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Beneficial effect of organic and inorganic forms of selenium on yield and nutritional characteristics of beetroot

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Abstract: Beetroot (garden beet) is an essential component of the human diet and a source of biologically active compounds, valuable for the pharmaceutical and food industry. To increase the content of essential nutrients and develop a functional food product with enhanced antioxidants, the effect of foliar sodium selenate and selenocystine supply on yield and biochemical characteristics of two table beet cultivars (Marusia and Nezhnost) was assessed. Compared to the untreated control, foliar application of 26.4 mM sodium selenate increased root yield by 1.20-1.25 times, monosaccharide content by 1.49-2.25 times, betalain pigments by 1.56-2.17 times and total antioxidant activity (AOA) by 1.38-1.79 times, whereas the selenocystine supply increased the same parameters by 1.44-1.85, 1.64-3.4, 1.28-1.50 and 1.31-1.33 times, respectively. Compared to pulp, root peel demonstrated 2-2.6 times higher levels of betalain pigments, 1.9-2.4 times higher levels of polyphenols (TP), and 1.5-2.2 times higher antioxidant activity. Significant varietal differences in biochemical characteristic changes due to organic and inorganic Se supply were recorded. Taking into account the relatively low Se biofortification levels of roots (3-3.5 in pulp and 7-12 in peel), the results of the present research prove the importance of Se application mostly to improve beetroot yield, antioxidant content, including betalain pigments, and root peel utilization as a significant source of pharmaceuticals.

Key words: *Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*; antioxidant status; betalain pigments; selenate; selenocystine; yield

Ugodni učinki organskih in anorganskih oblik selena na pridelek in prehranske lastnosti rdeče pese

Izvleček: Rdeča pesa je bistvena sestavina človeške prehrane in vir biološko aktivnih snovi, ki so pomembne za farmacevtsko in prehrambeno industrijo. Za povečanje vsebnosti esencialnih hranil in razvoj funkcionalnih prehrambenih produktov s povečano vsebnostjo antioksidantov je bil ocenjen vpliv foliarnega dodajanja natrijevega selenata in selenocistina na pridelek in biokemične lastnosti dveh sort rdeče pese (Marusia in Nezhnost). V primerjavi s kontrolo je foliarno dodajanje 26,4 mM natrijevega selenata povečalo pridelek rdeče pese za 1,20-1,25 krat, vsebnost monosaharidov za 1,49 do 2,25 krat, vsebnost betalaina za 1,56-2,17 krat in celokupno antioksidacijsko aktivnost (AOA) za 1,38-1,79 krat. Dodajanje selenocistina je iste paramatre povečalo za 1,44-1,85, 1,64-3,4, 1,28-1,50 in 1,31-1,33 krat. V primerjavi s pulpo je olupek rdeče pese izkazal 2-2,6 krat večje vsebnost betalaina, 1,9-2,4 krat večje vsebnosti polifenolov (TP) in 1,5-2,2 krat večjo antioksidacijsko aktivnost. Pri dodatkih organskega in anorganskega Se so bile ugotovljene tudi značilne razlike v biokemičnih lastnostih med sortama rdeče pese. Glede na relativno majhno kopičenje Se v koreninskem gomolju rdeče pese (3-3,5 krat v pulpi in 7-12 krat v olupu) rezultati raziskave dokazujejo pomen dodajanja selena, predvsem za povečanje pridelka, vsebnosti antioksidantov vključno z betalainom in uporabo olupkov kot pomemben vir farmacevtsko zanimivih snovi.

Ključne besede: *Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*; stanje antioksidantov; betalaini; selenate; selenocistin; pridelek

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1 INTRODUCTION

Beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*) is one of the most important root vegetables, an outstanding source of biologically active compounds and nutrients, such as carbohydrates, betalain pigments (Da Silva et al., 2019), polyphenols and vitamins (Wruss et al., 2015). Among sugar beet, fodder beet, mangold (leaf beet) and beetroot, the latter plays a special role in human nutrition, health maintenance and pharmacology (Zhang et al., 2016).

Betalains are known to be the main antioxidants in beetroot (Czapski et al., 2009), and have important effects such as cardio-protective and analgesic effects (Lundberg & Weitzberg, 2005; Sadowska-Bartosz & Bartosz, 2021), as well as antimicrobial, anticancer, antilipidemic activity, hepato- and neuroprotective, antidiabetic, anti-inflammatory and immuno-modulatory, reduce systolic and diastolic blood pressure, and normalize blood glucose level (Fu et al., 2020). Furthermore, betalain pigments of beetroot are highly valued as food coloring pigments (Chikara et al., 2019), thus contributing to quality improvement. Different factors affect beetroot yield and quality, such as genetic peculiarities, environmental conditions, light intensity, soil characteristics, fertilization technique including microbial supply, water availability, etc. (Agic et al., 2018), phytohormones and growth stimulators, humic and amino acids (El-Gamal et al., 2016), soil bacteria (Rašovský et al., 2022).

In recent years, much attention has been paid to growth stimulation and antioxidant properties of Se compounds (Hegedűsová et al., 2021; Khan et al., 2023). At low concentrations, Se may promote photosynthesis, enhance protein synthesis, protect plants against biotic and abiotic stresses, elicit monosaccharide increase, and activate the formation of phytohormones (Liu et al., 2023).

Biofortification of beetroot with Se has not been a common technique so far. Only Sentkowska & Pyrzynska (2023) described the accumulation of SeMet in beetroot juice, though the Se biofortification conditions have not been indicated. Besides, in previous experiments, low efficiency of foliar Se biofortification of root vegetables, such as carrots, was recorded (De Oliveira et al., 2018). According to the Periodic Table of Elements, Se mimics the chemical properties of S, forming appropriate salts (selenates Se^{+6}, and selenites Se^{+4}) and Se-amino acids (Se^{-2}): selenomethionine (SeMet) and selenocysteine (SeCys). Among them, selenates are the most mobile, selenites the most toxic and Se-amino acids are characterized by the highest bioavailability to plants (Dinh et al., 2019). Up to date, selenates have been used most frequently for plant biofortification (Malagoli et al., 2015), whereas Se-amino acid application was restricted because of their high cost. In 2022, an effective non expensive synthesis of selenocystine ($SeCys_2$) was developed (Poluboyarinov et al., 2022), providing the opportunity to investigate this compound's efficiency for plant biofortification more intensively.

The present work aimed to evaluate the efficiency of sodium selenate and selenocystine ($SeCys_2$) foliar supply on yield and nutritional quality of beetroot.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

The research was conducted on beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*) in 2022-2023 at the experimental fields of Federal Scientific Vegetable Center, Russia (55°39.510 N, 37°12.230 E). The mean values of monthly temperature and humidity during the crop cycles are presented in Table 1.

Plants were grown in a loam sod-podzolic soil with the following characteristics: pH 6.2; 2.12 % organic matter; 1.32 mg-eq 100 g^{-1} hydrolytic acidity; 18.5 mg kg^{-1} mineral nitrogen; 21.3 mg kg^{-1} ammonium nitrogen; sum of the absorbed bases as much as 93.6 %; 402 mg kg^{-1} mobile P; 198 mg kg^{-1} exchangeable K; 1 mg kg^{-1} S; 10.95 mg kg^{-1} Ca; 2.05 mg kg^{-1} Zn; 0.86 mg kg^{-1} B; and 220 µg kg^{-1} Se. The soil quality was assessed using the certified methods described in the agrochemical workshop (Carter & Gregorich, 2008), and its mineral composition was determined by an AAS Shimadzu GFA-7000 spectrophotometer (Shimadzu, Kyoto, Japan).

Seeds were sown on 8-10 May with a density of 2.9 plants per m^2 (50 × 70 cm). The experimental protocol was based on the factorial combination between two red beetroot cultivars selected at the Federal Scientific Vegetable Center (Nezhnost and Marusia) and two Se treatments (50 mg l^{-1} of sodium selenate solution, 26.4 mM;

Table 1: Mean values of monthly temperature and precipitation in 2022 and 2023

	Temperature (°C)		Precipitation (mm)	
Month	2022	2023	2022	2023
May	10.7	12.7	61	35
June	18.9	16.8	42	71
July	20.7	18.5	91	151
August	21.9	19.7	4	63
September	10.1	15.0	75	6
October	7.2	5.5	58	114

87 mg l^{-1} of $SeCys_2$ solution, 26.4 mM) plus an untreated control (water foliar spray). The plants were sprayed with the mentioned solutions twice: at the stage of beetroot formation (10-13 July) and 14 days later (1-4 August). A split plot design was used for the treatment distribution in the field, with three replicates, and each experimental unit covered a 9.8 m^2 surface area. Before sowing, the soil was accurately ploughed at 40 cm depth, and during the growing season, hoeing and manual weeding were carried out according to the needs determined through constant monitoring. The fertilization was performed twice during the crop cycles (30 June and 30 July) using 30 kg ha^{-1} of $N_{15}P_{15}K_{15}$. The irrigation was activated when the soil humidity dropped to 80 % of the available water capacity at 20 cm depth. Plants were harvested on 4-8 October.

2.2 SAMPLE PREPARATION

After harvesting and removing soil particles, the roots were separated from the aerial parts of 10 plants, washed with distilled water, dried with filter paper, and 0.5 mm peel was removed using a special knife. Both root pulp and peel were homogenized and used to determine betalain pigments, nitrates, and total dissolved solids (TDS). The remainder of the beetroot fractions was dried at 70 ˚C to constant mass and homogenized, and the resulting powders were used to determine the total antioxidant activity (AOA), total polyphenols (TP), and carbohydrate content.

2.3 DRY MATTER

The dry matter content was determined gravimetrically by drying beetroot samples at 70 °C for 72 hours. The results were expressed as a mass/mass percentage of dry matter (% m/m).

2.4 NITRATES

Nitrates in beetroot pulp samples were assessed using ion-selective electrode with an ionomer Expert-001 (Econix Inc., Moscow, Russia).

2.5 TOTAL DISSOLVED SOLIDS (TDS)

Total dissolved solids were analyzed on water extracts of beetroot pulp using a portable conductometer HM Digital TDS-3 (South Korea, Seoul). The results were expressed in mg kg^{-1} d. m.

2.6 SELENIUM

The selenium content was measured using the micro-fluorimetric method based on the acidic digestion of dried homogenized samples with a mixture of nitric and perchloric acids, subsequent conversion of selenate (Se^{+6}) to selenite (Se^{+4}) using a solution of 6 N HCl and fluorescence value determination of piazoselenol, formed as a result of a condensation between Se^{+4} and 2,3-diaminonaphtalene (Alfthan, 1984). The analysis was performed in hexane at λ emission 519 nm and λ excitation – 376 nm. As an external standard, Se-fortified mitsuba stem powder with a Se content of 1865 μg kg^{-1} (Federal Scientific Vegetable Center) was used. The results were expressed in μg kg^{-1} d m, as mean of three replications.

2.7 TOTAL POLYPHENOLS (TP)

The total polyphenols (TP) were determined in 70 % ethanol extracts of dried peel/pulp samples using the Folin–Ciocâlteu colorimetric method with some modifications (Golubkina et al., 2020). The extraction of samples was performed at 80 °C (1 h.) using a 70 % ethanol/water solution while the condensation with Folin–Ciocâlteu reagent was managed in the presence of saturated Na_2CO_3 solution at room temperature. The polyphenol concentration was calculated based on the absorption value of the resulting mixture at 730 nm by a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA) using the external standard solution of 0.02 % gallic acid. The results were expressed as mg of gallic acid equivalent per g of dry mass (mg GAE g^{-1} d. m).

2.8 ANTIOXIDANT ACTIVITY (AOA)

The antioxidant activity of beet roots and leaves was assessed on 70 % ethanolic extracts of dry samples using a redox titration method (Golubkina et al., 2020). The values were expressed in mg gallic acid equivalents (mg GAE g^{-1} d. m.).

2.9 BETALAIN PIGMENTS

The betalain pigment analysis was carried out spectrophotometrically on water extracts of homogenized beetroot pulp and peel using the absorption values at 535 nm (betacyanins, extinction 60,000) and 485 nm (betaxantins, extinction 48,000) according to Bucur et al. (2016). The results were expressed in mg g^{-1} f. m.

2.10 SUGARS

The monosaccharides were determined using the ferricyanide colorimetric method, based on the reaction of monosaccharides with potassium ferricyanide (Swamy, 2008). Total sugars were analogically determined after acidic hydrolysis of water extracts with 20 % hydrochloric acid. Fructose was used as an external standard. The results were expressed in % per dry mass.

2.11 STATISTICAL ANALYSIS

The data were statistically processed using the analysis of variance (ANOVA), and the mean separations were performed through the Duncan's test at $p < 0.05$ probability level, using the SPSS software version 29 (IBM, Armonk, NY, USA).

3 RESULTS AND DISCUSSION

3.1 YIELD AND BIOMETRICAL PARAMETERS

Our previous investigation regarding Savoy cabbage biofortification with Se demonstrated a significantly higher growth stimulation effect of selenocystine than sodium selenate (Antoshkina et al., 2023). The present results were in accordance with the mentioned observation indicating high prospects of selenocystine supply in beetroot production (Table 2, Figure 1). Furthermore, the possibility of increasing beetroot yield by 1.44-1.66 times using selenocystine and by 1.20-1.26 times *via* foliar application of sodium selenate was recorded under low Se concentration not exceeding 26.4 mM. In this respect, similar beneficial effect of inorganic forms of Se was recorded on the two cultivars studied and significant varietal differences in the case of selenocystine application (Figure 1). The data presented in Table 2 also indicate the increase of root marketability due to Se supply with higher efficiency of organic Se compared to sodium selenate. No significant differences in the dry matter content between control and Se-treated plants were recorded.

Table 2: Yield, biometrical and growth parameters of beet roots

Parameter	Treatment	'Nezhnost'	'Marusia'
Mass (g)	Control	157 ± 15 c	165 ± 16 b
	Se^{+6}	197 ± 19 b	198 ± 19 a
	$SeCys_2$	290 ± 28 a	237 ± 25 a
Yield (t ha $^{-1}$)	Control	46.9 ± 4.7 c	49.5 ± 4.8 c
	Se^{+6}	59.1 ± 6.0 b	59.4 ± 5.8 b
	$SeCys_2$	78.0 ± 7.7 a	71.2 ± 7.0 a
Marketability level (%)	Control	91.0 ± 0.9 e	94.0 ± 0.9 cd
	Se^{+6}	93.5 ± 0.9 d	96.2 ± 0.9 ab
	$SeCys_2$	95.6 ± 0.9 bc	98.2 ± 0.9 a
Length (cm)	Control	12.2 ± 1.0 a	5.1 ± 0.5 a
	Se^{+6}	12.6 ± 1.0 a	5.5 ± 0.5 a
	$SeCys_2$	12.2 ± 1.0 a	5.5 ± 0.5 a
Diameter (cm)	Control	5.0 ± 0.5 b	5.1 ± 0.5 b
	Se^{+6}	5.2 ± 0.5 a	5.4 ± 0.5 b
	$SeCys_2$	5.9 ± 0.5 a	6.0 ± 0.6 a
Dry mass (%)	Control	18.8 ± 1.7 a	18.8 ± 1.8 a
	Se^{+6}	18.4 ± 1.6 a	19.0 ± 1.8 a
	$SeCys_2$	17.5 ± 1.6 a	18.5 ± 1.8 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

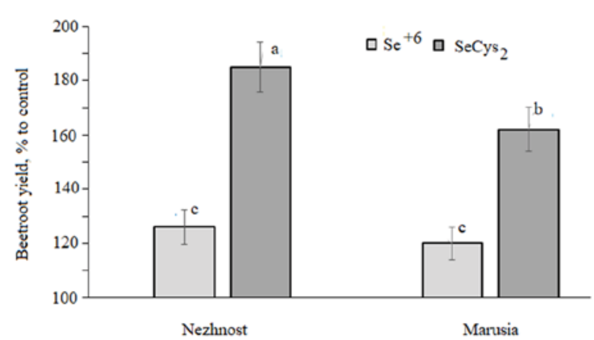


Figure 1: Changes in beetroot yield due to Se application. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

3.2 CARBOHYDRATES, NITRATES AND TOTAL DISSOLVED SOLIDS (TDS)

The accumulation of carbohydrates is one of the most important characteristics of beetroot. In the present study, Se treatment did not change the total sugar content in roots but significantly increased the concentration of minor monosaccharides (Table 3).

The results were in accordance with the corresponding phenomenon of monosaccharide increase for environmental stress alleviation, improving photosynthesis, osmotic homeostasis, protein synthesis and membrane stabilization (Sami et al., 2016). De-

Table 3: Content of carbohydrate, nitrate and total dissolved solids in beetroot roots

Parameter	Treatment	cv. Nezhnost	cv. Marusia
Monosaccharides (% d. m.)	Control	3.76 ± 0.33 c	2.58 ± 0.22 b
	Se^{+6}	8.15 ± 0.80 b	3.85 ± 0.34 a
	$SeCys_2$	12.79 ± 1.12 a	4.22 ± 0.40 a
Total sugar (% d. m.)	Control	72.50 ± 7.01 a	73.10 ± 7.00 a
	Se^{+6}	68.00 ± 6.62 a	67.80 ± 6.55 a
	$SeCys_2$	66.80 ± 6.45 a	66.20 ± 6.51 a
Nitrates (mg kg^{-1} d. m.)	Control	1055 ± 110 a	1282 ± 120 a
	Se^{+6}	1204 ± 110 a	1442 ± 140 a
	$SeCys_2$	1159 ± 110 a	1429 ± 140 a
TDS (%)	Control	3.97 ± 0.40 a	3.80 ± 0.35 a
	Se^{+6}	4.28 ± 0.40 a	4.14 ± 0.40 a
	$SeCys_2$	3.76 ± 0.38 a	4.37 ± 0.42 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

spite the significant varietal differences in Se effect on monosaccharide accumulation, $SeCys_2$ showed higher efficiency than selenate, increasing the monosaccharide levels by 1.8-3.4 times compared to 1.5-2.2 times recorded under sodium selenate application. Nevertheless, considering the low monosaccharide accumulation levels in beetroot, the mentioned phenomenon did not have a significant effect on disaccharides whose concentration was over 10 times higher than the levels of monosaccharides.

Beetroot belongs to a group of vegetables capable of accumulating high concentrations of nitrates, thus being highly valuable in supporting human health, preventing hypertension and protecting against cardiovascular diseases (Brzezinska-Rojek et al., 2023; Dos S.Baião et al., 2020) via regulation of gene expressions of proteins and enzymes involved in the nitric oxide synthesis. Selenium is known to affect nitrogen metabolism, usually decreasing the nitrate content in plants (Golubkina et al., 2018; Pilon-Smits & Quinn, 2010). In the present investigation, we did not record significant differences between beetroot plants supplied with Se and control plants regarding nitrate accumulation.

The lack of significant differences in total dissolved solids (TDS) between control and Se treated plants corresponds to the same situation referring to total sugars in roots.

3.3 BETALAIN PIGMENTS

Among beetroot natural antioxidants, betalain pigments are considered the most valuable (Fu et al., 2020; Sadowzka-Nartosz & Nartosz, 2021). The results of the present investigation revealed that sodium selenate supply increased the total betalain content by 2.16 times ('Marusia') and 1.55 times ('Nezhnost'), while selenocystine produced a lower effect, with 1.5 and 1.28 times increase, respectively (Table 4, Figure 2). Contrary, though the initial levels of betalain pigments were much higher in peel than in pulp, the pigment content changes due to Se application were lower in 'Nezhnost' (1.18-1.19 for both forms of Se) and higher in 'Marusia' (1.39 ($SeCys_2$)-1.64 (Se^{+6}) times). Betalain peel/pulp ratio in control and $SeCys_2$ treated plants of both cultivars was equal to 2.54, whereas a slightly lower value was recorded for selenate supplied plants (2.27).

Furthermore, the varietal differences between the two cultivars tested revealed that the magnitude of betalain pigment increase upon Se supply may be due to either differences in the intensity of pulp or peel pigment accumulation (Figure 2).

According to literature data, red pigments betacyanins account for approximately 75–95 % of beetroot pigments, the remaining 5–25 % being yellow betaxanthins (Delgado-Vargas et al., 2000; Ninfali & Angelino, 2013). Betaxanthin content in roots of investigated cultivars reached 67-68 % and showed a slight tendency to the increase up to 70-71 % under

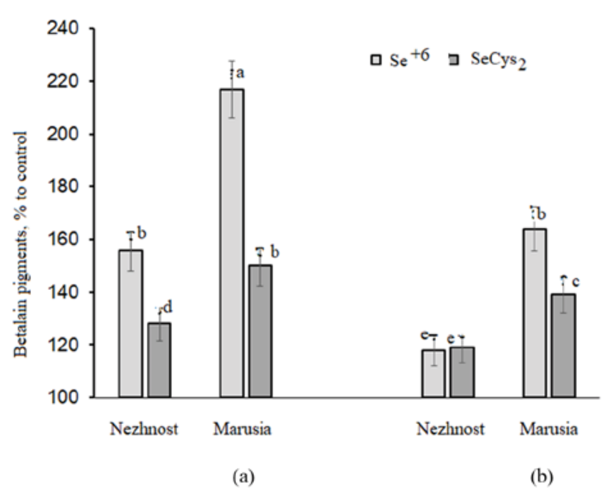


Figure 2: Effect of selenium biofortification on betalain pigment content in pulp (a) and peel (b) of beetroot. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

Se supply (Table 4). The betacyanin/betaxantin ratios were close to 1.9:1 in peel and 2.2:1 in pulp and were not significantly affected by Se supply.

The predominant accumulation of betalain pigments in beet root peel is well documented (Kujala et al., 2002; Slatnar et al., 2015), indicating great prospects of peel utilization as a valuable source of natural pigments.

The significance of high betalain pigment accumulation in peel and pulp of beetroot relates to the nutritional value increase due to Se supply and the possibility of betalain utilization as food colorant (Calva-Estrada et al., 2022). Indeed, betalains are widely used as food colorants due to their prominent and consistent colours at pH 3–7. The development of packaging films incorporated with betalains is used as the colorimetric indicators and smart packaging films capable of improving the functional properties of packaging films, including higher water resistance, tensile strength, elongation at break, and antioxidant and antimicrobial activities (Abedi-Firoozjah et al., 2023).

3.4 TOTAL ANTIOXIDANT ACTIVITY (AOA) AND POLYPHENOLS (TP)

According to literature data, beet roots contain a significant amount of catechins and polyphenolic acids, including ferulic, protocatechuic, vanillic, caffeic and others, providing increased antioxidant activity of root ethanolic extracts (Kavalcova et al., 2015; Platosz et al., 2020). The main beet root flavonoids are rutin, kaempherol, rhamnetin, rhamnecitrin and astragalin (Sentkowska & Pyrzynska, 2020).

The analysis of the total antioxidant activity (AOA) using 70 % ethanolic extracts of beet roots revealed significant increase in the parameter due to Se supplementation (Table 4, Figure 3).

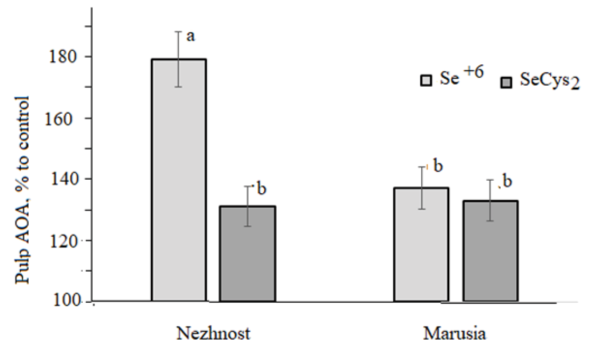
Contrary, TP levels did not differ significantly between control and Se treated plants, reaching 10.9-12.8 mg GAE g^{-1} d. m. in pulp and 23.7- 26.2 mg GAE g^{-1} d. m. in peel. The beneficial effect of Se on beetroot antioxidant activity was greater in 'Nezhnost' than 'Marusia' (Figure 3), contrary to water soluble betalain pigments whose level was higher in cultivar Marusia roots, compared to 'Nezhnost' (Figure 3 a,b).

Furthermore, 'Marusia' did not demonstrate differences in the AOA between organic and inorganic Se supply, while 'Nezhnost' showed more intensive AOA increase under selenate application. The latter phenomenon may be connected with varietal differences in polyphenol content of roots. However, there is no data about the mechanism of Se effect on polyphenol accumulation

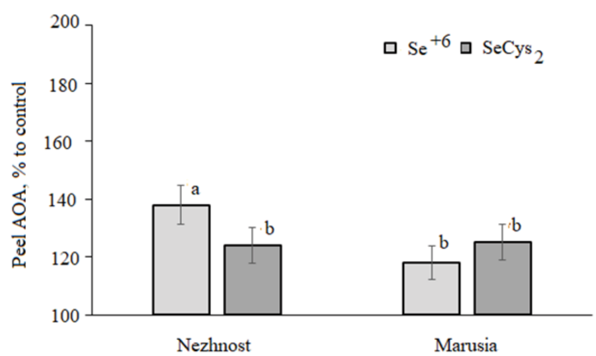
Table 4: Antioxidant levels in beetroot as affected by organic and inorganic forms of Se

		'Nezhnost'		'Marusia'	
Parameter	Treatment	Pulp	Peel	Pulp	Peel
Betacyanin	Control	1.34 ± 0.15 b	3.40 ± 0.31 a	1.20 ± 0.11 c	3.07 ± 0.29 b
(mg g^{-1})	Se^{+6}	2.13 ± 0.20 a	3.98 ± 0.36 a	2.73 ± 0.24 a	5.10 ± 0.49 a
	$SeCys_2$	1.81 ± 0.17 a	4.16 ± 0.40 a	1.89 ± 0.16 b	4.34 ± 0.41 a
Betaxantin	Control	0.64 ± 0.06 b	1.79 ± 0.16 a	0.60 ± 0.05 c	1.67 ± 0.15 b
(mg g^{-1})	Se^{+6}	0.94 ± 0.09 a	2.14 ± 0.20 a	1.18 ± 0.10 a	2.69 ± 0.24 a
	$SeCys_2$	0.72 ± 0.07 b	2.02 ± 0.19 a	0.81 ± 0.08 b	2.27 ± 0.20 a
Total betalain pigments	Control	1.97 ± 0.16 b	5.19 ± 0.50 b	1.80 ± 0.17 c	4.74 ± 0.44 b
(mg g^{-1})	Se^{+6}	3.07 ± 0.29 a	6.12 ± 0.60 a	3.91 ± 0.36 a	7.79 ± 0.75 a
	$SeCys_2$	2.53 ± 0.23 a	6.18 ± 0.60 a	2.70 ± 0.25 b	6.61 ± 0.62 a
AOA	Control	20.6 ± 2.0 c	40.5 ± 3.8 b	19.4 ± 1.5 b	41.9 ± 4.0 b
(mg GAE g^{-1} d. m.)	Se^{+6}	36.8 ± 3.3 a	55.9 ± 5.2 a	26.8 ± 2.4 a	49.4 ± 4.0 a
	$SeCys_2$	27.0 ± 2.5 b	52.3 ± 5.0 a	25.8 ± 2.3 a	52.4 ± 5.0 a
TP	Control	11.1 ± 1.0 a	25.8 ± 2.2 a	11.4 ± 1.0 a	25.2 ± 2.3 a
(mg GAE g^{-1} d. m.)	Se^{+6}	11.3 ± 1.0 a	22.7 ± 2.0 a	13.1 ± 1.0 a	24.8 ± 2.3 a
	$SeCys_2$	10.4 ± 0.9 a	22.7 ± 2.0 a	13.8 ± 1.0 a	28.6 ± 2.4 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.



(a)



(b)

Figure 3: Effect of organic and inorganic forms of Se on antioxidant activity of beet root pulp (a) and peel (b). Values with the same letters do not differ significantly according to Duncan test at $p < 0.05$

and further studies are needed to reveal the mechanism of these changes.

3.5 SE ACCUMULATION

Our previous investigations on Se biofortification of shallot (Golubkina et al., 2019) and Savoy cabbage (Antoshkina et al., 2023) demonstrated higher biofortification levels under $SeCys_2$ supply, compared to the values recorded in selenate treated plants. The present results indicate that the mentioned effect greatly depends on plant part and varietal differences in beetroot. Indeed, beet leaves were the most sensitive to Se supply, able to accumulate up to 1200 µg Se kg^{-1} d. m. in case of $Secys_2$ treated plants, with 1.35 times lower values in case of selenate supplementation (Table 5).

A less pronounced effect was recorded in beetroot where Se accumulated predominantly in root peel. The

Table 5: Selenium accumulation in beet roots under organic and inorganic Se supply (µg Se kg^{-1} d. m.)

Plant part	Treatment	'Nezhnost'	'Marusia'
Pulp	Control	33 ± 3 d	44 ± 4 c
	Se^{+6}	120 ± 10 ab	133 ± 13 a
	$SeCys_2$	100 ± 9 b	128 ± 11 a
Peel	Control	38 ± 3 d	50 ± 5 c
	Se^{+6}	345 ± 30 b	361 ± 33 b
	$SeCys_2$	458 ± 41 a	355 ± 31 b
Leaves	Control	29 ± 2 d	39 ± 3 c
	Se^{+6}	900 ± 79 b	910 ± 76 b
	$SeCys_2$	1220 ± 98 a	1235 ± 100 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

lowest biofortification levels were recorded in root pulp reaching only 3-3.5 times (Figure 4).

3.6 CORRELATION ANALYSIS

The correlation analysis between the parameters tested indicated a significant beneficial effect of pulp monosaccharide content and peel Se levels on beet root yield and a positive effect of Se on betalain accumulation in pulp and peel (Table 6).

Furthermore, antioxidant activity of fat-soluble antioxidants and root yield were significantly affected by Se accumulation both in pulp and peel. Indeed, Se supply demonstrated a significant relationship with root yield,

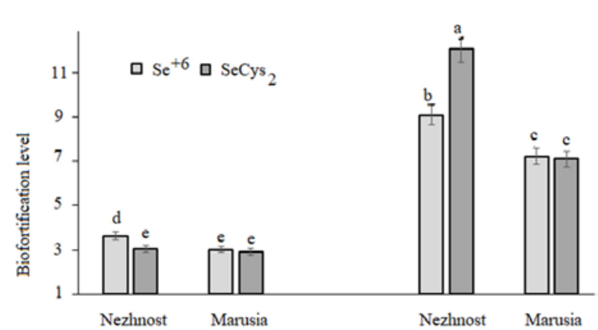


Figure 4: Selenium biofortification levels of beet root pulp (a) and peel (b). Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

Table 6: Correlations between beetroot quality parameters

	Betalains		Ms	AOA		Se	
Yield	pulp	peel	pulp	pulp	peel	pulp	peel
Yield	0.240	0.411	0.769 a	0.332	0.670 d	0.561 f	0.858 a
Pulp betalains		0.947 a	0.031	0.617 e	0.628 c	0.860 a	0.674 d
Peel betalains			0.044	0.464	0.605 e	0.880 a	0.742 b
Monosaccharides				0.505	0.578 f	0.241	0.644 e
Pulp AOA					0.888 a	0.717 c	0.685 d
Peel AOA						0.884 a	0.906 a
Se pulp							0.880 a

Ms: monosaccharides; AOA: total antioxidant activity; *p*: a) < 0.001; b) < 0.002; c) < 0.005; d) < 0.01; e) < 0.02; f) < 0.05.

betalain pigment accumulation and total antioxidant activity. It is interesting that antioxidant characteristics (including Se) of beet root peel directly correlate both with root yield values and accumulation of water-soluble betalain pigments, and indicate the importance of the 'edge' effect for plant adaptation (Golubkina et al., 2023).

4 CONCLUSION

From research carried out on selenium biofortification of beetroot, it arose that the organic and inorganic Se supply to beetroot plants significantly enhanced yield and quality of roots, compared to control plants, though showing low efficiency in producing beetroots with Se levels suitable to compensate Se deficiency in humans. Sodium selenate had a significantly higher beneficial effect on betalain pigment levels and fat-soluble antioxidant content, in comparison with $SeCys_2$, which led to higher root yield and monosaccharide levels. Overall, the outcome obtained from the present study allows to infer that Se biofortification of beetroot plants is a useful strategy to encourage yield and nutritional quality of roots.

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6 AUTHOR CONTRIBUTIONS

Conceptualization: N.G. and G.C.; formal analysis: N.G., A.S., O.C.M., and A.V.T.; investigation: N.G., V.Z., P.P. and Z.A.; methodology: N.G., A.S., O.C.M., and A.V.T.; validation, N.G., A.S., O.C.M., and G.C.; draft manuscript writing, N.G., O.C.M., and A.V.T.; manuscript revision and final editing, N.G., A.S., and G.C. All authors have read and agreed to the published version of the manuscript.

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