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Hormetic effect of UV-C radiation on red mustard microgreens growth and chemical composition

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ABSTRACT

Brassica microgreens are rich in phytochemicals and are attractive crops for controlled vertical farming systems where the light spectrum can be precisely manipulated. Understanding the effects of pre-harvest hormetic UV-C light doses on plant composition and growth parameters represents a novel and largely unexplored area for precision agriculture and nutrition. Therefore, the objective of this work was to investigate the impact of exposing red mustard microgreens to low/hormetic doses of UV-C radiation on their growth, chemical composition and colour. Plants were grown in a controlled environment and exposed to 0.3 kJ m^{-2}, 254 nm UV-C radiation at the end of the cultivation period. Treatments included a single pulse on day 7, or three pulses at days 7, 8, and 9 and harvest on day 10. UV-C radiation presented a hormetic effect, while 1 pulse of UV-C stimulated growth and productivity without significant colour changes in microgreens, 3 pulses of UV-C radiation to did not show significant effects when compared to controls (no UV-C exposure). Moreover, strong negative correlations were observed between growth parameters and chemical composition ($p < 0.05$). Microgreens with enhanced growth parameters showed a decrease in phenolic compounds content and antioxidant activity. Interestingly, regardless of quantification, untargeted metabolomics using UHPLC-Q-Orbitrap-MS/MS revealed that the secondary metabolites profile remained similar between control and microgreens treated with UV-C radiation.

1. Introduction

Microgreens are young plants with vibrant colours, flavours, and potential health benefits owing to their enhanced phytochemicals content, representing a great opportunity to enhance fresh vegetable consumption [1,2]. These vegetables of high economic value are typically grown in sustainable food production systems under controlled climate conditions (both in greenhouses and vertical farms) and are harvested at the cotyledonary stage when the first true leaves begin to appear [2,3]. Vertical farms are frequently installed in urban areas, increasing the supply of urban environments with a low carbon footprint [4].

Lighting is a crucial factor for the development and quality of plants. Energy-efficient artificial lighting technology has been widely employed in these systems due to the precise control of several advantages such as wavelength specificity, adjustable light quality, and intensity [5,6]. Ultraviolet (UV) light can affect plant growth and development, the effects being highly dependent on the wavelength, irradiance and intensity [7,8]. UV-C radiation presents shorter wavelengths (200–280

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nm) compared to other types of UV light, being the most intense and energetic one [9,10]. Having germicidal properties against fungi, bacteria and virus, UV-C radiation has traditionally been used in the post-harvest stage as an abiotic stress, safe and nonintrusive, mainly aimed at preventing microbiological infections and extending shelf-life [10–12]. Properly managed, UV-C exposure in early plant growth stages can be effective in stimulating the physiological responses of the plant and enhancing its plant defence mechanisms. The application of preharvest UV-C light has shown its potential to improve the resistance to diseases, antioxidant status and phenolic compounds of young spinach plants using hormetic doses, from 0.3 to 0.9 kJ m^{-2} [11]. Nonetheless, this should be investigated and tailored to each species and stage of plant development. This novel treatment has barely been explored in pre-harvest stages and has not yet been applied in microgreens, which are one of the youngest stages of plant growth, thus a low intensity of 0.3 kJ m^{-2} of UV-C radiation was chosen, to induce stimulation but prevent plant degradation. The *Brassicaceae* family is one of the most cultivated families of microgreens, generally recognized for containing substantial amounts of phytochemicals [13]. Specifically, mustard microgreens are a promising source of glucosinolates and polyphenols, although comprehensive information on the profile of such compounds is still scarce [13,14].

The main goal of this study was to assess the effect of pre-harvest exposure to low, hormetic doses of UV-C radiation on red mustard microgreens by evaluating i) agricultural parameters related to their growth and physiological state; (ii) chemical composition, including minerals (by ICP-OES), phytochemicals and antioxidant activity (spectrophotometric analyses), and secondary metabolites profiling (using ultrahigh-performance liquid chromatography coupled to Q-Orbitrap high-resolution mass accuracy spectrometry – UHPLC-Q-Orbitrap-MS/MS); and (iii) colour changes (using the CIELAB system – L*a*b coordinates).

2. Materials and methods

2.1. Plant material, treatments, and sample processing

Seeds of red mustard microgreens (*Brassica juncea*) were sown in an organic germination substrate (SIRO, Portugal) in 24 × 16 cm trays (3.2 g seeds per tray) under dark conditions (with a temperature of 22 °C and relative humidity of 60 %) until radicle emergence (4 days after sowing). Following that stage, a photoperiod of 16 h was applied using white LED lamps (450 μmol s^{-1} m^{-2} of photosynthetic photon flux density measured at the plant level; model SE-004, Systion, Portugal), and the temperature was changed to 22 °C during the day and to 19 °C during the night.

The UV-C light supplementation (0.3 kJ m^{-2}, 254 nm) was applied seven days after emergence, right after the end of the photoperiod, using three frequencies: control (no UV-C application, coded as Control); one pulse (day 7, codded as UV-C 1) or three pulses (day 7, 8, 9, codded as UV-C 3). The UV-C equipment was formed by two UV-C tubes with a power of 15 W each (295 × 76 mm length, VL-215.C, Vilber LourmatTM, France). On day 10, right after the cotyledonary leaves expanded and the first true leaf appeared, the hypocotyl height of 30 microgreens, randomly selected, per tray was measured. Then, microgreens were harvested at the substrate level, with sharp and sterile scissors, and weighed to determine the yield per tray. Part of the microgreens from each tray were then dried to a constant weight in a forced-air oven at 65 °C and weighed to determine their relative water content (RWC) and respective dry weight. The other part was immediately freeze-dried (Telstar Cryodos freeze dryer, Telstar Industrial S.L.) for 48 h. Freeze-dried microgreens were milled with a knife mill (Grindomix GM 200, Retsh, Hann, Germany), kept protected from the light and frozen at −80 °C, until further analyses.

2.2. Reagents and equipment

For the *in vitro* spectrophotometric assays: gallic acid (≥98 % purity), sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH•) solution, trolox (97 % purity), 2,4,6-tri-pyridyl-s-riazine (TPTZ), sodium acetate, ferric chloride, ferrous sulfate, sodium carbonate, sodium nitrite, sodium acetate, aluminium chloride, gallic acid, catechin and cyanidin-3-*O*-glucoside were acquired from Sigma-Aldrich Corp. (St. Louis, MO, USA). Folin-Ciocalteau (F-C), sodium hydroxide, hydrochloric acid (HCl), potassium chloride solution and sodium acetate were purchased from Merck (Darmstadt, Germany). Potassium chloride was obtained from Panreac (Barcelona, Spain). Methanol was purchased from Honeywell, Riedel-de-Haën (Seetze, Germany). Solvents utilized were HPLC-grade. Ultrapure water was prepared in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

For the UHPLC-Q-Orbitrap-MS/MS analyses. Methanol, HPLC-grade, was purchased from Honeywell, Riedel-de-Haën (Seetze, Germany. Formic acid (>98 % of purity), from Fisher Scientific (Erembodegem, Belgium) was used. Ultrapure water was obtained in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The chromatographic system used in this study was composed by a Thermo Fisher Scientific Vanquish Flex Quaternary LC, equipped with a Hypersil GOLD column (100 × 2.1 mm, 1.9 μm particle size) and operating at a column temperature of 30 °C. The system was coupled to a Thermo Scientific Q-Exactive Orbitrap hybrid mass spectrometer, functioning in both positive and negative ionization modes.

To calibrate LC-Q-Orbitrap, a mixture of acetic acid, caffeine, methionine-arginine-phenylalanine-alanine-acetate salt, and Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive) and another mixture of acetic acid, sodium dodecyl sulfate, taurocholic acid sodium salt hydrate, and Ultramark 1621 (fluorinated phosphazenes) (ProteoMass LTQ/FT-HybridESI negative) from Thermo Fisher Scientific were utilized.

2.3. Lipid peroxidation

Lipid peroxidation (LP) was estimated in frozen ground samples of microgreens (ca. 200 mg FW) and quantified following the procedure of Machado et al. [15]. Briefly, the material was homogeneized in 0.1 % (w/v) trichloroacetic acid, and then the malondialdehyde (MDA) content was determined using the molar extinction coefficient (ε) of 155 mM^{-1} cm^{-1} and expressed in $nmol^{-1}$ g FW. Absorbances were read at 532 and 600 nm, with the second being subtracted from the first to avoid the effects of non-specific turbidity.

2.4. Extraction of compounds for spectrophotometric analyses

The extraction of compounds from freeze-dried red mustard microgreens was performed using a solvent system of two cycles: 4 mL of methanol:water 80:20 (*v/v*) were added to 100 mg of milled freeze-dried samples, homogenized in vortex for 1 min, and thoroughly shaken for 1 h. The extract was then centrifuged at 5000×*g* for 5 min at 4 °C, and the supernatant was collected into a 15 mL tube. Then, the remaining sample was extracted again with 4 mL of methanol:water 80:20 (*v/v*) repeating agitation and centrifugation. After centrifugation, the supernatant was added to the previously collected extract. Two replicates of extraction were performed for each biological replicate. Extracts were filtered using a PTFE membrane of 0.45 μm, kept protected from the light and frozen at −80 °C until further analyses.

2.5. Quantification of total: phenolics, flavonoids, anthocyanins and antioxidant activity

Quantification of total phenolic compounds (TPC) content of red mustard microgreens were determined using the Folin–Ciocalteu (F-C) method, using the conditions described by Magalhães et al. [16] using

spectrophotometric detection and 96-well microplates, with minimal modifications and using a SPECTROstar Nano Microplate Reader (BMG Labtech, Ortenberg, Germany) at 765 nm. Gallic acid was used as a standard, therefore TPC were expressed as mg gallic acid equivalents (GAE). Assays were performed in triplicate for all extracts, standards and blank, at room temperature (25 ± 1 °C).

Total flavonoids (TFlav) content of red mustard microgreens extracts was determined based on the method reported by Peixoto et al. [17]. Absorbance was then recorded at 510 nm in a SPECTROstar Nano Microplate Reader (BMG Labtech, Ortenberg, Germany). Catechin was used as a standard, therefore the total flavonoids content was expressed as μg g^{-1} of catechin equivalents (CEq). The assay was performed in triplicate for all extracts, standards and blank, at room temperature (25 ± 1 °C).

Total monomeric anthocyanins (TMA) content was determined by the pH differential spectrophotometric method (AOAC Official Method 2005.02), according to Lee et al. [18], adapted to 96-well microplates. This method is based on the reversible structural rearrangements of anthocyanins at different pH: coloured at pH 1 with a maximum absorption between 490 and 540 nm, and colourless at pH 4.5. This way, the absorbance is recorded at pH 1 and pH 4.5 at the λvis-max (ca 520 nm), as well as at 700 nm (to correct for haze). Microplates were incubated in the dark for 30 min, and then the absorbance was measured in a SPECTROstar Nano Microplate Reader (BMG Labtech, Ortenberg, Germany). TMA content was expressed as mg of equivalents of cyanidin-3-*O*-glucoside (C3G). Assays were performed in triplicate for all extracts at room temperature (25 ± 1 °C).

The interaction with the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical method was performed according to Magalhães et al. [19] and Viegas et al. [20]. Trolox (550 μM–15.63 μM) was used as standard, thus the antioxidant activity was expressed in mg of trolox equivalents. Assays were performed in triplicate for all extracts, standards, DPPH• controls and blank controls, at room temperature (25 ± 1 °C).

The Ferric reducing antioxidant power (FRAP) assay was performed according to Peixoto et al. [17], with minor modifications. Ferrous sulfate was used as the calibration standard (25–500 μM), therefore the results were expressed as mg of ferrous sulfate equivalents (FSE). Assays were performed in triplicate for all extracts, standards and blank (distilled water), at room temperature (25 ± 1 °C).

2.6. Extraction and quantification of minerals

To determine the minerals content (Na, K, Mg, P, Ca, Mn, Cu, Zn, Fe), microgreens samples were mixed with 12.5 mL of 65 % nitric acid and 2.5 mL of hydrofluoric acid in a Teflon reaction vessel (MARSXpress, CEM Corporation). Afterwords, the mixture was heated in a microwave in a closed vessel microwave digestion system (Mars ONE, CEM Corporation; 175 °C, 15 min of ramp time, holding time of 15 min at 0 psi and power of 900 W). After digestion, the solution was diluted with 50 mL with ultrapure H_2O, filtered (0.45 μm filters) and analyzed for mineral composition by inductively coupled plasma-mass spectrometry (ICP-OES Optima 7000 DV, PerkinElmer, USA) with a radial configuration.

2.7. UHPLC-Q-orbitrap-MS/MS

The chromatographic fingerprint of phytochemicals from microgreens was obtained by UHPLC-Q-Orbitrap-MS/MS. The mobile phase was water containing 0.1 % formic acid (A) and methanol (B). The flow rate was set at 0.3 mL/min. Gradient elution started at 95 % of A. After 1 min %, the composition of A was linearly decreased to 70 % in 3 min and then to 0 % in 4 min. This percentage was kept during 2 min to later recover initial conditions in 0.5 min, and it was re-equilibrated during 4.5 min. Total run time was 15 min. Injection volume was 10 μL. The following MS parameters were used in the experiment: a spray voltage of 4 kV, sheath gas (N2, 95 %) at 35 (arbitrary units, au), auxiliary gas (N2, 95 %) at 10 (au), S-lens RF level at 50 (au), heater temperature at 305 °C, and capillary temperature at 300 °C. To acquire the mass spectra, full MS scans were done using ESI+ and ESI- without fragmentation (with HCD collision cell off) with a mass resolving power of 70,000 FWHM at *m/z* 200 and an AGC target of 10^6. The mass range was set to *m/z* 60−900. Additionally, data independent mass spectrometry fragmentation (DIA-MS/MS) was done using ESI ±with HCD on and collision energy of 30 eV. The mass resolving power was 35,000 FWHM at *m/z* 200, with an AGC target of 2×10^5 and an isolation window of 50 *m/z*. The obtained outcomes were analyzed using Xcalibur version 4.3.73, featuring Quan Browser and Qual Browser (Thermo Fisher Scientific, Les Ulis, France), as well as TraceFinder 5.1 (Thermo Fisher Scientific) for suspected evaluation. Additionally, nontargeted evaluation was carried out using Compound Discoverer v3.2 (Thermo Fisher Scientific). All samples were injected on the same day.

2.7.1. Suspect screening and unknown analysis

For compound annotation, a comprehensive tentative metabolic fingerprinting analysis of the red mustard microgreens was carried out. This was achieved using a two-pronged, untargeted metabolomics approach under two levels of levels of evidence: suspect screening and unknown analysis corresponding to levels 3 and 4, respectively, as defined by Schymanski et al. [21].

For suspect screening, a curated, in-house database containing approximately 100 phenolic and organosulfur compounds, including information on the precursor ion and two fragment ions together with elemental composition. This information was extracted from a previously published study and other works from our research group [22], and data was processed using TraceFinder 5.1. In addition, Xcalibur (specifically Qualbrowser) was used to meticulously scrutinize full-scan data and spectra analysis. For unknown analysis, raw files obtained from each run were processed with Compound Discoverer™ v3.2, by means of mzCloud™ and ChemSpider™ on-line libraries, as well as a comprehensive database created by us combining information from the indexed databases FoodB [23], PubChem [24], and PhenolExplorer [25], comprising over 41,325 compounds, for broader search.

Different actions were carried out to reduce the number of peaks and avoid false positives. Signals obtained in solvent blanks were eliminated in the samples. Retention time window was of 10.5 min, and peaks whose names were not defined, were removed. An intensity threshold was set at 1e7 to eliminate interferences, which could appear in the samples at lower signals. Peaks spectra and chromatograms were studied independently, and only the ones that provided suitable peak shape and isotopic pattern were chosen. Selected compounds were chosen with a mass error lower than 5 ppm, according to López-Ruiz [26].

2.8. Colour measurements

Characterisation of red mustard microgreens colour was performed using the L*, a*, b* system proposed by the International Commission on Illumination (CIE) in the work of Papadakis et al. [27]. Colour analyses were performed in the fresh red mustard microgreens right before harvesting, using a Minolta CR-400 colourimeter (Konica-Minolta, Osaka, Japan), previously calibrated with a white standard tile. Colour measurements were performed using as concordance criteria a RSD <7 % for each parameter. ΔE was also assessed using the formula $\Delta E^* = \sqrt{((\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)}$.

2.9. Data treatment strategies

Data analyses were performed using R version 4.2.3 (R Project for Statistical Computing). Descriptive analysis was conducted and Pearson's Correlations at every level of treatment, for all the parameters that were measured, both growth and chemical composition.

Z-scores normalization was also performed before the unsupervised chemometric techniques (principal component analysis (PCA) followed

by K-means clustering) using growth and chemical composition as variables. PCA was computed using the FactoMineR package [28]. Ggplot2 [29] and Factoextra [30] packages were also used to visualize the results. NbClust package was used to determine the optimal number of clusters [31].

Differences between the control and UV-C treated plants were assessed by one-way ANOVA followed by the Tukey's HSD (honestly significant difference) *post hoc* test.

The treatment of the data from LC-Q-Orbitrap-MS/MS involved normalization and scaling, namely through percentage normalization of the peak areas.

3. Results and discussion

3.1. Data overview: effect of UV-C treatments on growth and chemical composition

The results obtained from the growth parameters (Yield, Fresh weight, Dry weight, Hypocotyl length, RWC) and chemical composition (TPC, TFlav, TMA, DPPH, FRAP, macro- and microminerals) are summarized in Fig. 1a and Fig. 1b, respectively.

One-way ANOVA followed by the Tukey's *post hoc* test was applied to assess the effects of UV-C radiation in plants. UV-C radiation applied one time (UV-C 1) had positive and significant impact ($p < 0.05$) on all agricultural parameters measured, namely on the yield, fresh and dry weight, relative water content and hypocotyl length (Fig. 1a). However, applying UV-C 3 times was not statistically significant. Together with no significant changes ($p < 0.05$) in lipid peroxidation across treatments, this reveals a positive hormetic effect induced by UV-C radiation on red mustard microgreens' growth, since hormesis is a dose-response phenomenon, characterized by stimulation at low-doses and inhibition a higher-doses. Accordingly, a single application of UV-C radiation improved plants' biomass, while maintaining a good physiological state, however, when applied three times, the response returned to the control level, suggesting a return to normal conditions or even a potential inhibitory effect at higher doses, since despite lipid peroxidation did not show significant differences ($p < 0.05$) across samples, values show an increasing trend with 3 applications of UV-C, which could suggests that the plant could be suffering some oxidative damage, which should be further explored in the future with larger sample sizes.

In regard to chemical composition, the contrary trend was observed: the total phenolic, total flavonoids and total anthocyanins content, as well as the antioxidant capacity from the DPPH and FRAP assays all decreased ($p < 0.05$) when UV-C radiation was applied 1 time (UV-C 1) and returned to values similar to control ($p < 0.05$) when UV-C radiation was applied 3 times (UV-C 3) as can be observed in Fig. 1b. Interestingly, Cu and Fe are the only minerals significantly modulated by UV-C radiation ($p < 0.05$), despite presenting similar trends in the response, UV-C significantly decreases Cu with one application and increases Fe with 3 applications (Fig. 1b). It is plausible that the observed variations in these minerals are a response to the stress caused by radiation in an attempt to protect the plant, given the tight relationship between Fe and Cu homeostasis and their central roles in redox control and electron transport [32]. These variations, together with seemingly higher lipid peroxidation levels with UV-C 3, although not significant, also suggest that 3 pulses of UV-C could already be causing some damage to the plant. To explore the relationship between the growth parameters and chemical composition of red mustard microgreens, correlation analysis was performed, and the resulting correlation matrix is depicted in Fig. 2. Each cell in the matrix represents the correlation between two variables, and the colour of the cell indicates the strength and direction of the correlation.

Among growth parameters, strong positive correlations were observed between the yield and fresh weight ($R^2 = 0.99$), dry weight ($R^2 = 0.78$), hypocotyl length ($R^2 = 0.69$), relative water content ($R^2 = 0.80$); between fresh and dry weights ($R^2 = 0.78$); and between relative water content and hypocotyl length ($R^2 = 0.69$), and fresh weight ($R^2 = 0.80$). In general, as yield increases, fresh and dry weights, its relative water content, and the length of its hypocotyl increases. Strong correlations between fresh and dry weights, and hypocotyl length, suggest overall biomass accumulation.

Additionally, strong positive correlations were also observed among chemical parameters: DPPH was strongly correlated with FRAP ($R^2 = 0.82$), TPC ($R^2 = 0.79$), TFl ($R^2 = 0.74$), TMA ($R^2 = 0.78$). FRAP was also strongly correlated with TPC ($R^2 = 0.74$), TMA ($R^2 = 0.85$), and TFl were additionally strongly correlated with both TPC ($R^2 = 0.75$), and TMA ($R^2 = 0.72$). These results are justified by the significant contribution of the phenolic compounds (including total flavonoids and anthocyanins) to the overall antioxidant capacity of the samples, in accordance to what has been reported by several authors [33–36]. Regarding correlations between growth and chemical parameters, an opposite direction of the correlation was observed. Strong negative correlations were found between RWC, TPC ($R^2 = -0.68$) and some minerals, namely Mg ($R^2 = -0.84$), Fe ($R^2 = -0.78$), Ca ($R^2 = -0.78$), P ($R^2 = -0.66$) and Cu ($R^2 = -0.73$), inferring a dilution effect, as well as between both yield and fresh weight, DPPH ($R^2 = -0.68$), TPC ($R^2 =$

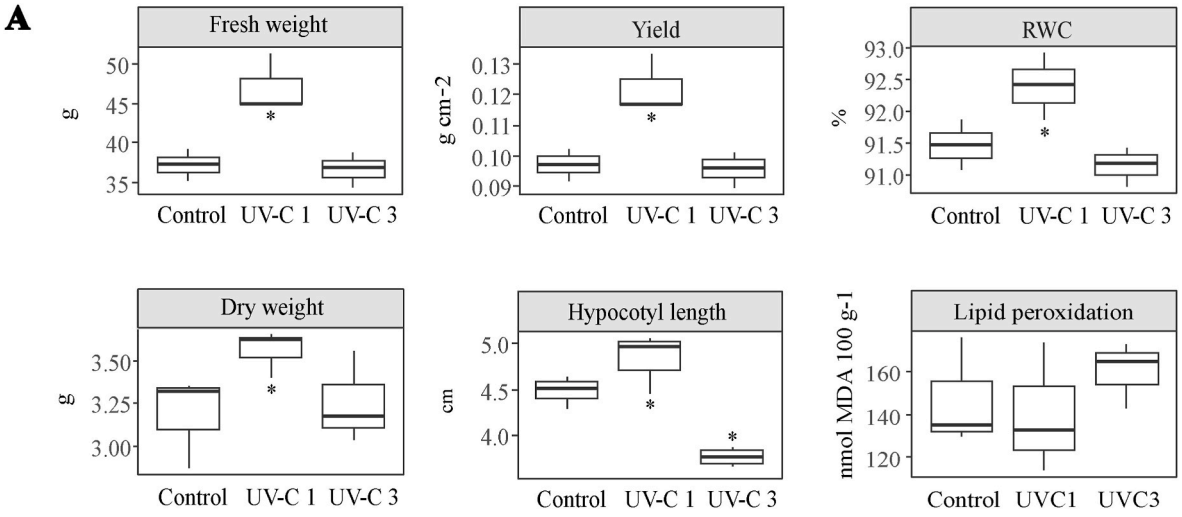

Fig. 1a. Boxplots reflecting the growth parameters of red mustard microgreens. RWC: Relative Water Content. DW: Dry weight. MDA: malondialdehyde. Statistical differences are indicated as asterisks ($p < 0.05$) by one-way ANOVA followed by the Tukey's HSD (honestly significant difference) *post hoc* test.

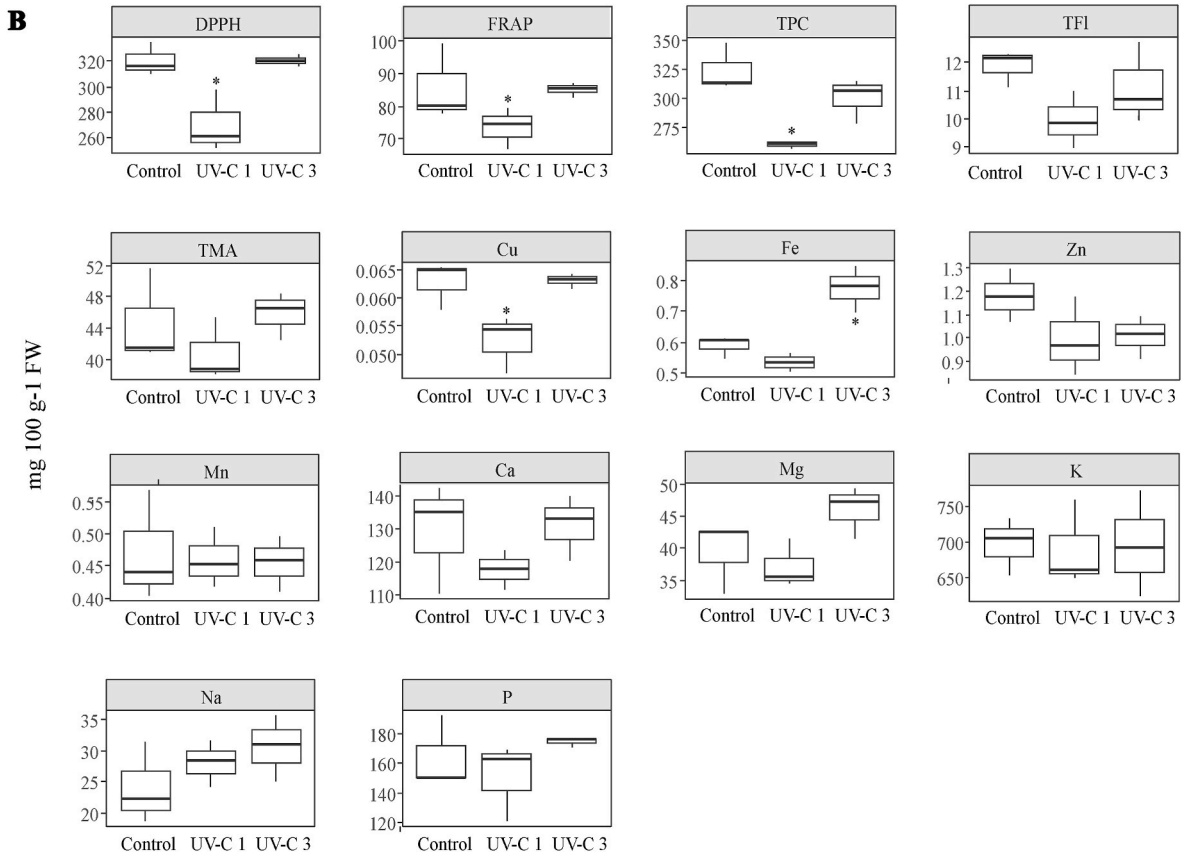

Fig. 1b. Boxplots reflecting the chemical composition of red mustard microgreens. DPPH: 1,1-diphenyl-2-picrylhydrazyl. FRAP: Ferric reducing antioxidant power. TPC: total phenolic compounds. TFlav: Total flavonoids. TMA: Total monomeric anthocyanins.

−0.67), Cu ($R^2 = -0.91$). These results indicate that an increase in the yield and/or fresh weight is associated with a decrease of antioxidant activity, total phenolics content and Cu. These results suggest that red mustard microgreens that grew more tended to have lower levels of defensive compounds, since our samples increased biomass while decreasing the amount of defensive compounds and antioxidant activity. As noted by Fayezizadeh M. et al., 2024 [35], photosynthesis is the main driver of biomass production in plants, therefore it appears that plants are prioritizing photosynthesis over secondary metabolite and minerals production. This trend has been described as the growth-defence trade-off hypothesis, that describes how plants could have a limited amount of energy which they invest in either growing or defence, showing that plants may allocate resources preferentially to growth and primary metabolism in certain conditions [35,37].

To further overview the global variation among red mustard microgreens based on the growth and chemical composition, PCA analysis was performed, followed by K-means clustering analysis. PCA required 4 dimensions to explain at least 80 % of the variance of the data. Together components 1 and 2 were able to explain 59.3 % of the variance and allowed a clear separation between growth and chemical composition parameters mostly by component 1, whereas component 2 allowed a general differentiation between minerals and secondary metabolites (Fig. 3a). Moreover, the results indicate that plants treated with 1 pulse of UV-C separated from the others and were mostly associated with increased growth parameters: yield, fresh weight, hypocotyl length and dry weight, and on the other hand all the chemical composition variables were more associated with the controls and the plants treated with 3 pulses of UV-C (Fig. 3b). This clear separation between the UV-C1 samples and the others was also observed in the K-means clustering analysis, where two distinct clusters were also identified (Fig. 3c).

3.2. *Effect of UV-C treatments on secondary metabolites fingerprint*

Untargeted metabolomics profiling revealed a total of 73,104 peaks, firstly reduced to 3,227, then to 277, and ultimately 94 candidates provided suitable peak shape and isotopic pattern to successfully achieve compound annotation. Among these, 38 were classified as secondary metabolites and are described in Table 1. The remaining were classified 56 as metabolites from the plant's regular functioning and metabolic processes (Supplementary Table 1). Compounds were classified according to their main biosynthetic origin and chemical structure, in line with the framework proposed by Frank et al. [38]. The broad spectrum of annotated compounds found in all samples reflect the complex metabolic profile of the young plants, critical to support plant growth and development [1,39,40].

The profile of secondary metabolites was determined by relative abundance of compounds. Regardless of previous quantification. As summarized in Fig. 4 the profile of secondary metabolites of UV-C treated microgreens was very similar to the control plants.

The relative abundance of compounds' was 96 % for glucosinolates and isothiocyanates, which are highly relevant sulphur-rich compounds found in *Brassica* plants linked to various health benefits, as well as fungicidal, nematocidal and bactericidal properties [41]. Specifically, sinigrin was the most relatively abundant compound, followed by neoglucobrassicin and glucobrassicin, which is in agreement with the literature for leafy mustard [14,42]. Other glucosinolates tentatively identified included progoitrin, gluconapin and glucoiberin, gluconasturtiin and glucotropaeolin. The remaining secondary metabolites were phenolic compounds which represented around 4 %, and the rest of compounds represented less than 1 % (Fig. 4). These results suggest that using low doses of UV-C as a sustainable treatment had no appreciable effect on the secondary metabolites profile, remaining consistent across

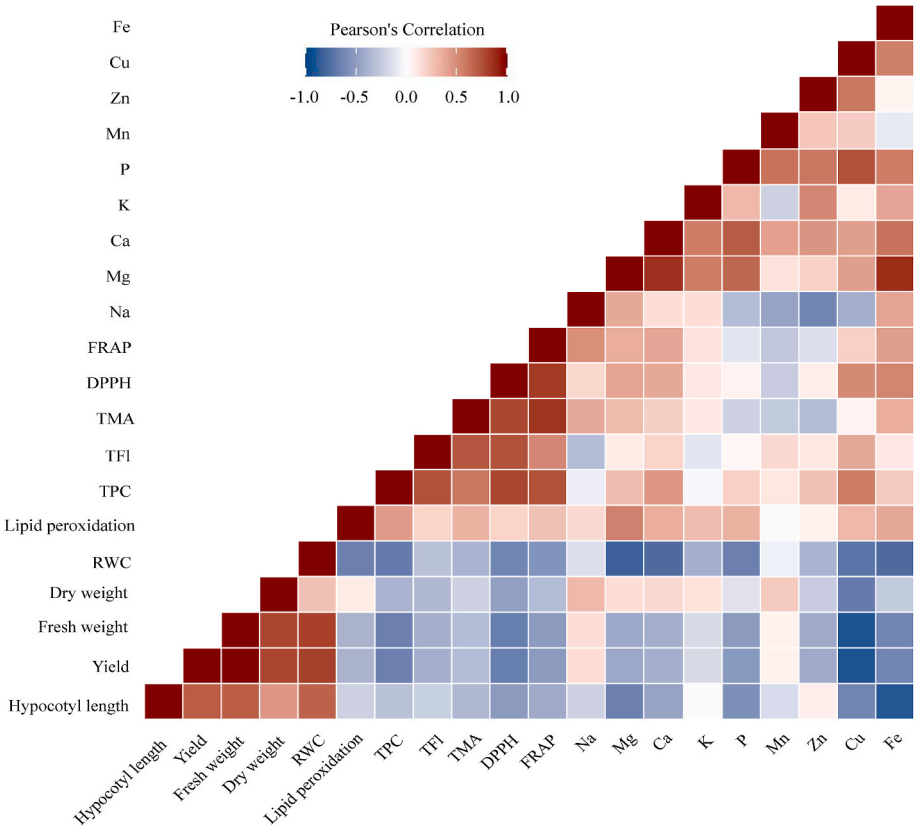

Fig. 2. Diagonal Correlation Matrix depicting the relationships between growth parameters and chemical composition parameters. RWC: Relative Water Content. TPC: total phenolic compounds. TFlav: Total flavonoids. TMA: Total monomeric anthocyanins. DPPH: 1,1-diphenyl-2-picrylhydrazyl. FRAP: Ferric reducing antioxidant power.

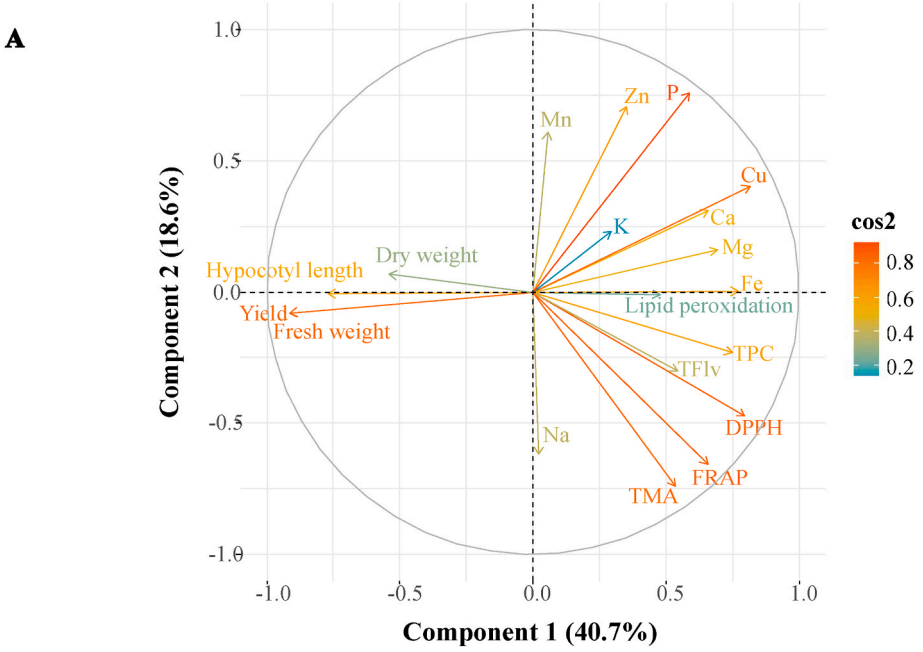

Fig. 3a. Variables Factor Map of the growth and chemical composition parameters of red mustard microgreens. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

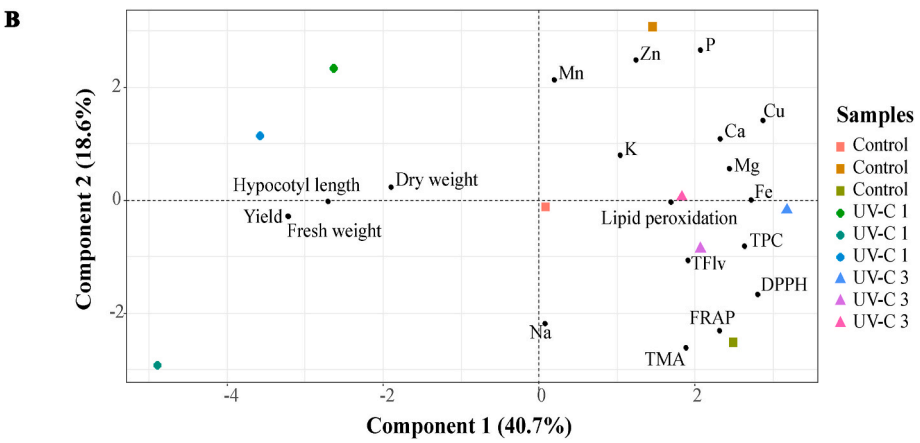

Fig. 3b. Principal component analysis biplot of red mustard microgreens samples based on growth and chemical composition parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

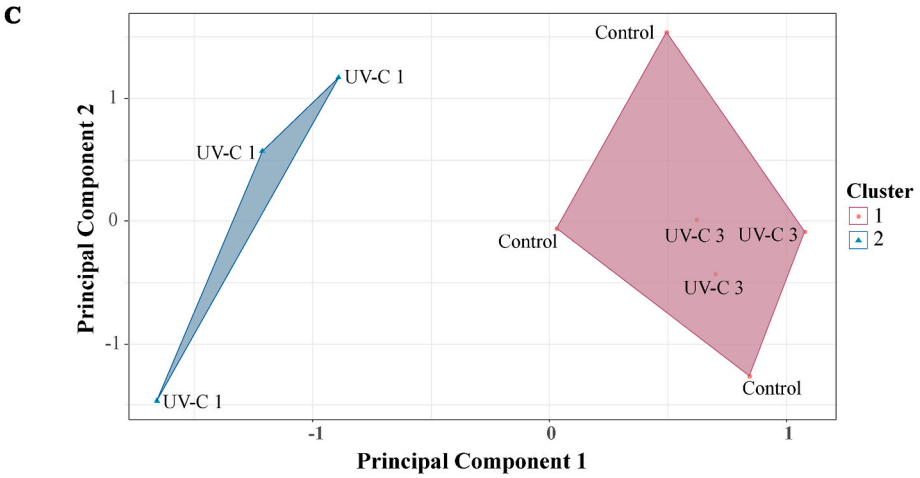

Fig. 3c. K-means Cluster Map of red mustard microgreens based on the PCA of their growth and chemical composition parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

control and treatment groups.

3.3. Effect of UV-C treatments on colour

Colour parameters were not significantly affected by the treatments (Table 2) and the ΔE^*, a widely used index for determining colour changes perceptible by human eye, presented no perceptible differences between the treated plants and the controls (ranging from 1.07 ± 0.66 and $\Delta E = 1.10 \pm 0.54$ for UV-C 1 and UV-C 3, respectively).

4. Conclusions

In conclusion, a thorough understanding of the effects of applying sustainable techniques such as UV-C irradiation at the pre-harvest stage of crops such as red mustard microgreens is innovative and relevant to ensure the continuous production of vegetables packed with health-promoting secondary metabolites, which are of increasing importance in modern food systems. Optimising light conditions to produce microgreens with ideal growth and chemical composition is challenging due to species-specific characteristics and the complex interplay between plant growth and secondary metabolite production. The present work shows that a single application of hormetic doses of UV-C radiation to red mustard microgreens stimulated growth and productivity without significant colour changes in the microgreens, whereas three doses of UV-C radiation showed no significant effects compared to controls (no UV-C exposure). The microgreens with improved growth parameters showed a decrease in phenolic content and antioxidant activity. Interestingly, regardless of the quantification of phytochemicals, the profile of secondary metabolites remained similar between control and UV-C treated microgreens. Despite the positive effect on microgreen growth, farmers, nutritionists, consumers and other stakeholders should carefully weigh this potential benefit against the trade-off in phytochemical content. The effects encountered here should encourage future studies in other Brassica microgreens, focusing on more in-depth studies and mechanistic analyses to optimize growing conditions and maximize consumer benefits.

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CRediT authorship contribution statement

Marta Silva: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Joana Machado:** Methodology, Investigation, Formal analysis, Data curation. **Rosalía López-**

Table 1
LC-Q-Orbitrap-MS/MS Data and Tentative Identification of Secondary Metabolites found in red mustard microgreens.

Tentative identification	Family	Molecular formula	Exact mass (*m/z*)	Ionization mode
Sinigrin[a]	Glucosinolates	$C_{10}H_{17}NO_9S_2$	163.0122	Negative
Gluconapin[a]	Glucosinolates	$C_{11}H_{19}NO_9S_2$	372.04285	Negative
Glucoiberin[a]	Glucosinolates	$C_{11}H_{21}NO_{10}S_3$	422.0254	Negative
Glucotropaeolin[a]	Glucosinolates	$C_{14}H_{17}NO_{10}S_2$	422.02211	Negative
Gluconasturtiin[a]	Glucosinolates	$C_{15}H_{21}NO_9S_2$	422.0585	Negative
Glucobrassicin[a]	Glucosinolates	$C_{16}H_{19}N_2O_9S_2$	463.04866	Negative
Neoglucobrassicin[a]	Glucosinolates	$C_{17}H_{22}N_2O_{10}S_2$	477.06431	Negative
Progoitrin[a]	Glucosinolates	$C_{11}H_{19}NO_{10}S_2$	389.04504	Negative
Iberin	Isothiocyanates	$C_5H_9NOS_2$	164.01949	Positive
Isoferulic acid[a]	(Poly)phenolic compounds	$C_{10}H_{10}O_4$	193.05013	Negative
Kaempferol-3-*O*-rutinoside[a]	(Poly)phenolic compounds	$C_{27}H_{30}O_{15}$	593.15179	Negative
Rutin[a]	(Poly)phenolic compounds	$C_{27}H_{30}O_{16}$	609.14661	Negative
4-Hydroxybenzoic acid[a]	(Poly)phenolic compounds	$C_7H_6O_3$	137.02348	Negative
Gallic acid[a]	(Poly)phenolic compounds	$C_7H_6O_5$	169.0135	Negative
Vanillic acid[a]	(Poly)phenolic compounds	$C_8H_8O_4$	168.04226	Negative
p-Coumaric acid[a]	(Poly)phenolic compounds	$C_9H_8O_3$	163.03932	Negative
m-Coumaric acid	(Poly)phenolic compounds	$C_9H_8O_3$	163.03925	Negative
Caffeic acid[a]	(Poly)phenolic compounds	$C_9H_8O_4$	179.03432	Negative
trans-Caffeic acid[a]	(Poly)phenolic compounds	$C_9H_8O_4$	180.04226	Negative
Scopoletin	(Poly)phenolic compounds	$C_{10}H_8O_4$	193.04911	Positive
Sinapic acid	(Poly)phenolic compounds	$C_{11}H_{12}O_5$	223.06076	Negative
Kaempferol	(Poly)phenolic compounds	$C_{15}H_{10}O_6$	287.05408	Positive
Luteolin	(Poly)phenolic compounds	$C_{15}H_{10}O_6$	287.05393	Positive
Quercetin	(Poly)phenolic compounds	$C_{15}H_{10}O_7$	303.04902	Positive
(+)-Catechin	(Poly)phenolic compounds	$C_{15}H_{14}O_6$	291.08634	Positive
Hesperetin	(Poly)phenolic compounds	$C_{16}H_{14}O_6$	303.08647	Positive
4-*p*-Coumaroylquinic acid	(Poly)phenolic compounds	$C_{16}H_{18}O_8$	339.10667	Positive
Chlorogenic acid	(Poly)phenolic compounds	$C_{16}H_{18}O_9$	355.10182	Positive
Isoquercetin	(Poly)phenolic compounds	$C_{21}H_{20}O_{12}$	465.10139	Positive
Quercetin-3-β-D-glucoside	(Poly)phenolic compounds	$C_{21}H_{20}O_{12}$	465.10143	Positive
Flavaprin	(Poly)phenolic compounds	$C_{26}H_{30}O_{10}$	503.18977	Positive
Petunidin 3,5-*O*-diglucoside	(Poly)phenolic compounds	$C_{28}H_{32}O_{17}$	641.16918	Positive
Pyrogallol	(Poly)phenolic compounds	$C_6H_6O_3$	127.0388	Positive
Quinic acid	(Poly)phenolic compounds	$C_7H_{12}O_6$	191.05543	Negative
Acetophenone	(Poly)phenolic compounds	C_8H_8O	121.06468	Positive
4-Hydroxycoumarin	(Poly)phenolic compounds	$C_9H_6O_3$	163.03856	Positive
(6s)-Vomifoliol OR Methyl jasmonate	Terpenes/Jasmonates	$C_{13}H_{20}O_3$	225.14792	Positive
Cuminaldehyde	Terpenoids	$C_{10}H_{12}O$	149.09577	Positive

[a] Secondary metabolites tentatively identified through a database containing the information on precursor ion and two fragment ions, previously identified with standards [22].

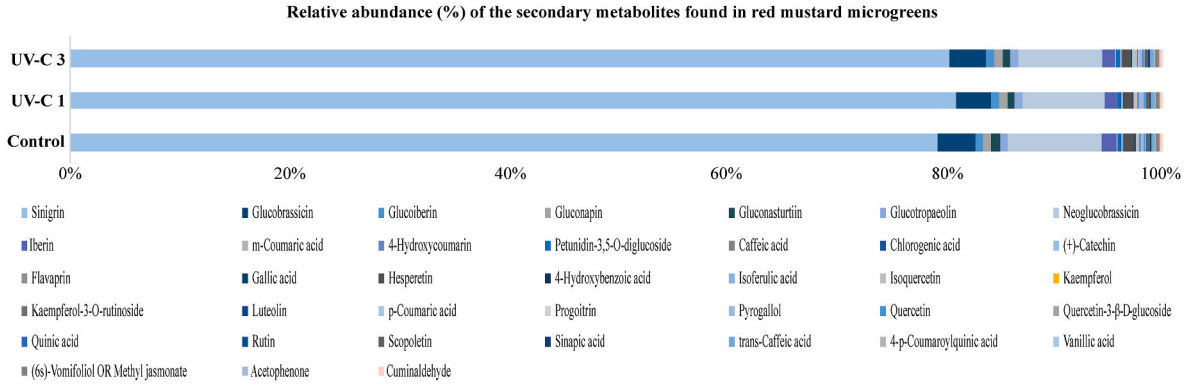

Fig. 4. Relative abundance (%) of secondary metabolites found in red mustard microgreens, determined by LC-Q-Orbitrap-MS/MS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Ruiz: Writing – review & editing, Methodology, Investigation, Formal analysis. **Jesús Marín-Sáez:** Writing – review & editing, Methodology, Formal analysis. **Olga Viegas:** Writing – review & editing. **Miguel Faria:** Writing – original draft. **Roberto Romero-Gonzalez:** Writing – review & editing, Funding acquisition. **Antonia Garrido-Frenich:** Writing – review & editing, Funding acquisition. **Susana M.P. Carvalho:** Writing – review & editing, Funding acquisition, Conceptualization. **Isabel M.P.L.V.O. Ferreira:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marta Silva reports financial support was provided by Foundation for Science and Technology. Susana Carvalho reports financial support was provided by Foundation for Science and Technology. Roberto Romero reports financial support was provided by Andalusian Association for Research and Industrial Cooperation. If there are other authors, they

Table 2
Effect of hormetic UV-C radiation treatments on the and colour parameters (L*, a*, b* coordinates and ΔE) of red mustard microgreens.

	L*	a*	b*	Δ*E*
Control	21.80 ± 0.26 a	5.76 ± 0.42 a	3.82 ± 0.72 a	–
UV-C 1	21.65 ± 0.53 a	5.69 ± 0.68 a	3.71 ± 0.71 a	1.07 ± 0.66
UV-C 3	21.55 ± 0.67 a	5.26 ± 0.35 a	3.03 ± 0.10 a	1.10 ± 0.54

L* (brightness); a* (red-green); b* (yellow-blue); ΔE, colour differences between treatment and control.
Values are means of three replicates ± standard deviation (SD). Means followed by different letters in the same column represent significant differences ($p \leq 0.05$) according to Tukey's HSD (Tukey's honestly significant difference) test.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101416.

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