partial pressures up to 10 d at 4 °C in darkness. Samples were collected for analysed on days 0, 4, 7, and 10 of the refrigerated shelf-life.

2.4. Analyses and determinations

2.4.1. Sprouts growth parameters

The sprouts were horizontally disposed nearby a ruler and photographed. The length of the hypocotyl (H) and root (R) of 3 replicates of 10 sprouts from each treatment, and on each sampling day, was determined using the ImageJ program (Wayne Rasband, Maryland, USA). Results were presented in cm. Growth speed was calculated by dividing H (mm) by the growing days and presented in mm d^{-1}.

2.4.2. Extraction of phytochemical compounds from samples

A volume of 3 mL methanol:water (80:20, v/v) were added to 25 mg of freeze-dried sprouts. The samples were vigorously shaken an orbital shaker (Stuart, Stone, UK) for 1 h inside a polystyrene box in darkness with an ice bed. The extracts were centrifuged at 3220 g for 10 min at 5 °C. The supernatant was recovered for Total Phenolic Content (TPC), Total Antioxidant Capacity (TAC), and individual phenolic content analyses.

The extraction of GLs was performed according to

Table 1
Growth parameters of UV-B treated (CTRL; 5; 10, and 15 kJ m^{-2}) kale sprouts during 10 growing d at 20 °C in darkness.

Growing days	Treatment	Hypocotyl length (cm)	Sprout length (cm)	H/R Ratio	FW/DW Ratio	Growth rate (mm d^{-1})
3	CTRL	0.71 ± 0.28^{c}	1.89 ± 0.53^{c}	0.58 ± 0.13^{b}	$2.51 \pm 0.01^{A\ b}$	2.36 ± 0.93^{a}
	UVB5	0.85 ± 0.16^{b}	1.94 ± 0.22^{c}	0.78 ± 0.11^{b}	$2.42 \pm 0.01^{B\ d}$	2.84 ± 0.53^{ab}
	UVB10	0.73 ± 0.13^{c}	2.18 ± 0.18^{d}	0.51 ± 0.10^{c}	$2.43 \pm 0.01^{B\ d}$	2.44 ± 0.45^{c}
	UVB15	0.61 ± 0.09^{c}	1.63 ± 0.19^{c}	0.60 ± 0.08^{b}	$2.42 \pm 0.01^{B\ d}$	2.04 ± 0.31^{b}
5	CTRL	$1.17 \pm 0.09^{B\ c}$	$2.41 \pm 0.02^{B\ bc}$	$0.95 \pm 0.17^{AB\ ab}$	$3.24 \pm 0.30^{B\ b}$	$2.34 \pm 0.18^{B\ a}$
	UVB5	$1.14 \pm 0.19^{B\ b}$	$3.22 \pm 0.34^{A\ b}$	$0.55 \pm 0.06^{B\ b}$	$4.71 \pm 0.44^{A\ c}$	$2.28 \pm 0.38^{B\ b}$
	UVB10	$1.74 \pm 0.15^{A\ b}$	$3.51 \pm 0.01^{A\ c}$	$1.00 \pm 0.17^{AB\ bc}$	$4.67 \pm 0.74^{A\ c}$	$3.49 \pm 0.30^{A\ ab}$
	UVB15	$1.80 \pm 0.34^{A\ b}$	$3.47 \pm 0.15^{A\ b}$	$1.10 \pm 0.32^{A\ b}$	$5.26 \pm 0.07^{A\ c}$	$3.60 \pm 0.69^{A\ a}$
7	CTRL	$2.14 \pm 0.34^{B\ b}$	3.73 ± 0.77^{ab}	1.38 ± 0.17^{a}	6.42 ± 2.05^{a}	$3.06 \pm 0.49^{B\ a}$
	UVB5	$2.50 \pm 0.05^{AB\ a}$	4.28 ± 0.24^{a}	1.42 ± 0.16^{a}	7.95 ± 0.14^{b}	$3.57 \pm 0.08^{AB\ a}$
	UVB10	$2.84 \pm 0.24^{A\ a}$	4.32 ± 0.19^{b}	1.93 ± 0.30^{a}	8.05 ± 0.26^{b}	$4.05 \pm 0.34^{A\ a}$
	UVB15	$2.61 \pm 0.24^{AB\ a}$	4.05 ± 0.22^{b}	1.86 ± 0.42^{a}	7.81 ± 1.00^{b}	$3.73 \pm 0.35^{AB\ a}$
10	CTRL	2.98 ± 0.42^{a}	5.18 ± 0.69^{a}	1.37 ± 0.18^{a}	$5.27 \pm 1.20^{B\ ab}$	2.98 ± 0.42^{a}
	UVB5	2.66 ± 0.37^{a}	4.29 ± 0.42^{a}	1.65 ± 0.29^{a}	$11.94 \pm 0.08^{A\ a}$	2.66 ± 0.37^{ab}
	UVB10	2.99 ± 0.12^{a}	5.20 ± 0.12^{a}	1.37 ± 0.20^{b}	$11.66 \pm 0.67^{A\ a}$	2.99 ± 0.12^{bc}
	UVB15	3.13 ± 0.33^{a}	5.57 ± 0.79^{a}	1.30 ± 0.13^{ab}	$12.32 \pm 0.06^{A\ a}$	3.13 ± 0.33^{ab}

H/R: Hypocotyl/Root. FW/DW: Fresh weigh/Dry weight. Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment. Absence of letters indicates that there are no significant differences ($p < 0.05$).

Moreira-Rodríguez et al. (2017b) with slight modifications. Briefly, a sample of 0.2 ± 0.01 g of kale sprouts powder were placed in glass tubes and 10 mL of ethanol/water (70:30, v/v) previously heated at 70 °C in a bath. Samples were incubated at 70 °C for 30 min and vortexed at 0, 10, and 20 min to ensure myrosinase inactivation. After that, the extracts were rapidly cooled on an ice bath and centrifuged at 18,000×*g* for 10 min at 4 °C (L-90K Ultracentrifuge Beckman Coulter, rotor type 45 70Ti). The supernatant was collected for GLs analysis. Just after the extraction, GLs were desulphated and purified using disposable polypropylene columns (Thermo Fisher Scientific, Waltham, MA, USA). The preparation of columns was carried out by adding 500 μL of MilliQ water, followed by 500 μL of Sephadex A-25 and 500 μL of MilliQ water. Then, 3 mL of ethanolic extract were added into a prepared column and allowed to drip through slowly. After that, two aliquots of 500 μL of MilliQ water were added followed by others two of 500 μL of 0.02 M sodium acetate. Purified sulfatase (75 μL) was added to each sample and left at room temperature overnight (16 h). Finally, 1.25 mL of water were added into columns to recover desulfoglucosinolates and the extracts were kept at −80 °C until analysis.

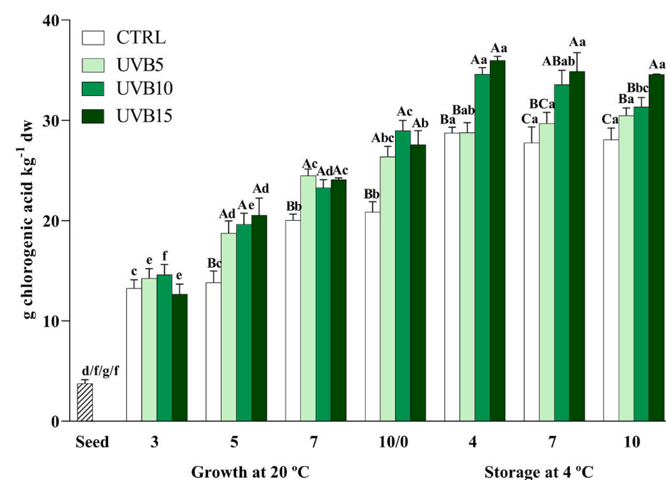


Fig. 3. Total phenolic content of kale sprouts treated with UV-B (CTRL, 5, 10, and 15 kJ m-2) during 10 growing d at 20 °C in darkness and stored up to 10 d at 4 °C as a minimally processed product. Different capital letters indicate significant differences among treatments at $p < 0.05$ based on Tukey's test. Different lowercase letters indicate significant differences among time of analysis of the same treatment at $p < 0.05$ based on Tukey's test.

2.4.3. Total phenolic content

TPC was determined following the method described by Singleton and Rossi (1965). Briefly, 19 μL sample extract were placed on a 96-well plate (Greiner Bio-One; Frickenhausen, Germany) and 29 μL of 1 mol L^{-1} Folin–Ciocalteu reagent were added. After 3 min incubation in darkness at room temperature (RT), 192 μL of 0.4% Na_2CO_3 2% NaOH were added. The absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200, Männedorf, Switzerland) after 1 h incubation in darkness at RT. The TPC was expressed as g chlorogenic acid equivalents (CAE) kg^{-1} dw. Each sample was analysed in triplicate.

2.4.4. Individual phenolic content

A sample of 1 mL of the methanolic extract was filtered using 0.2 μm PTFE membrane filters. Analysis and identification of individual phenolic compounds were based on Castillejo et al. (2021). An Ultra High 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 Gemini C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Torrance CA, USA). The results were quantified using standard patterns and expressed as g gallic acid equivalents kg^{-1} dw for gallic acid hexoside I and gallic acid; as g chlorogenic acid equivalents kg^{-1} dw for 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, and 1,2-diferuloyl-gentobiose; as mg caffeic acid equivalents kg^{-1} dw for caffeic acid and caffeic acid derivative; as g hydroxycinnamic acid equivalents kg^{-1} dw for hydroxycinnamic acid; and as g rutin equivalents kg^{-1} dw for kaempferol-3,7-di-*O*-glucoside and kaempferol-3-*O*-sinapoylglucoside-7-*O*-glucoside. Identified compounds were also checked by their retention time and spectra, based on previous publications (Castillejo et al., 2021; Martínez-Zamora et al., 2021; Moreira-Rodríguez et al., 2017a, 2017b). Identified phenolic compounds are shown in Fig. 1 and Fig. S1 shows the PDA spectra of each compound. Each sample was analysed in triplicate.

2.4.5. Total antioxidant capacity

TAC was analysed by using two different methods described by Castillejo et al. (2021): DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method) and FRAP (Ferric Reducing Antioxidant Power) assays. For DPPH assay, 194 μL of DPPH (0.7 mM) solution were added to 21 μL of sprout extract. The mixture was incubated for 30 min at RT in darkness. The TAC by DPPH was measured by changes in absorbance at 515 nm. FRAP method was carried out using a daily reaction solution (10:1:1; v/v/v) containing sodium acetate buffer (pH 3.6), 10 mM TPTZ

Table 2
Individual and total phenolic content of UV-B treated (CTRL; 5; 10, and 15 kJ m^{-2}) kale sprouts during 10 growing d at 20 °C in darkness and stored up to 10 d at 4 °C as a minimally processed product.

Treatment	Day of analysis	Gallic acid hexoside I	Gallic acid	3-*O*-caffeoylquinic acid	4-*O*-caffeoylquinic acid	5-*O*-caffeoylquinic acid	1,2-diferuloyl-gentobiose
Seed	0G	0.00 ± 0.00$^{e/d/g/f}$	0.00 ± 0.00$^{f/f/f/e}$	5.15 ± 0.20$^{a/a/a/a}$	0.00 ± 0.00$^{d/d/e/e}$	0.00 ± 0.00$^{f/e/e/f}$	0.40 ± 0.04$^{e/d/f/e}$
CTRL	3G	0.36 ± 0.04$^{B\ d}$	0.70 ± 0.05^{e}	4.06 ± 0.26^{b}	0.00 ± 0.00$^{B\ d}$	0.07 ± 0.01^{e}	2.65 ± 0.13$^{AB\ b}$
	5G	0.49 ± 0.00$^{B\ d}$	1.07 ± 0.06$^{C\ de}$	1.88 ± 0.17$^{A\ c}$	0.21 ± 0.00^{c}	0.10 ± 0.00$^{B\ d}$	3.31 ± 0.18^{a}
	7G	0.70 ± 0.10$^{B\ c}$	1.44 ± 0.08$^{C\ cd}$	1.09 ± 0.12$^{A\ d}$	0.25 ± 0.00^{b}	0.19 ± 0.01^{c}	2.29 ± 0.04^{c}
	10G/0S	0.80 ± 0.08$^{C\ c}$	1.96 ± 0.06$^{C\ c}$	0.41 ± 0.10$^{A\ ef}$	0.24 ± 0.01$^{B\ bc}$	0.19 ± 0.01$^{C\ c}$	1.20 ± 0.11$^{AB\ d}$
	10G+4S	1.03 ± 0.03$^{C\ b}$	3.51 ± 0.22$^{B\ b}$	0.80 ± 0.09$^{A\ de}$	0.27 ± 0.01$^{B\ ab}$	0.23 ± 0.01$^{C\ b}$	1.21 ± 0.06$^{B\ d}$
	10G+7S	1.29 ± 0.08$^{B\ a}$	5.06 ± 0.48$^{A\ a}$	0.36 ± 0.06^{f}	0.30 ± 0.03^{a}	0.26 ± 0.03$^{B\ a}$	1.23 ± 0.02$^{A\ d}$
	10G+10S	1.33 ± 0.10$^{B\ a}$	5.41 ± 0.25$^{A\ a}$	0.37 ± 0.05^{f}	0.29 ± 0.01^{a}	0.26 ± 0.02$^{B\ ab}$	1.17 ± 0.12$^{B\ d}$
UVB5	3G	0.46 ± 0.03$^{A\ c}$	0.68 ± 0.06^{e}	4.20 ± 0.25^{b}	0.00 ± 0.00$^{B\ d}$	0.07 ± 0.00^{d}	2.96 ± 0.18$^{A\ a}$
	5G	0.54 ± 0.00$^{B\ c}$	1.41 ± 0.08$^{AB\ d}$	0.94 ± 0.05$^{B\ c}$	0.21 ± 0.01^{c}	0.14 ± 0.02$^{A\ c}$	3.15 ± 0.22^{a}
	7G	1.13 ± 0.10$^{A\ b}$	1.91 ± 0.05$^{B\ cd}$	0.45 ± 0.04$^{B\ d}$	0.25 ± 0.01^{bc}	0.20 ± 0.03^{b}	2.27 ± 0.11^{b}
	10G/0S	1.20 ± 0.12$^{B\ b}$	2.44 ± 0.11$^{AB\ c}$	0.27 ± 0.01$^{AB\ d}$	0.26 ± 0.01$^{B\ b}$	0.23 ± 0.01$^{B\ b}$	1.13 ± 0.04$^{AB\ c}$
	10G+4S	2.37 ± 0.24$^{A\ a}$	7.22 ± 0.44$^{A\ a}$	0.34 ± 0.03$^{B\ d}$	0.29 ± 0.02$^{B\ ab}$	0.28 ± 0.01$^{B\ a}$	0.93 ± 0.02$^{C\ c}$
	10G+7S	2.32 ± 0.18$^{A\ a}$	3.83 ± 0.16$^{B\ b}$	0.35 ± 0.05^{d}	0.32 ± 0.04^{a}	0.28 ± 0.02$^{B\ a}$	1.01 ± 0.05$^{B\ c}$
	10G+10S	2.14 ± 0.20$^{A\ a}$	3.85 ± 0.28$^{C\ b}$	0.31 ± 0.03^{d}	0.32 ± 0.03^{a}	0.28 ± 0.01$^{B\ a}$	0.83 ± 0.05$^{A\ c}$
UVB10	3G	0.25 ± 0.02$^{C\ f}$	0.67 ± 0.03^{e}	4.26 ± 0.19^{b}	0.00 ± 0.00$^{B\ e}$	0.07 ± 0.01^{d}	2.72 ± 0.16$^{AB\ b}$
	5G	0.81 ± 0.00$^{A\ e}$	1.49 ± 0.05$^{A\ d}$	0.89 ± 0.14$^{B\ c}$	0.21 ± 0.00^{d}	0.15 ± 0.02$^{A\ c}$	3.48 ± 0.23^{a}
	7G	1.06 ± 0.05$^{A\ d}$	1.71 ± 0.18$^{BC\ cd}$	0.44 ± 0.03$^{B\ d}$	0.25 ± 0.01^{c}	0.21 ± 0.01^{b}	2.22 ± 0.09^{c}
	10G/0S	1.69 ± 0.08$^{A\ c}$	2.23 ± 0.16$^{BC\ c}$	0.32 ± 0.03$^{AB\ d}$	0.28 ± 0.01$^{A\ c}$	0.25 ± 0.01$^{A\ b}$	1.24 ± 0.14$^{A\ de}$
	10G+4S	1.78 ± 0.16$^{B\ bc}$	7.19 ± 0.39$^{A\ a}$	0.40 ± 0.01$^{B\ d}$	0.38 ± 0.01$^{A\ a}$	0.36 ± 0.03$^{A\ a}$	1.48 ± 0.10$^{A\ d}$
	10G+7S	2.38 ± 0.04$^{A\ a}$	4.26 ± 0.30$^{AB\ b}$	0.33 ± 0.01^{d}	0.35 ± 0.03^{ab}	0.34 ± 0.02$^{A\ a}$	1.13 ± 0.07$^{AB\ de}$
	10G+10S	1.91 ± 0.06$^{A\ b}$	4.08 ± 0.08$^{BC\ b}$	0.31 ± 0.02^{d}	0.34 ± 0.02^{b}	0.32 ± 0.02$^{A\ a}$	0.93 ± 0.08$^{A\ e}$
UVB15	3G	0.29 ± 0.02$^{BC\ e}$	0.73 ± 0.04^{d}	4.34 ± 0.42^{b}	0.22 ± 0.00$^{A\ cd}$	0.08 ± 0.02^{e}	2.43 ± 0.08$^{B\ b}$
	5G	0.81 ± 0.12$^{A\ d}$	1.24 ± 0.07$^{BC\ d}$	0.76 ± 0.04$^{B\ c}$	0.21 ± 0.01^{d}	0.12 ± 0.00$^{AB\ d}$	3.59 ± 0.03^{a}
	7G	1.28 ± 0.12$^{A\ c}$	2.36 ± 0.21$^{A\ c}$	0.41 ± 0.03$^{B\ cd}$	0.24 ± 0.01^{c}	0.21 ± 0.01^{c}	2.15 ± 0.25^{b}
	10G/0S	1.87 ± 0.02$^{A\ b}$	2.65 ± 0.11$^{A\ c}$	0.24 ± 0.03$^{B\ d}$	0.24 ± 0.00$^{B\ c}$	0.22 ± 0.01$^{B\ c}$	0.95 ± 0.05$^{B\ d}$
	10G+4S	1.71 ± 0.16$^{B\ b}$	7.07 ± 0.30$^{A\ a}$	0.40 ± 0.06$^{B\ cd}$	0.38 ± 0.02$^{A\ a}$	0.40 ± 0.02$^{A\ a}$	1.64 ± 0.14$^{A\ c}$
	10G+7S	2.41 ± 0.10$^{A\ a}$	4.43 ± 0.26$^{AB\ b}$	0.31 ± 0.02^{cd}	0.35 ± 0.01^{ab}	0.36 ± 0.02$^{A\ b}$	1.00 ± 0.07$^{B\ d}$
	10G+10S	2.15 ± 0.07$^{A\ a}$	4.73 ± 0.34$^{B\ b}$	0.29 ± 0.02^{cd}	0.33 ± 0.01^{b}	0.33 ± 0.02$^{A\ b}$	0.84 ± 0.04$^{A\ d}$

Treatment	Day of analysis*	Caffeic acid	Caffeic acid derivative	Hydroxycinnamic acid	Kaempferol-3,7-di-*O*-glucoside	Kaempferol-3-*O*-sinapoylglucoside-7-*O*-glucoside	Total phenolic content (g kg^{-1} DW)
Seed	0G	0.0 ± 0.0$^{f/f/e/e}$	38.2 ± 8.4$^{e/e/e/f}$	0.68 ± 0.11$^{e/c/e/f}$	2.2 ± 0.5$^{e/d/e/f}$	0.45 ± 0.00$^{e/e/d/f}$	8.9 ± 0.8$^{d/e/g/f}$
CTRL	3G	30.8 ± 4.9^{e}	63.2 ± 6.1^{cd}	1.17 ± 0.06^{de}	9.3 ± 0.6$^{B\ de}$	0.98 ± 0.18^{d}	19.4 ± 0.7$^{B\ c}$
	5G	63.8 ± 0.3^{d}	60.0 ± 3.2$^{B\ d}$	1.73 ± 0.22$^{B\ d}$	14.4 ± 0.1$^{B\ d}$	1.23 ± 0.01$^{C\ d}$	24.5 ± 0.3$^{C\ c}$
	7G	72.5 ± 1.7^{cd}	86.6 ± 7.2^{ab}	2.44 ± 0.12$^{B\ c}$	31.1 ± 1.7^{c}	1.92 ± 0.28^{c}	41.5 ± 1.2$^{B\ b}$
	10G/0S	78.7 ± 7.0^{bc}	81.0 ± 8.6^{bc}	2.58 ± 0.39$^{AB\ bc}$	33.9 ± 4.5$^{B\ bc}$	1.82 ± 0.08$^{C\ c}$	43.2 ± 5.1$^{B\ b}$
	10G+4S	90.9 ± 2.2$^{B\ ab}$	84.4 ± 1.0$^{C\ b}$	2.88 ± 0.15$^{B\ abc}$	40.7 ± 1.8$^{B\ ab}$	2.06 ± 0.08$^{D\ bc}$	52.9 ± 2.0$^{C\ a}$
	10G+7S	103.1 ± 8.7^{a}	90.8 ± 4.3$^{B\ ab}$	3.18 ± 0.24^{ab}	47.6 ± 5.1^{a}	2.29 ± 0.07$^{C\ b}$	61.7 ± 5.5^{a}
	10G+10S	100.6 ± 5.1^{a}	103.6 ± 8.1^{a}	3.29 ± 0.41^{a}	47.1 ± 4.6^{a}	2.96 ± 0.08^{a}	62.4 ± 5.3^{a}
UVB5	3G	31.2 ± 4.6^{e}	63.3 ± 1.3^{d}	1.27 ± 0.15^{b}	11.3 ± 0.8$^{A\ c}$	1.06 ± 0.15^{d}	22.1 ± 1.5$^{A\ d}$
	5G	62.9 ± 6.5^{d}	70.0 ± 5.0$^{AB\ d}$	1.83 ± 0.13$^{B\ b}$	21.9 ± 2.9$^{A\ b}$	1.58 ± 0.18$^{B\ c}$	31.8 ± 3.3$^{B\ c}$
	7G	78.8 ± 4.4^{c}	93.6 ± 2.5^{bc}	2.95 ± 0.01$^{A\ a}$	38.0 ± 1.6^{a}	2.59 ± 0.18^{b}	50.0 ± 2.04$^{A\ b}$
	10G/0S	86.6 ± 1.1^{bc}	90.3 ± 4.5^{c}	2.99 ± 0.24$^{A\ a}$	45.5 ± 3.4$^{A\ a}$	2.96 ± 0.09$^{A\ ab}$	57.1 ± 3.6$^{A\ ab}$
	10G+4S	124.2 ± 3.9$^{A\ a}$	109.3 ± 2.0$^{A\ a}$	3.13 ± 0.09$^{B\ a}$	43.6 ± 0.5$^{B\ a}$	3.13 ± 0.03$^{C\ a}$	61.6 ± 1.3$^{B\ a}$
	10G+7S	99.5 ± 9.3^{b}	117.7 ± 4.0$^{A\ a}$	3.04 ± 0.29^{a}	42.0 ± 4.2^{a}	2.89 ± 0.24$^{B\ ab}$	56.2 ± 4.9^{ab}
	10G+10S	88.7 ± 7.3^{bc}	105.8 ± 4.6^{ab}	2.85 ± 0.37^{a}	39.8 ± 4.2^{a}	3.26 ± 0.14^{a}	53.9 ± 4.7^{ab}
UVB10	3G	26.1 ± 0.1^{d}	58.1 ± 2.2^{d}	1.14 ± 0.09^{e}	9.4 ± 0.1$^{B\ e}$	0.87 ± 0.07^{d}	19.5 ± 0.6$^{AB\ f}$
	5G	70.8 ± 2.6^{c}	78.4 ± 5.3$^{A\ c}$	2.44 ± 0.16$^{A\ d}$	26.0 ± 1.8$^{A\ d}$	1.99 ± 0.10$^{A\ c}$	37.6 ± 2.2$^{A\ e}$
	7G	76.1 ± 1.9^{c}	89.7 ± 8.1^{bc}	2.59 ± 0.33$^{AB\ d}$	32.6 ± 4.7^{cd}	2.28 ± 0.40^{c}	43.5 ± 5.2$^{AB\ de}$
	10G/0S	82.1 ± 6.1^{c}	90.0 ± 4.0^{bc}	2.91 ± 0.22$^{AB\ cd}$	37.3 ± 3.5$^{AB\ bc}$	2.52 ± 0.12$^{B\ bc}$	48.9 ± 3.7$^{AB\ cd}$
	10G+4S		99.3 ± 2.0$^{B\ ab}$	4.07 ± 0.22$^{A\ a}$	51.9 ± 3.7$^{A\ a}$	3.55 ± 0.16$^{B\ a}$	71.3 ± 4.0$^{A\ a}$

(*continued on next page*)

Table 2 (*continued*)

Treatment	Day of analysis*	Caffeic acid	Caffeic acid derivative	Hydroxycinnamic acid	Kaempferol-3,7-di-*O*-glucoside	Kaempferol-3-*O*-sinapoylglucoside-7-*O*-glucoside	Total phenolic content (g kg^{-1} DW)
		134.0 ± 7.1$^{A\ a}$					
	10G+7S	107.9 ± 6.3 ^{b}	113.8 ± 4.2$^{A\ a}$	3.58 ± 0.27 ^{ab}	48.4 ± 1.5 ^{a}	3.19 ± 0.18$^{AB\ a}$	64.2 ± 1.6 ^{ab}
	10G+10S	95.9 ± 6.5 ^{b}	108.7 ± 4.8 ^{a}	3.25 ± 0.30 ^{bc}	43.7 ± 4.2 ^{ab}	3.05 ± 0.21 ^{ab}	58.1 ± 4.6 ^{bc}
UVB15	3G	24.1 ± 1.5 ^{d}	58.8 ± 3.0 ^{e}	1.19 ± 0.05 ^{e}	9.1 ± 0.4$^{B\ e}$	0.92 ± 0.05 ^{e}	19.4 ± 0.9$^{B\ e}$
	5G	67.4 ± 4.0 ^{c}	66.9 ± 6.9$^{AB\ de}$	2.36 ± 0.08$^{A\ d}$	23.2 ± 1.4$^{A\ d}$	1.78 ± 0.08$^{AB\ d}$	34.2 ± 1.7$^{AB\ d}$
	7G	75.0 ± 4.9 ^{c}	79.0 ± 6.0 ^{cd}	3.06 ± 0.15$^{A\ c}$	34.7 ± 1.1 ^{c}	2.54 ± 0.07 ^{c}	47.1 ± 1.7$^{AB\ c}$
	10G/0S	74.9 ± 0.4 ^{c}	94.8 ± 5.0 ^{bc}	2.23 ± 0.16$^{B\ d}$	29.8 ± 1.5$^{B\ c}$	2.10 ± 0.16$^{C\ d}$	40.5 ± 1.5$^{B\ cd}$
	10G+4S	133.9 ± 8.8$^{A\ a}$	98.0 ± 6.7$^{B\ b}$	4.37 ± 0.24$^{A\ a}$	54.2 ± 4.1$^{A\ a}$	4.08 ± 0.17$^{A\ a}$	74.5 ± 4.4$^{A\ a}$
	10G+7S	106.6 ± 3.7 ^{b}	115.9 ± 4.7$^{A\ a}$	3.56 ± 0.07 ^{b}	46.8 ± 1.9 ^{b}	3.40 ± 0.22$^{A\ b}$	62.8 ± 2.4 ^{b}
	10G+10S	95.1 ± 1.2 ^{b}	117.1 ± 4.2 ^{a}	3.00 ± 0.31 ^{c}	41.2 ± 2.8 ^{b}	3.00 ± 0.26 ^{b}	56.1 ± 3.6 ^{b}

*G = growing days at 20 °C in darkness; S = shelf-life days at 4 °C as a minimally processed product. Concentrations are reported as g gallic acid equivalents kg^{-1}. dw for gallic acid hexoside I and gallic acid; as g chlorogenic acid equivalents kg^{-1}. dw for 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, and 1,2-diferuloyl-gentobiose; as mg caffeic acid equivalents kg^{-1}. dw for caffeic acid and caffeic acid derivative; as g hydroxycinnamic acid equivalents kg^{-1}. dw for hydroxycinnamic acid; and as g rutin equivalents kg^{-1}. dw for kaempferol-3,7-di-*O*-glucoside and kaempferol-3-*O*-sinapoylglucoside-7-*O*-glucoside. Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment. Absence of letters indicates that there are no significant differences ($p < 0.05$).

solution (in 40 mM HCl), and 20 mM $FeCl_3$ and incubated at 37 °C for 2 h in darkness. Then, 198 μL of FRAP solution were added to 6 μL of methanolic extract and incubated for 14 min at RT in darkness. The TAC by FRAP was measured by changes in absorbance at 593 nm. The results were expressed as g of Trolox Equivalents (TE) kg^{-1} dw. Each sample was analysed in triplicate.

2.4.6. Glucosinolates content

Analysis and identification of GLs were conducted according to Moreira-Rodríguez et al. (2017b) with slight modifications. An UHPLC (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 Gemini C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Torrance CA, USA). Separation of desulfoglucosinolates in the UHPLC system was achieved using water (phase A) and acetonitrile (phase B) as mobile phases. A flow rate of 1.5 mL min^{-1} and a gradient of 0/100, 28/80, 30/100 (min/% phase A) with an injection volume of 20 μL was performed. Desulfoglucosinolates were detected at 227 nm and identified compounds are shown in Fig. 2 and Fig. S2 shows the PDA spectra of each compound. Obtained data were quantified as g glucoraphanin kg^{-1} dw, and time retention and spectra absorption of each peak was used to identified them based on previous unpublished results and reported data by Moreira-Rodríguez et al. (2017a, 2017b), and Hahn et al. (2016). Each sample was extracted and analysed in triplicate.

2.5. Statistical analyses

The experiment was a two-factor (treatment × time) 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 $p \leq 0.05$, and Tukey's multiple range test was used to separate means.

3. Results and discussion

3.1. Effect of UV-B radiation on kale sprouts growth

The main growth parameters during 10 d at 20 °C under several UV-B doses are presented in Table 1. Kale sprouts were harvested when they reached an average hypocotyl and sprout length of 2.94 ± 0.31 cm and 5.06 ± 0.51 cm, respectively. In general, UV-B did not influence the hypocotyl and sprout length development, which is also shown in Figs. S3 and S4, where kinetics of hypocotyl and sprout length are presented. In fact, all treatments fitted to a lineal model with an adherence (R^2) higher to 0.900. The lineal kinetic of the hypocotyl growth was fitted to the following formula: $y = 0.7698\times - 0.011975$ ($R^2 = 0.9440$), while the whole sprout growth fitted to $y = 1.038475x + 0.95715$ ($R^2 = 0.9493$). In this sense, UVB15 was the treatment with the highest slope (0.8354 for hypocotyl growth and 1.2381 for sprout growth), which can be related to the highest growth rate (Table 1) of these samples during growth at 20 °C. After 10 d at 20 °C, the H/R Ratio was 1.42 ± 0.20 without differences between treatments. The FW/DW Ratio of 3-day kale sprouts was 2.45 ± 0.01, which increased at the end of the sprouting period in UV-B treated samples up to 11.97 ± 0.27, reporting a 127% increase compared to CTRL samples (5.27 ± 1.20). In addition, the growth speed after 5 d was 53% higher on UVB10 and UVB15 samples, and there was an increase of 17% after 7 d in UVB10–treated kale sprouts compared to CTRL samples. No differences were found among the remaining treatments on days 3 and 10 of the sprouting period.

These results reveal that plants under UV-B lighting can develop specific photomorphogenic responses, which results in an increase of the length of the kale hypocotyls (Escobar-Bravo et al., 2017). In fact, as shown in Table 1, plant growth is partially inhibited by UV-B lighting, as recently described by Dou et al. (2019) and Lim et al. (2020). This effect can be justified by the fact that UVB-resistance 8 receptors are able to trigger a specific response to inhibit the hypocotyl elongation (Tohge et al., 2011; Yadav et al., 2020) and, subsequently, the growth of the kale sprouts.

In addition, UV-B light quantity and quality has demonstrated to increase the temperature and stress conditions in plants, which can explain the reduction of sprout length of UV-B kale sprouts compared to

the CTRL treatment (Escobar-Bravo et al., 2017).

3.2. Effect of UV-B radiation on the phenolic content

The phenolic content of the UV-B treated, and untreated kale sprouts was totally and individually analysed, as well as its relation to the TAC. In fact, the TPC measured by U-HPLC was highly correlated to obtained TAC results by DPPH and FRAP methods (0.9478 and 0.9093, $p < 0.001$, respectively).

Fig. 3 shows the TPC results obtained by the Folin-Ciocalteu method. The TPC of kale seeds was 3.8 ± 0.4 g chlorogenic acid kg^{-1} dw, which increased 4.5-fold after 10 d of sprouting at 20 °C in darkness (2.8 g chlorogenic acid kg^{-1} dw). After 10 d of shelf-life period at 4 °C as a fresh-cut product, the TPC was enhanced 35% in CTRL kale sprouts compared to its amount at harvest. However, kale sprouts under UV-B treatments reported significant increases in the TPC compared to CTRL samples, during pre- and post-harvest periods. Indeed, during germination, all studied UV-B doses reported an increase of 22% compared to CTRL. At harvest (after 10 growing d), UVB5, UVB10, and UVB15 had 26, 39, and 32% higher concentration in TPC as regards CTRL, respectively. This tendency was also maintained during the postharvest shelf-life, TPC being 30, 33, and 35% increased by 5, 10, and 15 kJ m^{-2} UV-B doses, respectively.

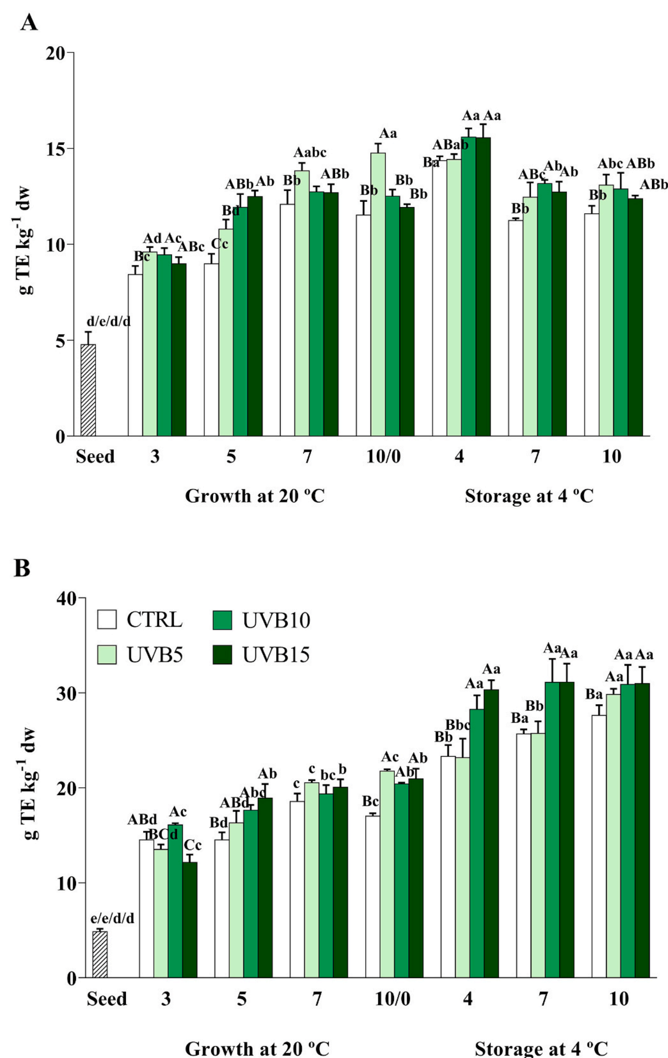


Fig. 4. Total antioxidant activity measured by DPPH (A) and FRAP (B) of kale sprouts treated with UV-B (CTRL, 5, 10, and 15 kJ m-2) during 10 growing d at 20 °C and 10 d stored at 4 °C. Different capital letters indicate significant differences among treatments at $p < 0.05$ based on Tukey's test. Different lowercase letters indicate significant differences among time of analysis of the same treatment at $p < 0.05$ based on Tukey's test.

These results showed a protective response carried out by the plant to protect itself from adverse environmental factors such as the radiation of very short wavelength of the light spectra, like the UV-B radiation. In this way, discontinuous treatments of 1.5 W m^{-2} UV-B for 1 h in 5-day peanut sprouts (each growing day) increased the TPC by 11.1% in comparison to untreated peanut sprouts (Nguyen et al., 2020). In addition, TPC measured by U-HPLC was highly correlated to kale sprouts development. In fact, TPC was correlated by 0.9305 to hypocotyl length, while 0.9178 to sprouts length ($p < 0.001$), which justified the relation between the accumulation of phenolics and the variation in the sprout growth.

As Fig. 1 and Table 2 show, eleven major phenolic compounds were identified in kale sprouts, including (1) Gallic acid hexoside I, (2) Gallic acid, (3) 3-*O*-caffeoylquinic acid, (4) 4-*O*-caffeoylquinic acid, (5) 5-*O*-caffeoylquinic acid, (6) 1,2-diferuloyl-gentobiose, (7) caffeic acid, (8) caffeic acid derivative, (9) hydroxycinnamic acid, (10) kaempferol-3,7-di-*O*-glucoside, and (11) kaempferol-3-*O*-sinapoylglucoside-7-*O*-glucoside. Differences among studied treatments after 10 growing d at 20 °C are shown in Fig. 5.

UV-B significantly induced the accumulation of nine of the eleven identified compounds (Table 2). Only 3-*O*-caffeoylquinic acid and 1,2-diferuloyl-gentobiose were not stimulated by discontinuous UV-B pre-harvest treatments. Of these stimulated compounds, kaempferol-3,7-di-*O*-glucoside was the major flavonoid found, followed by hydroxycinnamic acid, as the major phenolic acid identified in kale sprouts. This fact has been also shown by Duarte-Sierra et al. (2020), who reported an increase of hydroxyl-cinnamic acids after UVB radiation (1.5 and 7.5 kJ m^{-2}) on broccoli florets. Particularly, UVB10 was the main inductor of the biosynthesis of the rest of identified phenolics compounds, although all the UV-B doses positively affected to the concentration of these nutraceuticals. These results show as lower doses of UV-B can stimulate the shikimic acid pathway, from which gallic acid and cinnamic acids are synthetized, as well as the remaining phenylpropanoid compounds. This fact is also repeated in the flavonoid pathway from which kaempferol is derived. In fact, our hypothesis is that studied UV-B doses gives enough energy to increase the phenylalanine ammonia-lyase (PAL) and the chalcone synthase (CHS), both in charge to activate the biosynthesis of cinnamic acids and flavonoids. Nevertheless, this energy does not affect to caffeoyl-CoA, from which chlorogenic, ferulic, and sinapic acids derive. In this sense, further analyses to justify our theory are needed.

From a different point of view, 3-*O*-caffeoylquinic acid was the predominant phenolic compound found in kale seeds. In fact, its concentration decreased 12.6-fold after 10 d of sprouting at 20 °C in darkness. Besides, no differences were found from the 7th growing d to the end of the shelf-life period assayed.

In contrast, hydroxycinnamic acid, as the main phenolic acid, and kaempferol-3,7-di-*O*-glucoside, as the predominant flavonoid found in kale sprouts, increased their concentration during germination by 4 and 16-fold compared to kale seeds, respectively (Fig. 5). Such accumulation was also maintained during the postharvest storage. Focus on these compounds, the hydroxycinnamic acid content was increased in all UV treatments assayed. In fact, UVB5 and UVB10 treatments reported the higher content at harvest, with 16 and 13% increases compared to CTRL (Fig. 5). On the 4th d of the shelf-life period, the concentration of the hydroxycinnamic acid reached 4.37 ± 0.24 g kg^{-1} dw in UVB15 kale sprouts, which is 52% higher than that found in CTRL. Similarly, UVB5 reported an increase of 34% on the kaempferol-3,7-di-*O*-glucoside content, compared to CTRL. Nevertheless, UVB10 and UVB15 showed 27 and 33% higher content than CTRL, respectively. Those values did not vary from that moment to the end of the shelf-life period assayed.

Furthermore, discontinuous UV-B radiation highly enhanced the minor phenolic compounds content. For instance, at the end of the postharvest storage, gallic acid hexoside I content of UV-B treatments

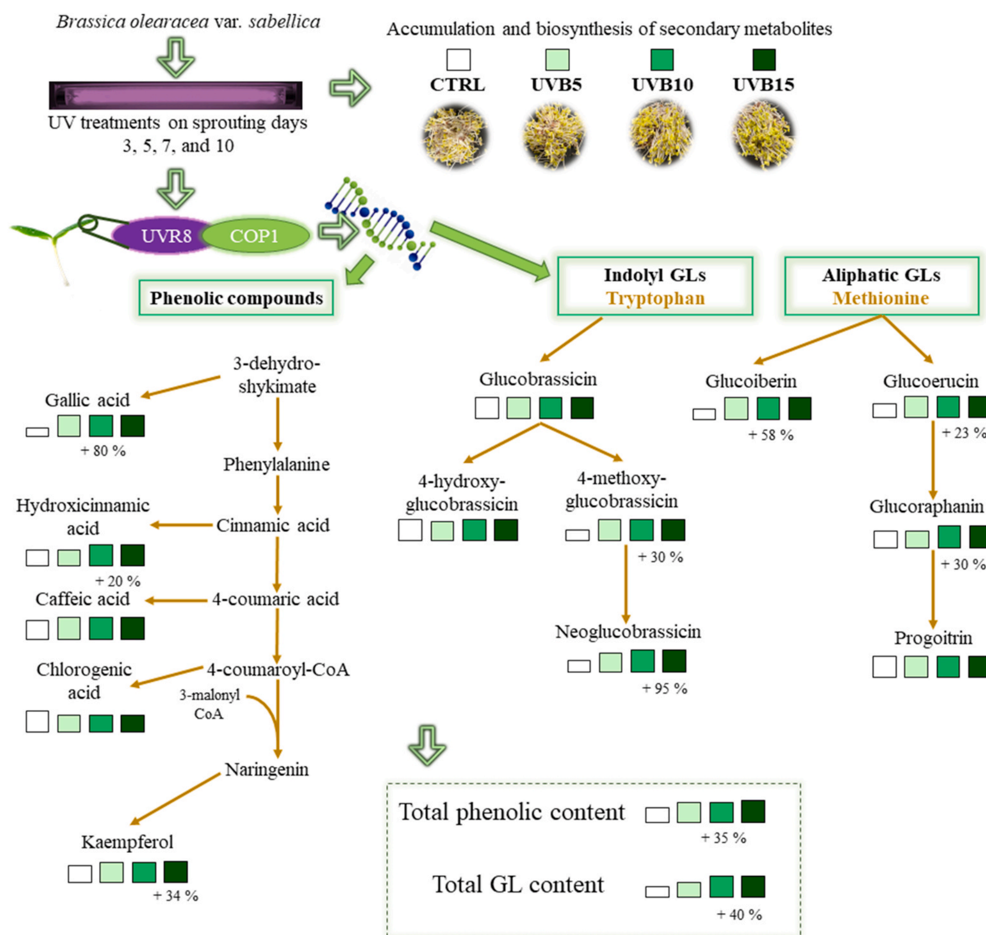


Fig. 5. Accumulation and biosynthesis of individual phenolic compounds and GLs in kale sprouts treated with UV-B light (CTRL, 5, 10, and 15 kJ m-2) during 10 growing days at 20 °C.

reported an increase of 55%, while gallic acid content increased by 78% compared to CTRL kale sprouts. The content of caffeic acid and its derivatives showed a similar behaviour, as well as the kaempferol derivatives.

Regarding the obtained results, UV-B induces genes involved in the phenylpropanoid biosynthesis pathway (Jenkins, 2009; Morales et al., 2010). In fact, when UV light is absorbed, bioactive compounds as flavonoids, hydroxycinnamic acids, and other phenolic compounds act as UV penetration reducers into the plant tissue to protect the plant from damage caused by ROS (Jenkins, 2009; Morales et al., 2010; Solovchenko and Merzlyak, 2008).

In fact, UV-B illumination, as an abiotic elicitor, is a good strategy to induce physiological changes and stimulate defense or stress-induced responses in plants. This kind of UV treatments trigger the synthesis of phytochemical compounds in young plants and vegetables during sprouting. This tendency agrees with Formica-Oliveira et al. (2017a), who studied the effect of the application of UV-B on the biosynthesis of phenolic compounds in fresh-cut carrots. Indeed, fresh-cut carrots treated with UV-B (1.5 kJ m^{-2}) reached a content of phenolic compounds 120% higher than untreated samples.

Furthermore, Moreira-Rodríguez et al. (2017a) showed an increase of gallic acid hexoside II (35.1%) in broccoli sprouts treated with a single dose of 7.16 W m^{-2} UV-B for 120 min (51.55 kJ m^{-2}). However, in the same study, the combination of UV-B lighting and 25 μM methyl jasmonate reported notable increases in the accumulation of phenolic compounds. Similarly, Moreira-Rodríguez et al. (2017b) reported increases in sinapoyl malate (12%), gallotannic acid (48%), and 5-sinapoyl-quinic acid (121%) content in broccoli sprouts exposed for 120 min to 3.34 W m^{-2} UV-B (24.05 kJ m^{-2}). These studies concluded that the biosynthesis of phenolic compounds was enhanced by low intensities, while it was inhibited by high intensities in Brassica sprouts. Such results justify applying the periodical application of low UV-B intensities during kale sprouting, as we did in our study.

3.3. Effect of UV-B radiation on the antioxidant activity

As it is widely known, the biosynthesis of phenolic compounds is directly related to the TAC of foods. The TAC of UV-B treated kale sprouts are presented in Fig. 4. DPPH values of CTRL kale sprouts at harvest increased by 142% in comparison to seeds, which was increased up to 25% during the first 4 d of the refrigeration storage period and subsequently decreased to the initial values found at harvest (Fig. 4A).

Moreover, the application of 5 kJ m^{-2} UV-B increased the TAC by 28% compared to CTRL at harvest, while no differences were found between UVB10, UVB15, and CTRL samples. In a similar way, UV-B radiation enhanced the TAC during the postharvest period. Indeed, after 4 d at 4 °C UVB10 and UVB15 showed 8% higher ability to scavenge DPPH radicals compared to CTRL and UVB5. Nevertheless, at the end of the shelf-life period, UVB5, UVB10, and UVB15 showed an increase by 13, 11, and 6% on the TAC compared to CTRL. In this sense, the scavenging percentage against free radicals during the postharvest period was 55% for CTRL kale sprouts, which was 15% higher under UVB5 and UVB10 treatments.

From other point of view, a different behaviour was shown in Fig. 4B regarding the TAC measured by the FRAP assay. FRAP values at harvest of CTRL kale sprouts increased by 250% compared to seeds. In this case,

Table 3
Glucosinolates content (g kg^{-1} dw) of UV-B treated (CTRL; 5; 10, and 15 kJ m^{-2}) kale sprouts during 10 growing d at 20 °C in darkness and stored up to 10 d at 4 °C as a minimally processed product.

Treatment	Day of analysis	Glucoiberin-dsg	Progoitrin-dsg	Glucoraphanin-dsg	4-hydroxy-glucobrassicin-dsg	Glucoerucin-dsg	Glucobrassicin-dsg	4-methoxy-glucobrassicin-dsg	Neoglucobrassicin-dsg	Total glucosinolate content
Seed	0G	$17.0 \pm 3.1^{a/a/a/a}$	$1.0 \pm 0.1^{ab/de/ab/ab}$	$43.0 \pm 6.4^{a/a/ab/a}$	$37.2 \pm 5.7^{a/a/ab/a}$	$16.2 \pm 1.8^{ab/ab/bc/bc}$	$2.2 \pm 0.2^{b/e/b/e}$	$0.6 \pm 0.1^{c/b/d/f}$	$0.3 \pm 0.0^{b/e/d/d}$	$117.4 \pm 12.8^{-/c/b/c}$
CTRL	3G	8.4 ± 0.6^{b}	1.3 ± 0.1^{a}	32.6 ± 1.2^{ab}	25.1 ± 2.5^{ab}	11.6 ± 1.6^{b}	5.1 ± 1.0^{b}	$6.6 \pm 0.4^{A\ b}$	3.7 ± 0.8^{b}	94.4 ± 12.5
	5G	$10.3 \pm 1.7^{B\ b}$	$1.4 \pm 0.1^{A\ a}$	$32.7 \pm 1.2^{B\ ab}$	$25.2 \pm 2.5^{B\ ab}$	17.8 ± 1.6^{a}	12.7 ± 1.3^{a}	10.3 ± 1.3^{ab}	$9.0 \pm 0.9^{B\ a}$	119.4 ± 15.9^{C}
	7G	9.1 ± 0.4^{b}	1.1 ± 0.2^{ab}	$26.7 \pm 1.1^{B\ b}$	28.6 ± 2.9^{ab}	$14.2 \pm 0.2^{C\ ab}$	12.2 ± 1.3^{a}	$10.0 \pm 1.0^{B\ ab}$	$11.8 \pm 1.3^{C\ a}$	113.7 ± 10.8^{B}
	10G/0S	$7.9 \pm 0.8^{B\ b}$	$1.3 \pm 0.1^{A\ a}$	$32.8 \pm 2.5^{B\ ab}$	$29.3 \pm 2.9^{A\ ab}$	$14.3 \pm 0.4^{B\ ab}$	$13.5 \pm 0.9^{A\ a}$	$14.5 \pm 1.0^{AB\ a}$	$12.3 \pm 0.3^{B\ a}$	125.9 ± 1.4^{BC}
	10G+4S	7.3 ± 0.3^{b}	1.0 ± 0.1^{ab}	24.0 ± 2.3^{b}	27.9 ± 0.6^{ab}	14.1 ± 1.5^{ab}	11.2 ± 1.9^{a}	$13.3 \pm 1.6^{B\ a}$	10.4 ± 1.6^{a}	110.1 ± 18.8
	10G+7S	$7.3 \pm 1.3^{B\ b}$	1.1 ± 0.2^{ab}	32.2 ± 3.5^{ab}	$32.8 \pm 2.8^{AB\ ab}$	$15.7 \pm 1.2^{B\ ab}$	12.7 ± 0.8^{a}	$10.1 \pm 0.6^{C\ ab}$	$10.0 \pm 1.2^{B\ a}$	121.8 ± 5.3^{B}
	10G+10S	$8.3 \pm 1.2^{AB\ b}$	0.8 ± 0.1^{b}	$23.2 \pm 2.5^{AB\ b}$	$19.5 \pm 2.3^{A\ b}$	$13.0 \pm 1.4^{AB\ ab}$	12.3 ± 1.4^{a}	11.1 ± 1.3^{ab}	$10.4 \pm 2.2^{AB\ a}$	98.6 ± 16.6^{AB}
UVB5	3G	13.4 ± 2.8^{ab}	1.7 ± 0.2^{a}	37.8 ± 3.8^{ab}	29.8 ± 2.7^{a}	14.9 ± 1.5^{ab}	6.1 ± 0.9^{d}	$3.4 \pm 0.4^{B\ b}$	5.9 ± 1.1^{d}	123 ± 20.1^{bc}
	5G	$15.1 \pm 1.0^{A\ a}$	$1.5 \pm 0.1^{AB\ ab}$	$43.7 \pm 4.3^{A\ a}$	$36.9 \pm 3.5^{AB\ a}$	19.4 ± 1.5^{ab}	13.1 ± 0.8^{ab}	13.9 ± 1.2^{a}	$23.8 \pm 1.5^{A\ a}$	$167.4 \pm 11.9^{AB\ a}$
	7G	14.3 ± 1.6^{ab}	1.3 ± 0.1^{bc}	$39.8 \pm 2.2^{B\ ab}$	35.5 ± 3.1^{a}	$20.3 \pm 0.4^{AB\ a}$	13.7 ± 1.6^{a}	$15.3 \pm 1.8^{B\ a}$	$25.1 \pm 1.7^{B\ a}$	$165.4 \pm 9.5^{A\ a}$
	10G/0S	$7.4 \pm 1.0^{B\ c}$	$0.8 \pm 0.1^{B\ d}$	$24.4 \pm 3.7^{B\ cd}$	$21.4 \pm 5.1^{AB\ b}$	$13.7 \pm 1.1^{B\ b}$	$10.6 \pm 0.5^{B\ bc}$	$13.0 \pm 1.2^{B\ a}$	$13.3 \pm 1.3^{B\ b}$	$104.6 \pm 16.5^{C\ c}$
	10G+4S	8.9 ± 1.0^{bc}	1.0 ± 0.0^{cde}	29.6 ± 2.0^{bc}	20.7 ± 2.5^{b}	15.5 ± 1.4^{ab}	11.6 ± 0.5^{abc}	$14.7 \pm 1.2^{AB\ a}$	11.0 ± 1.2^{bc}	113.0 ± 7.0^{c}
	10G+7S	$13.2 \pm 0.7^{A\ ab}$	1.3 ± 0.1^{bcd}	37.8 ± 0.3^{ab}	$33.8 \pm 2.5^{A\ a}$	$19.5 \pm 0.4^{AB\ ab}$	13.0 ± 1.3^{abc}	$14.7 \pm 1.8^{B\ a}$	$24.7 \pm 1.5^{A\ a}$	$158.1 \pm 4.8^{A\ ab}$
	10G+10S	$5.6 \pm 1.0^{B\ c}$	0.5 ± 0.1^{f}	$13.5 \pm 0.9^{B\ d}$	$9.9 \pm 1.3^{B\ c}$	$6.8 \pm 0.6^{B\ c}$	10.0 ± 0.3^{c}	11.3 ± 1.2^{a}	$7.0 \pm 1.0^{B\ cd}$	$65.0 \pm 12.1^{B\ d}$
UVB10	3G	12.0 ± 1.5^{ab}	1.4 ± 0.2^{a}	36.8 ± 4.3^{abc}	31.5 ± 3.1^{ab}	14.2 ± 1.2^{bc}	5.1 ± 1.0^{b}	$6.6 \pm 0.5^{A\ cd}$	4.1 ± 1.0^{d}	111.8 ± 14.2^{b}
	5G	$15.2 \pm 0.9^{A\ ab}$	$1.6 \pm 0.2^{AB\ a}$	$44.8 \pm 2.2^{A\ a}$	$40.3 \pm 4.1^{AB\ a}$	20.2 ± 0.1^{ab}	13.2 ± 1.3^{a}	12.6 ± 2.0^{c}	$24.3 \pm 1.6^{A\ b}$	$172.2 \pm 7.8^{A\ a}$
	7G	14.6 ± 0.6^{ab}	1.4 ± 0.3^{a}	$47.5 \pm 4.6^{A\ a}$	32.9 ± 3.2^{ab}	$19.3 \pm 0.5^{B\ ab}$	14.0 ± 1.5^{a}	$27.0 \pm 1.8^{A\ a}$	$24.8 \pm 2.3^{B\ b}$	$181.6 \pm 18.7^{A\ a}$
	10G/0S	$13.1 \pm 0.8^{A\ ab}$	$1.1 \pm 0.2^{AB\ ab}$	$46.2 \pm 4.5^{A\ a}$	$19.7 \pm 1.2^{AB\ c}$	$25.2 \pm 2.1^{A\ a}$	$13.5 \pm 0.9^{A\ a}$	$20.1 \pm 2.2^{A\ b}$	$35.3 \pm 1.4^{A\ a}$	$174.2 \pm 8.7^{A\ a}$
	10G+4S	9.1 ± 0.5^{b}	1.0 ± 0.1^{ab}	28.8 ± 4.2^{bc}	19.5 ± 1.3^{c}	16.1 ± 1.3^{bc}	12.0 ± 1.4^{a}	$19.3 \pm 1.1^{A\ b}$	11.3 ± 1.2^{c}	116.9 ± 17.0^{b}
	10G+7S	$12.6 \pm 0.9^{A\ ab}$	1.3 ± 0.1^{ab}	36.5 ± 3.3^{abc}	$27.7 \pm 1.1^{B\ bc}$	$18.9 \pm 1.6^{AB\ ab}$	13.6 ± 0.0^{a}	$29.0 \pm 0.6^{A\ a}$	$23.9 \pm 1.9^{A\ b}$	$163.6 \pm 8.1^{A\ a}$
	10G+10S	$9.6 \pm 2.6^{AB\ b}$	0.7 ± 0.1^{b}	$30.3 \pm 3.4^{A\ c}$	$17.6 \pm 1.7^{AB\ c}$	$11.7 \pm 1.3^{AB\ c}$	10.8 ± 1.0^{a}	12.5 ± 1.2^{c}	$10.6 \pm 0.0^{AB\ c}$	$100.2 \pm 15.3^{AB\ b}$
UVB15	3G	10.5 ± 1.1^{b}	1.4 ± 0.2^{a}	31.5 ± 2.2^{ab}	31.5 ± 3.1^{ab}	11.7 ± 1.7^{c}	5.1 ± 1.0^{de}	$5.1 \pm 0.9^{AB\ ef}$	4.1 ± 1.1^{d}	100.9 ± 21.6^{c}
	5G	$13.5 \pm 0.3^{A\ ab}$	$1.2 \pm 0.2^{B\ ab}$	$40.4 \pm 0.6^{AB\ a}$	$31.4 \pm 3.4^{AB\ ab}$	19.2 ± 0.5^{ab}	10.3 ± 1.3^{bc}	9.6 ± 1.1^{de}	$13.2 \pm 1.5^{B\ c}$	$138.8 \pm 13.3^{BC\ abc}$
	7G	13.9 ± 2.0^{ab}	1.2 ± 0.1^{ab}	$37.4 \pm 4.6^{AB\ ab}$	31.3 ± 0.7^{ab}	$23.0 \pm 2.2^{A\ a}$	14.8 ± 2.0^{a}	$22.9 \pm 2.3^{A\ a}$	$32.1 \pm 1.1^{A\ ab}$	$176.6 \pm 15.2^{A\ a}$
	10G/0S	$10.1 \pm 1.0^{AB\ b}$	$1.0 \pm 0.1^{AB\ ab}$	$26.4 \pm 4.4^{B\ b}$	$15.6 \pm 1.2^{B\ c}$	$18.0 \pm 1.5^{B\ abc}$	$8.4 \pm 0.8^{B\ cd}$	$20.7 \pm 2.2^{A\ ab}$	$35.1 \pm 3.1^{A\ a}$	$135.2 \pm 11.3^{B\ bc}$
	10G+4S	10.1 ± 0.1^{b}	1.1 ± 0.1^{ab}	30.8 ± 3.1^{ab}	21.7 ± 1.8^{bc}	16.2 ± 0.8^{bc}	11.4 ± 0.3^{abc}	$15.8 \pm 0.5^{AB\ bc}$	11.56 ± 0.6^{c}	118.6 ± 5.5^{bc}
	10G+7S	$12.9 \pm 1.7^{A\ ab}$	1.2 ± 0.1^{ab}	35.4 ± 2.8^{ab}	$35.6 \pm 1.6^{A\ a}$	$20.2 \pm 2.4^{A\ ab}$	12.5 ± 0.5^{ab}	$14.6 \pm 0.5^{B\ cd}$	$25.8 \pm 1.6^{A\ b}$	$158.4 \pm 10.1^{A\ ab}$
	10G+10S	$12.0 \pm 2.8^{A\ ab}$	0.6 ± 0.1^{b}	$33.6 \pm 4.9^{A\ ab}$	$15.9 \pm 1.5^{AB\ c}$	$14.1 \pm 1.1^{A\ bc}$	9.9 ± 0.6^{bc}	12.4 ± 1.1^{cd}	$11.4 \pm 1.8^{A\ c}$	$110.0 \pm 18.5^{A\ c}$

G = growing days at 20 °C in darkness; S = shelf-life days at 4 °C as a minimally processed product. Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment. Absence of letters indicates that there are no significant differences ($p < 0.05$).

the TAC increased by 62% after 10 d of storage at 4 °C in comparison with kale sprouts at harvest.

From a general overview, all the pre-harvest UV-B treatments applied were able to increase the iron reduction capacity of kale sprouts throughout the study. For instance, after 10 d growing, UVB5, UVB10, and UVB15 kale sprouts showed 28, 20, and 23% higher TAC measured by FRAP than CTRL, respectively. Besides, on the first analyses of the refrigeration period, UVB10 and UVB15 presented an increase by 21 and 30% on the 4th d, respectively, and by 21% on the 7th d compared to the CTRL in both cases. Similarly, on the 10th d of the postharvest period, UVB5 showed an increase by 8% compared to CTRL, while UVB10 did it by 12%. Furthermore, TAC was also highly correlated to kale sprouts growth, as related to phenolic biosynthesis. Indeed, TAC measured by DPPH was correlated by 0.8776 to hypocotyl length, while 0.9054 to sprouts length ($p < 0.001$). In a similar way, TAC measured by FRAP was also correlated by 0.8837 to hypocotyl length, while 0.9262 to sprouts length ($p < 0.001$). This behaviour justified the relation between the antioxidant ability of kale sprouts and the variation in their growth.

In a similar manner, Tsurunaga et al. (2013) reported increases of 1.6-fold in the scavenging capacity against DPPH free radicals in 6-day buckwheat sprouts treated with 10 μmol m^{-2} s^{-1} for 24 h (187.5 kJ m^{-2}) UV-B in comparison to sprouts kept in dark. Besides, the TAC was higher in the postharvest period compared to the germination process, as it has been shown in the present study. Similarly, broccoli florets exposed to 2 kJ m^{-2} UV-B reported an increase in the TAC after 6 and 18 h compared to the initial values at harvest (Darré et al., 2017), which also explained that abiotic stresses provoked by UV-B are able to increase the TAC in different stages of the plant development. Therefore, the described results have suggested that UV-B illumination is a valuable technological strategy to induce the TAC of minimally processed sprouts.

3.4. Effect of UV-B radiation on the glucosinolate content

Eight major GL compounds were identified in kale sprouts: (1) Glucoiberin, (2) Progoitrin, (3) Glucoraphanin, (4) 4-hydroxy-glucobrassicin, (5) Glucoerucin, (6) Glucobrassicin, (7) 4-methoxy-glucobrassicin, and (8) Neoglucobrassicin (Fig. 2 and Table 3). In general, glucoraphanin was the GL found in a greater proportion in kale sprouts and seeds, followed by 4-hydroxy-glucobrassicin (Fig. 2). Indeed, the total GL content of CTRL kale sprouts at harvest after 10 growing d was 125.9 ± 1.4 g kg^{-1} dw, Glucoraphanin content was the 26%, and 4-hydroxy-glucobrassicin the 23% of the total content. Differences among studied treatments after 10 growing d at 20 °C are shown in Fig. 5.

The individual and total GLs content, quantified as desulfoglucosinolates, was positively affected by UV-B treatments (Fig. 5). In fact, the content of some desulfoglucosinolates compounds was highly accentuated, such as glucoiberin and glucoraphanin, as aliphatic GLs, and 4-methoxy-glucobrassicin and neoglucobrassicin, as indolyl GLs. Nevertheless, the amount of progoitrin, 4-hydroxy-glucobrassicin, glucoerucin and glucobrassicin was not increased by discontinuous UV-B applications during the growth of kale sprouts.

The total GLs content started to increase from the first UV-B dose applied on the 3rd d of the growing period in darkness. In this way, the maximum amount was reached after 7 d at 20 °C, when UVB5, UVB10, and UVB15 treatments increased their synthesis of GLs by 32, 42, and 39%, respectively, compared to CTRL. Similarly, on the 7th d of the postharvest period at 4 °C, UVB5, UVB10, and UVB15 reported 18, 21, and 18% more GLs accumulation compared to CTRL, respectively.

Among the aliphatic GLs, glucoiberin content was increased by all the UV-B treatments during both preharvest and postharvest periods. However, UVB10-treated kale sprouts reported an increase at harvest by 58% as regards the CTRL treatment. In contrast, after 10 d at 4 °C of shelf-life, UVB15 increased by 26% the accumulation of glucoiberin in comparison to CTRL. Glucoraphanin content was also increased in a similar way as previously described. In fact, UVB10 and UVB15 reported at the end of the shelf-life period a higher content by 24 and 36% compared to CTRL, respectively.

Among the indolyl GLs, UVB10 and UVB15 kale sprouts presented at harvest an increase of 27 and 30% of their content in 4-methoxy-glucobrassicin, respectively (Fig. 5). Both treatments also increased the content on neoglucobrassicin by 31% compared to CTRL. After that, the indolyl GLs amount slightly decreased during the shelf-life period at 4 °C. Nevertheless, on the 7th d of shelf-life, the accumulation of 4-methoxy-glucobrassicin increased by 30, 120, and 29% on UVB5, UVB10, and UVB15 kale sprouts, respectively. In this sense, also neoglucobrassicin experimented an increase of 93, 89, and 100% on the same treatments in comparison with CTRL, respectively.

The obtained results show that the GL content was increased in UV-B treated kale sprouts. In fact, previous authors agree with this behaviour. Moreira-Rodríguez et al. (2017a) showed an increase of glucoraphanin (78%) and 4-methoxy-glucobrassicin (177%) in 7-day broccoli sprouts treated with 7.16 W m^{-2} UV-B for 120 min (51.55 kJ m^{-2}), which had a synergistic effect in combination with 25 μM methyl jasmonate. In the same way, Moreira-Rodríguez et al. (2017b) reported the highest GL accumulation in 7-day broccoli sprouts after 24 h 3.34 W m^{-2} UV-B treatment for 120 min (24.05 kJ m^{-2}). Indeed, that treatment increased by 170, 78, and 73% the biosynthesis of 4-methoxy-glucobrassicin, glucobrassicin, and glucoraphanin, respectively.

Furthermore, these results also agree with Mewis et al. (2012), who showed as UV-B radiation enhanced the biosynthesis of GLs, mainly 4-methoxy-glucobrassicin and glucoraphanin in 12-day broccoli sprouts 24 h after 0.3 and 0.9 kJ m^{-2} doses. Indeed, this exposure triggered the transcription of several genes (CYP71A and CYP71B families of Cyt P450 monooxygenases) related to GLs biosynthetic pathway. Likewise, FMO GS-OX5, involved in the oxidation of methylthioalkyl GLs into methylsulfinylalkyl GLs, which triggered the main pathway to the synthesis of aliphatic GLs (glucoiberin and glucoraphanin). Besides, UV-B lighting also induced transcription factor MYB51 expression, which also catalyzes the hydroxylation of 4-methoxy-glucobrassicin and neoglucobrassicin, as main indolyl GLs identified in Brassicaceae sprouts.

Considering that the consumption of uncooked Brassicaceae products resulted up to 17–77% of isothiocyanates (Johnson, 2002; Traka, 2016), 21–99 mg glucosinolates g^{-1} dw, or which is the same, 197–976 mg glucosinolates g^{-1} fresh kale sprouts could be converted into isothiocyanates, which have demonstrated their anticancer properties. In fact, following the recommendations of 100 μM isothiocyanates daily dose for healthy consumers provided by Egner et al. (2014) and Riedl et al. (2009), an intake of 18 g of fresh UVB10 or UVB15 kale sprouts per day can be proposed to enhance the detoxication of some airborne pollutants (Egner et al., 2014) and to potent an increase in antioxidant Phase II enzymes in airway cells (Riedl et al., 2009). Similarly, a consumption of 91 g of fresh UVB10 or UVB15 kale sprouts per day (500 μM isothiocyanates daily dose) may help to improve chemotherapy treatment in pancreatic cancer patients (Lozanovski et al., 2014).

4. Conclusions

In conclusion, periodical low UV-B treatments, up to a total received dose of 15 kJ m^{-2}, while the seeds are sprouting could be a useful technological tool to stimulate phytochemicals biosynthesis in kale sprouts, which were even enhanced during a refrigerated shelf-life period as a minimally processed product. In some cases, it can even accelerate the sprouting process. Accordingly, UV-B treated sprouts will become an important source of bioactive compounds with great potential health benefits for consumers.

Funding statement

Project financed by the Autonomous Community of the Region of Murcia nº 20849/PI/18 through the grant call for projects for the development of scientific and technical research by competitive groups, included in the Regional Programme for the Promotion of Scientific and

Technical Research (Action Plan, 2018) of the Seneca Foundation - Science and Technology Agency of the Region of Murcia (Spain).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Noelia Castillejo was funded by a predoctoral grant (FPU16/04763) from the Spanish Ministry of Education. Lorena Martínez-Zamora's contract (21322/PDGI/19) is part of the Regional Programme for research talent and its employability of the Seneca Foundation, co-financed by the European Social Fund (ESF) through the Youth Employment Initiative (YEI). The technical assistance of Francisca Andreo is highly appreciated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2021.05.022.

Author contributions statement

Noelia Castillejo: Methodology; Formal analysis; Investigation; Writing – Original Draft. Lorena Martínez-Zamora: Methodology; Formal analysis; Data curation; Investigation; Writing – Original Draft. Francisco Artés–Hernández: Conceptualization; Resources; Writing – Review & Editing; Supervision; Funding acquisition.

References

Artés, F., Gómez, P., Aguayo, E., Escalona, V., Artés-Hernández, F., 2009. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. Postharvest Biol. Technol. 51, 287–296. https://doi.org/10.1016/j.postharvbio.2008.10.003.

Baenas, N., García-Viguera, C., Moreno, D.A., 2014. Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19, 13541–13563. https://doi.org/10.3390/molecules190913541.

Barba, F.J., Mariutti, L.R.B., Bragagnolo, N., Mercadante, A.Z., Barbosa-Cánovas, G.V., Orlien, V., 2017. Bioaccessibility of bioactive compounds from fruits and vegetables after thermal and nonthermal processing. Trends Food Sci. Technol. 67, 195–206. https://doi.org/10.1016/j.tifs.2017.07.006.

Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F., Galieni, A., 2019. Sprouted grains: a comprehensive review. Nutrients 11, 421. https://doi.org/10.3390/nu11020421.

Castillejo, N., Martínez-Zamora, L., Gómez, P.A., Pennisi, G., Crepaldi, A., Fernández, J. A., Orsini, F., Artés-Hernández, F., 2021. Postharvest LED lighting: effect of red, blue and far red on quality of minimally processed broccoli sprouts. J. Sci. Food Agric. 101, 44–53. https://doi.org/10.1002/jsfa.10820.

Chen, M., Blankenship, R.E., 2011. Expanding the solar spectrum used by photosynthesis. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2011.03.011.

Clayton, W.A., Albert, N.W., Thrimawithana, A.H., McGhie, T.K., Deroles, S.C., Schwinn, K.E., Warren, B.A., McLachlan, A.R.G., Bowman, J.L., Jordan, B.R., Davies, K.M., 2018. UVR8-mediated induction of flavonoid biosynthesis for UVB tolerance is conserved between the liverwort Marchantia polymorpha and flowering plants. Plant J. 96, 503–517. https://doi.org/10.1111/tpj.14044.

Darré, M., Valerga, L., Ortiz Araque, L.C., Lemoine, M.L., Demkura, P.V., Vicente, A.R., Concellón, A., 2017. Role of UV-B irradiation dose and intensity on color retention and antioxidant elicitation in broccoli florets (Brassica oleracea var. Italica). Postharvest Biol. Technol. https://doi.org/10.1016/j.postharvbio.2017.02.003.

Dou, H., Niu, G., Gu, M., 2019. Pre-harvest UV-B radiation and photosynthetic photon flux density interactively affect plant photosynthesis, growth, and secondary metabolites accumulation in basil (Ocimum basilicum) plants. Agronomy 9, 434. https://doi.org/10.3390/agronomy9080434.

Duarte-Sierra, A., Munzoor Hasan, S.M., Angers, P., Arul, J., 2020. UV-B radiation hormesis in broccoli florets: glucosinolates and hydroxy-cinnamates are enhanced by UV-B in florets during storage. Postharvest Biol. Technol. 168, 111278. https://doi.org/10.1016/j.postharvbio.2020.111278.

Egner, P.A., Chen, J.G., Zarth, A.T., Ng, D.K., Wang, J.B., Kensler, K.H., Jacobson, L.P., Muñoz, A., Johnson, J.L., Groopman, J.D., Fahey, J.W., Talalay, P., Zhu, J., Chen, T. Y., Qian, G.S., Carmella, S.G., Hecht, S.S., Kensler, T.W., 2014. Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China. Canc. Prev. Res. https://doi.org/10.1158/1940-6207.CAPR-14-0103.

Escobar-Bravo, R., Klinkhamer, P.G.L., Leiss, K.A., 2017. Interactive effects of UV-B light with abiotic factors on plant growth and chemistry, and their consequences for defense against arthropod herbivores. Front. Plant Sci. 8, 1–14. https://doi.org/10.3389/fpls.2017.00278.

Fernandes, R. de P.P., Trindade, M.A., de Melo, M.P., 2018. Natural antioxidants and food applications: healthy perspectives. Altern. Replace. Foods 31–64. https://doi.org/10.1016/B978-0-12-811446-9.00002-2.

Formica-Oliveira, A.C., Martínez-Hernández, G.B., Aguayo, E., Gómez, P.A., Artés, F., Artés-Hernández, F., 2017a. A functional smoothie from carrots with induced enhanced phenolic content. Food Bioprocess Technol. 10, 491–502. https://doi.org/10.1007/s11947-016-1829-4.

Formica-Oliveira, A.C., Martínez-Hernández, G.B., Díaz-López, V., Artés, F., Artés-Hernández, F., 2017b. Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots. Postharvest Biol. Technol. 127, 99–104. https://doi.org/10.1016/j.postharvbio.2016.12.010.

Hahn, C., Müller, A., Kuhnert, N., Albach, D., 2016. Diversity of kale (Brassica oleracea var. sabellica): glucosinolate content and phylogenetic relationships. J. Agric. Food Chem. 64, 3215–3225. https://doi.org/10.1021/acs.jafc.6b01000.

Jenkins, G.I., 2014. The UV-B photoreceptor UVR8: from structure to physiology. Plant Cell 26, 21–37. https://doi.org/10.1105/tpc.113.119446.

Jenkins, G.I., 2009. Signal transduction in responses to UV-B radiation. Annu. Rev. Plant Biol. https://doi.org/10.1146/annurev.arplant.59.032607.092953.

Johnson, I.T., 2002. Glucosinolates: bioavailability and importance to health. Int. J. Vitam. Nutr. Res. 72, 26–31. https://doi.org/10.1024/0300-9831.72.1.26.

Klem, K., Holub, P., Štroch, M., Nezval, J., Špunda, V., Tříska, J., Jansen, M.A.K., Robson, T.M., Urban, O., 2015. Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress. Plant Physiol. Biochem. 93, 74–83. https://doi.org/10.1016/j.plaphy.2015.01.001.

Klug, T.V., Martínez-Hernández, G.B., Collado, E., Artés, F., Artés-Hernández, F., 2018. Effect of microwave and high-pressure processing on quality of an innovative broccoli hummus. Food Bioprocess Technol. 11, 1464–1477. https://doi.org/10.1007/s11947-018-2111-8.

Lim, Y.J., Jeong, H.Y., Gil, C.S., Kwon, S.J., Na, J.K., Lee, C., Eom, S.H., 2020. Isoflavone accumulation and the metabolic gene expression in response to persistent UV-B irradiation in soybean sprouts. Food Chem. 303, 125376. https://doi.org/10.1016/j.foodchem.2019.125376.

Lozanovski, V.J., Houben, P., Hinz, U., Hackert, T., Herr, I., Schemmer, P., 2014. Pilot study evaluating broccoli sprouts in advanced pancreatic cancer (POUDER trial) - study protocol for a randomized controlled trial. Trials. https://doi.org/10.1186/1745-6215-15-204.

Martínez-Hernández, G.B., Artés-Hernández, F., Gómez, P.A., Artés, F., 2013. Induced changes in bioactive compounds of kailan-hybrid broccoli after innovative processing and storage. J. Funct. Foods 5, 133–143. https://doi.org/10.1016/j.jff.2012.09.004.

Martínez-Zamora, L., Castillejo, N., Gómez, P.A., Artés, F., 2021. Amelioration effect of LED lighting in the bioactive compounds synthesis during. Carrot Sprouting 11, 1–17.

Mewis, I., Schreiner, M., Nguyen, C.N., Krumbein, A., Ulrichs, C., Lohse, M., Zrenner, R., 2012. UV-B irradiation changes specifically the secondary metabolite profile in broccoli sprouts: induced signaling overlaps with defense response to biotic stressors. Plant Cell Physiol. 53, 1546–1560. https://doi.org/10.1093/pcp/pcs096.

Morales, L.O., Tegelberg, R., Brosché, M., Keinänen, M., Lindfors, A., Aphalo, P.J., 2010. Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in Betula pendula leaves. Tree Physiol. https://doi.org/10.1093/treephys/tpq051.

Moreira-Rodríguez, M., Nair, V., Benavides, J., Cisneros-Zevallos, L., Jacobo-Velázquez, D.A., 2017a. UVA, UVB light, and methyl jasmonate, alone or combined, redirect the biosynthesis of glucosinolates, phenolics, carotenoids, and chlorophylls in broccoli sprouts. Int. J. Mol. Sci. 18, 1–20. https://doi.org/10.3390/ijms18112330.

Moreira-Rodríguez, M., Nair, V., Benavides, J., Cisneros-Zevallos, L., Jacobo-Velázquez, D.A., 2017b. UVA, UVB light doses and harvesting time differentially tailor glucosinolate and phenolic profiles in broccoli sprouts. Molecules 22. https://doi.org/10.3390/molecules22071065.

Nguyen, V.L., Dinh, T.D., Nguyen1, H.B., Nguyen, T.M.A., Vu, T.Y., Thieu, T.P.L., Nguyen, V.H., Lai, T.N.H., 2020. UVB exposure induced accumulation OF phenolics and resveratrol and enhanced antioxidant activities IN peanut sprouts. Carpathian J. Food Sci. Technol. 12, 89–101. https://doi.org/10.34302/crpjfst/2020.12.1.9.

Pennisi, G., Sanyé-Mengual, E., Orsini, F., Crepaldi, A., Nicola, S., Ochoa, J., Fernandez, J.A., Gianquinto, G., 2019. Modelling environmental burdens of indoor-grown vegetables and herbs as affected by red and blue LED lighting. Sustain. Times 11. https://doi.org/10.3390/su11154063.

Riedl, M.A., Saxon, A., Diaz-Sanchez, D., 2009. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. Clin. Immunol. https://doi.org/10.1016/j.clim.2008.10.007.

Robson, T.M., Klem, K., Urban, O., Jansen, M.A.K., 2015. Re-interpreting plant morphological responses to UV-B radiation. Plant Cell Environ. 38, 856–866. https://doi.org/10.1111/pce.12374.

Šamec, D., Pavlović, I., Radojčić Redovniković, I., Salopek-Sondi, B., 2018. Comparative analysis of phytochemicals and activity of endogenous enzymes associated with their stability, bioavailability and food quality in five Brassicaceae sprouts. Food Chem. 269, 96–102. https://doi.org/10.1016/j.foodchem.2018.06.133.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - a review. J. Funct. Foods 18, 820–897. https://doi.org/10.1016/j.jff.2015.06.018.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Solovchenko, A.E., Merzlyak, M.N., 2008. Screening of visible and UV radiation as a photoprotective mechanism in plants. Russ. J. Plant Physiol. https://doi.org/10.1134/S1021443708060010.

Taulavuori, K., Pyysalo, A., Taulavuori, E., Julkunen-Tiitto, R., 2018. Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. Environ. Exp. Bot. 150, 183–187. https://doi.org/10.1016/j.envexpbot.2018.03.016.

Thakur, M., Bhattacharya, S., Khosla, P.K., Puri, S., 2019. Improving production of plant secondary metabolites through biotic and abiotic elicitation. J. Appl. Res. Med. Aromat. Plants 12, 1–12. https://doi.org/10.1016/j.jarmap.2018.11.004.

Tohge, T., Kusano, M., Fukushima, A., Saito, K., Fernie, A.R., 2011. Transcriptional and metabolic programs following exposure of plants to UV-B irradiation. Plant Signal. Behav. 6 https://doi.org/10.4161/