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Iron and zinc biofortification and bioaccessibility in carrot 'Dordogne': Comparison between foliar applications of chelate and sulphate forms☆

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ABSTRACT

Hidden hunger is a worldwide problem, with iron (Fe) and zinc (Zn) deficiency being the most common causes of mineral deficiency. Vegetable biofortification is an effective strategy to fight mineral deficiency, especially when commonly consumed vegetables are utilized, as in the case of carrots. This biofortification study aimed to investigate the response of the off-season carrot cv. Dordogne to different forms of foliar applications of Fe and Zn. The crop received four applications of both minerals, either in the form of inorganic salt ($FeSO_4$ and $ZnSO_4$) or chelated forms (Fe-DTPA and Zn-EDTA), at a concentration of 6 mM of these elements. $FeSO_4$ was efficient in increasing the Fe concentration in carrots (by 52%), while Fe-DTPA caused no significant differences. Regarding Zn, both forms were effective in the biofortification, but Zn-EDTA proved to be more efficient in increasing root Zn concentration (+94%) than $ZnSO_4$ (+57%). Bioaccessibility data, measured after *in vitro* digestion, showed that biofortified carrots with the chelated forms retained a full bioaccessibility of the minerals (around 100% as in control carrots). However, the sulfate-biofortified carrots showed reduced bioaccessibility values (60% and 80% for Fe and Zn, respectively). The results also showed an increase in dry matter, total nitrogen content and antioxidant activity in plants treated with Zn-EDTA. This trend paralleled the increase of polyphenols and total carotenoids content, suggesting the overall benefit of biofortification strategies conducted in the field. In conclusion, our study revealed that chelated forms of both minerals are preferable in the biofortification programs of carrots.

1. Introduction

Zinc (Zn) and iron (Fe) are at the top of the mineral deficiency in the human diet and among the main determinants of the so-called "hidden hunger" (Stephenson et al., 2018). It affects broad population groups in many countries, including those economically developed (Beleggia et al., 2018; Mantadakis et al., 2020). Zinc is essential for many biochemical and immunological functions, as it is involved in the activity of more than 100 enzymes, besides playing a key role in the synthesis of nucleic acids and proteins (Hacisalihoglu, 2020). The main function of Fe is related to the synthesis of hemoglobin and myoglobin so that it is essential in the transfer of oxygen from the lungs to tissues. In addition, there are many Fe-dependent enzymes making this mineral essential to many metabolic processes (Zoroddu et al., 2019). The recommended daily allowance (RDA) of Zn ranges between 7 and 11 mg day^{-1} and the tolerable upper intake level (UL) for adults is 40 mg day^{-1}. The RDA of Fe ranges between 8 and 18 mg day^{-1}, whereas the UL for adults is 45 mg day^{-1} (Wishart, 2017). Wrong dietary patterns or scarce availability of adequate foods, could make it difficult to reach these RDA values, causing malnutrition problems (Means, 2020), in particular when regarding micronutrients as is the case of Fe and Zn (Buturi et al., 2021).

Vegetables are consumed worldwide and are natural sources of minerals, therefore they could be a good vehicle to increase the intake of these elements in the human diet, by implementing targeted biofortification strategies (Buturi et al., 2022a). In this view, the

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biofortification of vegetables consists in improving the mineral status of plant tissues (Ierna et al., 2020a). The success of this strategy depends on the market acceptance and consumption of the improved food products. Thus, choosing vegetables that are common in the human diet is crucial. This is the case of carrot (*Daucus carota* L.), one of the most popular vegetables worldwide, which is cultivated on a world surface area of approximately 1.13 million hectares and with a production of almost 41 million tons (FAOSTAT, 2022). The product is represented by the taproot of the plant, which is a versatile product that can be consumed fresh or processed in different ways, alone or as part of many recipes (Ierna et al., 2020b).

The biofortification strategy is based on increasing the concentration of essential mineral elements through the application of specific fertilizers on roots or leaves (Buturi et al., 2022b). Foliar sprays are commonly used in fertilization and are known for being more targeted than soil application. Indeed, foliar sprays could be effective in counteracting the low availability of minerals in soil caused by pH anomalies, besides being a simpler, more effective and convenient method, with often faster plant responses to the elements (Lawson et al., 2015; Smoleń et al., 2014). Once the nutrient solution is applied directly to leaves, micronutrients can penetrate the cuticle or enter directly through the stomata (Marschner, 2012; Shahid et al., 2017). However, biofortification efficiency varies depending on the chemical form of the fertilizers. Iron and Zn fertilization are usually based on sulfate or chelated forms and there are controversial debates about which one is more effective (Fernández and Ebert, 2005). In general, biofortification programs carried out through foliar application can benefit from the enhanced phloematic mobility of the minerals, because of the presence of chelating substances such as sugar or other organic metabolites, which facilitate the translocation from leaf to growing sinks as roots, fruits and grains (Gupta et al., 2016).

Iron biofortification studies show successful cases of foliar fertilization conducted using FeII ($FeSO_4$), as is the case of tomato and sweet potato (Carrasco-Gil et al., 2016; Sun et al., 2019). At the same time, similar studies were performed using FeIII in chelated forms, such as Fe-diethylene-triamine pentaacetic acid (DTPA), Fe-ethylenediamine tetraacetic acid (EDTA) or Fe-ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid) (EDDHA) (Kromann et al., 2017; Sida-Arreola et al., 2015). Some authors suggest that $FeSO_4$ is the only foliar fertilizer worth it (Rengel et al., 1999), while others indicate that chelated forms favor translocation and contribute to improving crop yield and activate antioxidant enzymes (Fernández and Ebert, 2005; Sida-Arreola et al., 2015).

Zinc biofortification studies are limited too, and Zn sulfate ($ZnSO_4$) seems to be the most applied inorganic source (Di Gioia et al., 2019; White et al., 2012). Some studies include the use of Zn nitrate (White et al., 2018), while some compare different organic Zn complexes (Almendros et al., 2015). Gupta et al. (2016) suggested that the most effective agronomic fertilizer is Zn-EDTA, but they highlighted the high cost of the molecule. On the other hand, there is still meager information about which forms of Fe and Zn are preferable to maximize, at the same time, their concentrations and bioaccessibility in edible portions, since the biofortification itself can modify the release of minerals from the food matrix (Renna et al., 2022).

For these reasons, the present study compared the efficacy of foliar applications of different chemical forms (chelated *vs*. sulfate) in increasing either Zn or Fe concentrations and the overall quality of biofortified carrots. Moreover, we comparatively investigated the amounts of these minerals released from the obtained food matrices during *in vitro* digestion, with the final aim to set up a targeted agronomic approach maximizing Zn and Fe bioaccessibility for absorption through the human intestine.

2. Materials and methods

2.1. Experimental site, plant material and crop management

A field trial was carried out during the 2019–2020 growing season at a commercial farm located at Ispica plain (Southeastern Sicily: 36°31′07.2″N 15°04′41.5″E, 42 m a.s.l.), one of the most typical areas for early carrot cultivation in Italy. The climate is semi-arid Mediterranean, with mild winters and hot, dry summers. Frost occurrence is virtually absent in winter. During the experiment (from December 15 to May 6) mean monthly maximum and minimum temperatures progressively decreased from December (16.8 and 14.7 °C, respectively) to January (15.1 and 9.1 °C), then increased up to May (22.0 and 15.2 °C).

The soil is a moderately deep, calcic brown on the basis of the USDA Soil Taxonomy Classification (USDA, 1999), with a sandy-loam texture, which, at the beginning of the experiment, comprised low N content (0.8 g kg^{-1}) and low organic matter (12.2 g kg^{-1}), P_2O_5 available (57 mg kg^{-1}), K_2O exchangeable (302 mg kg^{-1}), pH 7.4. All soil analyses were carried out according to the procedures approved by the Italian Society of Soil Science (Violante, 2000).

The experiment was arranged in a randomized blocks design with three replications, including foliar sprays of Fe and Zn either in an organic or chelated form (see below). The cultivar Dordogne was utilized, a hybrid of the Nantes type, which is well-adapted to the Mediterranean growing conditions, and is usually adopted for the production of early carrots.

Seeds were sown at $a \approx 1$ cm depth, through a precision seeder operating in triple rows (0.15 × 0.15 × 0.03 m) on 0.80 m wide ridges; soon after seeding, the ridges were rolled uniformly. The seeding rate was 1.5 million seeds ha^{-1}, whereas the theoretical density was 125 plants m^{-2}. The plot size was 3.6 m × 3.6 m, and consisted of 3 ridges, 3.6 m long. Tillage consisted in a preparatory work of deep ploughing (~40 cm) and ridges setting with a bed-maker for the formation of raised ridges, ≈2 weeks before sowing. One week before sowing, 70 kg ha^{-1} of P_2O_5 (as mineral superphosphate), 150 kg ha^{-1} of K_2O (as K sulfate) and 60 kg ha^{-1} of N (as ammonium nitrate) were applied. Other 60 kg ha^{-1} N were applied in early March. The crop coefficient of carrot adopted was 1.09 (da Silva et al., 2018). Crop water requirements, starting from early spring, were satisfied by rain irrigation, supplying 100% of crop maximum evapotranspiration, when the accumulated daily evaporation, estimated through the Penman–Monteith equation, reached 25 mm. Over the crop cycle, 170 mm of irrigation water were applied. Weeds and pests controls were performed by applying metribuzin and pirimicarb when needed.

2.2. Biofortification treatments

The biofortification protocols were implemented by leaf spraying aqueous solutions enriched with Fe or Zn, either in the form of inorganic salts ($FeSO_4$ and $ZnSO_4$) or chelated forms (Fe-DTPA (diethylenetriaminepentaacetic acid), and Zn-EDTA (ethylenediaminetetraacetic acid)), at a concentration of 6 mM of these elements.

In total, four applications were conducted: the first one was performed on March 4, at the plant stage of full vegetative growth (~30 cm height), while the remaining leaf applications were performed weekly. Leaf sprays were done using a hand pump pressure sprayer. For every treatment, the volume used was 0.25 L m^{-2}. The sprayed solutions contained the non-ionic surfactant Vector® (1 mL L^{-1}; Chimiberg, Caravaggio, BG, Italy) to improve spreading and sticking properties.

2.3. Root physical variables

Roots harvest was manually carried out on May 6 avoiding any damage to leaves. Within each experimental unit, harvested carrots were selected for uniform size and absence of defects, then arranged by hand in 20 bunches each containing 10 roots. Within 4 h from harvest,

all bunched carrots were brought to the laboratory, washed to remove soil particles and dried with paper towels.

In the laboratory, variables such as root length, fresh weight (FW), and dry weight (DW) were determined. Root average fresh weight was determined using an electronic gage (0.01 g accuracy). For the dry weight calculation and mineral content, samples of carrot roots were dried at 70 °C in a laboratory oven (Thermo Scientific-Herathermoven, Waltham, Massachusetts, US), with forced air circulation until a constant weight was reached. After dry weight registration, samples were grounded in a mill and stored at −80 °C for further analyses.

2.4. Root chromatic variables

The external root chromatic coordinates were determined on 3 fresh carrots for each replicate. Measurements were effected on 2 points per root (≈1 cm below the plant collar) through a tristimulus Minolta Chroma meter (model CR-200, Minolta Corp.) calibrated with a standard white tile (UE certificated) with illuminant D65/10∘, measuring color in terms of lightness (L*), green-red axis (a*) and blue-yellow axis (b*) (McGuire, 1992). Root color was described as L*, a*, b* and Chroma [as $(a^2 + b^2)^{½}$].

2.5. Root compositional variables

The determination of root composition included total carotenoids, total phenols and antioxidant assays. All these determinations were performed on lyophilized plant powder by using a Jeanway UV/Visible spectrophotometer (Stone, Staffordshire, UK).

2.5.1. Total carotenoids concentration

Total carotenoids concentration was determined according to Lichtenthaler and Wellburn (1983), with slight modifications. For the extraction, 50 mg of lyophilized carrot powder were mixed with 5 mL of ethanol 96% and vortexed, then the tubes were placed in the ultrasonic bath for 10 min and left overnight in the dark (at 10 °C). After that, tubes were centrifuged for 10 min, and samples were read in 1.5 mL plastic cuvette. Ethanol 96% was used as blank. Readings were done in the following wavelengths: 470, 649 and 665 nm and the absorbance values were applied in the following equations:

- $C_a = 13.95\,A_{665} - -6.88\,A_{649}$
- $C_b = 24.96\,A_{649} - -7.32\,A_{665}$
- $C_{x+c} = (1000\,A_{470} - -2.05\,Ca - -114.8\,Cb)/245$

where, C_a stands for chlorophyll A, C_b stands for chlorophyll B and C_{x+c} stands for total amount of carotenoids [xanthophyll (x) plus carotenes (c)]. Results are expressed in μg 100 g^{-1} fresh weight (FW).

2.5.2. Total phenolic content

Total phenolic content (TPC) was quantified using a modified Folin-Ciocâlteu method (Cicco et al., 2009). For the extraction, 100 mg of lyophilized carrot powder were mixed with 1 mL of 70% methanol and agitated for 1 h at room temperature, then samples were centrifuged at 5000 g for 5 min at 25 °C. 100 μL of extract solution were mixed with 100 μL Folin-Ciocâlteu reagent and allowed to react at room temperature for 2 min. Next, 800 μL of Na_2CO_3 (5% w/v) were added and tubes were left in a temperature bath at 40 °C for 20 min. Samples were read at 760 nm and TPC was reported as μmol gallic acid equivalents (GAE) 100 g^{-1} FW.

2.5.3. DPPH assay

The DPPH (α, α-diphenyl-β-picrylhydrazyl) radical scavenging activity of carrot extracts was determined according to Brand-Williams et al. (1995). For the extraction, 100 mg of lyophilized carrot powder were mixed with 5 mL of methanol (80%) and vortexed for 1 min. Samples were then submitted to 10 min of ultrasonic bath (below 10 °C) and centrifuged for 15 min at 4000 g (6 °C). For the reaction, 150 μL of supernatant was mixed to 1350 μL of DPPH solution (150 μM) vortexed and placed in the dark for 30 min. The decrease in the absorbance of methanolic solution of DPPH was read at 515 nm and DPPH was calculated from a standard curve prepared by plotting change in absorbance against different concentrations of Trolox and expressed as μmol Trolox equivalent (TE) 100 g^{-1} FW.

2.5.4. FRAP assay

The ferric reducing antioxidant power (FRAP) assay of carrot extracts was determined according to Benzie and Strain (1999). For the extraction, 200 mg of lyophilized carrot powder were mixed with 10 mL of methanol 100%, vortexed for 1 min and placed in the dark for 30 min. After that, samples were centrifuged for 10 min at 4500 g (6 °C). Preparation of FRAP reagent consisted of 10 mL of acetate buffer (300 mM, pH 3.1) mixed with 1 mL of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution (10 mM in 40 mM HCl) and 1 mL of $FeCl_3$ (20 mM). For the reaction, 150 μL of supernatant were mixed to 300 μL of ultrapure water, vortexed and added to 3 mL of FRAP reagent. Samples were placed in the dark at 20 °C for 10 min. The FRAP, based on the reduction of Fe(III) by the sample extract, was determined following the change in absorbance at 593 nm due to the formation of a blue-colored Fe(II)-tripyridyltriazine compound from colorless oxidized Fe(III) form in presence of a particular concentration of the sample. FRAP was calculated from a standard curve prepared by plotting change in absorbance against different concentrations of Trolox and expressed as μmol Trolox equivalent (TE) 100 g^{-1} FW.

2.5.5. Mineral analyses

Dry carrots were grounded and submitted to wet digestion before the ICP-MS measurement, according to May et al. (2019). The wet digestion was performed with an infrared-controlled and power-adjusted microwave (Go, Anton Paar, Graz, Austria) up to an end temperature of 180 °C (dwell time of 10 min) using approximately 100 mg of the oven-dried sample, 4 mL supra pure HNO_3 and 2 mL ultrapure water. The digested samples were transferred into polypropylene tubes (Greiner bio-one, Kremsmünster, Austria) and made up to 40 mL with water. Subsequently, an aliquot of 2.5 mL of the latter solution was made up to 10 mL with water under the addition of 100 μL internal standard (rhodium, 1 mg L^{-1} standard concentration prepared from a 1000 mg L^{-1} stock solution of $Rh(NO_3)_3$, Merck, Darmstadt, Germany), yielding 10 μg L^{-1} rhodium in the final solution. Each sample workup and digestion were done in duplicate.

The ICP-MS measurements were carried out with a NexION 300d (Perkin Elmer, Waltham, Massachusetts, USA) equipped with an S10 autosampler (Perkin Elmer), a Meinhard® concentric nebulizer, a cyclonic spray chamber, a quartz torch, and nickel cones. The following operating conditions and acquisition parameters were used: 1550 W RF (radio frequency) power; 15 L min^{-1} in plasma gas flow; 1.04 L min^{-1} nebulizer gas flow; 1.375 L min^{-1} auxiliary gas flow, and 5.2 L min^{-1} He gas flow in KED (kinetic energy discrimination) mode. Calibration was performed using a custom-made multi-element standard solution (Inorganic Ventures, Christiansburg, VA, USA) containing the target elements. The calibration standards were matrix-adjusted by adding HNO_3. To avoid possible polyatomic interferences, several elements were quantitated in KED mode (Mn, Fe, Ca, Na, Mg, and K). The reference material SRM 1570a (Trace Elements in Spinach Leaves, NIST, Gaithersburg, MD, USA) was used to control the accuracy of the method and as a daily quality control standard. LOQ was calculated based on the nine-fold standard deviation of a blank solution prepared and analyzed twelve times. The following LOQs were achieved: Ca: 0.5 mg kg^{-1}, Mg: 0.1 mg kg^{-1}, K: 1.0 mg kg^{-1}, Na: 0.2 mg kg^{-1}, Zn: 30 μg kg^{-1}, Cu: 15 μg kg^{-1} and Mn: 3 μg kg^{-1}. Nutrients were calculated and expressed either as mg 100 g^{-1} FW or μg 100 g^{-1} FW.

2.6. Digestion procedure and bioaccessibility assessment

Carrot samples were cut into small cubes of 2 mm x 2 mm before being milled with a Retsch Ball Mill MM 400 (Retsch, Haan, Germany) for 90 s at a frequency of 30 shakes per second. Five grams of carrot puree were put into a 50 mL Greiner tube. All samples were digested according to the INFOGEST protocol as described by Minekus et al. (2014).

To assess the bioaccessibility of the Zn and Fe from the carrot into the intestine, the mineral amount in the supernatant that was present after digestion was measured. After digestion, 15 mL of the sample was put into a 15 mL Greiner centrifuge tube. All the samples were centrifuged for 15 min at a speed of 4000 g at room temperature. Ten mL of the supernatant of the biofortified samples were taken and analyzed as described in 2.5.5 for the presence of Fe and Zn minerals. The results were obtained in mg 100 g^{-1} fresh carrot and compared to those present in the carrots before digestion.

2.7. Statistical procedures

Collected and calculated data were firstly subjected to Shapiro–Wilk's and Levene's tests, in order to check for normal distribution and homoscedasticity, respectively. Data were then subjected to a one-way analysis of (ANOVA). For all the variables, the comparison between means was performed by calculating the Fisher's protected least significant difference (LSD, $P \leq 0.05$). A Pearson's correlation analysis was also performed, to define possible relationships among mineral concentrations. All calculations were performed using Microsoft Excel® and Minitab version 19 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Carrot quality variables and chromatic coordinates

As shown in Table 1, when compared to control, the average fresh weight of roots was increased in plants treated with $FeSO_4$ (+25%) and with both forms of Zn (+20%, on average), while the dry matter was significantly higher only in the treatment with Zn-EDTA. Root diameter was not affected by any treatment, while roots treated with both Zn forms showed the highest length (+11%). None of the chromatic variables (L*, a*, b* and Chroma) were significantly affected by the biofortification treatments.

3.2. Biochemical variables

Total carotenoids content of roots increased (+4%) in plants treated with Zn-EDTA and decreased (−9%) in those treated with Fe-DTPA, in comparison to control roots. A significant difference in plants treated with Fe-DTPA was also noticed for TPC (−14%) DPPH (−20%) and FRAP (−11%), in comparison to untreated plants (Table 2).

Table 2
Biochemical traits of carrot as affected by Fe and Zn treatments.

Treatment	Total carotenoids (µg 100 g^{-1} FW)	TPC (GAE µmol 100 g^{-1} FW)	DPPH (TE µmol 100 g^{-1} FW)	FRAP (TE µmol 100 g^{-1} FW)
Control	8017 b	56.5 a	49.3 a	49.7 a
Fe-DTPA	7315 c	48.3 b	39.3 b	44.0 b
$FeSO_4$	7981 b	56.3 a	46.5 a	47.8 ab
Zn-EDTA	8337 a	57.3 a	47.0 a	50.7 a
$ZnSO_4$	8318 ab	55.8 a	44.9 a	45.4 ab
LSD	594	6.5	7.0	5.3
F-test	***	*	*	*

Different letters indicate significance fisher's protected LSD Test ($P = 0.05$)
*, **, ***: significance of $P \leq 0.05$, 0.01, 0.001, respectively. NS: not significant.

3.3. Iron and zinc biofortification of carrot roots

As shown in Fig. 1, the Fe content of carrots was promoted by the $FeSO_4$ application (+52% compared to control), whereas decreased in plants receiving $ZnSO_4$ (−35%) (Fig. 1A). On the other hand, Zn content was enhanced by all treatments, showing a 94% increase when submitted to Zn-EDTA and a 57% increase when submitted to $ZnSO_4$, in comparison to control (Fig. 1B).

3.4. Mineral composition of carrot roots

When total N was concerned, the strongest differences in the concentration were recorded among the Zn-EDTA and FeSO4 treatments (103.0 vs. 88.3 mg 100 g^{-1} FW), being the former able to maximize the P and K contents too (Table 3).

The Ca content of carrots submitted to both forms of Fe and Zn-EDTA showed a significant increase as compared to control ones, but no difference when compared to the $ZnSO_4$ plants (Table 3). The Mg content in Zn-EDTA-treated roots increased only in comparison to those receiving $FeSO_4$ (+28%). On the other hand, a higher root Na content than control was recorded in all biofortification treatments, except for the $FeSO_4$ (Table 3). Manganese content was boosted by the two forms of Zn (by 26%, on average) compared to the control. Meanwhile, the Ni content was reduced by Fe-DTPA (−29%) and promoted by $FeSO_4$ (+57%) (Table 3). Copper and molybdenum were not affected by any of the treatments applied.

3.5. Iron and zinc bioaccessibility of carrot roots

The amount of Fe obtained in the intestine fluid at the end of the INFOGEST procedure was 530 mg 100 g^{-1} for carrots biofortified with Fe-DTPA, 521 mg 100 g^{-1} for the biofortification with $FeSO_4$. The absolute values were not significantly different respect to the 610 mg 100 g^{-1} found in the non-biofortified control samples. However, the increased amount present in the $FeSO_4$ biofortified samples was not reflected in the bioaccessibility data (Fig. 2).

The picture is comparable for Zn biofortified samples: 270 mg 100 g^{-1} in carrot samples that were biofortified with Zn-EDTA and 160 mg

Table 1
Morphological traits of carrot as affected by Fe and Zn treatments.

Treatment	Root average fresh weight (g $plant^{-1}$)	Root dry matter (%)	Root diameter (mm)	Root length (cm)	L*	a*	b*	Chroma
Control	88 b	11.0 b	32.0	17.3 b	38.9	32.7	41.0	52.4
Fe-DTPA	98 ab	10.5 b	33.6	17.6 ab	37.6	31.9	40.2	51.4
$FeSO_4$	110 a	10.7 b	34.7	17.1 b	38.1	30.8	38.8	49.5
Zn-EDTA	103 a	11.8 a	33.6	19.2 a	37.9	31.6	39.1	50.2
$ZnSO_4$	108 a	11.0 b	32.5	19.2 a	37.7	32.2	40.7	51.9
LSD	14.4	0.8	3.1	1.6	1.3	1.8	1.7	2.0
F-test	*	*	NS	*	NS	NS	NS	NS

Different letters indicate significance fisher's protected LSD Test ($P = 0.05$)
*, **, ***: significance of $P \leq 0.05$, 0.01, 0.001, respectively. NS: not significant.

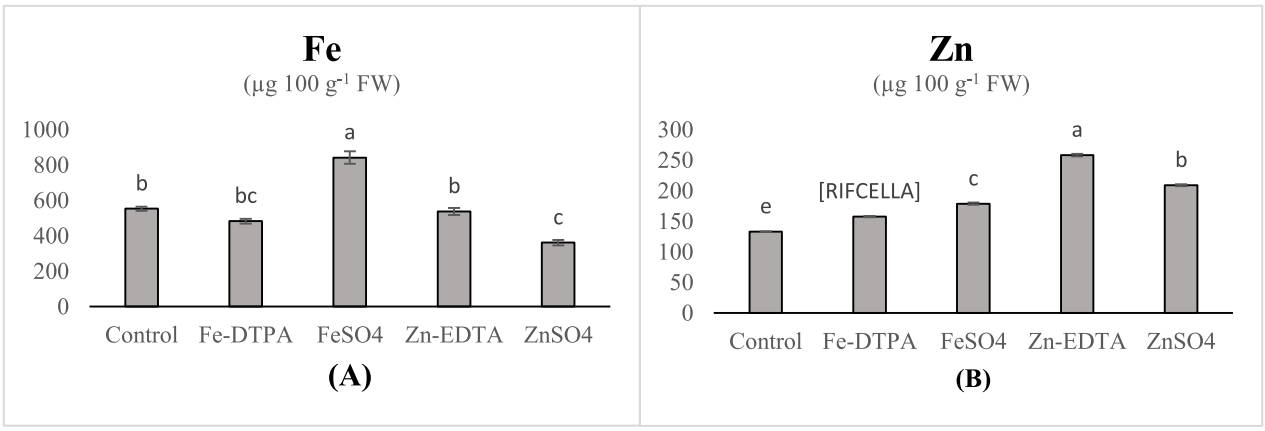

Fig. 1. Iron (A) and Zn (B) content of carrot roots as affected by Fe and Zn treatments. Different letters indicate significance Fisher's protected LSD Test ($P \leq 0.05$).

Table 3
Mineral composition of carrot roots as affected by Fe and Zn treatments.

Treatment	N	P	K	Ca	Mg	Na	Mn	Cu	Ni	Mo
	mg 100 g^{-1} FW						µg 100 g^{-1} FW			
Control	91.3 bc	23.9 d	321 b	30.5 b	10.8 ab	20.6 b	128 b	45.0	3.43 b	1.24
Fe-DTPA	90.9 bc	25.6 cd	322 b	34.7 a	11.9 ab	24.8 a	116 b	44.1	2.43 c	1.26
$FeSO_4$	88.8 c	26.6 bc	322 b	36.4 a	10.2 b	19.4 b	110 b	49.0	5.41 a	1.18
Zn-EDTA	103.3 a	29.4 a	394 a	36.6 a	13.1 a	24.4 a	157 a	45.7	3.48 ab	1.05
$ZnSO_4$	99.3 ab	27.8 ab	340 b	33.2 ab	12.5 ab	24.6 a	165 a	49.2	3.89 ab	1.09
LSD	9.5	1.7	38.3	3.7	2.4	1.7	221	8.0	1.9	0.2
F-test	**	***	**	***	*	***	**	NS	*	NS

Different letters indicate significance fisher's protected LSD Test ($P = 0.05$)
*, **, ***: significance of $P \leq 0.05$, 0.01, 0.001, respectively. NS: not significant.

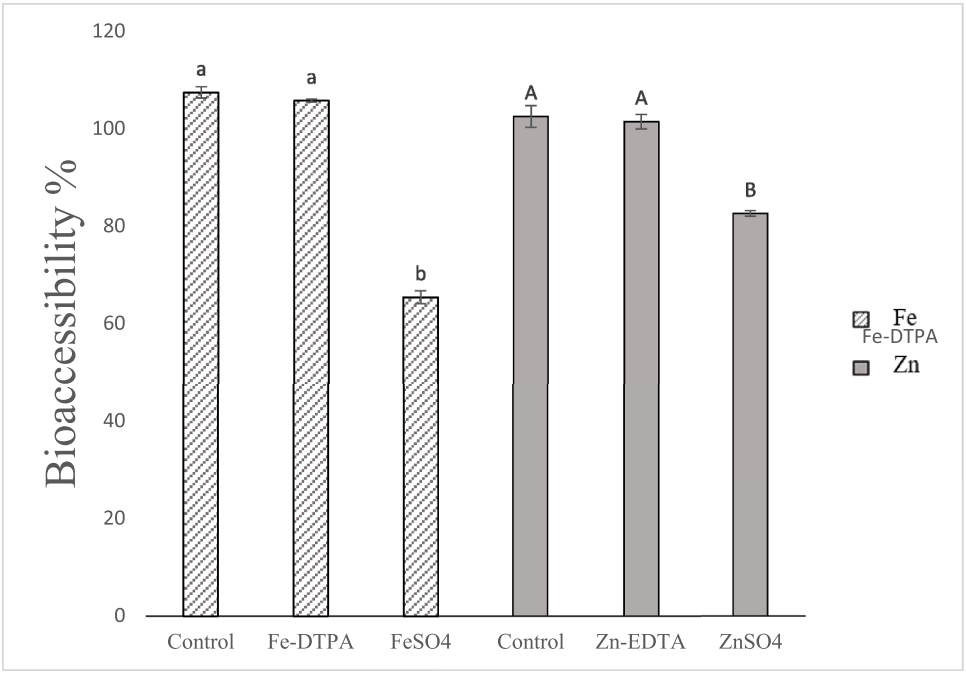

Fig. 2. Fe and Zn bioaccessibility of carrot roots as affected by Fe and Zn treatments. Different letters indicate significance fisher's protected LSD Test ($P = 0.05$). Lower cases represent differences among Fe treatments and upper cases represent differences among Zn treatments.

100 g^{-1} in $ZnSO_4$ biofortified carrots were recovered in the digestive fluid. In this case too, while control and chelated fortified samples have bioaccessibility value in line with the actual carrot content, the sulfate biofortified sample lost the advantage gained with the fortification procedure (Fig. 2).

4. Discussion

4.1. Yield and quality traits

Under the specific conditions of our experiment, the foliar

applications of both forms of Zn promoted roots' fresh weight and length, while the dry matter content was promoted by Zn-EDTA, suggesting a stimulatory effect of Zn on crop photosynthetic metabolism. This is consistent with the findings of Awad et al. (2021) in an experiment with carrots and foliar sprays of Zn-EDTA. After three applications of a 5.7 mM solution, the authors noticed an increase in the root fresh weight and dry matter equal to 39% and 25%, respectively. Mousavi et al. (2007) also reported an increase in tuber yield and dry matter content after treating potato plants with two foliar applications of $ZnSO_4$, in the concentration of 0.122 mM. The same stimulatory effect was observed by Almendros et al. (2015), after submitting onion plants to different forms of Zn, in the concentration of 0.15 mM through soil fertilization. In their experiment, the most efficient chemical form was Zn-EDTA.

In our study, the application of Zn-EDTA promoted multiple morphometric variables of carrots (mainly in terms of fresh weight, dry matter, root length and total N content). This could be explained by the importance of Zn in maintaining the plant's physiological status, through the stimulation of photosynthesis which increases leaf dry matter production leading to an improvement in the plant growth variables (Rizwan et al., 2019). At the same time Zn-EDTA caused a significant increase in the total carotenoids content but no increase in other biochemical parameters. On the other hand, after treating broccoli plants cultivar Parthenon with $ZnSO_4$ at 5 mM, Rivera-Martin et al. (2021) found that the antioxidant activity (as revealed by DPPH and ABTS) and TPC significantly increased compared to control. This suggests that the concentration of Zn-EDTA could be optimized to enhance carrots biochemical traits. Once more, these increases in the antioxidant variables demonstrate the potential of Zn biofortification in improving important quality parameters of vegetables (Barrameda-Medina et al., 2017; Blasco et al., 2015).

Regarding Fe, the effect of foliar applications of $FeSO_4$ stimulated carrot fresh weight, also improving the content of P and Zn. This positive effect suggests that Fe, in the form of $FeSO_4$, could stimulate plant growth, since Fe is involved in the synthesis of chlorophyll and it is also important to complete the enzyme functions that maintain plant's health (Marschner, 2012). In contrast to Fe-DTPA, which did not improve root FW, the stimulatory effect of $FeSO_4$ could be attributed to the presence of sulfur (S), since S application has been proved to improve carrot yield (Singh et al., 2016). However, it should be highlighted that excess of Fe can be toxic to plants, leading to the formation of reactive oxygen species (ROS) (Das et al., 2020). Chelated forms of Fe, as Fe-DTPA, can penetrate leaves more easily than the sulfate form (Ferrandon and Chamel, 1988), being more prone to phytotoxicity effects, a feature that could explain the inhibitory effects of Fe-DTPA on total carotenoids and total polyphenol content and the reduction in the antioxidant activities, looking at the DPPH and FRAP data.

4.2. Mineral biofortification

This stimulatory effect of Zn treatments in our study is also supported by the increase in root concentration of some elements (mainly P, Na, Fe, Zn, Mn), thus suggesting an improved root absorption capacity. This is in accordance with the results showed by Awad et al. (2021) who obtained carrots with a higher content of P, Fe, Mn, Zn and Cu, after foliar application of Zn-EDTA (5.7 mM). This stimulatory effect can be attributed to the fact that Zn plays a key role in increasing membrane function, cell elongation, protein synthesis and positively stimulates plants roots to exchange cations, increasing nutrients absorption (Andresen et al., 2018). On the other hand, White et al. (2017) reported that biofortification of potato with 1.96 g Zn m^{-2} as leaf spray, had little consequence for the concentration of other mineral elements in tubers.

4.2.1. Zn-EDTA vs. $ZnSO_4$

Regarding micronutrients, foliar sprays of Zn were shown to be effective in enhancing the Zn content in carrots, which is one of the main goals of the present biofortification study. In this sense, our results suggest that biofortification of carrot with Zn can be successfully performed with both forms of Zn (Zn-EDTA or $ZnSO_4$), at the concentration of 6 mM of the element. Moreover, among the two chemical forms, Zn-EDTA treatment proved to be more efficient, as carrots showed approximately double mineral concentration when compared to control. The reason why Zn-EDTA presented better results could be related to the fact that the chelated form is more soluble and available for the plant when compared to the sulfate form (Gupta et al., 2016). The positive results obtained in our study concerning Zn biofortification are coherent with those of Awad et al. (2021), as they were able to produce carrots having a Zn concentration 61% higher than those of untreated plants, after spraying with a solution 5.7 mM of Zn-EDTA. Kromann et al. (2017), in a similar experiment with potatoes, using chelated sprays (EDTA) of Zn (3.06 mM) obtained a 2.51-fold increase of the Zn concentration in tubers. Meanwhile, after applying a foliar spray with a lower concentration of Zn (0.122 mM), as $ZnSO_4$, Mousavi et al. (2007) obtained a lower increase (23%) in the tuber Zn concentration, in comparison to control. On the other hand, in a Zn biofortification study of carrots through soil applications, contrasting results were obtained by De Sousa Lima et al. (2015) after applying different doses of Zn (0–300 mg kg^{-1}). They observed no significant increase in the root concentration of Zn, which can be explained by the limited Zn mobility in the soil. This supports the hypothesis that the foliar spray strategy used in this study, could represent the best approach in the Zn biofortification of carrots, probably facilitated by the high solubility and translocation of this mineral in the phloem (Andresen et al., 2018).

4.2.2. Fe-DTPA vs. $FeSO_4$

In the present study, biofortification of carrots using $FeSO_4$ was successful, as the roots showed a 52% increase in their Fe content. The efficacy of $FeSO_4$ we observed for biofortification purpose is consistent with the results of Sun et al. (2019), after applying a Fe-enriched solution (6.6 mM) in the leaves of sweet potato, they obtained tubers with 43% more Fe than the untreated ones. Moreover, our observation is in accordance with that of Zhang et al. (2022), where Fe has been enriched in potato tubers by five foliar applications of sulfate forms of Fe (9 mM). In contrast, the inefficiency of Fe-DTPA in the Fe biofortification observed in our study was confirmed also by Kromann et al. (2017) after applying Fe-EDTA (6.71 mM) on potato leaves, they noticed no significant Fe increase in the tubers. A possible explanation why Fe biofortification was more effective using $FeSO_4$ than Fe-DTPA, could be the lower mobility of Fe chelated inside the leaf, when compared to $FeSO_4$; this was demonstrated by Rios et al. (2016), when tracing the uptake pathway of different forms of Fe in the leaves of *Prunus*. In the case of $FeSO_4$, Fe was found in the vascular areas of the leaf, whereas in the case of Fe(III) salts the stain remained in the stomatal areas. Differently, when using a nutrient solution enriched with Fe-DTPA (0.537 mM), carrots leaves showed a 18% increase in the Fe content, when compared to untreated plants. This suggests that Fe-DTPA could be more easily absorbed and translocated through the nutrient solution rather than through foliar sprays (Gupta and Chipman, 1976). In fact, the translocation of Fe chelate from roots to other organs of the plant, has been demonstrated by Sida-Arreola et al. (2015), supporting the hypothesis that biofortification using Fe-chelate could be more efficient when applied *via* roots using the fertigation rather than as foliar sprays.

According to Rengel et al. (1999), the chelated form of Fe limited the Fe biofortification of carrots, because of the high concentration of the solution: it is possible that the chelate competes with ionized groups in the cuticle. Furthermore, possible phytotoxic effects of the application of Fe-DTPA, at 6 mM, could have impaired Fe biofortification. This was not the first case in which $FeSO_4$ demonstrates to be more efficient than Fe-DTPA in translocating Fe from the leaves to the roots. Aciksoz and co-workers compared foliar sprays of two chemical forms (Fe-DTPA and $FeSO_4$) and showed that, in the case of biofortification, the sulfate form of Fe was more efficient in increasing Fe content in wheat grains

(Aciksoz et al., 2011). Another reason for the limited effectiveness of Fe biofortification of carrots could be the limited mobility of the mineral inside the plant (Kobayashi et al., 2019). Additional Fe biofortification studies in carrots should be performed in order to better comprehend if lower doses of the chelated form of Fe could be, as effective as the sulfate form, used in the biofortification of carrots through foliar applications.

4.3. Bioaccessibility: chelated vs. sulfate forms

Beside studying the fate of the minerals in plant and their accumulation in edible portions, it is extremely important to investigate if they are actually released from the food matrix during the digestion procedure and become bioaccessible for the absorption through the intestine. The data obtained in this study are summarized in Fig. 1 reporting the percentage increase of the bioaccessible minerals irrespective to the amount present in the carrot samples. In the control roots, the amount of minerals detected in the intestinal fluid is slightly above 100% of that present in carrots. This is not statistically significant, and it is possibly due to the enhanced solubility induced by the enzymatic treatments. Looking at the Fe data, similar results were obtained with Fe-DPTA; however, the bioaccessibility dropped to about 60% for the $FeSO_4$ samples suggesting that this type of biofortification, which was very effective in plant, do not provide an actual nutritional benefit. The figure is similar in Zn-biofortified samples: Zn-EDTA has a similar bioaccessibility percentage than the control but in the $ZnSO_4$ fortified samples the Zn bioaccessibility was only about 80% of the expected value.

If the percentage of the bioaccessible minerals had been similar for all samples the increase in the amount of minerals observed in the carrots would have been actually bioaccessible to the human body. These *in vitro* data suggest that this is true for chelate forms of Fe and Zn but not for the sulfate ones.

This result is not in line with a previous study which showed that biofortification with $ZnSO_4$ led to an increase in bioaccessibility of Zn (Zou et al., 2014). Also, another research showed that high ratios of EDTA: Zn even led to an inhibitory effect on the absorption of Zn (Hotz et al., 2005). Another factor that could play a role is the amount of Fe that was present in the Zn-biofortified carrots. For both Zn-EDTA as $ZnSO_4$ and control, carrots contained more Fe^{2+} than Zn^{2+}. In itself this might not pose a problem, as it is the goal to obtain more Fe and Zn; nevertheless, literature suggests that non-heme Fe in a ratio of Fe/Zn of 1:1 slightly inhibits Zn absorption, and a ratio of 2:1 substantially inhibits Zn uptake (Solomons and Jacob, 1981). As non-heme Fe is found in legumes and crops such as carrot or eggplant (Mauro et al., 2022), this antagonist relation between Zn and Fe should be further analyzed.

5. Conclusion

In conclusion, the present study demonstrates that the production of Zn- or Fe- enriched carrot is possible and that the limitation regarding the bioaccessibility of these minerals after human digestion can be countered by using chelated forms of fertilizers, which guarantee that almost 100% of the minerals are accessible for absorption. These results are encouraging because they contribute to the definition of important agronomic biofortification protocols that could allow the introduction of new premium vegetables in the market, that will ultimately improve human diet and, consequently, human health. Agronomic biofortification represents the best strategy in the case of vegetables, since genetic improvement programs would not be convenient due to the high rate of varietal turnover. The challenge for the future is the production of enriched vegetables with low-cost molecules that can also maintain the minerals bioaccessibility while avoiding possible phytotoxic effects that could limit crop yield. In this sense, studies involving different molecules and concentrations are recommended to optimize the efficiency of carrot biofortification.

CRediT authorship contribution statement

Camila Vanessa Buturi: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Rosario Paolo Mauro:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Vincenzo Fogliano:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Cherubino Leonardi:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Francesco Giuffrida:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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 results of this study.

Data availability

Data will be made available on request.

References

Aciksoz, S.B., Yazici, A., Ozturk, L., Cakmak, I., 2011. Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. Plant Soil 349, 215–225. https://doi.org/10.1007/s11104-011-0863-2.

Almendros, P., Obrador, A., Gonzalez, D., Alvarez, J.M., 2015. Biofortification of zinc in onions (*Allium cepa* L.) and soil Zn status by the application of different organic Zn complexes. Sci. Hortic. (Amsterdam). 186, 254–265. https://doi.org/10.1016/j.scienta.2015.02.023.

Andresen, E., Peiter, E., Küpper, H., 2018. Trace metal metabolism in plants. J. Exp. Bot. 69, 909–954. https://doi.org/10.1093/jxb/erx465.

Awad, A.A.M., Rady, M.M., Semida, W.M., Belal, E.E., Omran, W.M., Al-Yasi, H.M., Ali, E.F., 2021. Foliar nourishment with different zinc-containing forms effectively sustains carrot performance in zinc-deficient soil. Agronomy 11, 1–18. https://doi.org/10.3390/agronomy11091853.

Barrameda-Medina, Y., Blasco, B., Lentini, M., Esposito, S., Baenas, N., Moreno, D.A., Ruiz, J.M., 2017. Zinc biofortification improves phytochemicals and amino-acidic profile in *Brassica oleracea* cv. Bronco. Plant Sci. 258, 45–51. https://doi.org/10.1016/j.plantsci.2017.02.004.

Beleggia, R., Fragasso, M., Miglietta, F., Cattivelli, L., Menga, V., Nigro, F., Pecchioni, N., Fares, C., 2018. Mineral composition of durum wheat grain and pasta under increasing atmospheric CO_2 concentrations. Food Chem. 242, 53–61. https://doi.org/10.1016/j.foodchem.2017.09.012.

Benzie, I.F.F., Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299, 15–27. https://doi.org/10.1016/S0076-6879(99)99005-5.

Blasco, B., Graham, N.S., Broadley, M.R., 2015. Antioxidant response and carboxylate metabolism in *Brassica rapa* exposed to different external Zn, Ca, and Mg supply. J. Plant Physiol. 176, 16–24. https://doi.org/10.1016/j.jplph.2014.07.029.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Buturi, C.V., Mauro, R.P., Fogliano, V., Leonardi, C., Giuffrida, F., 2021. Mineral biofortification of vegetables as a tool to improve human diet. Foods 10, 223. https://doi.org/10.3390/foods10020223.

Buturi, C.V., Coelho, S.R.M., Cannata, C., Basile, F., Giuffrida, F., Leonardi, C., Mauro, R. P., 2022b. Iron biofortification of greenhouse cherry tomatoes grown in a soilless system. Horticulturae 8, 858. https://doi.org/10.3390/horticulturae8100858.

Buturi, C.V., Sabatino, L., Mauro, R.P., Navarro-León, E., Blasco, B., Leonardi, C., Giuffrida, F., 2022a. Iron biofortification of greenhouse soilless lettuce: an effective agronomic tool to improve the dietary mineral intake. Agronomy 12, 1793. https://doi.org/10.3390/agronomy12081793.

Carrasco-Gil, S., Rios, J.J., Álvarez-Fernández, A., Abadía, A., García-Mina, J.M., Abadía, J., 2016. Effects of individual and combined metal foliar fertilisers on iron- and manganese-deficient *Solanum lycopersicum* plants. Plant Soil 402, 27–45. https://doi.org/10.1007/s11104-015-2759-z.

Cicco, N., Lanorte, M.T., Paraggio, M., Viggiano, M., Lattanzio, V., 2009. A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchem. J. 91, 107–110. https://doi.org/10.1016/j.microc.2008.08.011.

da Silva, J.C., da Silva, C.B., dos Santos, D.P., dos Santos, M.A.L., de Oliveira, W.J., Reis, L.S., 2018. Evapotranspiration and crop coefficient of the irrigated carrot on the alagoano agrest. Rev. Ceres. 65, 297–305. https://doi.org/10.1590/0034-737X201865040001.

Das, U., Rahman, Md Motiur, Roy, Z.R., Rahman, Md Mominur, Kabir, A.H., 2020. Morpho-physiological retardations due to iron toxicity involve redox imbalance rather than photosynthetic damages in tomato. Plant Physiol. Biochem. 156, 55–63. https://doi.org/10.1016/j.plaphy.2020.08.034.

De Sousa Lima, F., Nascimento, C.W.A., Da Silva Sousa, C., 2015. Zinc fertilization as an alternative to increase the concentration of micronutrients in edible parts of vegetables. Rev. Bras. Ciencias Agrar. 10, 403–408. https://doi.org/10.5039/agraria.v10i3a5132.

Di Gioia, F., Petropoulos, S.A., Ozores-Hampton, M., Morgan, K., Rosskopf, E.N., 2019. Zinc and iron agronomic biofortification of Brassicaceae microgreens. Agronomy 9. https://doi.org/10.3390/agronomy9110677.

FAOSTAT. Faostat analytical brief 37 land cover statistics global, regional and country trends. FAOSTAT, 2022. Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL.

Fernández, V., Ebert, G., 2005. Foliar iron fertilization: a critical review. J. Plant Nutr. 28, 2113–2124. https://doi.org/10.1080/01904160500320954.

Ferrandon, M., Chamel, A.R., 1988. Cuticular retention, foliar absorption and translocation of Fe, Mn and Zn supplied in organic and inorganic form. J. Plant Nutr. 11, 247–263. https://doi.org/10.1080/01904168809363800.

Gupta, N., Ram, H., Kumar, B., 2016. Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. Rev. Environ. Sci. Biotechnol. 15, 89–109. https://doi.org/10.1007/s11157-016-9390-1.

Gupta, U.C., Chipman, E.W., 1976. Influence of iron and pH on the yield and iron, manganese, zinc, and sulfur concentrations of carrots grown on sphagnum peat soil. Plant Soil 44, 559–566. https://doi.org/10.1007/BF00011375.

Hacisalihoglu, G., 2020. Zinc (Zn): the last nutrient in the alphabet and shedding light on Zn efficiency for the future of crop production under suboptimal Zn. Plants 9, 1–9. https://doi.org/10.3390/plants9111471.

Hotz, C., DeHaene, J., Woodhouse, L.R., Villalpando, S., Rivera, J.A., King, J.C., 2005. Zinc absorption from zinc oxide, zinc sulfate, zinc oxide + EDTA, or sodium-zinc EDTA does not differ when added as fortificants to maize tortillas. J. Nutr. 135 https://doi.org/10.1093/jn/135.5.1102.

Ierna, A., Pellegrino, A., Mauro, R.P., Leonardi, C., 2020a. Micronutrient foliar fertilization for the biofortification of raw and minimally processed early potatoes. Agronomy 10, 11. https://doi.org/10.3390/agronomy10111744.

Ierna, A., Mauro, R.P., Leonardi, C., Giuffrida, F., 2020b. Shelf-life of bunched carrots as affected by nitrogen fertilization and leaf presence. Agronomy 10, 1982. https://doi.org/10.3390/agronomy10121982.

Kobayashi, T., Nozoye, T., Nishizawa, N.K., 2019. Iron transport and its regulation in plants. Free Radic. Biol. Med. 133, 11–20. https://doi.org/10.1016/J.FREERADBIOMED.2018.10.439.

Kromann, P., Valverde, F., Alvarado, S., Vélez, R., Pisuña, J., Potosí, B., Taipe, A., Caballero, D., Cabezas, A., Devaux, A., 2017. Can Andean potatoes be agronomically biofortified with iron and zinc fertilizers? Plant Soil 411, 121–138. https://doi.org/10.1007/s11104-016-3065-0.

Lawson, P.G., Daum, D., Czauderna, R., Meuser, H., Härtling, J.W., 2015. Soil *versus* foliar iodine fertilization as a biofortification strategy for field-grown vegetables. Front. Plant Sci. 6, 1–11. https://doi.org/10.3389/fpls.2015.00450.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Chlorophyll and carotenoid determination. Biochem. Soc. Trans. 11, 591–593.

Marschner, P., 2012. Marschner's mineral nutrition of higher plants, Third ed. Academic Press, London. https://doi.org/10.1016/C2009-0-63043-9.

Mauro, R.P., Stazi, S.R., Distefano, M., Giuffrida, F., Marabottini, R., Sabatino, L., Allevato, E., Cannata, C., Basile, F., Leonardi, C., 2022. Yield and compositional profile of eggplant fruits as affected by phosphorus supply, genotype and grafting. Horticulturae 8. https://doi.org/10.3390/HORTICULTURAE8040304.

May, B., Dreifke, T., Patz, C.D., Schütz, C.L., Schweiggert, R., Dietrich, H., 2019. Filter aid selection allows modulating the vanadium concentration in beverages. Food Chem. 300, 125168 https://doi.org/10.1016/j.foodchem.2019.125168.

McGuire, R.G., 1992. Reporting of objective color measurements. HortScience 27, 1254–1255. https://doi.org/10.21273/hortsci.27.12.1254.

Mantadakis, E., Chatzimichael, E., Zikidou, P., 2020. Iron deficiency anemia in children residing in high and low-income countries: risk factors, prevention, diagnosis and therapy. Mediterr. J. Hematol. Infect. Dis. 12, 1–12. https://doi.org/10.4084/MJHID.2020.041.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., MacIerzanka, A., MacKie, A., Marze, S., McClements, D.J., Ménard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W., Brodkorb, A., 2014. A standardised static *in vitro* digestion method suitable for food-an international consensus. Food Funct. 5, 1113–1124. https://doi.org/10.1039/c3fo60702j.

Means, R.T., 2020. Iron deficiency and iron deficiency anemia: implications and impact in pregnancy, fetal development, and early childhood parameters. Nutrients 12, 1–15. https://doi.org/10.3390/nu12020447.

Mousavi, S.R., Galavi, M., Ahmadvand, G., 2007. Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). Asian J. Plant Sci. 6, 1256–1260.

Rengel, Z., Batten, G.D., Crowley, D.E., 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. F. Crop. Res. 60, 27–40. https://doi.org/10.1016/S0378-4290(98)00131-2.

Renna, M., D'Imperio, M., Maggi, S., Serio, F., 2022. Soilless biofortification, bioaccessibility, and bioavailability: signposts on the path to personalized nutrition. Front. Nutr. 9, 1–16. https://doi.org/10.3389/fnut.2022.966018.

Rios, J.J., Carrasco-Gil, S., Abadía, A., Abadía, J., 2016. Using perls staining to trace the iron uptake pathway in leaves of a prunus rootstock treated with iron foliar fertilizers. Front. Plant Sci. 27, 893. https://doi.org/10.3389/fpls.2016.00893.

Rivera-Martin, A., Reynolds-Marzal, D., Martin, A., Velazquez, R., Poblaciones, M.J., 2021. Combined foliar zinc and nitrogen application in broccoli (*Brassica oleracea* var. *italica* L.): effects on growth, nutrient bioaccumulation, and bioactive compounds. Agronomy 11, 548. https://doi.org/10.3390/agronomy11030548.

Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A., Zia ur Rehman, M., Waris, A.A., 2019. Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. Chemosphere 214, 269–277. https://doi.org/10.1016/j.chemosphere.2018.09.120.

Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., Niazi, N.K., 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: a comparison of foliar and root metal uptake. J. Hazard. Mater. 325, 36–58. https://doi.org/10.1016/j.jhazmat.2016.11.063.

Sida-Arreola, J.P., Sánchez-Chávez, E., Ávila-Quezada, G.D., Zamudio-Flores, P.B., Acosta Muñíz, C.H., 2015. Iron biofortification and its impact on antioxidant system, yield and biomass in common bean. Plant, Soil Environ. 61, 573–576. https://doi.org/10.17221/643/2015-PSE.

Singh, D.P., Singh, H., Ali, J., Singh, S.P., 2016. Productivity, profitability and nutrient uptake in carrot (*Daucus carota*) and radish (*Raphanus sativus*) crops under sulphur nutrition. Indian J. Agric. Sci. 86, 1577–1580.

Smoleń, S., Kowalska, I., Sady, W., 2014. Assessment of biofortification with iodine and selenium of lettuce cultivated in the NFT hydroponic system. Sci. Hortic. (Amsterdam). 166, 9–16. https://doi.org/10.1016/j.scienta.2013.11.011.

Soil taxonomy - A basic system of soil classification for making and interpreting soil surveys, 1999. Superintendent of Documents, Second. USDA (United States Department of Agriculture, Washington, DC, https://doi.org/10.1007/BF01574372.

Solomons, N.W., Jacob, R.A., 1981. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. Am. J. Clin. Nutr. 34 https://doi.org/10.1093/ajcn/34.4.475.

Stephenson, J., Heslehurst, N., Hall, J., Schoenaker, D.A.J.M., Hutchinson, J., Cade, J.E., Poston, L., Barrett, G., Crozier, S.R., Barker, M., Kumaran, K., Yajnik, C.S., Baird, J., Mishra, G.D., 2018. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. The Lancet 391, 1830–1841. https://doi.org/10.1016/S0140-6736(18)30311-8.

Sun, Y., Mi, W., Wu, L., 2019. Effects of foliar Fe and Zn fertilizers on storage root Fe, Zn, and beta-carotene content of sweet potato (*Ipomoea batatas* L.). J. Plant Nutr. 42, 16–26. https://doi.org/10.1080/01904167.2018.1510505.

Violante, P., 2000. Metodi Di Analisi Chimica Del Suolo, 1st ed. Franco Angeli Editori, Milano, Italy.

White, P.J., Broadley, M.R., Hammond, J.P., Ramsay, G., Subramanian, N.K., Thompson, J., Wright, G., 2012. Bio-fortification of potato tubers using foliar zinc-fertiliser. J. Hortic. Sci. 87, 123–129. https://doi.org/10.1080/14620316.2012.11512842. Biotechnol.

White, P.J., Pongrac, P., Sneddon, C.C., Thompson, J.A., Wright, G., 2018. Limits to the biofortification of leafy brassicas with zinc. Agriculture 8, 9–11. https://doi.org/10.3390/agriculture8030032.

White, P.J., Thompson, J.A., Wright, G., Rasmussen, S.K., 2017. Biofortifying Scottish potatoes with zinc. Plant Soil 411, 151–165. https://doi.org/10.1007/s11104-016-2903-4.

Wishart, K., 2017. Increased micronutrient requirements during physiologically demanding situations: review of the current evidence. Vitam. Miner. 6, 166. https://doi.org/10.4172/2376-1318.1000166.

Zhang, R., Zhang, W., Kang, Y., Shi, M., Yang, X., Li, H., Yu, H., Wang, Y., Qin, S., 2022. Application of different foliar iron fertilizers for improving the photosynthesis and tuber quality of potato (*Solanum tuberosum* L.) and enhancing iron biofortification. Chem. Biol. Technol. Agric. 9, 79. https://doi.org/10.1186/s40538-022-00346-8.

Zoroddu, M.A., Aaseth, J., Crisponi, G., Medici, S., Peana, M., Nurchi, V.M., 2019. The essential metals for humans: a brief overview. J. Inorg. Biochem. 195, 120–129. https://doi.org/10.1016/j.jinorgbio.2019.03.013.

Zou, T., Xu, N., Hu, G., Pang, J., Xu, H., 2014. Biofortification of soybean sprouts with zinc and bioaccessibility of zinc in the sprouts. J. Sci. Food Agric. 94, 3053–3060. https://doi.org/10.1002/jsfa.6658.