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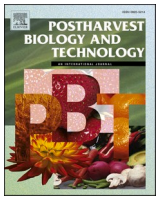

UVB treatments of packaged ready-to-eat salads: Induced enhancement of quercetin derivatives in baby-leaf lettuce (*Lactuca sativa* L.) and wild rocket (*Diplotaxis tenuifolia* L.)

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ABSTRACT

Ready-To-Eat (RTE) salads conveniently pre-prepared in bags can promote the intake of natural bioactive compounds, including antivirals such as quercetin. However, the content of these compounds in the species used for RTE salads is usually low due to limited solar UV exposure under tunnels and greenhouses in which they are usually cultivated. To address this, we treated commercial fresh-cut lettuce (*Lactuca sativa* L.) and wild rocket (*Diplotaxis tenuifolia* L.) leaves using a narrow-band UVB lamp directly through sealed polypropylene bags during storage (5–6 °C, 80% RH). The bagged leaf samples were kept under 20 μmol m^{-2} s^{-1} of white light with a 12 h photoperiod for 6 d. Half of the samples were additionally treated, 9 h d^{-1}, during the first 3 d by UVB narrow-band lamps delivering 2.8–3.6 μmol $m^{-2}s^{-1}$ of UVB and 0.8–1.0 μmol $m^{-2}s^{-1}$ of UVA radiation. The effects of the UVB treatments on epidermal phenolics, chlorophyll and photosynthetic parameters were monitored daily by non-destructive fluorescence sensors over a 6-d storage period. At the end of the experiment, destructive HPLC-DAD analysis of phenolics and photosynthetic pigments, antioxidant capacity assays and fresh weight loss determinations were conducted. The UVB-treatment increased the epidermal phenolics (EPhen) Index with respect to unirradiated controls, while not affecting chlorophyll and carotenoids levels as well as photosynthetic efficiency. For both species, the EPhen Index changes were detected 15 h after the first UVB application. Then, wild rocket responded faster than baby-leaf lettuce and reached the maximal phenolic level with less than 1/3 of the energy dose needed by lettuce. UVB-treated samples exhibited higher flavonoid concentrations (mainly quercetin derivatives) compared to controls (48–67% and 37–66% in lettuce and wild rocket, respectively). Leaf chlorophyll and carotenoid contents were not affected by both UVB treatment and storage. We proved, for the first time, that it is possible to treat RTE salad leaves using through-packaging UVB radiation and enhance their total phenolic and quercetin derivative contents. We also provided more insights concerning the dynamics of the UVB-elicitation of phenolic compounds in postharvest leaves. Our results are propaedeutic for the optimization of potential UVB-treatments; selection of the most efficient wavelengths, intensity, single/multiple doses and proposals for application in the food industry.

1. Introduction

The intake of natural bioactive molecules can be promoted by the consumption of fresh fruits and vegetables. This habit is rapidly increasing due to the growing health concerns of the consumers. Freshly eaten products such as Ready-To-Eat (RTE) salads are becoming more and more popular in today's fast-paced society (Lorente-Mento et al., 2022). RTEs are fresh-cut products subjected to minimal processing before packing; therefore providing convenient nutritious food without requiring time-consuming preparation (Nicola and Fontana, 2014; Teng

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et al., 2023). In particular, due to rapid urbanization and increasing demand for healthy and diverse plant-based diets, the RTE salads market is growing. In 2022, the fresh-cut vegetables represented a global market of $ 346.05 billion (Teng et al., 2023). In Italy the consumption of RTE salads reached over 84 10^6 kg with 550 M€ revenue in 2019 (Lodi, 2020).

In terms of composition and nutritional importance, RTE salads are low in calories, rich in fibre, vitamins, minerals, antioxidants, and other phytochemicals (Saini et al., 2017). Some of the RTE salads, such as red and green lettuce, pea shoots, wild rocket, watercress and red mustard, also contain significant amounts of quercetin derivatives (Santos et al., 2014). These compounds are particularly relevant since quercetins were recently proven to have antiviral properties preventing the spread and replication of dangerous viruses, such as SARS-CoV-2 (Abian et al., 2020; Colunga Biancatelli et al., 2020). Quercetin binds the 3CLpro and PLpro virus proteins, interfering with SARS-CoV-2 replication (Colunga Biancatelli et al., 2020; Derosa et al., 2020) and preventing virus cell entering (Colunga Biancatelli et al., 2020). These benefits for human health highlight the importance of choosing species for RTE salads that produce the highest concentration of quercetin derivatives.

Baby-leaf lettuce (*Lactuca sativa* L.) and wild rocket (*Diplotaxis tenuifolia* L.) are the two most common leafy vegetables present in RTE salad packs. Lettuce is a major vegetable crop grown worldwide and is known to be highly nutritious when consumed raw (Mulabagal et al., 2010). Wild rocket belongs to the Brassicaceae family and has a pleasant bitter taste (Romano et al., 2022). Both species possess beneficial antioxidant phytonutrients, including quercetin derivatives (Romano et al., 2022; Santos et al., 2014). In different baby leaf vegetables analyzed by Santos et al. (2014), the largest amount of flavonols appeared in ruby red lettuce (286 g kg^{-1} of dry weight), followed by green lettuce (15 g kg^{-1} of dry weight) and wild rocket (7.2 g kg^{-1} of dry weight). The content of these compounds has been shown to be remarkably stable during storage at low temperatures (Santos et al., 2014). Furthermore, because of their benefits to consumer health, there is great interest in extending the shelf-life of these RTE vegetables, and enhancing their bioactive molecules content (Teng et al., 2023).

It must be emphasized that most of the plant species used in producing RTE salads are grown under greenhouse or plastic tunnel conditions. This practice has the drawback of producing plants with a lower content of health-promoting compounds; especially phenolics, due to the low intensity or lack of natural UV-radiation during plant cultivation (Lee et al., 2021; Tsormpatsidis et al., 2010). Ultraviolet radiation stimulates the accumulation of flavonoids, mainly flavonols that have strong antioxidant properties (Guidi et al., 2016). Accordingly, the flavonoid content in several green and red salad cultivars was found to be higher in full sunlight grown plants compared to those cultivated under greenhouse conditions (Sytar et al., 2018) or under tunnels covered by plastic film transmitting different portion of the UV radiation (Lee et al., 2021; Tsormpatsidis et al., 2008). In this context, supplying artificial UV radiation under greenhouse cultivation conditions can elicit the synthesis of plant bioactive compounds. For instance, an increase in quercetin was observed in green and red lettuce grown in greenhouses under supplemental UVB irradiation (Assumpção et al., 2019; Weiland et al., 2023).

Ultraviolet radiation treatments to improve quality aspects of fresh-cut vegetables can also be applied postharvest (Teng et al., 2023). For example, a 5-min irradiation with an unspecified spectral emission band of UVB lamps for 3 d stimulated an increase of flavonoids in freshly harvested leaves of spinach, radish and parsley during the following 3 d of storage (Kanazawa et al., 2012). However, the irradiance of 98 μmol m^{-2} s^{-1} needs further validation as it was measured using a LI-190SA Quantum Sensor (LI-COR. Inc., Lincoln, NE) which has limitations in detecting UV radiation. An increase in flavonols was also observed in broccoli inflorescences, irradiated postharvest by visible light (19 μmol m^{-2} s^{-1}) combined with low irradiance (0.23 W m^{-2}) from a broad-band UVB lamp (Rybarczyk-Plonska et al., 2016). Harbaum-Piayda et al. (2016) showed also a low-dose UVB broad-band lamp induction of quercetin-triglycoside in cabbage. Moreover, UVB was a good postharvest elicitor of flavonoids in bell peppers (Castillejo et al., 2022), apples (Assumpção et al., 2018) and tomatoes (Castagna et al., 2013). Recently, Romano et al. (2022) also showed the positive effects of 45 s of postharvest UVB broad-band lamp irradiation on the leaf phenolic content of wild rocket.

Application of UV radiation both pre- or postharvest to horticultural crops certainly represents an effective method to increase the plant nutraceutical content (Jacobo-Velázquez et al., 2022). Therefore, it can be considered a process of fruit and vegetable biofortification, according to the new definition suggested for this term (Jacobo-Velázquez, 2022).

Despite strong evidence for the stimulation of bioactive compounds by UV light, the application of UV radiation on RTE packaged products, such as RTE salads, has been poorly investigated. Information on what are the best protocols regarding UV emission bands, intensity and application timing are unknown. Furthermore, the manipulation of short UV wavelengths, such as the UVB band (280–315 nm) is not trivial from a safety and technical standpoint, especially considering postharvest and packaging conditions. The harmful effects of excess UVB on plants are well known (Centritto et al., 2014; Jansen et al., 1998); therefore, the development of new UV-application protocols must consider possible side effects on the products. In this regard, low doses of UVB radiation, applied during a specific time and under well-established conditions, can be used to trigger the accumulation of important secondary metabolites without producing side effects (Schreiner et al., 2016) such as reduced shelf-life. The synthesis of these important nutraceutical compounds in response to UV radiation can also be evaluated and monitored using non-destructive methods, such as optical sensors to measure changes in the epidermal flavonol content of leaves. Those are promising tools for industrial and commercial applications (Julkunen-Tiitto et al., 2015; Nascimento et al., 2020).

Considering the above premise, this study aims to:

- Evaluate the efficacy of a postharvest UVB through-packaging irradiation treatment in enhancing antioxidative active phenolic compounds in RTE salads (baby-leaf lettuce and wild rocket) during storage;
- Demonstrate the applicability of a non-destructive tool (the Multiplex sensor) in monitoring phenolic compounds in packaged RTE salads during UVB treatments and storage, while gaining more insight into the dynamics of the elicitation process;
- Test single-day or daily-repeated UVB treatments for the elicitation of phenolics;
- Assess the possible effects of the UVB treatments on salad quality aspects, with a special focus on photosynthetic pigments and parameters.

2. Material and methods

2.1. Plant material and sample preparation

Commercially produced green baby-leaf lettuce (*Lactuca sativa* L., cultivar Luna Verde) and wild rocket (*Diplotaxis tenuifolia* L., cultivars Marte and Naples) Ready-To-Eat (RTE) salads were used in the present study. The plants were grown under high tunnels at to different sites in Piana del Sele – Bellizzi (Salerno, South Italy) (40°37'12.0"N 14°56'52.4"E) during February-March 2022, used for a first trial (Trial 1) and Bagnolo Mella (Brescia, North Italy) (45°25'46.6"N 10°11'10.0"E) during April-May 2022, used for Trial 2. Seeds were sown in silty-loam or loam soils at a density of 20–23 kg h^{-1} and 5–6 kg h^{-1} for lettuce and wild rocket, respectively, following organic basal dressing. Sprinkler irrigation was applied after sowing and at mid-growth cycle. Harvest occurred 35 and 28 days after sowing (DAS) in Trial 1 and Trial 2, respectively. Yields resulted 0.8–0.9 kg m^{-2} for lettuce and 0.5–0.6 kg m^{-2} for wild rocket. In Trial 1, the average daily global radiation

ranged from 39 to 145 W m^{-2} (mean 111.4 W m^{-2}), the average air temperature was 5.3 – 18.2 °C (mean 11.4 °C) and the relative humidity was 45 – 100% (mean 79.2%). In Trial 2, the average daily global radiation ranged from 53 to 324 W m^{-2} (mean 233 W m^{-2}), the average air temperature was 7.6 – 24.4 °C (mean 14.6 °C) and the relative humidity 41.2 – 95.7% (mean 61.2%). These data were obtained by Regional meteorological stations close to the cultivation sites: the Centro Agrometeorologico Regionale (Regione Campania, Napoli, Italy) for Trial 1 and the Agenzia Regionale per la Protezione dell'Ambiente (ARPA) Lombardia (Milano, Italy) for Trail 2. The covering of tunnels consisted of PATILUX® ethylene vinyl acetate films (Idromeccanica Lucchini, Guidizzolo – MN, Italy) transmitting as average 3% of solar UVB radiation.

Freshly cut leaves were obtained the day after packaging from a commercial producer of minimally processed vegetables (La Linea Verde, Manerbio (BS), Italy). The leaves were packaged as 125 g of salad products in polypropylene bags, then transported under refrigerated conditions from the production site to our laboratory where they were immediately placed in a dark cold room (5–6 °C and 80% RH, sensor TFA Dostmann, Wertheim, Germany).

For each species, samples were prepared by weighing a few undamaged leaves (6–10 leaves weighing 3.5–6 g) and allocating them to small trays made of recycled PET (R-PET) (Model H26, size 140×117×26 mm, Carton Pack, Rutigliano (BA), Italy). The trays were packaged inside 25 μm thick BOPP Antifog Kemilen film (Kemiplast, Calenzano (FI), Italy) bags and sealed with a heat sealer (LA FELSINEA S. R.L., Piazzola Sul Brenta (PD), Italy). The BOPP film had an oxygen permeability of 2150 cm^3 m^{-2} 24 h^{-1} (at 23 °C, 0% R.H) and a water vapour permeability of 7 g m^{-2} 24 h^{-1} (at 37.8 °C, 100% RH). Sample preparation was performed inside the cold room at 5–6 °C to avoid any cold chain interruption to the salad products and the formation of vapour condensation within the packages. After sealing the sample bags, we ensured that RH within the bags had reached saturation using the TFA RH (Dostmann, Wertheim, Germany) sensor. Samples were arranged within two boxes made of UV-blocking LEE 226 plastic film (Lee Filters, Andover, UK), one containing the fluorescent UVB lamps (see Section 2.2) for the radiation treatments and the other for the control treatment (see Figure S1 of the Supplementary Material). Two white LEDs lamps were placed above both boxes (see Section 2.2) to provide a minimal amount of photosynthetically active radiation (PAR) to the leaf samples during the daily cycles. The two boxes containing the samples were equipped with openings to allow for air circulation. The temperature and relative humidity in the cold room was continuously monitored by an EL-USB-2-LCD EasyLog datalogger (Lascar Electronics, Whiteparish, England).

2.2. Postharvest UVB treatment and lighting conditions

In Trial 1, three samples of baby-leaf lettuce (*L. sativa*) and three samples of wild rocket (*D. tenuifolia*) were irradiated at a 0.5 m distance by the UVB-lamps during the light period of the first three consecutive days (3d-UVB). This protocol was chosen on the basis of previous studies on other species (Kowalski et al., 2021; Rybarczyk-Plonska et al., 2016). Trial 2 aimed to test the efficiency of the UVB elicitation as function of the number of daily treatments. Twelve samples per species were divided in triplicates and then exposed to three different durations of UVB-lamp treatment in addition to the light period: 1d-UVB) a single application on day 1; 2d-UVB) two consecutive days of UVB (days 1 and 2); 3d-UVB) three consecutive applications of UVB (days 1, 2 and 3), this last treatment corresponds to Trial 1. For both Trial 1 and Trial 2, control triplicates were kept under the same storage conditions as the treated samples, exposed only to the white light. The postharvest UV treatments were performed using two narrow-band UVB fluorescent lamps (Philips UV-B Narrowband PL-L 36 W/01, Signify, Eindhoven, The Netherlands) with emission peaked at 313 nm (the emission spectrum is reported in the Supplementary Figure S2). The lamps also emitted a small fraction of UVA radiation. Therefore, the total UV irradiance provided by the radiation source consisted of 80% UVB (280–315 nm) and 20% UVA (316–400 nm). White light of about 20 μmol m^{-2} s^{-1} of PAR (400–700 nm) was also supplied to both the UV treatment and control boxes by two LED lamps (LumiGrow Pro 650 SP, Emeryville, CA, USA).

The white light photoperiod during storage was 12 h light (09:00–21:00) and 12 h dark (21:00–9:00). The UVB treatment started at 12:00 am and was applied for 9 h d^{-1} (12:00–21:00) at an irradiance of 1.1–1.4 W m^{-2} (2.84–3.62 μmol $m^{-2}s^{-1}$); the UVA fraction was within 0.2–0.3 W m^{-2} (0.81–1.03 μmol $m^{-2}s^{-1}$).

Treatments could be performed on packaged samples since the BOPP film of the bags did not affect the emission spectrum of the UVB lamps. This was proved by the flatness of the total transmittance spectrum of the BOPP film between 300 and 800 nm (Fig. S3) as measured by a spectrophotometer equipped with an integrating sphere (Jasco UV/VIS V-770 UV-Visible/NIR Spectrophotometer, Jasco International Co., Ltd., Tokyo, Japan). The film caused an 8% attenuation of both UVB radiation and white light that was taken into consideration when calculating the actual irradiance received by the samples.

Polypropylene material can undergo photooxidation when exposed to UV radiation (Girois et al., 1996), with the release of potentially toxic compounds. However, in the present study this event was unlikely since the maximal energy dose of UV radiation used was several dozen lower than that required to start polypropylene photooxidation (François-Heude et al., 2014). We also tested the integrity of the BOPP film after exposure to the UVB lamps at an energy dose of 200 kJ m^{-2}, significantly higher than the maximal energy dose (136 kJ m^{-2}) used to treat the leaf samples. As shown in Figure S3, the BOPP film total transmittance spectrum after UVB exposure was not distinguishable from that before exposure.

Storage and monitoring lasted for six days. Before and after the storage period, with or without the UVB treatment, samples were weighed, frozen in liquid nitrogen and then freeze-dried for extraction and HPLC-DAD analysis of leaf polyphenols and pigments. (For the timing of treatments and measurements see Figure S4 of the Supplementary Material).

2.3. Non-destructive monitoring

Non-destructive monitoring of the UVB induced effects on samples was performed using the Multiplex (Force-A, Orsay, France) fluorescence sensor, the LI-600 porometer fluorometer (LI-COR, Lincoln, Nebraska, USA), the Hansatech FMS-2 field-portable pulse-modulated chlorophyll fluorometer (Hansatech Instruments Ltd., Norfolk, UK) and the Imaging Pam M-series fluorimeter (Heinz Walz GmbH, Effeltrich, Germany).

2.3.1. Epidermal phenolic and chlorophyll indices

The Multiplex (Mx) sensor provides indices of the leaf content of epidermal phenolic (EPhen) compounds and chlorophyll (Chl), as described in detail elsewhere (Agati et al., 2011; Ghozlen et al., 2010). The indices used in the present work are defined as:

$$EPhen\ Index = \frac{FRF\_R}{FRF\_UV} \quad (1)$$

$$Chl\ Index = \frac{FRF\_R}{RF\_R} \quad (2)$$

where FRF_UV and FRF_R represent the chlorophyll fluorescence emitted in the far-red region (far-red fluorescence, FRF) under the excitation with UV at 375 nm and red (R) at 630 nm radiation, respectively. The RF_R is the chlorophyll red fluorescence excited by R. The EPhen Index is equal to 10^{FLAV} where FLAV is the Mx flavonoid index previously introduced (Agati et al., 2011).

The Mx measurements were taken daily at around 11:50 am, a few minutes before the start of the UVB treatment. Therefore, at the time of

measurements the samples had been adapted to white light for a period of 2 h 50 min, which is sufficient for stabilisation of the chlorophyll fluorescence signal after the dark period. Indices of chlorophyll and polyphenols of the salad leaves were measured using the Mx sensor directly without removing the leaves from the packaging. This was possible because the BOPP film does not affect the Mx measurements as the total transmittance of the BOPP film is uniform between 300 and 800 nm (Fig. S3). Moreover, the Mx fluorescence indices (Eqs. 1 and 2) measured on a fluorescence standard (Rosco Urban Blue 81, Rosco Laboratories, Stamford, CT, USA) gave the same values with or without the BOPP film placed above the standard.

2.3.2. Chlorophyll fluorescence parameters

The photosynthetic activity of sample leaves was evaluated by measuring the quantum efficiency of photosystem II photochemistry in the light adapted state, Φ_{PSII}, calculated as:

$$\Phi_{PSII} = \frac{F_m^{'} - F_s}{F_m^{'}} \quad (3)$$

where $F_m^{'}$ is the maximum chlorophyll fluorescence in the light and F_s is the steady-state chlorophyll fluorescence measured immediately before the saturating flash used to measure $F_m^{'}$ (Genty et al., 1989). This parameter was provided by the LI-600 porometer/fluorometer by measuring three leaves per sample, under 20 μmol m^{-2} s^{-1} of PAR inside the cold room, before and at the end of the 6-day storage period. During the UVB treatments and storage period, Φ_{PSII} was determined daily on packed samples in loco using the Hansatech FMS-2 fluorometer on three leaves per sample under 20 μmol m^{-2} s^{-1} of PAR. The spatial variability of Φ_{PSII} and the maximum photochemical efficiency of PSII (F_v/F_m) were measured using a WALZ IMAG-CM Imaging Pam (Heinz Walz GmbH, Pfullingen, Germany). F_v/F_m was determined after 30 min of dark adaptation, and Φ_{PSII} after 10 min exposure to actinic light of an identical intensity to that experienced during the treatment. For each image, the means of the parameters were calculated over the whole leaf area and then given as average (± SD) on three different samples per treatment.

2.4. Destructive determination of polyphenols by HPLC-DAD

2.4.1. Leaf extraction

After completion of the storage and UVB treatments, leaf material of both species was immediately collected and frozen in liquid nitrogen, and then lyophilized for 24 h. Ten mg of lyophilized leaves were ground and extracted with 3 × 600 μL ethanol 75% (pH 2.5 adjusted with formic acid) by an ultrasound-assisted extraction (UAE), following the method of Nascimento et al. (2020). The UAE was conducted in an ultrasonic ice-bath (BioClass CP104) using a constant frequency of 39 kHz and an input power of 100 W, over 30 min at 5 °C. After centrifugation (3 min, 5880 g), the supernatants were combined and partitioned with 3 × 600 μL of n-hexane to remove lipophilic compounds (chlorophylls and carotenoids). The ethanolic phase was reduced to dryness, weighed on a digital analytical balance, and resuspended in a methanol/water acidified solution (1:1 v/v, pH 2.5 adjusted with formic acid), to conduct the HPLC-DAD analysis.

2.4.2. HPLC-DAD analysis

Aliquots of the sample extracts (15 μL for rocket salad and 10 μL for lettuce) were injected into a Perkin Elmer Flexar liquid chromatograph equipped with a quaternary 200Q/410 pump and an LC 200 diode array detector (DAD) (all from Perkin Elmer, Bradford, CT, USA). The stationary phase consisted of an Agilent Zorbax SB-18 column (250 × 4.6 mm, 5 μm) (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy), kept at 30 °C. The eluents were (A) acidified water (at pH 2.5 adjusted with formic acid) and (B) acetonitrile (at pH 2.5 adjusted with formic acid). For each plant species, the following solvent gradient (v/v) was applied. For lettuce: 0–2 min (2% B), 2–37 min (2–80% B), 37–42 min (80% B), 42–44 min (80–2% B); while for rocket salad: 0–1 min (3% B), 1–46 min (3–40% B), 46–49 min (40% B), 49–50 min (40–3% B). The flow rate was 0.6 mL min^{-1}. Chromatograms were obtained at 280, 330 and 350 nm and the identification and quantification of the most abundant polyphenols were carried out based on the retention time, UV spectra and comparison with standards. Standard curves were used to quantify the compounds, as follows: caffeic acid, chlorogenic acid, chicoric acid and rutin were used for lettuce samples, while caffeic acid, luteolin and quercetin 3,4′ diglucoside were used for wild rocket samples. Amounts of compounds (g kg^{-1}) were expressed on a dry weight basis. The methods allowed determination of the qualitative and quantitative polyphenolic profile in UVB-treated and untreated samples after 6 d of storage. The Total Flavonoid Content (TFC) and the total HydroxyCinnamic Acid derivatives content (HCAs) were obtained as the sum of the content of the compounds belonging to these specific classes. The sum of all these classes corresponded to the Total Phenolic Content (TPC).

2.5. Destructive determination of photosynthetic pigments

For the identification and quantification of carotenoids and chlorophylls from lettuce and wild rocket, 10 mg of freeze-dried samples were extracted in duplicate in 500 μL of a 1:1 methanol/tetrahydrofurane (THF) mixture (v/v). The samples were vortexed at 20 °C for 10 min in a thermoshaker (Eppendorf, Hamburg, Germany) at the maximal speed. Samples were then centrifuged at 2268 g and 20 °C for 5 min, and the resultant supernatant collected. This procedure was repeated twice, resulting in a total of 1.5 mL extracted sample. The combined supernatants were evaporated to dryness in an RVC 2–25 CD plus vacuum centrifuge (Christ, Osterode am Harz, Germany). 100 μL of methyl tert-butyl ether (MTBE) was added to the dry residue to re-dissolve the sample. An additional 150 μL methanol (MeOH 100%) was added, resulting in a total volume of 250 μL. The samples were then passed through polytetrafluoroethylene filters with a pore size of 0.2 μm (ChromafilXtra, Macherey-Nagel, Düren, Germany) and the filtrate was used for further measurements on a Shimadzu prominence HPLC (Shimadzu, Kyoto, Japan) equipped with a DGU-20A5 degasser, LC-20AT pump, SIL-20AC autosampler, CTO-10AS column oven, and SPD-M20A photodiode array detector. Photosynthetic pigments were separated on a C30 Carotenoid column (250 × 4.6 mm i.d.; 5 μm particle size) (YMC, Kyoto, Japan) protected by a YMC C30 guard cartridge (10 × 4.6 mm i.d.; 5 μm particle size). Eluents A and B consisted of methanol, MTBE, and water (80:18:2, v/v/v, eluent A; 8:90:2, v/v/v, eluent B. Gradient of eluent A was 90–40% (0–30 min), 40–0% (30–35 min), isocratic at 0% (35–37 min), 0–90% (37–40 min), followed by an isocratic step at 90% (40–45 min) at a flow rate of 0.6 mL min^{-1} at an oven temperature of 30 °C (Bayer et al., 2022). Carotenoids were detected at a wavelength of 450 nm and chlorophyll a and b were measured at 663 and 647 nm, respectively. Furthermore, UV/vis spectra were recorded between 200 and 600 nm. For identification and quantitation, authentic standards of β-carotene (Roth), lutein, zeaxanthin (Extrasynthese, Lyon, France), chlorophyll a, and chlorophyll b (Sigma-Aldrich) were used. Furthermore, spectra of carotenoids and chlorophylls were compared to those reported previously (Schex et al., 2018). Concentrations (g kg^{-1} or mmol kg^{-1}) were expressed on a dry weight basis.

2.6. Extraction and analysis of glucosinolates

Twenty milligrams of freeze-dried powder were used for the extraction of glucosinolates from wild rocket leaves following Bayer et al. (2022). The powder was extracted with 750 μL of hot methanol (70%; 70 °C) and subsequently shaken at the maximal speed in a thermoshaker (Eppendorf, Hamburg, Germany) for 10 min. Afterwards, the sample was centrifuged at 2268 g for 5 min, the supernatant was

collected and the pallet was reextracted twice with 500 µL hot 70% methanol under the same conditions. Samples were also spiked with 75 µL sinigrin standard solution, used as an internal standard. Solid phase extraction (SPE) was performed in Pasteur pipettes, filled with glass wool on which 500 µL of a DEAE Sephadex (Cytiva, Marlborough, MA, USA) suspension was pipetted. The SPE column was pre-conditioned twice with 1 mL of imidazole solution and subsequently washed twice with 1 mL ultrapure water. The methanolic extract was then added to the column. Afterwards, each test tube was rinsed twice with ultrapure water to ensure transfer of all residues. Subsequently, absorber columns were rinsed twice with 1 mL of sodium acetate buffer (pH 4.3). 75 µL of purified arylsulfatase (EC 3.1.6.8) (Merck, Darmstadt, Germany) was added, and for at least 16 h enzymatic desulphurization was conducted. Desulfoglucosinolates were eluted twice with 500 µL of ultrapure water and transferred to Spin-X/Filter tubes with a 0.22 µm cellulose acetate membrane (Corning Costar Spin-X, Sigma Aldrich, St. Louis, MI, USA) and were centrifuged at 1792 g at 20 °C for two min before HPLC measurement. Glucosinolates were quantitated based on a previously published method (Bayer et al., 2022), using relative response factors and calculations suggested by Clarke et al. (2010). Concentrations (mmol kg^{-1}) were expressed on a dry weight basis. For separation a Jasco 4000 series HPLC, equipped with a PU-4185 pump, AS-4250 autosampler, UV-4070 UV/vis detector, and CO-4060 column oven was used. A NUCLEODUR Sphinx RP (150 ×4.6 mm i.d.; 5 µm) column (Macherey-Nagel, Düren, Germany) with an NUCLEODUR Universal RP 4 × 3 mm i.d.; 5 µm) guard column (Macherey-Nagel) column was used for separation at a flow rate of 0.6 mL min^{-1}. Eluents were ultrapure water (eluent A) and acetonitrile (eluent B). The applied gradient (eluent B) was 1–20% (0–20 min), isocratic 20% (20–25 min), 20–1% (25–27 min), and isocratic 1% (27–35 min). Detection wavelength was 229 nm. For identification, samples were measured with an Agilent 1290 Infinity II HPLC system with a G6545A Q-TOF, a G7104A pump, G7116B column compartment and a G7167A multisampler (Agilent Technologies, Waldbronn, Germany), coupled to an Agilent 6545 LC/Q-TOF mass spectrometer. Measurement was performed in ESI positive mode in a range from *m/z* 100–920 with a rate of 4 spectra s^{-1}. Gas temperature was set to 320 °C at a flow rate of 8 L min^{-1}. Nebulizer gas was at a pressure of 35 psi and sheath gas temperature and flow rate were 350 °C and 11 L min^{-1}, respectively. Source parameters were a Capillary voltage of 3500 V, a nozzle voltage of 1000 V, and a fragmentor voltage of 150 V. Injection volume was 5 µL.

2.7. Antioxidant TEAC and DPPH assays

Methanolic extracts of the leaf material, obtained according to Engelhardt et al. (2022), were used to evaluate the antioxidant activity. The Trolox equivalent antioxidant capacity (TEAC) and the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) assays were performed on 96 microwell plates. Both assays used individual calibration series of Trolox on each plate. For the TEAC assay, a 10 µL sample or a Trolox standard solution was mixed with a 150 µL working solution in a 96 micro-well plate and then after six minutes were measured in a Synergy HTX microwell plate reader (BioTek Instruments, Bad Friedrichshall, Germany) at a wavelength of 734 nm. For the DPPH assay, a 20 µL sample or a Trolox standard solution was mixed with 180 µL DPPH solution. After incubation for 30 min under darkness, plates were measured at a wavelength of 515 nm (Engelhardt et al., 2022).

2.8. Fresh weight loss

For the evaluation of the fresh weight loss rate, the pooled leaves of each sample were weighed (fresh weight) in a precision balance (KERN Mod. PCB 250–3, 1 mg readability) before and after 6 d of storage and expressed as a percentage of the difference between the two values with respect to the initial values. To avoid loss of water by evaporation, weighing was performed in the cold room (5 °C, 80% RH) just before packaging and after unpackaging.

2.9. Data analysis

Statistical analysis and curve fitting of data were carried out using SigmaPlot for Windows Version 14.0 software (Systat Software, San Jose, CA). Mean values underwent comparison by t-test or one-way analysis of variance (ANOVA) and by the all pairwise multiple comparison Holm–Sidak test; $p \leq 0.05$ values were considered to be statistically significant.

3. Results

3.1. Postharvest UVB-induced enhancement of phenolic compounds

3.1.1. Phenolic composition and quantification

The main baby-leaf lettuce phenolic compounds consisted of chicoric, caffeic, and chlorogenic acid, and quercetin glucosides (Supplementary Figure S5). In wild rocket, we found quercetin triglucosides, acylated quercetin glycosides and caffeic acid derivatives (Supplementary Figure S6).

Six days of storage at low temperature and high RH without the UVB treatment did not change the concentrations of phenolic compounds in both baby-leaf lettuce and wild rocket salad samples (Tables S1 and S2, respectively). Notably, for both species, the concentrations of phenols in the samples used in Trial 1 was higher than those in the Trial 2 samples. The UVB treatment induced no change in the qualitative phenolic composition of either species. However, the 3d-UVB application increased the content of specific classes of compounds compared to controls (Fig. 1). In Trial 1 of baby-leaf lettuce, HCAs, TFC and TPC increased by 35%, 48% and 44%, respectively, in the UVB-treated samples with respect to controls. In the second trial, 3d-UVB induced a rise of about 67% in the concentration of all compounds compared to controls. UVB radiation did not change the content of HCAs in wild rocket, but enhanced TFC by 37% and 66% and TPC by 35% and 57% in Trial 1 and Trial 2 (3d-UVB), respectively.

Comparing the TPC and the Mx EPhen Index at the end of storage, a strong positive relationship between these two parameters was observed for both species (Fig. 2). Due to the high biological variability in the leaf phenolic content between Trial 1 and 2, the data set of Trial 2 was rescaled to the one of Trial 1, using the ratio between the average concentrations of the two sets as a rescaling factor. Similar results were observed for the relationship between TFC and the EPhen Index (Figure S7 A,B of the Supplementary Material). On the other hand, for the HCAs a significant linear regression with the EPhen Index was observed only in baby-leaf lettuce (see Figure S7 C,D of the Supplementary Material). The Pearson correlation analysisP (Table S3 of the Supplementary Material) further supported this observation, indicating strong correlations between the TPC and the EPhen Index for both baby-leaf lettuce and wild rocket ($r = 0.839$ and 0.862, respectively, $p \leq 0.001$). Additionally, both species showed a strong correlation between the TFC and the EPhen Index ($r > 0.80$, $p \leq 0.001$).

3.1.2. Non-destructive monitoring

The time course of the EPhen Index values, relative to the initial (time zero) value, recorded during storage of UVB-treated and control samples are reported in Figs. 3A and 3B for baby-leaf lettuce and wild rocket, respectively. The results are grouping data from Trial 1 and from the 3d-UVB treatment of Trial 2.

In both species, the EPhen Index of RTE samples treated by UVB radiation significantly ($p \leq 0.001$) increased by 25% with respect to the initial value, while the index of controls remained unchanged. The EPhen Index started to increase after the second day of the UVB treatment. Between the 3rd and the 4th day of storage, the rate of the EPhen Index increase declined as levels reached a plateau. This behaviour was especially evident in wild rocket (*D. tenuifolia,* Fig. 3B).

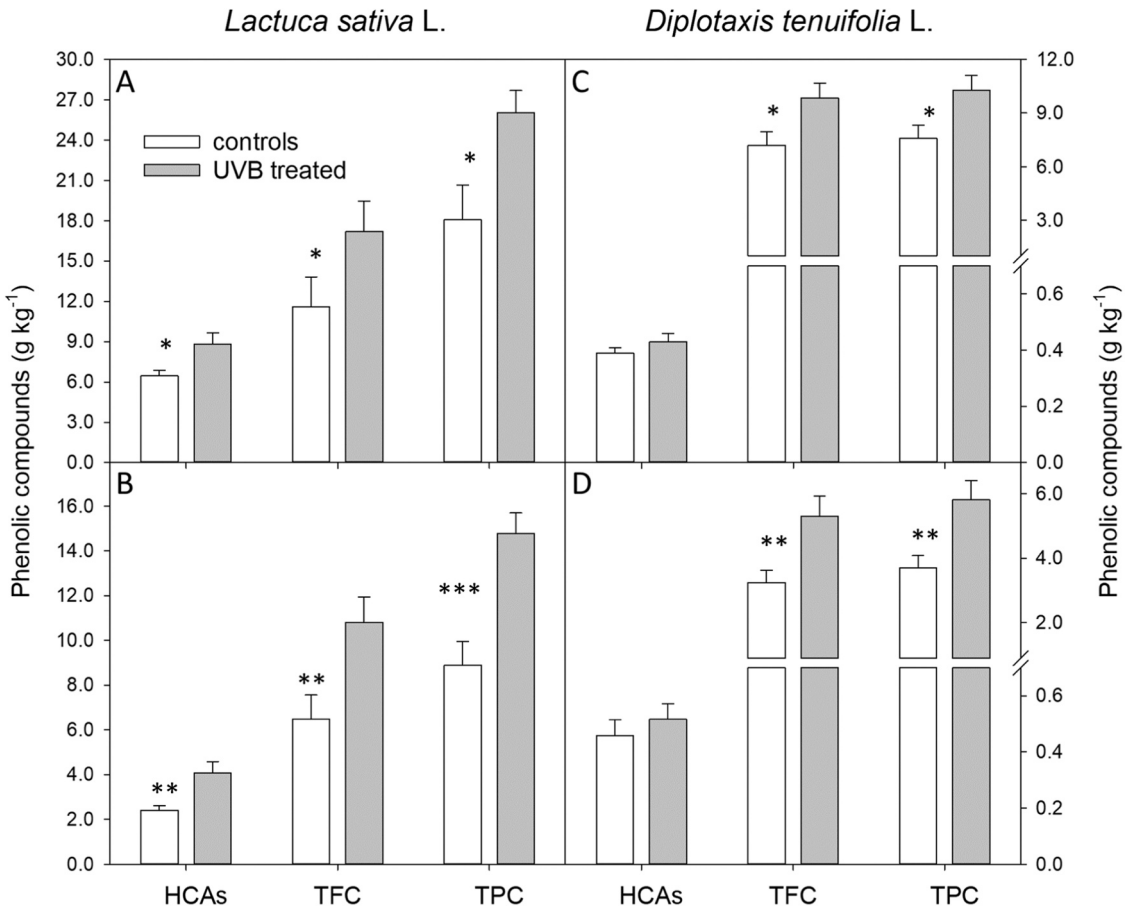

Fig. 1. Polyphenolic content (g kg^{-1} on a dry weight basis) of baby-leaf lettuce (left panels) and wild rocket (right panels) leaves determined on RTE samples treated (grey bars) or untreated (white bars, control) with 3d-UVB radiation after 6 d of storage for Trial 1 (A, C) and Trial 2 (B, D). Compounds are grouped as total HydroxyCinnamic Acid derivatives (HCAs), Total Flavonoid Content (TFC) and Total Phenolic Content (TPC). For each class of compounds, significant difference between UVB-treated and controls is indicated by asterisks with $p \leq 0.05$ * , $p \leq 0.01$ * * or $p \leq 0.001$ * ** , according to the Student's t-test.

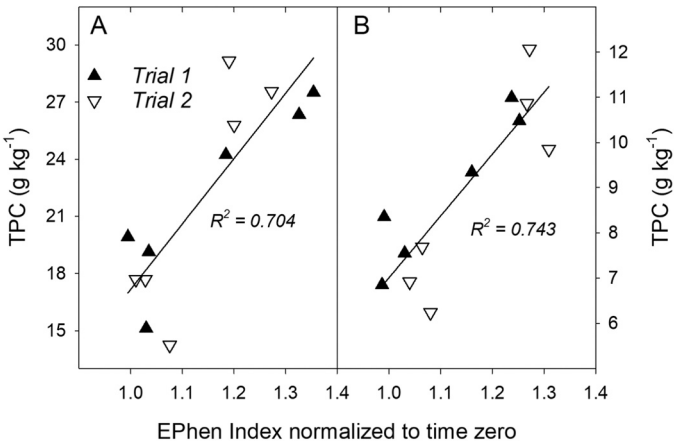

Fig. 2. Relationship between the TPC (expressed on a dry weight basis) and the EPhen Index at the end of storage, from samples with and without UVB treatment, for baby-leaf lettuce (*L. sativa*; A) and wild rocket (*D. tenuifolia*; B). The data set of Trial 2 (3d-UVB) was rescaled to the one of Trial 1, using the ratio between the average phenolic concentrations of the two sets as a rescaling factor.

In Trial 2, the effects of single-day and multiple-days UVB treatments were compared. A progressive increase in both the EPhen Index and TPC was observed as the duration of the UVB treatment extended from 1 to 3 days (Table 1, Fig. 4). Baby-leaf lettuce showed a significant increase in TPC after three days of UVB exposure, while in the case of wild rocket, treatments for 1, 2, or 3 days were comparable in inducing the synthesis of phenolic compounds (TPC), which were significantly higher compared to control samples (Table 1).

Wild rocket leaves responded more rapidly to the UVB treatment than baby-leaf lettuce as evidenced by comparing the rate of change in the EPhen Index over single-day or multiple-days of the UVB application (Fig. 4). In wild rocket, a significant change in the EPhen Index was already observed on day 1, with a pronounced increase on day 2, for all three UVB treatments. The EPhen Index values induced by the 2-d and 3-d UVB treatments were notably higher than those induced by the 1-d UVB exposure. By day 3, the rates of the EPhen Index decayed to their initial values, remaining almost constant till the end of the storage period. On the other hand, baby-leaf lettuce showed a minimal increase in the EPhen Index on day 1. On day 2, all the three UVB treatments (1-d, 2-d or 3-d) yielded similar rates of increase. The EPhen Index values continued to rise and reached a maximum on day 3. Subsequently, these values decreased to a constant level in the case of the 1-d UVB treatment, while they remained higher for the 2-d treatment and even higher for the 3-d (Fig. 4).

3.2. *UVB effects on RTE salad quality aspects*

3.2.1. *Photosynthetic pigments*

The non-destructive Chl Index did not change with storage time or treatment in both salad species (Fig. 5). These results were confirmed by the destructive HPLC analysis of the total Chl in leaf extracts from samplings at the end of the storage period (Figure S8).

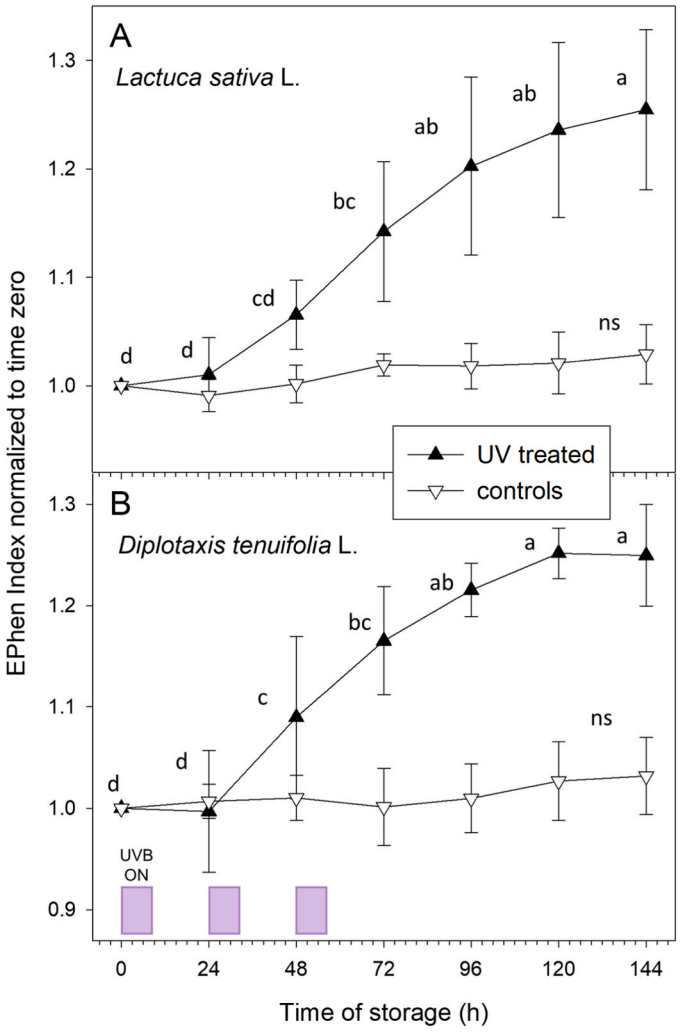

Fig. 3. Evolution of the leaf epidermal phenolic (EPhen) Index, normalized to the initial (time zero) value, from samples of baby-leaf lettuce (*Lactuca sativa*, A) and wild rocket (*Diplotaxis tenuifolia*, B) under storage with (black triangles) or without (white triangles, control) the UVB treatment. Violet boxes indicate periods of irradiation by the UVB lamp (3 consecutive days, 9 h d^{-1}). Within each treatment, mean values tagged by a different letter are significantly different ($p \leq 0.05$) according to the All Pairwise Multiple Comparison Procedures (Holm-Sidak method), ns = not significant. Mean values (± SD) of 6 replicates (3 from Trial 1 and 3 from Trial 2).

Table 1
Total phenolic content (TPC), g kg^{-1} based on dry weight, and the EPhen Index values determined after 6 d of storage as function of the number of daily UVB treatments.

UVB treatment	Baby-leaf lettuce		Wild rocket	
	EPhen Index	TPC (g kg^{-1})	EPhen Index	TPC (g kg^{-1})
None (control)	1.04 ± 0.03c	8.9 ± 1.1 b	1.06 ± 0.02 b	3.7 ± 0.4 b
1-day	1.08 ± 0.03 bc	9.3 ± 1.4 b	1.12 ± 0.05 b	6.4 ± 0.6 a
2-days	1.14 ± 0.04 ab	10.6 ± 2.1 b	1.26 ± 0.02 a	6.9 ± 0.7 a
3-days	1.22 ± 0.04 a	14.8 ± 0.9 a	1.28 ± 0.02 a	5.8 ± 0.6 a

Notes. Within each column, mean values tagged by a diverse letter are significantly different ($p \leq 0.05$) according to the Holm-Sidak test for All Pairwise Multiple Comparison.

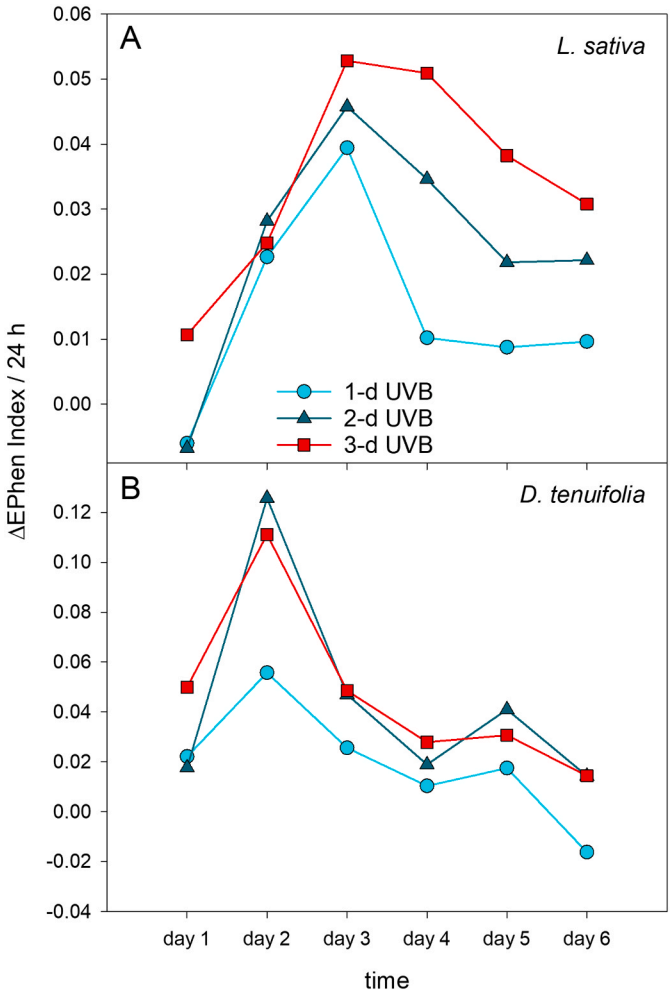

Fig. 4. Rates of the changes in the EPhen Index during storage for the three different UV treatments (1-d UVB – cyan colour, 2-d UVB – green colour, and 3-d UVB – red colour) in baby-leaf lettuce (*L. sativa*, A) and wild rocket (*D. tenuifolia*, B). The rate was calculated as the difference between two consecutive measurements taken at 24 h-intervals.

Analogously, the total carotenoids determined from the same leaf extracts were not affected by the UVB additional radiation (Figure S8). Concerning the single photosynthetic pigment compounds, concentrations of Chl a and Chl b, violaxanthin, antheraxanthin, zeaxanthin (VAZ pool), luteolin and β-carotene were similar between UVB-treated and control samples of both species (Table S4). Also, the Car/Chl ratio was not affected by the UVB irradiation.

3.2.2. Fresh weight

In wild rocket, there was no difference in the decrease of the weight associated with storage between UVB-treated and control samples, for both trials. Conversely, a significant weight loss was observed in lettuce in response to the Trial 1 3-d UVB treatment (Table 2). Nevertheless, in Trial 2, the 2-d and 3-d UVB applications induced the same loss of leaf weight as the controls, while the 1-d UVB treatment resulted in the lowest leaf weight decrease.

3.2.3. Photosynthetic efficiency

For both species, the photosynthetic efficiency over the whole leaf area was not affected by the UVB treatment, as assessed by F_v/F_m and Φ_{PSII} at the end of storage (Fig. 6 and Figure S9). Furthermore, no changes in Φ_{PSII} were observed during storage when comparing the initial and final values in the Trial 1 UVB-treated and untreated samples (Figure S10 of the Supplementary Material), or during the whole storage

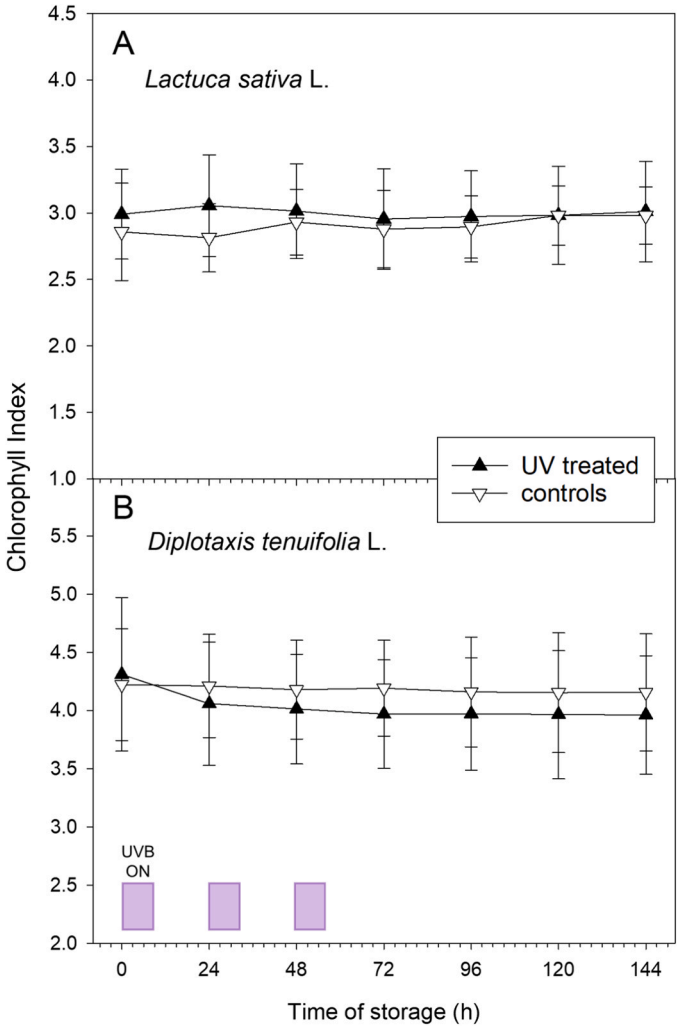

Fig. 5. The leaf Chlorophyll Index (Eq. 2) of baby-leaf lettuce (A) and wild rocket (B) samples under storage with (black triangles) or without (white triangles, control) 3d-UVB treatment. Violet boxes indicate periods of irradiation by the UVB lamp. Within each treatment, mean values were not significantly different ($p > 0.05$) according to ANOVA. Means between UVB-treated and controls at the same storage time were not significantly different according to the Student's t-test. Mean values (± SD) on 6 replicates (3 from Trial 1 and 3 from Trial 2).

Table 2
Leaf weight loss (%) during 6 d of storage (5–6 °C, 80% RH) with and without UVB treatments.

		Baby-leaf lettuce	Wild rocket
	UVB treatment	%	%
Trial 1	None (control)	3.0 ± 0.6 b	1.3 ± 0.1
	3-days	4.6 ± 0.6 a	1.7 ± 0.5
Trial 2	None (control)	7.3 ± 0.6 a	3.9 ± 1.1
	1 day	4.6 ± 0.8 b	4.3 ± 0.6
	2 days	5.9 ± 0.5 ab	5.0 ± 0.5
	3 days	7.0 ± 0.7 a	5.7 ± 0.4

Notes. Within each species and trial, mean values tagged by a diverse letter are significantly different ($p \leq 0.05$) according to the Student's t-test or the Holm-Sidak test for All Pairwise Multiple Comparison. Data are average ± SD, n = 3

period detected in Trial 2 (Table S5).

3.2.4. Glucosinolates

The main glucosinolates (GSLs) identified in the wild rocket leaf extracts, in order of average amounts, were glucosativin, dimeric 4-mercaptobutyl glucosinolate, 4-(Cystein-S-yl)butyl glucosinolate, glucoraphanin, neoglucobrassicin and diglucothiobeinin. Glucosativin and its dimeric form accounted for the 77% of total GSLs. The total GSLs average leaf concentration among trials and treatments was 28.75 ± 5.62 mmol kg^{-1} (12.9 ± 2.5 g kg^{-1} on a dry weight basis). A large variability in the content of the single GSLs between the two trials was observed, with a coefficient of variation (CV) ranging from 19% to 55%. No significant difference in the amount of GSLs between UVB-treated and untreated samples could be found (Figure S11 of the Supplementary Material). However, the main glucosinolate glucosativin was more concentrated in UVB treated plants in the first study than in untreated leaves. In the second study the concentration of glucosativin was higher in the untreated leaves. Differences in concentrations of other glucosinolates were not observed.

3.2.5. Antioxidant activity

Overall, there were no noticeable differences in the antioxidant activity of the extracts, measured by TEAC and DDPH assays, between the control samples and those treated with 3-d UVB for both species (Trials 1 and 2). However, a trend towards higher antioxidant activity in the UVB-treated samples with respect to controls was observed for both species (Figures S12). Notably, only the baby-leaf lettuce from Trial 1 exhibited a significant 50% increase in the antioxidant activity for the UVB samples compared to controls ($p \leq 0.05$), in the DPPH assay. Despite the lack of significance in the antioxidant capacity resulting from the UVB exposure, the antioxidant results for both DPPH and TEAC assays showed a positive and significant linear relationship with the TPC in both species (Figure S13 in Supplementary Material).

4. Discussion

4.1. Elicitation of phenolic compounds by postharvest UVB irradiation

The results of our investigation clearly show that the postharvest treatment of packaged RTE salad samples by a narrow-band UVB lamp enhanced the content of phenolic compounds in both *L. sativa* and *D. tenuifolia*. This was confirmed by both destructive (Fig. 1) and non-destructive (Fig. 3) analyses. To date only a single study has been undertaken to investigate the effect of postharvest UVB irradiation on *D. tenuifolia* (Romano et al., 2022), with no equivalent studies on *L. sativa*. Indeed, studies on the UV treatment of lettuce in postharvest are limited to the application of UVC radiation to reduce microbial load (Collazo et al., 2019; Sonntag et al., 2023). In addition, it is worth mentioning that no studies were conducted until now on the application of UVB treatments on sealed/packaged salads or other vegetables. This application can be an effective and practical approach to enhance health beneficial secondary plant metabolites without damaging leafy vegetables. Several studies have instead been conducted on UV effects on lettuce plants during growth, reporting the increase in phenolic compounds production due to the UV exposure (Assumpção et al., 2019; Tsormpatsidis et al., 2010; Weiland et al., 2023). On the other hand, negative effects of UV radiation in term of reduced biomass, limited plant growth and physiological disorders were also observed (Lee et al., 2014; Tsormpatsidis et al., 2010). Furthermore, the comparison of these *in planta* studies with one another and with postharvest investigations is complex due to the wide range of experimental designs adopted and the frequent absence of key protocol parameters, such as the spectral emission of the UV radiation sources used, the irradiance applied and duration of the treatments.

Postharvest UVB effects on phenolic production in vegetables have been focused on other species, such as broccoli (Darré et al., 2017; Duarte-Sierra et al., 2020; Martínez-Zamora et al., 2021; Rybarczyk-Plonska et al., 2016) and cabbage (Harbaum-Piayda et al., 2016; Kowalski et al., 2021). These studies indicate that continuous low-irradiance UVB irradiation for a long time (hours per day) and

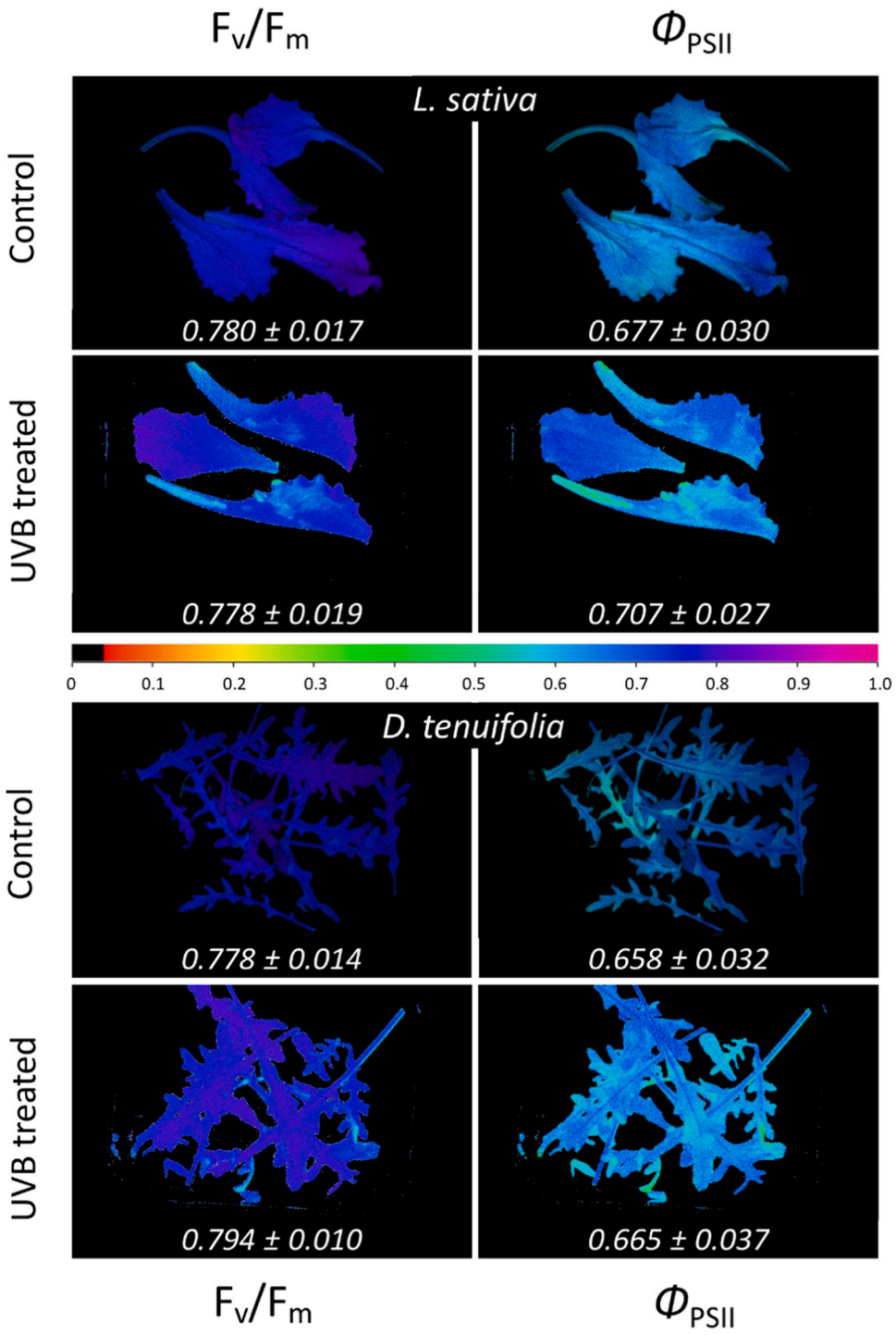

Fig. 6. Pseudo-colour fluorescence images acquired by the Imaging PAM to calculate F_v/F_m (left panels) and Φ_{PSII} (right panels) of UVB-treated and controls of baby-leaf lettuce (*L. sativa*) and wild rocket (*D. tenuifolia*) leaves after 6 d of storage (Trial 1). For each image, the reported means (± SD) were calculated on triplicates and for each sample the average values over the whole fluorescing pixels were considered.

repeated during cold storage may be more efficient in increasing phenolic compounds than acute short treatments at high irradiance. Concerning wild rocket salad, in a study conducted by Romano et al. (2022) leaves exposed to postharvest irradiation (45 s at about 4.5 W m^{-2} from a broad band UVB lamp) showed a 20% increase in TPC. This effect was more pronounced on plants previously exposed to UVB solar radiation during growth (irradiance not reported). Our study demonstrated a much higher increase in TPC compared to control samples. The differences in the experimental setup likely contributed to these variations. Indeed, our narrow-band UVB lamp contained much less UVA radiation than the broad-band UVB lamp employed by Romano et al. (2022) (about 20% versus 60% of total UV radiation). We also used approximately 4-times lower UVB irradiance and a much longer treatment period.

It is well known that the accumulation of phenolic compounds in plants can be mediated by specific pathways involving UVB photoreceptors (UVR8) (Jenkins, 2009) or non-specific pathways involving radiation-generated reactive oxygen species (ROS) (Tan et al., 2023). In

addition, the presence of different proportions of UVB and UVA components in the irradiation source emission, as well as the spectral distribution of radiation in the UVA range, can significantly impact the induction of flavonoid and phenolic biosynthesis. In fact, it was recently shown that the two plant photoreceptors perceiving UVB and UVA/blue radiation and mediating the plant photomorphogenic responses, namely UV RESISTANCE LOCUS 8 (UVR8) and Cryptochromes (CRYs), respectively, can interact with each other in a competitive way (Rai et al., 2019). The extent of this negative interaction is expected to be proportional to the amount of UVA radiation in the long wavelength range (above 350 nm) that more closely overlaps the CRYs absorption spectra (Rai et al., 2021, 2020). This process may explain part of the quantitative differences that can be observed in the induction of phenolic compounds between narrow-band and broad-band UVB lamps.

The UVB-induced increase of flavonoids observed in the present study could likely be due to the up-regulation of genes encoding key enzymes of the flavonoid biosynthetic pathway, such as the phenylalanine ammonia-lyase (PAL) (Lee et al., 2014) and the chalcone synthase (CHS) (Harbart et al., 2023). This regulatory mechanism should take place in postharvest UVB-treated RTE salad leaves analogously as observed in plants during pre-harvest UV treatments (Harbart et al., 2023; Lee et al., 2014).

The stress induced by mechanical injuries on leaves at harvest may also contribute to increase PAL activity and phenolic accumulation (Reyes et al., 2007). However, the lack of variation in the EPhen Index and phenolic content we observed in our control samples indicates that there was no effect of leaf injuries (due to the minimal cut in baby leaves) or storage conditions on the leaf phenolic compounds of the RTE salads.

4.2. Non-destructive detection of phenolic compounds

The non-destructive optical detection of phenolic compounds conducted here was performed through the sealed bag of the salad samples since the polypropylene film of the packaging did not affect our Multiplex sensor measurements. In this way, we did not alter the storage conditions of the samples because the leaves remained sealed within the packaging and measured in situ inside the cold room. This technique could serve further as a quality control method for future applications.

The Mx fluorescence sensor was previously used on the lettuce species to monitor the changes in UV absorbing compounds during the shift of plants from greenhouse to full-sun conditions (Sytar et al., 2018). A positive relationship between total phenolics and the Mx FLAV Index (=log FRF_R/FRF_UV, that is related to EPhen Index reported here) on lettuce plants during growth was observed (Zivcak et al., 2017). This kind of relationship was confirmed in our study when considering the leaf TPC and TFC versus the EPhen Index in postharvest after storage and UVB treatments. However, in wild rocket the correlation between the EPhen Index and HCAs was weaker. Similarly, no significant correlation between the HCA concentration and the epidermal absorbance in the UVA spectral region was found in rye (Burchard et al., 2000) or okra (Neugart et al., 2021) leaves. It is important to mention that the Mx sensor reveals compounds localized in the epidermis of the leaves, just above the first chlorophyll parenchyma layer, and measures the in vivo absorbance at 375 nm. Therefore, the accuracy of the Mx in determining the content of particular compounds depends on the tissue localization of the target compound and its absorption properties. HCAs, with absorption maxima at 300–330 nm, have a limited contribution to the EPhen Index, unless they are present at high concentration in the epidermis. Thus, the low content of HCAs found in *D. tenuifolia* leaves explains the reduced correlation between these compounds and the EPhen Index.

The EPhen Index also provided non-destructive time-resolved information on the dynamics of the phenolic production process and distinguished the different responsiveness to UVB radiation between *D. tenuifolia* and *L. sativa*. It appears that the UVB-induced accumulation of phenolic compounds is an energy dose-dependent process. In wild rocket, the maximal TPC was already obtained with about 40 kJ m^{-2} of the UVB radiation, while in lettuce the highest TPC was found after about 140 kJ m^{-2}.

Our results indicate that the EPhen Index can serve as a robust and reliable non-destructive proxy for TPC in stored RTEs leaves.

4.3. Effects of the UVB treatments on salad quality aspects

The visual appearance of the RTE salad samples remained unaffected throughout the 6-d storage period, regardless of the presence or absence of UV treatment. No signs of browning or damage were observed (see Fig. S14), in accordance with unchanged concentration of chlorophyll and carotenoids and leaf photosynthetic activity before and after storage. Therefore, both storage conditions and UVB exposure did not have detrimental effects on the visual quality of the samples. In accordance with our data, a previous study showed that the total chlorophyll and carotenoids were unaltered in *D. tenuifolia* stored for 7 d at 5 °C under continuous application of 35 μmol m^{-2} s^{-1} of white light (Pennisi et al., 2021). Chlorophyll was also unaffected in lettuce during a 9-d storage period under 12 h light/12 h darkness cycles with 120 μmol m^{-2} s^{-1} of PAR (Liu et al., 2015). The use of a light/dark cycle during storage helped to preserve quality and phytochemicals in the RTE samples. In fact, light has been shown to maintain the integrity of chloroplasts and decrease protein degradation (Wada and Ishida, 2009). In addition, light exposure in fresh-cut lettuce has been found to reduce cut-edge browning during storage, potentially by inhibiting polyphenol oxidase (PPO) activity (Charles et al., 2018; Zhan et al., 2012). This suggests that the absence of browning in UVB-treated samples, despite having higher levels of the phenolic PPO substrate, can be attributed to the compensatory effect of additional white light, which counteracted the expected increase in PPO activity. In contrast, a negative effect of storage under a 12-h light period was observed in terms of weight loss in the leaves. This effect was more pronounced in *L. sativa* compared to *D. tenuifolia*. The extent of leaf weight loss was found to be strictly related to the intensity of the photosynthetic active radiation (PAR) (data not shown), in accordance with previous observations (Charles et al., 2018; Martínez-Sánchez et al., 2011; Pennisi et al., 2021) due to the light-dependent opening of stomata (Martínez-Sánchez et al., 2011).

The loss of weight from leaves either increased or did not change when the UVB irradiation treatment was added to the white-light in stored samples. Our UVB fluorescent lamp contained about 3 μmol m^{-2} s^{-1} of blue light in addition to the white light irradiation. This could contribute to weight loss since the stomatal opening process can be induced even by small levels of PAR (Martínez-Sánchez et al., 2011) or blue light (Doi et al., 2015). Therefore, a compromise must be made when choosing the light level during storage to avoid browning, preserve chlorophyll and minimize water loss (Charles et al., 2018).

Photosynthetic activity of leaves was not affected either by the low storage temperature or the UVB treatment (Fig. 6, Table S5). Fluorescence image histograms showed a very similar spatial distribution of Φ_{PSII} over the whole leaf blade between UVB treated and control samples (Fig. S9). Likewise, F_v/F_m measured through packaging by the Imaging PAM fluorimeter on intact leaves of romaine lettuce remained widely unaffected or in some cases slightly declined after 13 d of storage at 6 °C (Hägele et al., 2016). Charles et al. (2018) also observed that F_v/F_m in lettuce remained stable for 7 d at 6 °C under darkness or 50 μmol m^{-2} s^{-1} light exposure. F_v/F_m did also not change significantly in green lettuce for 10 d of storage even at 16 °C, 70% RH (Chen et al., 2021).

The apparent consistency of Φ_{PSII} values throughout the storage period under both control and UVB treatments indicates that the generally stress sensitive thylakoid membranes were unaffected by the relatively low light levels and exposure to UVB radiation. This low radiation load may be conducive to the prevention of photo-oxidative damage that might induce an increase in dissipation of energy via non-photochemical quenching associated with increased synthesis of

protective compounds (Close and Beadle, 2003; Zhong et al., 2022). Higher PAR or UVB intensities might likely disrupt photosystem II function, leading to a decline in the actual quantum efficiency of Φ_{PSII} (Demmig and Björkman, 1987; Maxwell and Johnson., 2000). The maximum quantum efficiency of photosystem II (F_v/F_m) is generally less sensitive to abiotic stress (Killi et al., 2017), and may therefore be a less useful parameter in assessing the impact of storage or supplemental UVB treatment on commercially grown salad leaves. The similarity found in the VAZ pool as well as in the Car/Chl ratio between controls and UVB-treated samples further supports the interpretation that the UVB irradiation during storage did not induce any additional oxidative stress on both salad species (Table S4).

No significant impact of the UVB treatments on the GSLs concentration of wild rocket was observed. However, the real UVB-induced effect could be hidden by the large biological variability among the samples. Factors affecting the GSLs content in *D. tenuifolia* are the site and mode of cultivation (open field, soil, soilless, growth cabinets) (Bell et al., 2015; Di Gioia et al., 2018; Pasini et al., 2012), environmental temperature (Jasper et al., 2020), time of harvest and number of cuts (Bell et al., 2015; Jasper et al., 2020). Since our wild rocket samples came directly from the producer, there was no standardized control of growth conditions of the cultivation and of the number of cuts at harvest explaining the large variability we found in the GSLs concentrations between trials. Mechanical damage during harvest may have caused GSLs change or release in its aftermath. To better and definitely evaluate the impact that UVB radiation may have on wild rocket GSLs, a larger number or more homogeneous samples, from controlled cultivation and harvest conditions, should be investigated. However, our results suggest that there is no clear enhancement effect of postharvest UVB radiation on GSLs concentrations of wild rocket, which might lead to the positive outcome of not increasing the bitterness of salads.

We found that the antioxidant activity of the leaf extracts measured by the TEAC and DPPH methods gave similar results, for both species, without changes induced by the UVB treatment. This lack of changes in the antioxidant activity induced by UVB radiation was unexpected, especially considering the significant increase in TPC and TFC observed in both species as a response to UVB treatment. In addition, the strong linear relationship observed between the leaf extract antioxidant activity and TPC indicates that the higher content of phenolics would lead to a greater antioxidant capacity. Nevertheless, alongside phenolics several other compounds present in food samples, including salads, have antioxidant properties, such as vitamins A, C and E, and minerals. Some of these compounds respond to UVB, such as ascorbic acid (vitamin C), whose production has been shown to be affected by UVB radiation in lettuce plants (Zhou et al., 2023). Therefore, it is possible that unexamined changes in one or more of these compounds, resulting from the UVB treatment and/or storage conditions, could partially explain the lack of significant changes in the antioxidant capacity despite the higher TPC content of the samples. On the other hand, it is worth mentioning that the antioxidant capacity of in vitro tested antioxidants has no bearing on how effective they are in vivo (Martins et al., 2016). Consequently, while we did not observe a significant increase in the antioxidant activity in the UVB-treated sample extracts, a higher phenolic content (flavonoids and total concentrations) might ultimately enhance the antioxidant properties of these RTE salads for human tissue protection.

5. Conclusions

For the first time, we provide evidence for enhancing the content of bioactive molecules in RTE salads (*Lactuca sativa* L. and *Diplotaxis tenuifolia* L.) by postharvest UVB irradiation through sealed polypropylene bags during storage. These bioactive phenolic compounds, particularly quercetin glucoside derivatives, possess valuable antioxidant and antiviral properties. The production of RTE salads enriched in these compounds will have a positive impact on consumers, providing them with healthier vegetables.

We demonstrated that the UVB-induced synthesis of phenolic compounds in *Lactuca sativa* and *Diplotaxis tenuifolia* leaves can be non-destructively determined by a fluorescence sensor. Producers and retailers may adopt this technology to rapidly control the phenolic content and quality level of leafy vegetables. The optical assessment of TPC in salad samples combined with the application of a short postharvest UVB irradiation treatment may serve as a rapid method for selecting the species and cultivars most suited for the production of antioxidant compounds and find application in the production of health beneficial lettuce products. Optical TPC assessment can also represent an innovative tool for research studies aimed at understanding the effects and mechanisms of action of abiotic and biotic stresses in postharvest plant physiology. Our results showed that the UVB treatments did not affect several quality features of RTE salads, such as pigments, fresh weight, photosynthetic and antioxidant activities. The absence of downsides on these features due to the treatments guarantees a high marketable value of the products. Further research will evaluate the impact of the UV irradiation on microbiological parameters, the visual and organoleptic properties and the shelf-life of products. Because of the antioxidant and antimicrobial activities of phenolics induced by UV radiation, treated RTE species are expected to have a prolonged shelf-life. This would be an additional advantage for retailers and consumers in term of the reduction of vegetable waste.

The results of the present research are propaedeutic for further studies aimed at optimizing UVB-treatments of RTE salads and proposing a real application for the food industry. In the future, the most efficient UV emission wavelengths (achieved by employing narrow-band UV LEDs sources) or a combination of wavelengths (along with the optimal intensity, duration and mode, continuous versus intermittent, of single or multiple treatments) should be defined. UVB irradiation applied directly to packaged products will allow repetition of elicitor treatments during storage and retail periods, or even inside the home fridge, maintaining the health-beneficial compounds in RTE vegetables until few days before consumption.

CRediT authorship contribution statement

Luana Beatriz dos S. Nascimento: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Antonella Gori**: Data curation, Investigation, Methodology, Validation Writing – review & editing. **Lucia Cavigli:** Investigation, Data curation, Formal analysis. **Giovanni Marino:** Investigation, Data curation. **Cecilia Brunetti:** Verification, Resources, Supervision, Writing – review & editing. **Matthew Haworth:** Data curation, Formal analysis, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. **Filippo Micheletti:** Resources, Software. **Tobias Pöhnl:** Investigation, Data curation. **Susanne Neugart:** Supervision, Resources. **Giovanni Agati:** Conceptualization, Funding acquisition, Project administration, Data curation, Visualization, Writing – original draft, Writing – review & editing. All authors contributed to the article and approved the submitted version.

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.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2023.112606.

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