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The Antioxidant Properties and Biological Quality of Radish Seedlings Biofortified with Iodine

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Abstract: Iodine is an essential trace element for humans, and iodine deficiency is a significant health problem. In this study, an improved method for iodine biofortification based on seed germination was established. Solutions of KI (0.15, 0.30, 0.75 and 1.5 mg·g^{-1} of seeds) were applied to germinating radish seeds of two cultivars *Raphanus sativus* L. var. sativus: Warta and Zlata. Compared with the control (seeds treated with water) the iodine content (in the radish sprouts produced by germinating seeds treated with KI were approximately 112.9–2730 times higher. The application KI rates did not adversely affect the biological quality of the radish sprouts. Regarding the biological quality of the iodine-enriched seedlings, we determined their length, dry mater, protein, soluble sugars, chlorophylls, total phenol, ascorbic acid, thiol group content and total antioxidant capacity. The effect of potassium iodide on the selected parameters of their biological quality varied depending on the KI doses and radish cultivars. The results showed that the most appropriate biofortification application rates were 0.15 and 0.30 mg KI per g seeds, because the enriched seedlings had excellent biological quality parameters.

Keywords: seed germination; radish; iodine phytoaccumulation; biofortification; biological quality

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1. Introduction

Iodine is an essential nutrient in the human diet. It is involved in thyroid hormone production and thereby affects the entire metabolism. Excessive iodine intake, which is relatively rare, can cause hyperthyroidism. Insufficient iodine in the diet leads to hypothyroidism, manifested as weakness, weight gain, and an enlarged thyroid gland. Daily iodine intake has been defined for various age groups by the World Health Organization [1]. Depending on age, the recommended daily allowance (RDA) ranges from 50 to 250 µg/day, and is 150 µg/day for adults. Iodine deficiency poses a risk in particular to the health of pregnant and nursing women, who need 250 µg iodine/day [2]. Developing infants and children require adequate iodine intake for normal brain function, and iodine deficiencies lead to mental retardation. This nutrient is not stored in the human body and must always be present in the diet. One means of preventing iodine deficiency is iodization of table salt; however, excessive intake of sodium chloride can lead to cardiovascular disease, particularly hypertension. The resulting recommendation to restrict intake of table salt negatively affects the prevention of iodine deficiencies [3]. Despite systemic measures undertaken on a global scale, such as iodization of table salt or other food products, iodine deficiency remains a problem affecting large populations, including in Europe. Many population groups in industrialized countries suffer from iodine deficiencies. Iodine prophylaxis must be adjusted to the changing dietary preferences of consumers. Vegans and vegetarians are a good example, as their dietary restrictions may put them at risk of iodine deficiency. Vegans have an increased risk of low iodine levels, deficiency and inadequate intake compared to adults whose diet is less restrictive [4]. There is a need to develop new solutions supporting iodine prophylaxis by promoting alternative sources of iodine. One possible solution is the biofortification of plants with iodine during their development and growth. Iodine is not an element essential to plants, but its content in plant-based food can be increased by fertilization with iodine. One factor limiting the development of agrotechnical methods for enriching plants with iodine is insufficient knowledge of the effect of fertilization with iodine on the growth, metabolism and quality of plants. The complexity of this subject encompasses the choice of means of fertilization—the optimal amount and chemical form of the compound and the application method (soil, foliar spraying or hydroponics), as well as the accumulation of iodine in the edible parts of the plants [5]. Ideally, iodine-enriched food would be natural, easily and quickly produced, and have valuable health-promoting properties, such as high content of antioxidants. The results of our previous research indicate that young seedlings of vegetables such as lettuce and radishes meet these criteria [6]. Furthermore, they can be produced quickly and in large quantities in controlled laboratory and industrial conditions. They can be consumed directly or added to other dishes. Therefore, it is an important task to test the predispositions of various genotypes of vegetables to fortification with iodine. The aim of the study was to increase the iodine content in seedlings of previously untested radish varieties, so that they will be safe to eat and approach the recommended daily intake of iodine, without reducing their biological quality or content of other health-promoting substances.

2. Materials and Methods

2.1. Cultivation of Plants Biofortified with Iodine

Two cultivars of radish *Raphanus sativus* L. var. sativus were chosen for the study: Warta and Zlata. The seeds were planted in Petri dishes 150 mm in diameter, on filter paper. The plants were watered once on the first day of the experiment with a potassium iodide solution or water. The source of potassium iodide was KI solutions applied in the amount of 0.15 mg, 0.30 mg, 0.75 mg and 1.5 mg per g of seeds. Next, on the second and fourth day, all seedlings were watered with water (5 cm^3/g of seeds). The seedlings grew under a grow light. The photoperiod was 12 h/12 h, and the temperature was 25 °C/22 °C during the day and night, respectively. After six days of growth, the plants were collected for analysis.

2.2. Assessment of Seedling Growth Rate

The following criteria were used to assess the growth rate of the seedlings: length, fresh weight, and dry weight of seedlings. The length of the entire seedling was measured. The biomass of the seedlings was determined by the gravimetric method. The dry weight of the seedlings was determined by the oven-drying method at 105 °C under normal pressure.

2.3. Determination of Iodine Content by ICP-MS

Iodine content in the test material was determined following incubation with TMAH (tetramethyl ammonium hydroxide) by ICP-MS (inductively coupled plasma mass spectrometry) according to PN-EN 15111:2008P [7]. The analyses were performed in the Central Laboratory of Agroecology (CLA) of the University of Life Sciences in Lublin, which is accredited by the Polish Centre for Accreditation (PCA), no. AB 1375.

2.4. Preparation of Extracts of Plant Material for Laboratory Analysis

Fresh plant material was weighed out and extraction solution was added (1:10 *w*/*w*), after which it was homogenized in a homogenizer for 2 min. The homogenate was centrifuged for 5 min at 3500 rpm. The supernatant was used for biochemical assays. In this way aqueous, methanol, acetone and TCA extracts were prepared. The solvents for preparing the extracts were water, 10% methanol in water [8], 80% acetone in water [9] and 10% trichloroacetic acid (TCA) in water [10].

2.5. Determination of Content of Protein and Soluble Sugars in Aqueous Extracts of Seedlings

Protein in the extract was determined according to Lowry [11]. The absorbance value was read after 30 min at 750 nm. Protein content in the seedling extracts was determined based on a standard curve for bovine serum albumin and expressed in mg·g^{-1} FW. The concentration of soluble sugars was determined by colorimetry according to Miller with 3,5-dinitrosalicylic acid (DNS) [12]. Absorbance was measured at 575 nm. The content of reducing sugars in the seedling extracts was determined based on a standard curve for glucose and expressed in mg·g^{-1} FW.

2.6. Determination of Chlorophyll a, Chlorophyll b and Chlorophyll a + b in Acetone Extracts of Seedlings

Chlorophyll content in the seedlings was determined by spectrophotometry in acetone extracts [9]. Absorbance was measured at 663 nm (chlorophyll a), 645 nm (chlorophyll b) and 652 nm (chlorophyll a + b). Chlorophyll content was expressed in mg·g^{-1} FW.

2.7. Determination of Total Antioxidant Capacity in Methanol Extracts of Seedlings by the DPPH Method

A methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added to a methanol extract of seedlings according to Brand-Williams et al. [13]. After 30 min, the decrease in absorbance relative to the control sample was measured at 515 nm. Total antioxidant capacity was expressed as equivalent of the synthetic antioxidant Trolox in 1 g FW.

2.8. Determination of Total Antioxidant Capacity in Aqueous Extracts of Seedlings by the ABTS Method

A concentrated solution of the ABTS$^{\cdot+}$ radical cation was obtained from 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) by oxidation of potassium persulfate. Diluted ABTS$^{\cdot+}$ solution was added to an aqueous extract of seedlings according to Re et al. [14].

The absorbance of the reaction mixture was measured after 30 min at 414 nm. Total antioxidant capacity was expressed as Trolox equivalent in 1 g FW of seedlings.

2.9. Determination of Total Content of Phenolic Compounds in Methanol Extracts of Seedlings

The total content of phenolic compounds in methanol extracts of seedlings was determined by spectrophotometry using Folin–Ciocalteu phenol reagent. Absorbance was measured after 1 h at 765 nm [15]. The results were expressed as gallic acid equivalent in 1 g FW of seedlings.

2.10. Determination of Ascorbic Acid Content in TCA Extracts of Seedlings

Content of ascorbic acid (AA) in the seedling extracts was determined using Folin–Ciocalteu reagent in TCA extract. Absorbance was measured after 10 min at 750 nm relative to a blank sample. The content of ascorbic acid in the sample was calculated using a standard curve and expressed in mg AA g^{-1} FW [10].

2.11. Determination of Concentration of Thiol Groups in Alcohol Extracts of Seedlings

The concentration of thiol groups (-SH) was determined using reagent, containing 5,5′-dithiobis(2-nitrobenzoic acid, DTNB) according to the Ellman method [16]. Dodecyl sodium sulphate solution (SDS) and phosphate buffer were added to seedling extract, and the absorbance of the sample was measured at 412 nm. Then DTNB solution was added and the reaction mixture was incubated at 3 °C for 1 h, after which the absorbance was measured again. The content of thiol groups in the sample was calculated as the difference in the absorbance between the test sample and the value obtained for a blank sample. The concentration of thiol groups in the sample was expressed as reduced glutathione (GSH) equivalent in 1 g FW of seedlings.

2.12. Statistical Analysis

Data for statistical computations were taken from at least three independent experiments, each performed in three replications. Statistical analysis was performed using one-way analysis of variance (ANOVA) in STATISTICA 10.0 software, at a significance level of $p < 0.05$. Homogeneous groups were determined using the Tukey test.

3. Results

Radish seedlings proved to be a good material for biofortification with iodine. The control radish seedlings had low iodine content (Table 1).

Table 1. Effect of level of KI application on the content of iodine in radish seedlings.

KI level ($mg·g^{-1}$ of Seeds)	Iodine Content ($mg·kg^{-1}$ FW of Seedlings)		Iodine Content (%) *		Amount of Seedlings (g) Containing 150 µg of Iodine	
	Warta	Zlata	Warta	Zlata	Warta	Zlata
0	0.72 a	0.11 a	100 a	100 a	214.29 a	1500.00 a
0.15	7.91 b	5.12 b	1129 b	5000 b	18.99 b	30.00 b
0.30	16.14 c	9.02 c	2286 c	9000 c	9.38 c	16.67 c
0.75	35.52 d	19.41 d	5071 d	19,400 d	4.23 d	7.73 d
1.50	96.09 e	27.30 e	13714 e	27,300 e	1.56 e	5.49 e

Means designated with the same letters for the same cultivars do not differ significantly at $p < 0.05$. * Iodine content expressed in % of the control.

Radish seedlings that developed from seeds germinated in the presence of KI contained much more of this element. Accumulation of iodine in the fortified seedlings was found to depend on the amount of KI applied. Both radish cultivars showed a capacity to accumulate iodine in large amounts, with a higher iodine concentration noted in the Warta seedlings than in the Zlata seedlings. The highest level of KI application caused a very high increase in iodine content in the seedlings, by nearly 137 times for Warta and 273 times in the case of Zlata.

The seedlings of both cultivars treated with KI showed rapid growth. At the highest level of 1.5 mg $KI·g^{-1}$ of seeds, the Warta seedlings were more than 31% shorter than the control seedlings (Table 2), while the Zlata seedlings biofortified with iodine did not differ statistically in size from the control seedlings.

Table 2. Morphometric and biochemical characteristics of radish seedlings biofortified with iodine.

KI Level ($mg·g^{-1}$ Seeds)	Length of Seedlings (mm)		Dry Matter (% FW)		Protein ($mg·g^{-1}$ FW)		Content of Soluble Sugars ($mg·g^{-1}$ FW)	
	Warta	**Zlata**	**Warta**	**Zlata**	**Warta**	**Zlata**	**Warta**	**Zlata**
0	111.13 a	110.22 a	6.74 a	6.15 a	2.30 b	2.20 a	12.50 a	14.53 a
0.15	102.90 a	110.46 a	6.62 a	5.91 a	2.52 a.b	2.20 a	11.59 a	14.25 a
0.30	91.81 a.b	99.48 a	6.66 a	6.18 a	2.42 b	2.16 a	10.91 a	13.61 a,b
0.75	94.62 a.b	96.34 a	6.75 a	6.14 a	2.52 a.b	2.12 a	12.30 a	12.53 a,b
1.50	76.41 b	92.93 a	6.86 a	6.40 a	2.66 a	2.11 a	12.87 a	11.46 b

Means designated with the same letters for the same cultivars do not differ significantly at $p < 0.05$.

The content of dry matter was not significantly changed in either cultivar of biofortified plants. In the case of the Zlata cultivar, enrichment of radish seedlings with iodine also caused no statistically significant changes in protein content (Table 2). In the Warta cultivar, biofortification with iodine was accompanied by an increase in protein content in seedlings; following the application of KI in the amount of 1.5 $mg·g^{-1}$ of seeds, the protein concentration increased by nearly 16% in comparison with the control seedlings. The content of soluble sugars in the biofortified seedlings of the Warta cultivar was similar to the control, while in the Zlata cultivar biofortification decreased the content of soluble sugars, which was lowest (by 21%) following application of KI at 1.5 mg·g^{-1} of seeds.

Another parameter indicative of the good biological quality and good physiological condition of the seedlings was their chlorophyll content. In the control seedlings, chlorophyll content was higher in the Warta cultivar (Table 3), and biofortification with iodine had no negative effect on its concentration.

Table 3. Chlorophyll content in radish seedlings biofortified with iodine.

KI Level (mg ·g^{-1} Seeds)	Chlorophyll a (mg·g^{-1} FW)		Chlorophyll b (mg·g^{-1} FW)		Chlorophyll a + b (mg·g^{-1} FW)	
	Warta	Zlata	Warta	Zlata	Warta	Zlata
0	0.209 a	0.123 b	0.104 a	0.075 b	0.320 a	0.190 b
0.15	0.194 a	0.146 a	0.101 a	0.086 a,b	0.302 a	0.221 ab
0.30	0.189 a	0.152 a	0.101 a	0.090 a,b	0.299 a	0.232 ab
0.75	0.205 a	0.149 a	0.106 a	0.092 a,b	0.315 a	0.231 ab
1.50	0.209 a	0.174 a	0.097 a	0.103 a	0.302 a	0.263 a

Means designated with the same letters for the same cultivars do not differ significantly at $p < 0.05$.

Radish seedlings of the Zlata cultivar responded to biofortification with iodine with greater chlorophyll synthesis. Even after the lowest level of KI application, there was an increase in chlorophyll content. Following application of KI at a concentration of 1.5 mg·g^{-1} of seeds, the content of chlorophyll a increased by over 41%, that of chlorophyll b by nearly 37%, and content of chlorophyll a + b by nearly 39% (Table 3).

The content of antioxidant substances was tested by several methods: measurement of total antioxidant capacity, the ABTS and DPPH methods, the content of ascorbic acid, which commonly occurs in plants, the total content of phenolic compounds, and content of compounds containing thiol groups.

The methods used to measure total antioxidant capacity make it possible to distinguish hydrophilic and hydrophobic antioxidants. Methanol extracts were prepared for the DPPH assays and aqueous extracts for the ABTS assays. In the control seedlings, a total antioxidant capacity was higher in the DPPH method in the Zlata seedlings but higher in the ABTS method in the Warta seedlings (Table 4).

Table 4. Content of antioxidant substances in radish seedlings biofortified with iodine.

Level of KI (mg·g^{-1} Seeds)	TAC DPPH (mM Trolox·g^{-1} FW)		TAC ABTS (mM Trolox·g^{-1} FW)		TPC (mM GA·g^{-1} FW)		AA (µg·g^{-1} FW)		TC (µmol GSH·g^{-1} FW)	
	Warta	Zlata	Warta	Zlata	Warta	Zlata	Warta	Zlata	Warta	Zlata
0	1.54 a	2.31 a	79.27 a	67.57 a	334.03 a	264.80 a	66.51 b	73.45 a	3.36 c	3.36 a
0,15	1.67 a	2.33 a	79.69 a	66.61 a	311.23 a,b,c	279.95 a	66.98 a,b	72.56 a	3.80 a	3.41 a
0.30	1.57 a	2.32 a	79.96 a	70.27 a	328.90 a,b	279.13 a	69.48 a,b	79.91 a	3.68 a,b	3.36 a
0.75	1.73 a	2.18 a	79.73 a	66.44 a	293.30 c	272.61 a	74.15 a	74.15 a	3.44 b,c	2.74 b
1.50	1.58 a	2.22 a	79.54 a	66.47 a	298.39 b,c	278.97 a	74.74 a	78.04 a	3.40 c	2.56 b

TAC—total antioxidant capacity, TPC—total phenolic content, AA—ascorbic acid content, TC—thiol group content; Means designated with the same letters for the same cultivars do not differ significantly at $p < 0.05$.

Biofortification caused no significant changes in TAC in the iodine-enriched seedlings.

The antioxidant properties of extracts are largely influenced by the presence of phenolic compounds. The total content of phenolic compounds in the control seedlings of the cultivars differed significantly; it was about 23 µg·g^{-1} FW higher in the Warta seedlings than in the Zlata seedlings. Biofortification with iodine did not reduce their concentration in the radish seedlings, except for the Warta seedlings treated with 0.75 and 1.5 mg·g^{-1} of seeds, by about 13% and 19%.

Vitamin C content in 1 g of control radish seedlings was 73.45 µg of vitamin C in the case of the Zlata cultivar and 66.51 µg for Warta (Table 4). Enrichment of radish seedlings with iodine caused no significant changes in the content of this vitamin in the

Zlata seedlings, while in Warta seedlings growing in the presence of 0.75 and 1.5 mg KI·g^{-1} of seeds, an increase of about 12% was noted relative to the control.

An increase in the content of compounds containing thiol groups was noted following biofortification of Warta radish with the two lowest concentrations of KI, i.e., 0.15 and 0.3 mg·g^{-1} of seeds, by about 13% and 10%, respectively. In the case of the Zlata radish seedlings, application of KI reduced the content of thiol groups, especially at 0.75 and 1.5 mg KI·g^{-1} of seeds, by about 19% and 24%.

Radishes of both cultivars can be biofortified with iodine, producing a high-quality yield after just a few days of growth. The significant increase in iodine content, high content of antioxidants, good biological quality, and high content of antioxidants make them an attractive source of nutrients and health-promoting substances.

4. Discussion

Iodine deficiency in the human diet and the need to enrich food with this nutrient justify research aimed at finding the most suitable species for biofortification with iodine [2]. The proper choice of plant species and amount of fertilizer are of key importance for the practical use of biofortification for food production. Biofortification of various plants using various forms and amounts of iodine has been described: spinach [17], lettuce [18,19], tomato and potatoes [20], carrot [21] and radish [22]. Accumulation of iodine in vegetables is largely dependent on transport through the xylem, which means that it is not always possible to achieve an adequate concentration in the edible parts of plants. Various amounts of iodine have been applied in soil; 2.5 mg of iodine kg^{-1} of soil can be regarded as a potential level of supplementation. In our experiment, satisfactory effects of biofortification were obtained with a much lower level—0.15 mg KI·g^{-1} of seeds. Iodine fertilizer is not fully used by plants [23]. This creates the risk of environmental pollution and uncontrolled reactions in the environment, e.g., with ozone in the atmosphere [24]. Attempts to enrich seedlings with microelements have been described in the literature, e.g., selenium [25,26], iron [27] and zinc [28]. The biofortification method used in our study enables strict control over the amount of iodine applied and results in high concentrations of this nutrient in plants (Table 1).

In our experiment, seedlings with high iodine content were obtained in the commercial maturity stage. A relationship was demonstrated between the amount of fertilizer applied and the concentration of iodine in the seedlings. Our previous research and that of other authors has also confirmed a positive correlation between the amount of iodine compounds applied and the iodine concentration of in plants—in lettuce [6], spinach [17], potato tubers and tomatoes [20].

In the present study, potassium iodide was used for biofortification of seedlings, but the literature contains descriptions of experiments using iodine salts in iodide and iodate form. Both salts are taken up well by plants. Other authors have shown that when plants are watered with iodide, biofortification is more effective than after application of iodate. This was observed in the case of biofortification of potato tubers, tomato fruits [20], and lettuce [19]. Studies by other authors indicate that the iodide form is more phytotoxic than the iodate form, but results in a higher iodine concentration in the plant, because it is taken up more easily [17,18,20,29]. Plants taking up iodate first reduce it to iodide; therefore, in our opinion, appropriate levels of iodide enable effective control of the biofortification process.

The aim of biofortification of plants is to increase the content of minerals so that the daily requirement for a given element is successfully provided by the diet [30]. We calculated the weight of radish seedlings that would meet the daily intake of 150 µg of iodine recommended by the WHO for an adult [1]. In our opinion, the lowest level of iodine fertilizer, i.e., 0.15 mg KI·g^{-1} of seeds, is optimal for biofortification of radish seedlings, as just 19 g of fresh Warta sprouts or 30 g of Zlata sprouts satisfies the recommended daily intake of iodine. This is a small portion of radish sprouts that can be eaten at one time. Raw sprouts can be added to salads or sandwiches. Moreover, by using varying amounts of potassium iodide for biofortification, we can obtain sprouts with varied iodine content. Research on biofortification of vegetables with iodine has mainly been conducted on spinach, lettuce, tomatoes, soybean sprouts, and broccoli sprouts. Some authors suggest about 50 g of lettuce biofortified with selenium and iodine [31] as portions providing the RDA of these microelements. Other authors suggest that a good option for direct consumption is iodized tomatoes containing 30 mg I·kg^{-1} FW [32], or even tomatoes with lower iodine content of 10 mg I·kg^{-1} FW [33].

In our experiment, despite the low levels of KI fertilizer, seedlings with high content of iodine as well as good biological quality were obtained (Tables 2 and 3).

The biofortified seedlings in our study developed at a similar rate to that of the control seedlings and showed no signs of chlorosis or necrosis, or any visible symptoms indicating that the amount of potassium iodide applied was toxic. A decrease in the length of the seedlings but not in the content of dry matter was observed in both cultivars of radish enriched with the highest levels of KI. In our opinion, the concentration of 1.5 mg KI·g^{-1} of seeds may be too high for biofortification, but it is not toxic for this organism. Only a decrease in seedling length was observed in the plants, but none of the symptoms of toxic effects characteristic of excessive application of iodine, as described for other biofortified plants [18,34]. In this context, it is worth noting that the high concentration of iodine in the Warta and Zlata radishes was not accompanied by changes in parameters determining the consumer and biological quality of the seedlings.

Seedling length, dry matter content, and chlorophyll content were chosen as parameters for assessment of the growth rate of seedlings in the presence of potassium iodide. The levels of potassium application higher than the amount we recommend for biofortification (0.15 mg·g^{-1} of seeds) generally caused a decrease in the average length of the seedlings, but did not cause changes in dry matter (Table 2). A reduction in biomass yield is often described as an unfavourable consequence of biofortification of plants, such as lettuce [18] or spinach [17]. Other authors stress that when an appropriate amount of iodine is properly applied to the plant, biofortification does not significantly affect dry matter content [35]. Content of sugars and protein in the edible parts of vegetables affects their quality, taste attributes, and nutritional value. The effect of the level of KI application on the concentration of protein and sugars varies depending on both the radish cultivar and the amount of potassium iodide applied. In the Warta cultivar, the protein concentration increased, while the concentration of sugars did not change significantly. Fortification of Zlata radish seedlings with iodine had a minor effect on protein content and reduced the sugar content in the samples biofortified with high levels of iodine. In lettuce plants, biofortification with iodine does not affect carbohydrate metabolism [34]. Other experiments have obtained iodized lettuce with increased accumulation of sugars [35]. In the case of mature radishes grown in soil, the form of iodine (KI and KIO_3) and means of application (soil or foliar) did not affect the content of soluble sugars [36]. In carrot roots as well, biofortification with iodine had no significant effect on the content of soluble sugars [37].

Content of chlorophyll, the pigment conditioning photosynthesis, is a sensitive indicator of plant physiology that enables diagnosis of biotic and abiotic stressors in plants [38]. The addition of KI caused generally small, generally statistically non-significant changes in chlorophyll content, and in the case of the Zlata cultivar, an increase (Table 3). An increase in chlorophyll content in iodized seedlings can have health benefits resulting not only from iodine supplementation but also from chlorophyll intake. Extracts rich in chlorophyll effectively alleviate weight gain and mild inflammation, improve glucose tolerance, and beneficially modify the composition of the gut microbiota in experimental animals [39]. Chlorophyll contains phytol, a molecule that takes part in cholesterol metabolism and has anti-inflammatory properties. Its presence in food reduces the risk of cardiovascular disease and chronic inflammatory diseases [40].

Biochemical processes taking place in seeds during germination alter the nutritional value and sensory traits of plants. Brassicaceae sprouts are rich in substances with health benefits, especially antioxidant compounds [41]. Cruciferous vegetables in the form of sprouts provide unique flavour and additional health benefits, as the content of bioactive substances in young Brassicaceae seedlings or sprouts can be 2 to 10 times higher than in mature vegetables [41]. Radish seedlings have varied composition and content of antioxidant compounds (Table 4), as indicated by TAC measurement in both the DPPH and ABTS methods. Owing to the specific characteristics of the reaction of the indicator substance with antioxidants, the numerical values obtained in the assays using different methods (ABTS and DPPH) are different [8]. Iodization of radish sprouts did not affect the total antioxidant capacity of hydrophilic antioxidants measured by the ABTS method or hydrophobic antioxidants measured by the DPPH method in either cultivar (Table 4). Blasco et al. [42] reported an increase in TAC in lettuce iodized with KI in a pot experiment.

The major antioxidant compounds in plants are phenolic compounds, ascorbic acid, and compounds containing thiol groups. As biofortification with iodine can affect the antioxidant system of plants and cause oxidative stress [18], in our experiment we tested the content of these compounds in iodized radish seedlings. Biofortification with high levels of iodine reduced the content of phenolic compounds in the Warta cultivar, but their concentration was still higher than in the Zlata cultivar. The content of these compounds in the Zlata cultivar did not change significantly (Table 4). Phenolic compounds affect the quality of food of plant origin, especially flavour characteristics (e.g., pungency), and contribute to health benefits associated with the consumption of Brassicaceae species, such as anti-cancer and anti-aggregation effects and activation of detoxifying enzymes [43]. There have been experiments on biofortification with iodine in which an increase in the concentration of phenolic compounds in plants was obtained. In lettuce, an increase in the concentration of phenolic compounds was achieved following application of iodide fertilizer [42]. In mature radish, foliar and soil application of iodine compounds also caused a significant increase in the concentration of these compounds [36]. Vitamin C is one of the antioxidants abundant in plants. The human body must ingest it with food. It takes part in the metabolism of carbohydrates, proteins, and fats as well as in synthesis of haemoglobin, erythrocytes, and some hormones [44]. The concentrations of potassium iodide used in our experiment did not significantly affect the content of ascorbic acid in the Zlata radish seedlings, whereas its concentration increased in the Warta seedlings. Other authors have also reported an increase in vitamin C content in plants enriched with iodine: in prickly pear (*Opuntia ficus-indica* L.) [45], water spinach [46].

Among the antioxidant substances tested, it is worth noting the high content of thiol compounds in the radish seedlings (Table 4). The concentrations of KI applied in the present study had varied effects on the content of compounds containing thiol groups. In the case of fertilization with 0.15 and 0.30 mg KI$\cdot$g^{-1} of seeds, which ensures effective biofortification, the concentration of thiol compounds in the Warta cultivar is higher than in the control, while in the Zlata cultivar it is similar to the control seedlings (Table 4). Iodized radish seedlings can thus provide both iodine and beneficial thiol compounds with strong reducing properties. These compounds are components of thiol-disulphide redox buffer, free radical scavengers, and chelators of metal ions [47].

5. Conclusions

In this study, we introduce a practical method using seed germination to enhance iodine accumulation in radish sprout. The results showed that a single application of potassium iodide fertilizer in a range of 0.15–1.50 mg$\cdot$g^{-1} of seeds effectively increases iodine content in seedlings. Both analysed radish cultivars accumulate iodine well, and practically every variant of the experiment could be used to obtain seedlings of good quality. Our observations indicate that the best concentrations of KI fertilizer for satisfying the RDA would be 0.15 and 0.30 mg$\cdot$g^{-1}, at which biofortification minimally affected the content of nutritionally valuable and health-promoting substances. Significantly high iodine content accompanied by unaffected high antioxidant activity and high content of phenolic compounds, ascorbic acid, and thiol compounds is indicative of the phytochemical stability of the biofortified sprouts, and thus of better food quality. The results of the study indicate that iodized radish sprouts have many advantages and merit particular attention as a source of iodine and phytochemicals with health-promoting properties.

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