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WW was involved in the study design, participated in the laboratory analyses, was responsible for the graphic presentation and statistical analysis of the results, and wrote the paper; BHN designed the study, participated in the laboratory analyses, and participated in the writing of the manuscript

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ORIGINAL RESEARCH PAPER

Selected antioxidant properties of alfalfa, radish, and white mustard sprouts biofortified with selenium

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Abstract
The aim of this study was to evaluate the effect of application of two mineral selenium forms (selenite Se^{4+} or selenate Se^{6+}) on the accumulation of this element by alfalfa (*Medicago sativa*), radish (*Raphanus sativus* var. *sativus*), and white mustard (*Sinapis alba*) at early stages of plant development for biofortification of sprouts with selenium, and the impact of this process on selected phytochemical traits. For this purpose, selenium-biofortified sprouts were analyzed for the contents of L-ascorbic acid and anthocyanin as well as their antioxidant activity. Additionally, the concentration of selenium in the biomass was determined. It was demonstrated that the application of selenium contributed to increased bioaccumulation of the element in the sprouts, constituting an effective method for the production of selenium-biofortified food. Selenate was accumulated less efficiently than was selenite. It was found that a concentration of 20 µmol L^{-1} Se in the form of both selenate and selenite was an optimal dose for enrichment of the sprouts with this element. Biofortification of the experimental species with selenium (20 µmol L^{-1}) generally increased accumulation of anthocyanins but did not significantly alter the level of L-ascorbic acid and free radical scavenging activity. Therefore, it seems that consumption of selenium-biofortified sprouts can be an effective way to supplement low-selenium diets with this element.

Keywords
antioxidants; anthocyanin; functional food; selenium biofortification

Introduction

Contemporary society is becoming increasingly aware of the influence of an appropriate well-balanced diet on health. There is therefore now a growing demand for high-quality food with both desirable palatability and health-enhancing properties that will be less processed and convenient in use. To meet consumers' requirements, producers offer an increasingly wide range of food products, including the so-called "functional foods" enriched with a variety of nutrients and bioactive compounds with a targeted and scientifically documented effect on the organism. Natural compounds that are normally present in food in limited amounts and consumed in functional foods do not induce side-effects and are better absorbed by the organism than are their synthetic equivalents [1,2]. Food is fortified with bioactive compounds to eliminate nutrient deficiencies and prevent the so-called "civilization diseases", e.g., obesity, cardiovascular diseases (atherosclerosis and ischemic heart disease), cancers, diabetes, osteoporosis, and other illnesses [3].

In order to function properly, the human organism requires at least 22 mineral elements, which are mainly supplied by a properly balanced diet. It is estimated that >60%, >30%, 30%, and 15% of the population worldwide suffer from iron, zinc, iodide, and selenium deficiencies, respectively. Additionally, calcium, magnesium, and copper

deficiency are frequently reported from many developed and developing countries [4]. The deficit of some micronutrients in the diet is mainly associated with their reduced levels in food originating from agricultural production in regions characterized by low levels or low bioavailability of essential elements in the soil. Another cause is the use of plant varieties that accumulate insufficient quantities of micronutrients in their edible parts to meet human requirements [5,6]. It is therefore important to increase production of food that will not only ensure an adequate supply of calories and productivity but will also provide all essential nutrients. Simultaneously, this involves global combat against the so-called "hidden hunger", i.e., vitamin or mineral deficiency, which does not cause obvious symptoms but exerts an adverse effect on the organism health [7].

Selenium is a micronutrient with great importance for cell metabolism, especially for antioxidant reactions. Concurrently, it exhibits a relatively narrow spectrum of physiological activity, as both the deficiency and excess of the element exert a negative effect on organism function [8]. As an antioxidant, it protects cells from excessive accumulation of reactive oxygen species (ROS), thereby reducing the risk of cancer and cardiovascular diseases. In the human organism, selenium is usually present in combination with amino acids, cysteine (selenocysteine SeCys), methionine (selenomethionine SeMet), and proteins (selenoproteins). Selenium is part of approximately 20 enzymes. Glutathione peroxidase (GPx; EC 1.11.1.9), one of the most important selenium-containing enzymes, is involved in the metabolism of H_2O_2 and protects cell membranes against lipid peroxidation. Selenoenzymes catalyze reactions in many biochemical pathways, e.g., lipid and amino acid transformations, and the pentose phosphate pathway; they also stabilize biological membranes [9]. Selenium has been demonstrated to prevent the development of atherosclerosis and reduce the risk of heart attack and stroke. Together with vitamin E, it delays aging processes and accelerates cell regeneration. The element is involved in the metabolism of thyroid hormones, has anti-inflammatory and antiviral properties (limits the development of AIDS), and plays an important role in the transmission of nerve impulses in the central nervous system. Additionally, it has organism-detoxifying properties. It forms weakly-soluble selenides with toxic trace metals and metalloids, e.g., cadmium, lead, mercury, and arsenic [10]. Selenium deficiency in an organism can cause numerous diseases and is mainly associated with myocardial damage (Keshan disease) and osteoarticular changes (Kashin–Beck disease). Furthermore, there are a number of recent reports on the effect of this element on the development of neoplastic, cardiovascular, immune, and nervous diseases [11]. Meat, fish, cereal products, dairy products, some fruits and vegetables are the basic sources of selenium in the human diet. The highest content of selenium has been detected in Brassicaceae vegetables (broccoli and white cabbage), bulb vegetables (garlic and onion), asparagus, and legumes (lentils) [12]. Both in Poland and in many European and Asian countries, soils are characterized by low contents of this microelement [13]. Therefore, it is important to develop and implement new methods for enrichment of food with selenium to prevent its deficiencies.

Biofortification is the most promising method for enrichment of plants with minerals, vitamins, and nutrients and thus production of functional foods. Biofortification of plants with nutrients differs from fortification, i.e., simple enrichment of food products. The former method focus primarily on production of plants with increased levels of these components rather than the production of processed food enriched with nutrients at the final stage of manufacture. Consumption of biofortified food helps to counteract the causes as well as the symptoms of microelement deficiency. This is therefore a long-term and prophylactic strategy requiring lower financial input than fortification or supplementation [14]. Biofortification focuses on production of plants (conventionally bred or via genetic engineering) with an increased amount of micronutrients in their edible parts. Another method involves cultivation of plant varieties that contain lower levels of compounds inhibiting micronutrient absorption [15].

Plants can take up selenium as selenate, selenite, or organic selenium compounds; however, these chemical forms are characterized by a diversified metabolism, bioavailability, and their mobility within plant. Selenate is easily translocated from roots to shoots, whereas selenite, or its metabolic products, tends to accumulate in roots [16]. Therefore, in some Se biofortification programs, it is suggested to use selenate instead of selenite, especially when selenium is introduced to the soil or into a nutrient solution [17]. On the other hand, it is suggested that selenite-biofortified plants can accumulate

higher amounts of Se-methylselenocysteine (MeSeCys), a nonprotein monomethylated selenoamino acid with confirmed chemo-preventive and anticarcinogenic properties [18].

Sprouts, which are becoming increasingly popular with consumers, are an ideal candidate for the production of biofortified food. This is relatively easy and the chemical composition can be modified by appropriately selected technological processes, which yield products rich in the desirable components. The present study was focused on the possibility of selenium biofortification of three species of sprouts, and assessment of the effect of this element used in two chemical forms (selenate or selenite) on selected parameters of the oxidative status of plants.

Material and methods

Plant materials, seed sprouting, and experimental design

Commercially available seeds of varieties specially designed for production of sprouts were the research material. The study was carried out on alfalfa (*Medicago sativa* L.), radish (*Raphanus sativus* L. var. *sativus*), and white mustard (*Sinapis alba* L.) seeds (Polan Company, Poland).

Dry seeds (15 g) were submerged in distilled water and left to swell for ca. 2 hours. The liquid was decanted and the seeds were washed again and placed in special sprouters (Vilmorin, France). The seed containers were supplemented with 40 mL of distilled water (control) or 40 mL of a selenium solution in the specified form and concentration. Inorganic selenium was used in two chemical forms: selenite (Na_2SeO_3; Sigma-Aldrich, USA) or selenate (Na_2SeO_4; Sigma-Aldrich, USA) at concentrations of 0, 20, or 200 μmol L^{-1}. The experimental design was randomized in a 2 × 3-factorial scheme (two selenium chemical forms with combinations of three selenium concentrations).

The germination process was carried out in an air-conditioned phytotron at a temperature of ca. 22–23°C for 5 days (alfalfa and radish) or 6 days (white mustard). During the sprouting process, the sprouts were sprayed with distilled water as necessary. After the specified time, the fresh mass of the sprouts was analyzed to determine the concentrations of anthocyanins, L-ascorbic acid (vitamin C) and free radical scavenging activity (FRSA). Next, the plant material was rinsed three times with distilled water and dried at a temperature of 80°C to constant mass in order to determine the total selenium content in dry weight (DW).

Concentration of L-ascorbic acid

The Tillman's titration method modified by Pijanowski [19] was used to determine the concentration of L-ascorbic acid (AsA). To prepare samples for the analysis, the plant material was homogenized in a 2% solution of oxalic acid. The homogenate was filtered and titrated with a standard solution of 2,6-dichloroindophenol (Sigma-Aldrich, USA). The total concentration of L-ascorbic and L-dehydroascorbic acids were expressed in mg per 100 g fresh weight (FW).

Content of anthocyanins

The content of anthocyanins was determined with the modified Harborne technique described in detail by Hawrylak-Nowak [20]. Anthocyanin pigments were extracted from the sprouts by 24-h maceration of tissues in a mixture of methanol and HCl (99/1, v/v). The extract was decanted into test tubes and centrifuged (10 min, 10,000 rpm). Absorbance was read at wavelengths of 527 nm and 652 nm using a Cecil CE 9500 spectrophotometer (Cecil Instruments, UK). The concentration of anthocyanins in the biomass was calculated using the molar extinction coefficient for cyanidin-3-monoglucoside, taking into account the molecular mass of the compound and the dilution of the sample [21], and expressed in mg per 100 g FW.

Free radical scavenging activity

The ability of the plant extracts to reduce radicals was assessed with the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The extracts were prepared by homogenization of 0.25 g of the plant material with 5 mL of 95% ethanol and centrifuged (10 min, 10,000 rpm). Next, 100 µL of the ethanolic supernatant were added to 2 mL of a 100 µmol L^{-1} DPPH solution. 100 µL of 95% ethanol were added to the control sample and absorbance at 515 nm (A0) was measured immediately. The absorbance of the analyzed samples was read after 15 min (A15) using a Cecil CE 9500 spectrophotometer (Cecil Instruments, UK). The ability of the extracts to counteract the oxidation reaction was calculated with the following formula [22]:
% of *DPPH reduction* = 100 (*A*0 − *A*15)/*A*0
where: *A*0 – absorbance of the control sample, *A*15 – absorbance of the sample at the specified time point.

Determination of the selenium concentration

The plant material was wet-ashed by HNO_3–$HClO_4$ digestion (ratio of 4:1 v/v) at a temperature of 210°C. The total selenium concentrations in the digests were determined by the hydride generation atomic absorption spectrometry (HG-AAS) technique. The measurements were performed at a wavelength of 196 nm (Perkin Elmer 1100B fitted with Perkin Elmer MHS-10) according to a method described previously in detail [23].

Statistical analysis

The experiment involved six treatments and three replications per treatment. It was independently repeated twice under the same conditions to ensure authenticity of the results. Values are given as means ±*SD* of six measurements (from two independent repetitions). All variables were tested with two–way analysis of variance (ANOVA) followed by Tukey's test at the 0.05 probability level. The results of the statistical analysis presented in the figures represent the interactive effect of the Se form and concentration.

Results

Effect of selenium on the L-ascorbic acid content

The lowest concentration of AsA in the fresh weight of the control sprouts (germinating without selenium addition) was detected in the alfalfa (10.4 mg 100 g^{-1} FW). An almost threefold higher level was determined in the mustard sprouts (28.7 mg 100 g^{-1} FW) and the highest value was noted in the radish sprouts (65.9 mg 100 g^{-1} FW) (Fig. 1). The application of selenium did not exert a significant effect on the AsA concentration in the alfalfa and radish sprouts, despite the slight increase in its level (13–15%) in the presence of selenite (Fig. 1A,B). In turn, the enrichment of the mustard sprouts with a 200 µmol L^{-1} selenium solution, regardless of the chemical form, resulted in ca. 34–39% reduction of the AsA level compared with the control (Fig. 1C).

Effect of selenium on the anthocyanin content

The content of anthocyanins in the fresh mass of the control plants was as follows: the highest concentration was detected in the mustard sprouts (0.89 mg 100 g^{-1} FW), whilst the alfalfa (0.20 mg 100 g^{-1} FW) and radish (0.16 mg 100 g^{-1} FW) sprouts exhibited lower levels of the pigments (Fig. 2). There was an increase in the anthocyanins in the alfalfa and radish sprouts (by 29% and 19%, respectively, relative to the control) after the application of the 20 µmol L^{-1} selenite solution. In turn, the application of 200 µmol Se L^{-1}, regardless of the chemical form, resulted in a significant decline in the

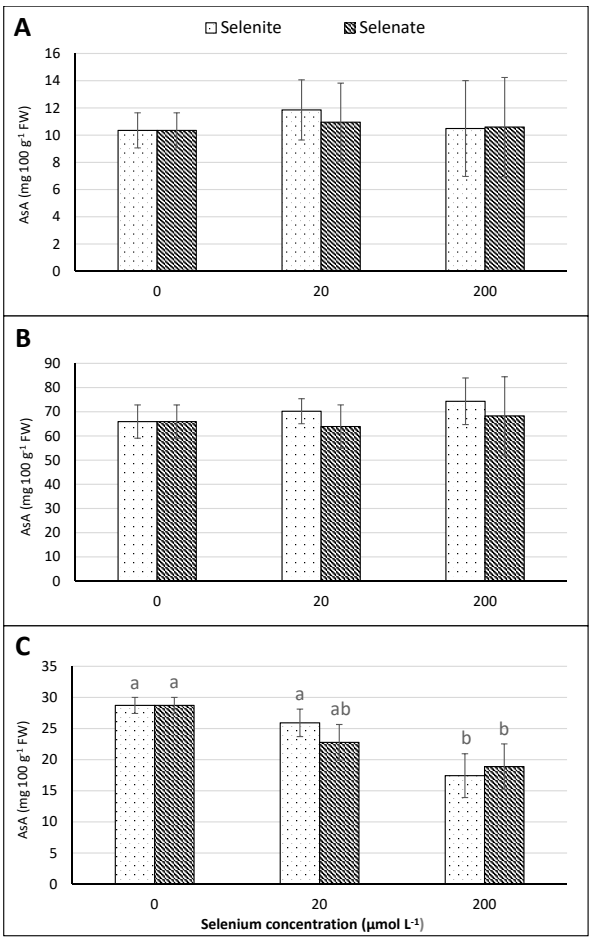

Fig. 1 Effects of selenium biofortification with selenite or selenate on L-ascorbic acid (AsA) concentrations in the sprouts of alfalfa (**A**), radish (**B**), and white mustard (**C**). Means (±*SD*; $N = 6$) with different letters on top of the bars are significantly different ($p < 0.05$) according to Tukey's LSD test.

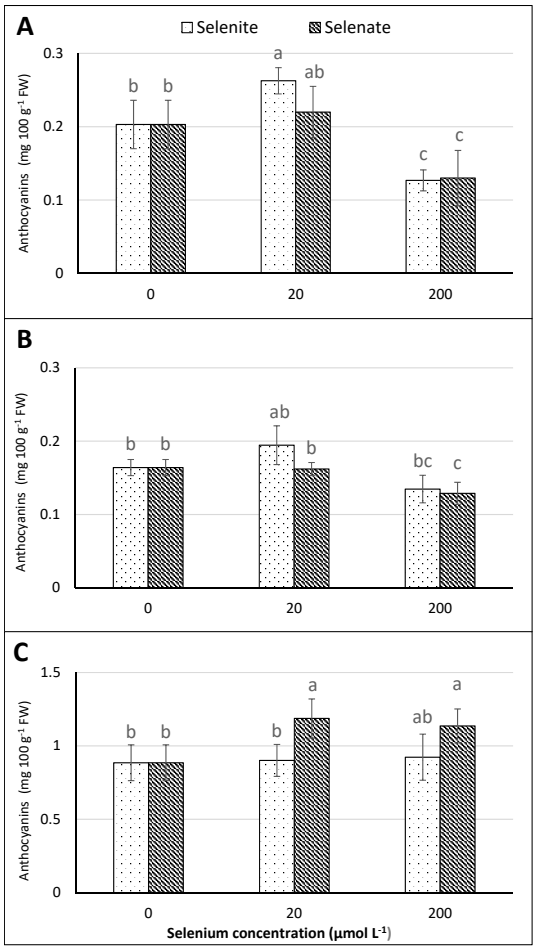

Fig. 2 Effects of selenium biofortification with selenite or selenate on anthocyanin concentrations in the sprouts of alfalfa (**A**), radish (**B**), and white mustard (**C**). Means (±*SD*; $N = 6$) with different letters on top of the bars are significantly different ($p < 0.05$) according to Tukey's LSD test.

anthocyanin concentrations in the alfalfa and radish sprouts, by 36–38% and 18–21%, respectively, in comparison to the control (Fig. 2A,B).

In the mustard sprouts, an increase in the level of anthocyanin pigments (by 34%) was noted after the use of 20 µmol Se L^{-1} in the form of selenate. A similar increase in the anthocyanin concentration (by 28%) was also induced by the application of selenate at the concentration of 200 µmol L^{-1}. In contrast, selenium in the form of selenite did not exert a significant effect on the concentration of these pigments in the mustard sprouts, regardless of the treatment concentration (Fig. 2C).

Free radical scavenging activity of the Se-biofortified sprouts

The highest FRSA was determined for the extract from the radish sprouts, whereas the lowest was exhibited by the alfalfa extract (Fig. 3). The application of 20 µmol L^{-1} of selenium had no significant effect on the antioxidant activity of the alfalfa sprout extracts. However, the selenium concentration in the solution increasing up to 200 µmol L^{-1}, irrespective of the chemical form, contributed to a slight decrease in the FRSA value in this species (Fig. 3A). The 200 µmol L^{-1} concentration of selenium, again regardless of its chemical form, increased the FRSA of the mustard and radish sprout extracts. The ability to reduce the DPPH radical increased from 21% to ca. 25–26% in the radish and from 13% to 17–18% in the mustard. The sprouts of these species treated with selenium at the concentration of 20 µmol L^{-1} did not exhibit significant fluctuations in the FRSA value (Fig. 3B,C).

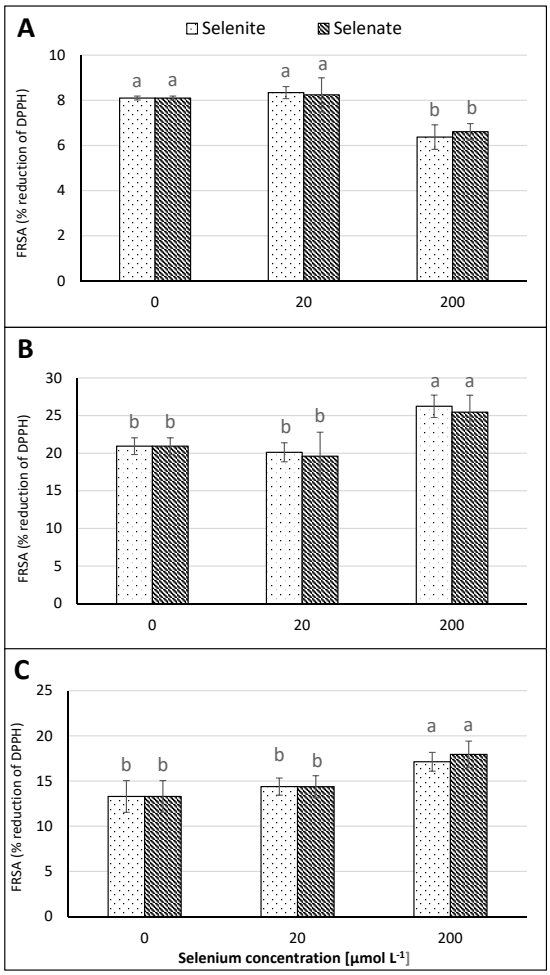

Fig. 3 Effects of selenium biofortification with selenite or selenate on free radical scavenging activity (FRSA) in the sprouts of alfalfa (**A**), radish (**B**), and white mustard (**C**). Means (±*SD*; *N* = 6) with different letters on top of the bars are significantly different ($p < 0.05$) according to Tukey's LSD test.

Bioaccumulation of selenium

The lowest concentration of selenium in the control conditions was detected in the radish and mustard sprouts, whereas the level of this element was slightly higher in the alfalfa sprouts (Tab. 1). The application of selenium contributed to a significant increase in the level of this element in the biomass. The application of selenite (20 µmol L^{-1}) yielded the highest concentration of selenium in the mustard (4.85 mg Se kg^{-1} DW) and alfalfa sprouts (3.20 mg Se kg^{-1} DW), and the lowest value was detected in the radish sprouts (2.15 mg Se kg^{-1} DW). After the application of selenate (20 µmol L^{-1}), the highest selenium concentration was recorded in the alfalfa and radish sprouts (2.74 and 2.55 mg Se kg^{-1} DW, respectively), whereas the selenium concentration was approximately twofold lower in the mustard biomass (1.20 mg Se kg^{-1} DW) (Tab. 1).

The increase in the selenium concentration in the applied solution to 200 µmol L^{-1} contributed to a significant increase in the concentration of the element in the sprouts. In terms of the ability of the analyzed species to bioaccumulate selenium, the highest value was exhibited by the alfalfa supplemented with 200 µmol L^{-1} Se in the form of selenite. In such conditions, the concentration of this element in the dry matter reached 38 mg Se kg^{-1} DW, which is a higher value than that detected in the radish (28.75 mg Se kg^{-1} DW) and mustard (25.25 mg Se kg^{-1} DW). No such high accumulation was found in the alfalfa sprouts when selenium was supplemented as selenate at a dose of 200 µmol L^{-1} Se. The concentration of the element in this species was 21.50 mg kg^{-1} DW, which was comparable with that determined in the radish sprouts (18.25 mg Se kg^{-1} DW) and lower than the concentration of selenium in the mustard biomass (28.00 mg Se kg^{-1} DW) (Tab. 1).

Discussion

Biofortification plays an important role in the production of functional foods. This process yields products enriched with easily absorbable minerals, e.g., iron, zinc, copper, calcium, magnesium, selenium, and iodine, whose levels are usually insufficient in diets. Selenium is one of the micronutrients

Tab. 1 Effects of selenium biofortification with selenite or selenate on total selenium content in the sprouts of alfalfa, radish, and white mustard.

Chemical form of selenium in the solution	Selenium concentration in the solution (mg L^{-1})	Total selenium concentrations in the biomass (mg kg^{-1})		
		Alfalfa	Radish	White mustard
Selenite	0	0.23 ±0.05 ^{d}	0.17 ±0.08 ^{d}	0.12 ±0.07 ^{e}
	20	3.20 ±0.29 ^{c}	2.15 ±0.26 ^{c}	4.85 ±0.11 ^{c}
	200	38.00 ±1.91 ^{a}	28.75 ±0.79 ^{a}	25.25 ±1.21 ^{b}
Selenate	0	0.23 ±0.05 ^{d}	0.17 ±0.08 ^{d}	0.12 ±0.07 ^{e}
	20	2.74 ±0.27 ^{c}	2.55 ±0.89 ^{c}	1.20 ±0.13 ^{d}
	200	21.50 ±1.97 ^{b}	18.25 ±1.41 ^{b}	28.00 ±1.41 ^{a}

Means (±*SD*; *N* = 4) in each column with different letters are significantly different ($p < 0.05$) according to Tukey's LSD test.

with a supply that does not fulfil the organism requirements in many countries. It has been estimated that over a billion people worldwide suffer from a deficiency of this element [24,25]. Consumption of biofortified vegetable food products can be an effective method for counteracting the selenium deficit. The present study was focused on assessment of the effects of selenium phytofortification on some antioxidant parameters and bioaccumulation of the element in sprouts. The assessment of the effect of the two chemical forms (selenite or selenate) and concentrations of selenium (20 or 200 μmol L^{-1}) used for biofortification on the total concentration of this element in the biomass revealed that the exogenous application contributed to a significant increase in the level of bioaccumulation of the element. Regardless of the plant species and the selenium form, the concentration of this element in sprouts biofortified with 20 μM Se μmol L^{-1} was in the range of 1.2–4.8 mg kg^{-1} DW and this level was substantially higher than that in the control plants (0.12–0.23 mg Se kg^{-1} DW). Furthermore, the tenfold increase in the selenium concentration in the solution (up to 200 μmol L^{-1}) resulted in a further increase in selenium accumulation in biomass (up to 18–38 mg kg^{-1} DW). After application of 200 μmol Se L^{-1}, the highest selenium bioaccumulation potential was exhibited by the alfalfa sprouts. There were no visual symptoms of selenium toxicity in the biofortified seedlings. Moreover, we found that application of selenite led, in general, to a higher accumulation of selenium in sprouts compared to selenate, which probably results from the faster incorporation of selenite into organic Se-compounds in comparison with selenate [16,26]. In similar experiments carried out on alfalfa, wheat and sunflower sprouts biofortified with selenate (0.78–800 mg L^{-1}), the selenium concentration in the plant biomass was found to increase as well [27]. The study demonstrated that the sunflower was the most resistant species to the increasing concentrations of selenate. However, selenium remained in the seedling biomass as nonmetabolized selenate. In turn, in the less resistant alfalfa and wheat biofortified with a low level of selenate (selenium content was 1–2 mg kg^{-1} DW), only approx. 20% of selenate remained in the form of inorganic selenium, which indicates a high degree of metabolism of this element. However, a further increase in the selenium level in the tissues of these species reduced their ability to metabolize this element. At a high selenium level in the biomass, 40–50% of its total content remained in a non-metabolized form [27]. Similarly, increased selenium concentration in the biomass of biofortified tomatoes was reported by Castillo-Godina et al. [28]. Application of selenite at a concentration of 2 or 5 mg L^{-1} significantly increased the selenium concentration in tomato leaves and stems. In the case of fruits, there were differences only after application of a solution containing 5 mg Se L^{-1}. The concentration of this element in the tomato fruits (35.8 μg Se g^{-1}) was 53% higher than in the control plants. Experiments conducted on buckwheat sprouts by Cuderman et al. [29] demonstrated that the total selenium content in the plants increased along the increasing selenium concentrations in the solutions used for seed imbibition. Three selenium compounds were used in the experiments: selenomethionine (10 mg Se L^{-1}), selenite and selenate (5, 10, or 20 mg Se L^{-1}). Buckwheat sprouts germinating from seeds kept in solutions supplemented with 5 or 10 mg Se L^{-1} accumulated a similar amount of selenium, whereas a concentration of 20 mg L^{-1} caused an approximately twofold increase in its concentration. Irrespective of the type of solutions used for the imbibition process, extracts from biofortified sprouts contained selenate (23–30% in seedlings fortified with selenate and trace amounts in seedlings fortified with selenite or selenomethionine), selenomethionine (2–8%), and selenite (trace amounts). This suggests a more efficient bioconversion of selenite into organic selenium forms as compared to selenate, caused by the differences in uptake and metabolism of different forms of selenium by plants. Selenite uptake is an active process partly mediated by phosphate and/or silicon transporters. Furthermore, selenate is absorbed by sulfur transporters and distributed within the plants in a rather unchanged chemical form at much a faster rate as compared to selenite. Moreover, selenite is more rapidly bioconverted to protein seleno-amino acids and thus more bioaccumulated in comparison to selenate, which affects the higher phytotoxicity of selenite [30].

The present experiments have shown that the application of selenium did not exert a significant effect on the total concentration of AsA in the alfalfa and radish seedlings. In turn, enrichment of the mustard seedlings with selenium applied at the concentration of 200 μM, irrespective of its form, contributed to reduction (by 34–39%) of the level of this compound, in comparison with the control plants. There are only few studies which

have focused on the impact of selenium on the AsA content in plants. An increase in the concentration of this compound in green tea leaves was noted after foliar fertilization with selenite or selenate [31]. Similarly, Rìos et al. [32] reported a significant increase in the AsA level in lettuce induced by increasing concentrations of selenite or selenate even in the presence of toxic concentrations of these compounds. Similar results to the findings of the present study were reported by Lee and Park [33], who noted reduction in the level of AsA in the presence of increasing concentrations of selenite. Moldovan et al. [34] analyzed the effect of selenium applied as selenite (5 or 10 ppm) on the AsA concentration in seedlings of various wheat, barley, and oat cultivars. The AsA concentration in the biomass of these species was shown to be directly proportional to the selenium concentration used in the germination medium. Although the level of AsA was higher than in the control seedlings in each species studied, the greatest increase was demonstrated in wheat sprouts. In contrast, a recent study conducted by Hawrylak-Nowak et al. [35] has shown that selenium biofortification of lamb's lettuce via foliar or soil application of selenate had no impact on the level of AsA in the plants. Thus, literature reports confirm the species specificity of the changes in the AsA level induced by selenium, which in the present study may additionally be determined by the very intense metabolism of the germinating plants.

The analysis of the ability of the plant extracts to inhibit DPPH radicals revealed the highest antioxidant capacity of the radish sprouts. The application of selenium in the form of selenite or selenate at the concentration of 200 μmol L^{-1} induced a significant increase in the FRSA value in the mustard and radish sprouts. In the case of alfalfa, the application of selenium did not cause significant changes in this parameter. Similarly, the biofortification of the seedlings with the 20 μmol L^{-1} selenium solution did not generally change the ability of the extracts to inhibit the DPPH radical. Similar experiments conducted by Bachiega et al. [36] at different stages of development of broccoli plants biofortified with a selenate solution (50 μmol L^{-1}) demonstrated an increase in the antioxidant potential in the extracts from broccoli sprouts and seedlings, compared with selenium-untreated plants. In turn, the application of selenate induced a slight decline in the antioxidant activity in the inflorescences. Field studies conducted on the common onion by Põldma et al. [37] demonstrated a significant effect of selenium on antioxidant activity. Extracts from onions receiving foliar selenate were characterized by higher antioxidant potential than the control plants. It has been suggested that, as in the human and animal organisms, selenium can exert the antioxidative effects in plants and thus increase their resistance to the oxidative stress caused by external factors and internal metabolic processes [37]. Investigations carried out by Xu et al. [38] confirmed that tea foliar treated with selenium salts was characterized by a higher antioxidant capacity than control plants. Additionally, the selenate-biofortified tea exhibited a higher ability to inhibit the DPPH radical than the tea fertilized with selenite. Rìos et al. [32] showed an increase in FRSA in selenite- and selenate-biofortified lettuce and, similar to the present study, these differences were noted at higher (>40 μM) concentrations of the selenium compounds.

Biofortification of the seedlings with selenite used at a concentration of 20 μmol L^{-1} had a positive effect on the concentration of anthocyanins in the alfalfa and radish sprouts. In turn, selenium applied at 200 μmol L^{-1}, regardless of its form, inhibited the accumulation of anthocyanins in these species. In the case of mustard, the concentration of anthocyanin increased after the application of selenate, irrespective of its concentration, whereas selenite did not change the concentration of these compounds. Similar results were obtained by Abbas [39] in a study of the effect of selenium on physiological changes in sorghum cultivated under low temperature. Before sowing, seeds of this species were soaked in a selenate solution (3–12 Se mg L^{-1}). The study has shown that the 3 or 6 mg Se L^{-1} induced an increase in the anthocyanin accumulation, whereas the highest concentration (12 mg Se L^{-1}) contributed to reduction of the concentration of these pigments. Hajiboland and Keivanfar [40] investigated the effect of selenate on the levels of anthocyanin in canola (family Brassicaceae). These authors found that the selenium application did not exert a significant effect on the content of these compounds in canola leaves. In turn, study conducted by Hawrylak-Nowak [20] demonstrated that selenium applied in mineral (selenate) and organic (selenomethionine) forms induced accumulation of anthocyanins in maize leaves and selenomethionine was found to be a more effective inducer than was selenate. In recent years, Tian et al. [41] conducted

investigations of sprouts of various broccoli cultivars and showed that application of both selenite and selenate (100 μmol L^{-1}) contributed to an increase in the anthocyanin content, with the highest achieved via supplementation with selenate. The increase in anthocyanin concentration may be one of the symptoms of selenium phytotoxicity [20] or may indicate a selenium-induced changes in plant oxidative status although, on the other hand, the presence of anthocyanin in food is desirable due to its strong antioxidant and nutraceutical potential [42].

Conclusions

Selenium biofortification of selected sprout species contributed to an increase in the accumulation of this element in the biomass. Consumption of selenium-biofortified sprouts could therefore be an effective way to supplement low-selenium diets with this element. In comparison with the widespread wheat biofortification program, this method is substantially cheaper, faster and less labor-intensive. However, in terms of the nutraceutical value of sprouts obtained with this method, it will be necessary to carry out further analyses of selenium speciation in biomass enriched with this element. Given a significant increase in the selenium content in biomass, the absence of disturbances in the AsA concentration and FRSA level, and simultaneous increase in anthocyanin accumulation (in the presence of selenite), the 20 μmol L^{-1} concentration of selenium applied as either selenite or selenate seems to be an optimal for enrichment of seedlings with this element.

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Wybrane właściwości antyoksydacyjne biofortyfikowanych selenem kiełków lucerny, rzodkiewki oraz gorczycy białej

Streszczenie

Celem pracy była ocena wpływu dwóch mineralnych form selenu (seleninu – Se^{4+} lub selenianu – Se^{6+}) na akumulację tego pierwiastka przez lucernę (*Medicago sativa*), rzodkiewkę (*Raphanus sativus* var. *sativus*) oraz gorczycę białą (*Sinapis alba*) na etapie wczesnych faz rozwoju roślin w aspekcie biofortyfikacji kiełków selenem oraz wpływu tego procesu na wybrane cechy fitochemiczne. W tym celu w biofortyfikowanych selenem kiełkach określono zawartość kwasu L-askorbinowego, antocyjanów jak również ich aktywność antyoksydacyjną. Ponadto oznaczono stężenie selenu w biomasie. Wykazano, że aplikacja selenu powodowała jego zwiększoną bioakumulację w kiełkach, stanowiąc efektywny sposób wytwarzania żywności biofortyfikowanej selenem. Selenian był akumulowany mniej efektywnie niż selenin. Stwierdzono, że optymalne dla wzbogacenia kiełków w selen jest stężenie 20 µmol L^{-1} Se, zarówno w odniesieniu do selenianu jak i seleninu. Biofortyfikacja badanych gatunków selenem (20 µmol L^{-1}) na ogół wywoływała wzrost akumulacji antocyjanów, bez istotnych wahań poziomu kwasu L-askorbinowego oraz zmian aktywności przeciwutleniającej. Dlatego wydaje się, że spożycie biofortyfikowanych selenem kiełków może być skutecznym sposobem uzupełniania diety ubogiej w ten pierwiastek.