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Selenium biofortification of microgreens: Influence on phytochemicals, pigments and nutrients

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ABSTRACT

Kale (*Brassica oleracea* L. var. *sabellica* L.), kohlrabi (*Brassica oleracea* L. var. *gongylodes* L.) and wheat (*Triticum aestivum* L. cv. Bancal) microgreens were cultivated in presence of selenium 20 μmol L^{-1} as sodium selenite and sodium selenate mixture. The influence of this biofortification process was evaluated in terms of biomass production, total Se, macro- and micronutrients concentration, polyphenols, antioxidant activity, chlorophylls and carotenoids levels and total soluble proteins content. The results obtained have shown a significant concentration of total Se in the biofortified microgreens of kale (133 μg Se·g^{-1} DW) and kohlrabi (127 μg Se·g^{-1} DW) higher than that obtained for wheat (28 μg Se·g^{-1} DW). The Se uptake in all the species did not produce oxidative damage to the plants reflected in the bioactive compounds, antioxidant capacity or pigments concentration. These Se-enriched microgreens may contribute to the recommended intake of this nutrient in human diet as to overcome Se-deficiency.

1. Introduction

Microgreens are young seedlings that are typically harvested 1–3 weeks after germination when they are only a few centimeters tall and the first true leaf has appeared (Partap et al., 2023). These small plants are produced from the seeds of vegetables, herbs, grains, ornamental, or even wild species. In recent years, microgreens have gained popularity as a new culinary trend due to their variety of flavors, vibrant colors, appearance, and textures, allowing them to be use in salads, soups, or many other dishes. Furthermore, due to their high nutritional value, microgreens have earned the title of "superfood" or "functional foods" as they contain considerably higher concentrations of phytonutrients and secondary metabolites, such as amino acids, enzymes, pigments, vitamins, polyphenols, and antioxidants, than their mature plant counterparts (Nair and Lekshmi, 2023). This unusual combination of high levels of macro- and micronutrients is related to their important role in health, functioning as antioxidants, and immunomodulators, against inflammation, obesity, cardiovascular diseases, cancer, diabetes, among others (Sharma et al., 2022).

In addition to the short growing time, microgreens also require less space, water, and substrate for large-scale production than mature plants. The production of microgreens ranges from innovative hydroponic techniques to improved greenhouses and vertical farms where virtually no pesticides or herbicides are needed (Kyriacou et al., 2016). These crops can therefore be considered environmentally friendly and can be produced in densely populated areas. With these advantages and nutraceutical values, microgreens are promising targets for increasing the level of essential mineral nutrients in plant foods through biofortification techniques.

Biofortification is a process aimed to improve the nutritional value of crops by increasing the concentration of essential micronutrients in edible portions without sacrificing agronomic characteristics such as yield, or resistance to pests and drought (Dhaliwal et al., 2022). Among the different strategies for obtaining biofortified crops are agronomic or genetic approaches. The latter through conventional breeding, where plant varieties with higher nutrient content are selected and crossed, or using transgenic programs that involve biotechnology studies such as the genetic modification of species to obtain a plant with specific

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characteristics (Sheoran et al., 2022). However, agronomic biofortification has several advantages and has been more widely applied due to its cost-effectiveness and ease of scaling (Teklu et al., 2023). Other approaches, but addressed to a lesser extent, are biofortification through nanotechnology, where nanomaterials are applied to plants alone or as a component of conventional fertilizers (e.g. Zn, Fe or graphene nanoparticles), and green technologies, which involve the use of microorganisms to improve the nutrient status of the soil and the accessibility of nutrients to plants (Dhaliwal et al., 2022). The success of biofortification programs depends on a combination of factors. These include the availability of highly nutritious crop varieties adapted to local conditions, strategies to promote awareness and acceptance among communities, address regulatory issues, ensure sustainable production, and foster public-private partnerships to facilitate widespread adoption of biofortified crops (Van Ginkel and Cherfas, 2023).

Selenium (Se) is an essential trace element for humans and a component of biologically important Se-proteins, such as antioxidant enzymes (Guardado-Félix et al., 2020). However, Se-deficiency in diets currently affects 15 % of the world's population, and its prevalence may increase with climate change (Schiavon et al., 2020). One of the solutions aimed at increasing the Se content in foods produced in Se-deficient areas is the biofortification of plants with this element. Biofortification of crops with this nutrient in open field is generally achieved through soil fertilization or foliar application of Se treatments. The most widely used Se compounds are inorganic Se salts (e.g., sodium selenite and/or sodium selenate) due to their low cost and because plants can transform these inorganic forms of Se into bioavailable Se-amino acids (Trippe and Pilon-Smits, 2021). These chemical forms of Se are involved in the maintenance of the immune system, regulation of thyroid function, cognitive function of the brain, antioxidant, and detoxification capacity, anticancer, and antiviral effects (Zhou et al., 2020). Therefore, the combination of Se with bioactive compounds and phytochemicals in microgreens is considered a new trend in the development of functional foods (Islam et al., 2020; Mezeyová et al., 2022; Newman et al., 2021; Pannico et al., 2020). Phenolic compounds, carotenoids, and other secondary metabolites with antioxidant capacity can scavenge free radicals and protect against high oxidative stress and related diseases. The high bioavailability of these compounds in microgreens may have anti-cancer, anti-microbial, anti-inflammatory, and antidiabetic properties (Zhang et al., 2021).

The aim of the present study is the production of microgreens biofortified with Se suitable for human consumption. To this end, three microgreens (kale, kohlrabi, and wheat) were selected based on their nutritional values and high demand in the food market. These plants were exposed to the combination of selenite and selenate ions throughout their growth time, since it is well known that selenate is more easily transported from roots to shoots where it can accumulate but selenite metabolism to bioavailable organic species is faster and consumes less energy for plants. In previous works, the mixture of both species resulted in a modulation of these two important parameters: the accumulation and the toxic effect of each specie separately and the increase in the production of desired selenoamino acids in the consumable parts of the plants. Other analyses focused on the Se uptake and its influence on macro- and micronutrients levels and on the bioactive compounds, pigments and total soluble proteins produced by microgreens.

2. Materials and methods

2.1. Chemicals

Sodium selenate (Na_2SeO_4, 98 %), nitric acid (HNO_3, 69 %) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox reagent) were obtained from Thermo Fisher Scientific Inc. (Barcelona, Spain). Sodium selenite (Na_2SeO_3), Folin–Ciocalteau reagent and sodium carbonate (Na_2CO_3) were from VWR International (Barcelona, Spain). Hydrogen peroxide (H_2O_2, 30 %), methanol (HPLC grade) and acetone were acquired in Scharlab (Barcelona, Spain). Gallic acid and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) were purchased from Sigma-Merck (Schnelldorf, Germany).

2.2. Plant material and growth conditions

The experiment was carried out according to a randomized design in a factorial arrangement (2 × 3), with two concentrations (0 or 20 μmol L^{-1}) of a mixture of Na_2SeO_3 and Na_2SeO_4 (1/1, v/v) in tap water and three species of microgreens (Ramos et al., 2011; Woch and Hawrylak-Nowak, 2019). Seeds of kale (*Brassica oleracea* L. var. *sabellica* L.- InstaGreen SL, Spain), kohlrabi (*Brassica oleracea* L. var. *gongylodes* L. - InstaGreen SL, Spain) and wheat (*Triticum aestivum* L. cv. Bancal- Fitó S. A., Spain), were sown in cellulose sheets (7.5 × 11.5 cm) on plastic cups (10 × 14.5 × 5 cm) distributed in trays with a capacity for 40 cups. The plants were exposed to the solution with or without Se for 15 min twice a day. The study was carried out in a controlled environment of temperature (25.8 ± 0.6 °C) and relative humidity (59 ± 7 %) and considering a photoperiod of 16 h day/8 h night with a light intensity of 35 μmol $m^{-2}{\cdot}s^{-1}$. The average of pH and conductivity value of the solutions used was 7.6 ± 0.1 and 1.5 ± 0.3 mS cm^{-1}, respectively. After the specific growth period for each microgreen (Table 1), the shoots were cut at a height of ~1.0 cm from the cellulose sheet. The growth of microgreens was analyzed to confirm the effects of different concentrations of Se. Biomass yield (g per cup) was calculated in terms of fresh weight (FW) and dry weight (DW). To determine DW, the plants were dried in an oven at 50 °C until constant weight. The experiment was repeated twice.

2.3. Total selenium, macro- and micronutrients

The dry plant material (200 mg) was digested with 10 mL of a mixture of HNO_3/H_2O_2 (7:3, v/v) in a closed vessel of HP500 PFA at 180 °C and 1.9 atm for 45 min using a microwave digestion system (Mars 5, CEM, USA). The digested samples were filtered using 0.22 μm syringe filters and diluted until 3 % HNO_3. The samples were analyzed for Se, sulfur (S), sodium (Na), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7900 ICP-MS system (Agilent Technologies, Inc., USA).

2.4. Total polyphenolic compounds

The extraction of phenolic compounds was carried out following the method reported by Newman et al. with some modifications (Newman et al., 2021). For extraction, 0.5 g of fresh weight microgreen samples were mixed with 5 mL of methanol/water (80/20, v/v), stirred for 2 h in the dark, sonicated in an ice bath for 15 min and then centrifuged at 2200 rpm for 10 min. The supernatant was collected, and the extraction process was repeated. Supernatants were combined and stored at −20 °C until analysis. The analysis of total polyphenolic compounds (TPC) was carried out according to the procedure of Singleton et al. (1999) with the Folin–Ciocalteau reagent. In this case, 100 μL of the plant extracts and

Table 1
Cultivation characteristics of the microgreen species.

Common name	Scientific name	Density of seeds per cup (g)	Cultivation days in:	
			Darkness	Light
Kale	*Brassica oleracea* L. var. *sabellica* L.	2.0	5	3
Kohlrabi	*Brassica oleracea*. L. var. *gongylodes* L.	2.3	7	3
Wheat	*Triticum aestivum* L. cv. Bancal	20	5	8

500 μL of the Folin–Ciocalteau reagent (0.2 N) were mixed and incubated for 5 min in the dark at room temperature. Then, 400 μL of sodium carbonate (75 g L^{-1}) was added and the solution was incubated again for 2 h under the same conditions as before. Finally, 200 μL of the solution was added to a 96-well microplate and the absorbance was measured at 760 nm with an Infinite M200Pro microplate reader (Tecan Trading AG, Switzerland). TPC were determined using a gallic acid standard curve (0–150 mg L^{-1}) and were expressed in mg of gallic acid equivalents (GAE) per gram of FW.

2.5. Antioxidant activity

Antioxidant activity was measured using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Brand-Williams, Cuvelier, and Berset (Brand-Williams et al., 1995). The analysis was done following the method of (Villalva et al., 2020) with some modifications. Briefly, 2.925 mL of DPPH solution in methanol (0.1 mmol L^{-1}) was mixed with 0.075 mL of methanolic extracts of microgreens, vortexed for 10 s, and incubated in the dark for 30 min at room temperature. The DPPH remaining in the solution after this time was measured at 515 nm in the 96-well microplate. Trolox standard solutions (0–1.25 mmol L^{-1}) were used to obtain the DPPH inhibition curve following the same treatment of the samples. The results were expressed as μmol of Trolox equivalent antioxidant capacity (TEAC value) per gram of FW.

2.6. Chlorophylls and carotenoids

Pigments were determined as total chlorophylls (T Chl) and total carotenoids (T Car). Pigment extraction was performed by adding 10 mL of acetone/water (80/20, v/v) to 100 mg of lyophilized and ground plant tissues. The mixture was stirred for 10 min, centrifuged at 3500 rpm for 10 min, and the supernatant was filtrated through a 0.45 μm syringe filter. The solution was made up to 25 mL with the acetone solution. The absorbance of the solution was analyzed at 440, 646 and 663 nm wavelengths (UV double-beam spectrophotometer from UNICAM, model UV-2 200, USA). Pigment concentration was determined using the equations for chlorophylls (equations (1)–(3)) of Lichtenthaler and Welburn (Lichtenthaler and Wellburn, 1983) and the equation for carotenoids (equation (4)) of Holm (1954), where A is the absorbance of the samples at the corresponding wavelengths. The results were expressed as mg pigment 100 g DW^{-1}.

$$Chl_A\ (\mu g{\cdot}mL^{-1}) = 12.21 \times A_{663} - 2.81 \times A_{646} \quad (1)$$

$$Chl_B\ (\mu g{\cdot}mL^{-1}) = 20.13 \times A_{646} - 2.81 \times A_{663} \quad (2)$$

$$T_{Chl}\ (\mu g{\cdot}mL^{-1}) = Chl_A + Chl_B \quad (3)$$

$$T_{Car}\ (\mu g{\cdot}mL^{-1}) = 4.69 \times A_{440} - 0.268 \times T_{Chl} \quad (4)$$

2.7. Total soluble proteins

Total soluble protein concentration was determined using the Bradford assay as described by D'Amato et al. (2018). Initially, 0.1 g of dry shoots were mixed with 25 mL of 0.05 M Tris-HCl buffer (pH 9.0). After centrifugation at 12,000 g for 20 min at 4 °C, the supernatant containing the extracted soluble proteins was collected. To quantify the protein concentration, 30 μL of the supernatant was mixed with 1500 μL of Bradford reagent and vortexed. After a 5-min incubation to allow color development, absorbance was measured at 595 nm. Bovine serum albumin (BSA) was used as a standard for calibration, and protein concentration in the sample was expressed as mg of BSA per gram of dry weight (mg BSA·g^{-1} DW).

2.8. Data analysis

All analyses were performed for the two experiments with three replicates of each microgreen treated with and without Se (n = 6). Data analysis was performed with OriginPro 9.0 software using one-way analysis of variance (ANOVA) to determine the differences between treatments. The comparison of means was done by the Tukey test at the 5% significance level (p-value ≤ 0.05).

3. Results and discussion

3.1. Biomass yield

The observed differences in biomass yield between the different species of microgreens, expressed as FW or DW per cup (Fig. 1A), are due to their specific physiological characteristics, the different cultivation time of each species and/or the density of seeds per cup. In general, the growth of the different biofortified microgreens did not change in terms of biomass produced compared to the control plants. This indicates that biomass yield was not affected by the addition of Se. These results are consistent with the fact that the plants showed no visible symptoms of toxicity. Similar results were obtained for other short-term crops biofortified with different Se concentrations, such as broccoli (Ramos et al., 2011), basil (Chomchan et al., 2017; Pannico et al., 2020) or rice (Puccinelli et al., 2019) where biomass yield was not affected by biofortification. In the present work, we suggest that this effect is probably due to the fact that the plants are able to tolerate this Se concentration and eliminate the excess by transforming Se compounds into the volatile forms dimethyselenide and dimethydiselenide to avoid toxicity as Chomchan et al. (2017) stated. These volatilized forms of Se are about 600 times less dangerous than inorganic Se.

3.2. Total selenium, micro- and macronutrients concentration

The total selenium concentration, expressed on a DW basis, was significantly higher in the Se-enriched microgreens compared to their respective controls (Fig. 1B). Similar Se uptake was observed in kale (133 mg Se·kg^{-1} DW) and kohlrabi (137 mg Se·kg^{-1} DW) microgreens on average, while it was significantly lower for wheat (28 mg Se·kg^{-1} DW).

The results obtained demonstrate the effectiveness of the experiment performed in the bioaccumulation of Se, achieving the biofortification of these microgreens in accordance with previous works. Sprouts of green cabbage, kale and other Brassica species (Ávila et al., 2014) showed a similar accumulation of Se (approximately 160 mg kg^{-1} DW) when treated with 50 μmol L^{-1} of sodium selenate. In our findings, kale and kohlrabi microgreens accumulate more Se than wheat, confirming the known ability of Brassica species to accumulate large amounts of S (Table 2) and concomitantly Se due to their chemical similarity in terms of properties. The uptake, translocation, and metabolism of Se mimic those of S which plays the same role in biochemical systems. Therefore, the substitution of sulfur for selenium results in selenium-analogous compounds that increase the selenium content.

In another study (Islam et al., 2020), biofortification of wheat microgreens was performed under hydroponic conditions, but with different selenite concentrations, a lower Se dose (12.7 μmol L^{-1} Se), and higher total Se concentrations (140 mg kg^{-1} DW) if compared to our experiment. The increase in Se accumulation could be related to the absence of competition between Se and essential ion transporters in a nutrient-deficient growth medium. In the present work, additional nutrients from tap water (Table S1), especially phosphate and sulfate ions, could affect Se uptake by wheat roots (Nothstein et al., 2016).

For other microgreens or sprouts, the total Se concentration found is directly related to the ability of the plants species to uptake and accumulate Se in their shoots, the Se species used in biofortification (selenite or selenate), the number of days of exposure to the treatment and

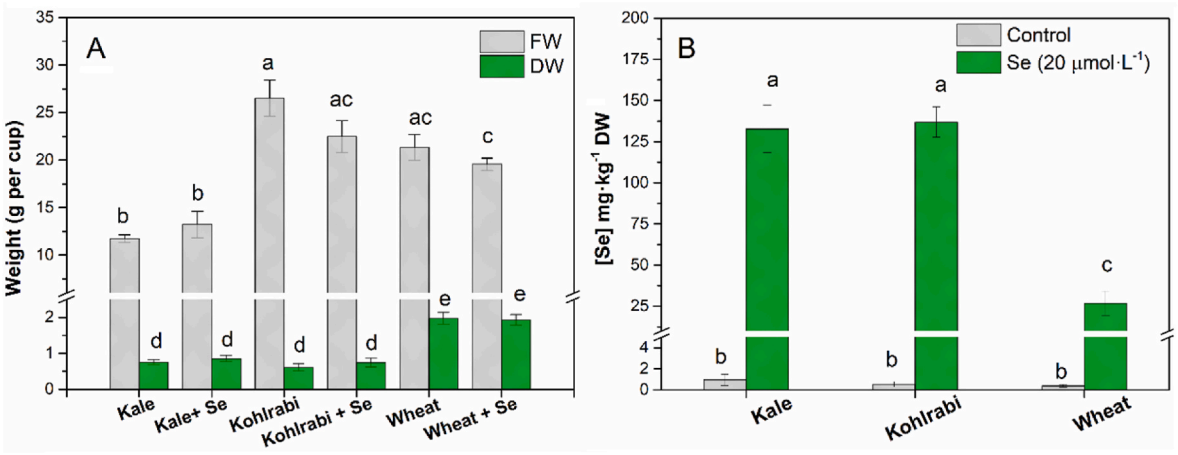

Fig. 1. (A) Fresh weight (FW -green bars) and dry weight (DW- grey bars) and (B) Total Se concentration obtained for kale, kohlrabi and wheat microgreens treated with selenium (Se) and their respective controls. Bars indicate the mean ± standard deviation (SD) of three replicates from two different experiments (n = 6). Tukey's significance at $p \leq 0.05$ among treatments is indicated by different letters within the species of microgreens.

Table 2
Macro (g·kg^{-1} DW) and micronutrients (mg·kg^{-1} DW) concentration in biofortified kale, kohlrabi and wheat microgreens and the corresponding controls.

Macronutrients (g·kg^{-1} DW)	Mg	P	S	K	Ca
Kale	5.7 ± 0.5 b	10 ± 1 a	21 ± 2 a	23 ± 3 a	11 ± 2 b
Kale + Se	5.9 ± 0.3 b	10.7 ± 0.9 a	23 ± 1 a	22 ± 4 a	12 ± 1 b
Kohlrabi	7.6 ± 0.7 a	10.9 ± 0.6 a	22 ± 1 a	25 ± 4 a	19 ± 3 a
Kohlrabi + Se	7.4 ± 0.3 a	11.0 ± 0.6 a	24 ± 3 a	26 ± 4 a	19 ± 3 a
Wheat	3.2 ± 0.1 c	8.7 ± 0.1 b	3.77 ± 0.05 b	12.3 ± 0.4 b	4.1 ± 0.2 c
Wheat + Se	3.1 ± 0.4 c	9.1 ± 0.5 b	4.0 ± 0.3 b	12.7 ± 0.7 b	3.8 ± 0.6 c

Micronutrients (mg·kg^{-1} DW)	B	Mn	Fe	Ni	Cu	Zn	Mo
Kale	52 ± 8 a	43 ± 9 ab	93 ± 15 ab	1.7 ± 0.1 ab	13 ± 2 a	60 ± 6 ab	0.63 ± 0.09 b
Kale + Se	51 ± 6 a	48 ± 6 a	108 ± 15 a	2.1 ± 0.5 ab	10 ± 2 b	63 ± 5 a	0.65 ± 0.08 b
Kohlrabi	56 ± 7 a	38 ± 4 b	77 ± 7 b	1.6 ± 0.1 b	11 ± 2 ab	56 ± 3 ab	0.67 ± 0.09 b
Kohlrabi + Se	57 ± 10a	39 ± 4 b	82 ± 10 b	2.2 ± 0.2 a	7 ± 1 c	52 ± 5 b	0.58 b ± 0.09 b
Wheat	58 ± 6 a	52.8 ± 0.2 a	79 ± 6 b	1.8 ± 0.2 ab	13.2 ± 0.6 a	36 ± 1 c	1.36 ± 0.04 a
Wheat + Se	42 ± 4 a	48 ± 4 a	80 ± 10 b	1.6 ± 0.2 ab	14 ± 2 a	44 ± 8 bc	1.50 ± 0.20 a

Values are expressed as the mean ± SD of 3 replicates from two different experiments (n = 6) of each microgreen. Tukey's significance at p ≤ 0.05 between treatments is indicated by different letters for each nutrient.

presence of nutrients in the medium (D'Amato et al., 2018; Newman et al., 2021; Puccinelli et al., 2019). Plants species differ strongly in the uptake and accumulation of Se in shoots and in their ability to tolerate high concentrations of Se in the roots and/or shoots.

Microgreen seeds and roots take up Se by passive diffusion using sulfate or phosphate carriers and there is a clear difference in the uptake of selenite or selenate. Selenate is actively taken up by the roots via sulfate channels (*SULTRs*) in their plasma membrane. Therefore, there is competition with sulfate, with high concentrations of S suppressing selenate uptake and low levels enhancing it. Selenite uptake is driven by other processes (phosphate transporters and aquaporins), as excess or deficiency of P effectively alters the uptake of Se and aquaporins involved in silicon transport. Furthermore, in addition to the presence of competing S and P, Se uptake rate also depends on the concentration of plant-available Se in the soil and conditions in the rhizosphere such as pH and redox potential, as Se ions are absorbed through the surface of the plasma membrane via an electrochemical gradient into the epidermal cells of the roots (Trippe and Pilon-Smits, 2021) .

Selenite is easily assimilated to organic forms in plant roots, limiting root-to-shoot translocation, while selenate is rapidly translocated. The presence of nutrients such as S has a protective effect against Se toxicity both through anionic competition during uptake in the roots and during subsequent transport of Se in the plant and in the incorporation of Se versus S into proteins (Zhou et al., 2020).

The macro- and microelement profiles obtained (Table 2) are comparable to those reported in previous works on microgreens or more mature plants, however the available data published on the mineral composition of microgreens are scarce, limiting the comparison (Lenzi et al., 2019; Mezeyová et al., 2022; Newman et al., 2021; Pinto et al., 2015).

In the case of macronutrients (Mg, P, S, K and Ca), the most abundant element was K followed by S, Ca, P, and Mg of the three species tested, the two Brassicaceae microgreens (kale and kohlrabi) had similar amounts of K, S, and P, but differed in Ca and Mg levels, with kohlrabi having the highest values. Wheat had the lowest macronutrient levels among microgreens. No significant differences were found between the microgreens biofortified with Se and their respective control in terms of the concentration of the above-mentioned elements.

Among them, it should be highlighted that the S concentration in the three crops studied was not affected by Se biofortification, considering that the preferential uptake of selenate or sulfate differs among plant species. The similarity between these two elements can lead to the substitution of S-amino acids by Se-amino acids, which results in negative changes in the tertiary structure of the protein and leads to Se toxicity for the plants (Gupta and Gupta, 2017).

In contrast, significant differences were observed between microgreens, for micronutrients, except for B. As expected, Fe was the microelement with the highest accumulation in microgreens, followed

by Zn, B, and Mn with values close to half and similar ranges between them. The micronutrients with the lowest values and in accordance with the need that plants have for them were Cu, Ni and Mo. The highest concentrations were found in kale (Fe, Mn, and Zn) and wheat (Cu and Mo) compared to kohlrabi, contrary to the values of the macronutrients. Statistically significant differences between the control and Se-enriched microgreens, were found only for Cu for kale and kohlrabi (Cu concentration decreases with biofortification) and Ni for kohlrabi (Ni concentration increases with Se uptake). Some works report the influence of Se on the transport and accumulation of micronutrients such as Cu or Ni (Cipriano et al., 2022) as Se taken up by plants can alter the ionic permeability coefficient in the cells of the plasma membrane, and thus the uptake of other ions such as micronutrients. Pazurkiewicz-Kocot et al. (2008) reported that Se reduces Cu concentration in the leaves of biofortified corn and suggested that these may be the first symptoms of the effects of Se on plants. Other possible explanations for reduced nutrient uptake are reduced root growth and poor root penetration under Se stress or competition between Se and nutrient transporters and bioligand sites, that inhibit the transfer of nutrient elements (Qu et al., 2023).

Therefore, it can be assumed that biofortification with Se (20 μmol L^{-1}) for does not affect the uptake and accumulation of macro- and micronutrients for these three plants species. These minerals are of crucial importance for the nutraceutical properties and consumption of these plants as foods.

Another aspect to consider is the recommended daily allowance (RDA) of 55 μg Se·day^{-1} for adults to maintain the proper functioning of metabolism and the expression of selenoproteins (Institute of Medicine and Food and Nutrition Board, 2000) The estimated dietary Se intake (EDI, μg·day^{-1}), from the consumption of Se-enriched kale, kohlrabi, and wheat microgreens, is shown in Table 3. These values were calculated on FW basis and assuming a daily consumption of 20 g of fresh microgreens per person (Lenzi et al., 2019; Pinto et al., 2015).

The values obtained indicate that the daily consumption of these plants could provide sufficient Se to meet the RDA criteria. In all cases, the results obtained are below the toxic threshold set at more than 400 μg·day^{-1} (Institute of Medicine and Food and Nutrition Board, 2000). However, it should be noted that other foods naturally rich in Se present in the diet can increase total Se intake.

3.3. Bioactive compounds: total polyphenolic compounds and antioxidant activity

Se-biofortification of microgreens is not only suitable for supplying humans and animals with the necessary amounts of this nutrient, but also for influencing the content of bioactive compounds produced by these plants, which have beneficial effects on health and metabolism (Pannico et al., 2020).

In our study, the total polyphenolic compounds (TPC) content and antioxidant activity of the three microgreens were not affected by Se treatment (Fig. 2). In contrast to antioxidant activity, no significant differences were found in TPC between the species. The TEAC value was higher in kohlrabi + Se (10.3 ± 0.6 μmol Trolox·g^{-1} FW), followed by kale + Se (8.3 ± 0.7 μmol Trolox·g^{-1} FW) and the lowest value was obtained in wheat + Se (7.3 ± 0.2 μmol Trolox·g^{-1} FW). In a study conducted by (Tomas et al., 2021), kale and kohlrabi showed similar trends in antioxidant activity, but the values largely depended on the antioxidant activity assay and the cultivation parameters of the microgreens.

Considering that the accumulation of polyphenols in plants is usually associated with stress conditions during the growth cycle (Gupta and Gupta, 2017) , such as the presence of Se in excessive doses, our results suggest that the Se concentration of 20 μmol L^{-1} used for biofortification does not induce abiotic stress in microgreens species. Other studies have reported comparable results when similar concentrations of Se were used in different microgreens species (Newman et al., 2021; Puccinelli et al., 2021). Due to its antioxidant effect, Se counteracts oxidative stress at low concentrations by inhibiting lipid peroxidation and increasing the activity of glutathione peroxidase (GSH-Px) with a positive effect on plant growth at low concentrations. Increased antioxidation associated with an increase in GSH-Px activity can delay plant senescence and decrease postharvest losses. On the contrary, at high concentrations of Se, particularly selenite, it can function as a pro-oxidant, causing oxidative stress in leafy vegetables, by increasing H_2O_2, malondialdehyde (MDA) content and lipid peroxidation (Puccinelli et al., 2017). Sometimes, when Se does not induce changes in total phenolic compounds, its effect may be on a specific compound and a phenolic compound profile must be performed. There is, however, disagreement in the literature about the impact of selenium (Se) on phenolic content and antioxidant activity. These differences can be related to plant species, growth stage, and Se treatments.

3.4. Pigments: chlorophylls and carotenoids

The values of photosynthetic pigments are presented in Table 4. The concentration of chlorophyll A was the highest in wheat microgreens (651 ± 92 mg·100 g^{-1} DW) and similar in kale and kohlrabi. Chlorophyll B and total chlorophyll values had no significant differences between the three species. The level of carotenoids was also similar between species. These results suggest that the pigment content is variable between microgreens species, considering that the concentration of these phytochemical compounds increases during leaf development.

However, no significant changes were observed in the concentration of Chls and Car between the biofortified microgreens and their respective controls, except for carotenoids in kohlrabi which increases from 96 to 130 mg·100 g^{-1} DW with Se supply.

Biofortification with Se could influence the biosynthesis of chlorophylls and carotenoids in microgreens by promotioning electron flow in the respiratory chain, protecting chloroplast enzymes and producing radical oxygen species (ROS), according to Lanza and Reis (2021). However, our results indicate that, in general, there is no significant influence of Se in the accumulation of chlorophyll or carotenoid pigments at the biofortification levels achieved for these species. The current findings are consistent with the concentration of bioactive compounds, as no indication of stress were observed.

The increased level of carotenoids in kohlrabi may be directly related to biofortification. In previous works on Se biofortification in wheat microgreens (Islam et al., 2020), the total carotenoid content increased compared to control plants. The authors suggested that this could be related to the high rate of photosynthesis and the application of Se in the restoration of chloroplasts damaged by environmental stress and ROS. In that work, biofortification was carried out with selenite in different concentrations and growth periods compared to the present work, which resulted in an increase in TPC and antioxidant activity, as well as pigments, but in a decrease of plant performance. When these results are compared to our own, they confirm that many parameters can influence the biofortification process and that plants can produce different amounts of phytochemical compounds at different Se concentrations. It is important to note that the effect of photoperiod on chlorophylls levels can vary depending on plant species and environmental conditions. Other factors, such as temperature and nutrient availability, can also

Table 3
Se concentration in FW and estimated daily intake (EDI) of Se from microgreens consumption.

Microgreens	Se concentration in FW (μg Se·g^{-1} FW)	Estimated dietary intake of Se (EDI, μg·day^{-1})
Kale + Se	8.6	172
Kohlrabi + Se	4.8	96
Wheat + Se	5.4	108

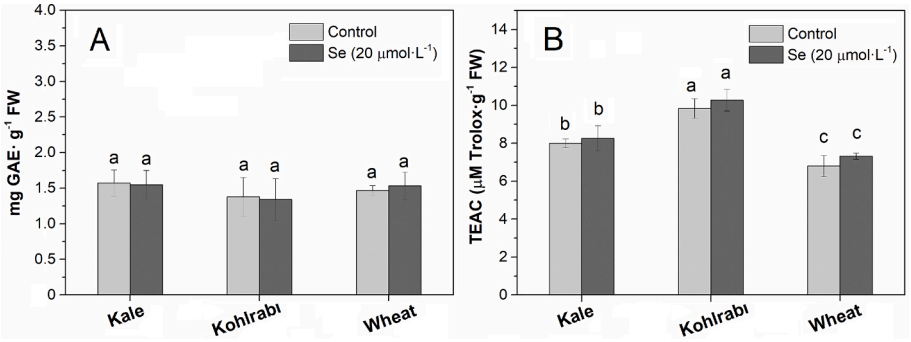

Fig. 2. Total polyphenolic compounds (A) and antioxidant activity (B) of kale, kohlrabi, and wheat microgreens biofortified with Se and their corresponding controls. Values are expressed as the mean ± SD of 3 replicates from two different experiments (n = 6) for each microgreen. Tukey's significance at $p \leq 0.05$ between treatments is indicated by different letters.

Table 4
Chlorophylls (Chl_A, Chl_B and T_{Chl}) and total carotenoids (T_{Car}) concentrations of kale, kohlrabi, and wheat microgreens biofortified with Se and their corresponding controls.

Microgreens	Chl_A (mg·100 g^{-1} DW)	Chl_B (mg·100 g^{-1} DW)	T_{Chl} (mg·100 g^{-1} DW)	T_{Car} (mg·100 g^{-1} DW)
Kale	456 ± 41 b	186 ± 15 a	642 ± 55 a	137 ± 9 ab
Kale + Se	457 ± 56 b	181 ± 18 a	638 ± 73 a	133 ± 10 a
Kohlrabi	394 ± 29 b	163 ± 12 a	557 ± 40 a	96 ± 12 c
Kohlrabi + Se	489 ± 76 bc	180 ± 17 a	669 ± 93 a	130 ± 23 ab
Wheat	651 ± 92 a	167 ± 11 a	818 ± 93 a	136 ± 31 ab
Wheat + Se	570 ± 58 ac	164 ± 10 a	734 ± 65 a	104 ± 20 bc

Values are expressed as the mean ± SD of 3 replicates from two different experiments (n = 6) for each microgreen. Tukey's significance at $p \leq 0.05$ between treatments is indicated by different letters.

affect chlorophyll production and accumulation in plants. Therefore, it is necessary to consider all these factors when studying the relationship between photoperiod and chlorophyll levels in plants.

3.5. Total soluble proteins

Total soluble protein (TSP) content was affected by selenium in kale and kohlrabi microgreens but not in wheat specie (Fig. 3). The increase of proteins with increasing selenium levels may be explained with the interference of Se on sulfur assimilation and thus on the selenoamino acids acid synthesis in these two cruciferous microgreens (D'Amato et al., 2018) . The reasons for the differential response in wheat microgreens warrant further investigation.

Overall, the results of this analysis shed light on the complex relationship between Se biofortification, differences between microgreens or plants species, and total soluble protein concentration. Understanding these variations is crucial to control the nutritional potential of microgreens and optimize their growth conditions to meet specific dietary requirements or nutritional preferences.

The most significant properties of each biofortified microgreen species are summarized in Fig. 4.

4. Conclusions

Selenium biofortification of kale, kohlrabi, and wheat microgreens seems to be a suitable alternative to increase the accumulation of this element in these highly nutritious crops using inorganic Se salts. High levels of Se were obtained in all microgreens with this methodology, especially for the two *Brassicaceae* species. The incorporation of Se was achieved without affecting plant biomass, the level of essential macro- and micronutrients, the production of bioactive compounds, antioxidant capacity, and pigment biosynthesis. Furthermore, for all three species, Se intake is within the recommended limits in the human diet to achieve

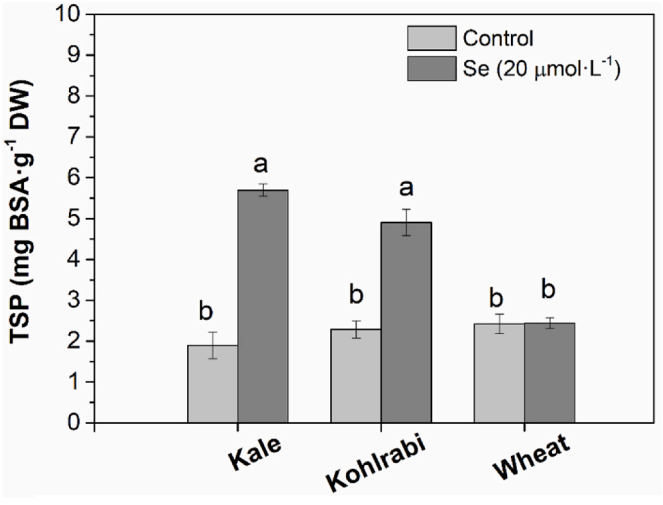

Fig. 3. Protein content in Se biofortified microgreens and their corresponding controls. Values are expressed as the mean ± SD of 3 replicates from two different experiments (n = 6) for each microgreen. Tukey's significance at $p \leq 0.05$ between treatments is indicated by different letters.

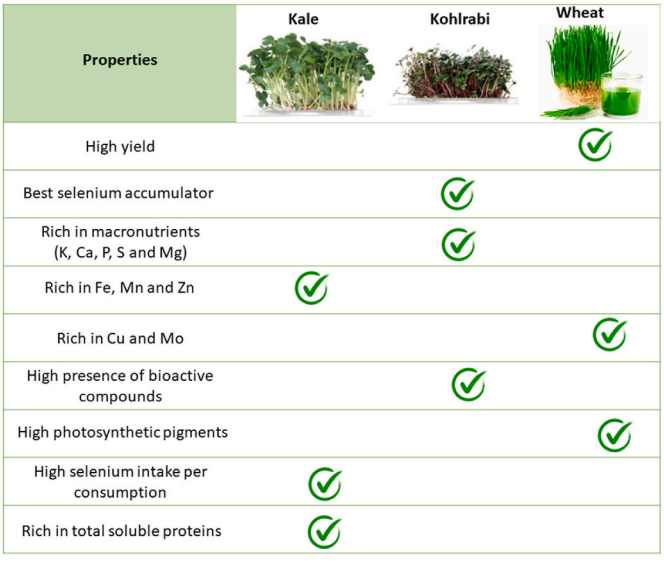

Fig. 4. Summary of the most significant properties of the Se-biofortified microgreens.

the health benefits associated with Se.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions

Marcia Viltres-Portales: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft. **María-Jesús Sánchez-Martín**: Conceptualization, Methodology, Supervision, Writing-Review & Editing. **Roberto Boada**: Conceptualization, Methodology, Writing - Review & Editing. **Mercè Llugany**: Conceptualization, Methodology, Writing - Review & Editing. **Manuel Valiente**: Conceptualization, Methodology, Supervision, Funding Acquisition, Writing - Review & Editing.

Declaration of competing interest

The authors declare no competing financial interests.

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.plaphy.2023.108283.

Abbreviations

Chl_A	Chlorophyll A
Chl_B	Chlorophyll B
DPPH	2,2-diphenyl-1-picrylhydrazyl free radical
DW	Dry weight
EDI	Estimated dietary intake
FW	Fresh weight
GAE	Gallic acid equivalents
RDA	Recommended dietary allowance
ROS	Radical oxygen species
T_{Car}	Total carotenoids
T_{Chl}	Total chlorophylls
TEAC	Trolox equivalent antioxidant capacity
TPC	Total polyphenolic compounds
TSP	Total soluble proteins

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