

Article

Chemical Composition of Lettuce (*Lactuca sativa* L.) Biofortified with Iodine by KIO_3 , 5-Iodo-, and 3.5-Diiodosalicylic Acid in a Hydroponic Cultivation

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Abstract: According to the recommendations of the World Health Organization (WHO), due to the increased risk of cardiovascular disease, the daily consumption of table salt should be reduced. To avoid the health consequences of iodine deficiency, it is necessary to include alternative food sources of this trace element in the human diet. One of the most effective ways of improving nutrition is the biofortification of crops with minerals and vitamins. The purpose of this study was to determine the influence of iodine biofortification (potassium iodate/ KIO_3 /, 5-iodosalicylic acid/5-ISA/and 3.5-diiodosalicylic acid/3.5-diISA/) on the chemical composition of lettuce (*Lactuca sativa* L. *capitata*) cv. ‘Melodion’. Plants were cultivated in a hydroponic system NFT (Nutrient Film Technique). We compared the effect of iodine fertilization on the basic chemical composition, fatty acid profile, macro- and micronutrients, content of sugars, nitrogenous compounds, chlorides, and iodine compounds. The results obtained in this research indicate that the application of iodine compounds has an influence on changes of concentration of iodine and other compounds in the treated samples. In lettuce, the main fatty acid was linolenic acid; however, fertilization with iodine did not affect the fatty acid profile in plants, except for concentrations of myristic and arachidic acids. We also found that iodine fortification has positive effects on concentrations of some micro- and micronutrients. Moreover, the application of 3.5-diISA decreased the concentration of nitrates as compared to control and other treatments. Therefore, it may be postulated that the production of lettuce fortified with iodosalicylates is worthy of consideration due to the fact that it may be a good source of iodine and other compounds in the human diet.

Keywords: biofortification; iodosalicylates; *Lactuca sativa*

1. Introduction

Iodine deficiency (ID) is regarded as one of the most important health problem that affects populations around the world [1]. The reduction of iodine concentration in soils can be caused by human activities that lead to soil erosion, the degradation of water, and deforestation. The level of iodine in the human organism is dependent on its content in soils, plants, and livestock products that are consumed, so people who live in mountainous areas are more exposed to risk of iodine

deficiency disorders [2]. Nowadays, it is estimated that two billion people suffer from ID, of which 50 million are reported clinical symptoms. Iodine is the micronutrient required for the synthesis of thyroxine T_4 and triiodothyronine T_3 , so the undersupply of this element in the diet may have serious implications for health due to the effect of decreasing the secretion of thyroid gland hormones [1–3]. These hormones play a critical role in the growth and differentiation of the brain and neural system during fetal development; therefore, to avoid permanent damage to the neural structure, it is necessary to have adequate iodine consumption [4]. Iodine deficiency is considered among the main causes of mental and physical disorders that can be prevented [5]. In addition, the intelligence quotient (IQ) is lower in children living in geographical areas without sufficient amounts of this trace element [6]. Iodine deficiency disorders (IDD) can affect all ages and include hypothyroidism, endemic goitre, cretinism, learning disabilities, and complications of pregnancy or stillbirth [7].

The main sources of iodine in the diet include iodized salt, which is considered to be a strategy that allows us to reach iodine sufficiency [8]. Besides table salt, important iodine sources in the human diet are natural products of marine origin (seaweed or shellfish), water, meat, and milk derived from animals fed food enriched with iodine [3,9]. According to the European Food Safety Authority (EFSA) guidelines, the recommended daily allowance (RDA) of iodine for adult women and men should be 150 $\mu\text{g}/\text{day}$ [10]. The obligatory iodization of table salt was introduced in Poland in 1997, and it was one of the most effective prevention programs of IDD [11]. This easy and economical method was implemented in Poland and many countries of the world; however, there are still places that currently do not have programs for the prevention and monitoring of IDD [3]. On the other hand, the high content of salt in the daily diet is regarded as a factor that increases the risk of cardiovascular diseases and malignant tumors [12].

Therefore, it is necessary to find new alternative strategies of increasing iodine contribution to the human diet. One of them is biofortification, which is a method of increasing the accumulation of micronutrients in crop plants. This technique is considered to be an economically viable manner of increasing trace elements of people suffering from a nutritional deficiency [13]. The iodine biofortification of plants can be an effective way of improving human health and nutrition. Therefore, many studies investigated the possibility of using cultivated plants that have been fertilized with iodine compounds. Lettuce belongs to vegetables that as a result of biofortification may accumulate greater amounts of iodine [14–16].

Lettuce belongs to family *Compositae* and it is a widely consumed crop. This low-calorie salad plant is a relatively good source of dietary fiber, folate, and vitamins (A, C, K). It is believed that consuming food high in dietary fiber has a positive effect on the proper functioning of the digestive system. Lettuce has antioxidant properties resulting from the high level of carotenoids and phenolic compounds, which are also present in lettuce. The content of phytochemicals can be differentiated for individual varieties; therefore, it is essential to choose those that are characterized by a high level of phytochemicals [17,18].

The hypothesis states that the availability of iodine in nutrient solutions in hydroponic cultures depends to a large extent on the chemical form of the applied compound. Iodine supplied to plants in organic form as iodosalicylates (5-iodosalicylic and 3,5-diiodosalicylic acid) is taken up and accumulated in the same or more effectively way by plants than the inorganic form of iodates.

The aim of the study was to determine the influence of iodine biofortification (potassium iodate/ KIO_3 , 5-iodosalicylic acid/5-ISA/, and 3,5-diiodosalicylic acid/3,5-diISA/) on the chemical composition of lettuce (*Lactuca sativa* L. *capitata*) cv. 'Melodion'.

2. Materials and Methods

2.1. Plant Material and Cultivation

In the spring seasons of 2018 and 2019, the hydroponic cultivation of lettuce *L. sativa* cv. 'Melodion' was conducted in an NFT (Nutrient Film Technique) system. The experiments were located in a

greenhouse of the Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków (50°05′04.1″ N 19°57′02.1″ E).

Each year, seed sowing was performed at the beginning of March (13 March 2018 and 4 March 2019). Seeds were sown into 112-cell propagation trays with 32 × 32 × 40 mm sized cells filled with peat substrate mixed with sand (1:1 *v/v*). Seedlings of 4–5 true leaves were transplanted into the NFT system (10 April 2018 and 2 April 2019). The substrate was thoroughly rinsed from the seedling root system with the use of tap water. Seedlings were placed into holes (spaced 25 cm apart) of styrofoam slabs filling NFT beds: a “dry hydroponic” method of cultivation without substrate. After transplanting, plants were watered for one minute every 5 min during the day between 5:00 and 19:00 and during the night between 1:00 and 2:00. The nutrient solution used for the cultivation contained the following amounts of macro- and micronutrients (mg·dm⁻³): N 150, P 50, K 200, Mg 40, Ca 120, Fe 2, Mn 0.55, Zn 0.33, B 0.33, Cu 0.15, and Mo 0.05. At the beginning of lettuce cultivation in the NFT system, the EC (electrical conductivity) of nutrient solution of all treatments in all the experiments was 1.75 mS·cm⁻¹, and the pH of all nutrient solutions was adjusted to 5.70 with the use of 38% nitric acid.

Mineral nutrients were introduced into the solution with the use of the following fertilizers: calcium nitrate, monopotassium phosphate, potassium nitrate, potassium sulfate, and magnesium nitrate (all produced/distributed by Yara, Szczecin, Poland) and potassium chloride (ICL Speciality Fertilizer, Warszawa, Poland). Micronutrients were introduced in the form of multi-element fertilizer ‘Mikro plus’ (Intermag, Olkusz, Poland). The composition of the nutrient solution was balanced based on the results of chemical analysis of water—150 mg N·dm⁻³ took into account the amount of nitrogen contained in water, fertilizers, and nitric acid.

The distinguishing factor of the experiment was the chemical form of iodine applied to nutrient solution: (1) Control; (2) KIO₃, (3) 5-iodosalicylic acid (5-ISA), and (4) 3,5-diiodosalicylic acid (3,5-diISA). Inorganic (KIO₃) and organic forms of iodine, i.e., 5-ISA and 3,5-diISA (all puriss. p.a., Sigma-Aldrich Co. LLC, St. Louis, MO, USA) were applied once in a concentration of 10 μM calculated per molar mass of a whole compound. The application of these compounds into nutrient solution was started when the plants were in the rosette phase (18 April 2018 and 12 April 2019). The iodine used in base nutrient solutions (control) was iodide I⁻ (25.52 μg I·dm⁻³) and iodate IO₃⁻ (0.29 μg I·dm⁻³). The content of iodine was natural (from water and dissolved fertilizers). The experiment was conducted in a randomized block design with four repetitions within one NFT set. Plants were cultivated in four replications of 15 plants (60 plants per treatments). The plants were harvested at the stage of head production by plants—15 May 2018 and 7 May 2019. The average head weight of lettuce head was measured during the plants’ harvest.

Lettuce heads were cut in half and mixed in order to obtain a representative sample of all leaves (old and young) from all heads in each treatment. Fresh lettuce leaves were analyzed to determine the concentrations of total sugars, sucrose, glucose and fructose, nitrates (V), nitrites (III), ammonium ion, chlorides, and free amino acids.

2.2. Lettuce Analysis after Sample Drying

Lettuce leaves were frozen at -20 °C and lyophilized with the use of a Christ Alpha 1-4 lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Samples of lyophilized leaves were ground in a laboratory grinder (FRITSCH Pulverisette 14, Idar-Oberstein, Germany) and stored in tightly closed polyethylene bags (at room temperature) until the analysis. The samples were subsequently analyzed to determine the concentrations of the following elements: iodine by the inductively coupled plasma (ICP)-MS/MS (Inductively coupled plasma—triple quadrupole mass spectrometry) technique; total nitrogen (N-total) by the Kjeldahl method, and P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Sr, Li, Ba, Al, V, and Cd by the inductively coupled plasma optical emission spectrometer (ICP-OES) technique; salicylic acid (SA), benzoic acid (BeA), and iodine metabolic compounds by the LC-MS/MS technique, as well as basic chemical composition and fatty acid profile.

2.3. Basic Chemical Composition

In freeze-dried lettuce leaves, the content of ash, crude fat, total proteins, and total dietary fiber were assayed. AOAC (Association of Official Analytical Chemists) methods were used to determine the basic chemical composition. The ash content of lettuce was determined by burning samples in a muffle furnace (AOAC procedure No. 930.05). The total protein content was assessed by using the Kjeldahl method (AOAC procedure No. 950.36). Crude fat was measured according to the Soxhlet extraction method (AOAC procedure No. 935.38). The total amount of dietary fiber was evaluated by a commercially available test kit (AOAC procedure No 991.43). The content of digestible carbohydrate was estimated with the following formula: $100 - \text{sum of the protein, fat, ash, and dietary fiber content}$.

2.4. Fatty Acid Profile

To 1 g of lyophilized lettuce, 30 mL of Folcha solution (chloroform–methanol (2:1) with the addition of 1 g/l butylated hydroxytoluene) was added [19]. After 24 h of incubation at RT (Room Temperature), the solution was filtered through a paper filter into a new tube. Then, 4 mL of 0.9% NaCl solution was added to the tube. The chloroform layer was transferred to a new tube and dried under a stream of nitrogen in a water bath at a temperature not exceeding 40 °C.

Extracted fatty acids were converted into their methyl derivatives using the method described by Morrison and Smith [20]. Saponification was carried out by adding 2 mL 0.5 M KOH solution to a 10 mg fatty acid mixture for 15 min at 60 °C. Then, fatty acids were methylated by adding 2 mL of BF₃/MeOH solution to the tube and incubating for another 15 min at 60 °C. Fatty acid methyl esters were extracted by adding 2 mL of hexane to the tube. The last stage of extraction was the addition of 2 mL of saturated NaCl solution. After separation of the layers, the hexane (upper) layer was taken for chromatographic analysis. Fatty acid profile analysis was performed using a gas chromatograph coupled with a Shimadzu QP 5050 mass spectrometer equipped with an SP-2560 capillary column (30 m long, 0.25 mm diameter, and stationary phase grain size 0, 25 µm (Sigma-Aldrich, St. Louis, MO, USA). Chromatographic analysis was carried out under the following conditions: carrier gas, helium; injection port temperature, −245 °C; injected sample volume, 1 mL; carrier gas flow through the column, 1.8 mL/min. An initial temperature of 60 °C, held for 5 min, was used to separate the various fatty acid methyl esters; then, the temperature was raised every 5 to 180 °C and held for another 16 min; then, it was raised every 5 to 220 °C and held for 20 min. Electron ionization (Electron Impact) at the full scanning spectrum from 40 to 500 m/z and an ionization energy of 70 eV were used to perform the mass spectrometry analysis. The identification of fatty acid methyl esters was performed using a reference mixture of these compounds (FAME Mixture, Larodan Fine Chemicals).

2.5. Determination of Macro- and Microminerals

N-total was determined by the Kjeldahl method [21] using an oven mineralization Foss Digestor 2020 by Tecator™ and Velp UDK 139 Semi Automatic Distillation Unit. Content of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Sr, Li, Ba, Al, V, and Cd was determined by the ICP-OES technique according to method described by Kalisz et al. [22]. Samples (0.5 g) were placed into 55 mL TFM (TFM - Modified Poly-Tetra-Fluoro-Ethylene (PTFE)) vessels and were mineralized in 10 mL 65% super pure HNO₃ (Merck no. 100443.2500) in a Mars 5 Xpress (CEM, USA) microwave digestion system. The following mineralization procedure was applied: 15 min achieving a temperature of 200 °C and 20 min maintaining this temperature. After cooling, the samples were quantitatively transferred to 25-mL graduated flasks with redistilled water. Concentration of the mentioned elements was determined using a high-dispersion inductively coupled plasma optical emission spectrometer (ICP-OES; Prodigy Teledyne Leeman Labs, USA).

2.6. Determination of Iodine

In order to evaluate the content of iodine in lettuce leaves, the alkaline extraction of samples with tetramethylammonium hydroxide (TMAH) was conducted according to the procedure described by Smoleń et al. [23]: 0.5 g air-dried leaf or root samples, 10 mL double-distilled water and 1 mL of 25% TMAH (Sigma-Aldrich) were put into 30 mL Falcon tubes. After mixing, samples were incubated for 3 h at 90 °C. After incubation, samples were cooled to a temperature of approximately 20 °C and filled to 30 mL with double-distilled water. After mixing, samples were centrifuged for 15 min at 4500 rpm. The measurements of iodine content using an ICP-MS/MS triple quadrupole spectrometer (TQ ICP-MS/MS Thermo Fisher Scientific) were conducted in the supernatant without decanting [24].

2.7. Determination of Content of Total Sugars, Sucrose, Glucose, and Fructose

The content of glucose, fructose, and sucrose was assessed in ethanol extracts as described by Smoleń et al. [25]. For sugar content determination, capillary electrophoresis (PA 800 Plus system with DAD /Diode-Array Detection/ detection) was used. Capillaries of \varnothing 25 μ m and a total length of 60.5 cm (50 cm to detector) were used. The positive power supply of 30 kV was applied, and the temperature of detection was 18 °C. The running buffer solution consisted of 3.6 mM Na₂HPO₄ and 0.2 mM β -cyclodextrin. The pH of buffer solution was set to 12.7 using 130 mM NaOH. The sum of the contents of glucose, fructose, and sucrose was presented as the total sugar content [25].

2.8. Determination of Content of Free Amino Acids, Nitrogenous Ions, and Chlorides

The content of free amino acids was assessed in ethanol-preserved samples. The level of free amino acids was determined spectrophotometrically after a reaction with ninhydrin. In order to analyze the level of NH₄⁺, NO₃⁻, and Cl⁻ in lettuce, sample extraction with 2% acetic acid was conducted. All the ions were determined by AQ2 Discrete Analyzer (Seal Analytical, USA) using the protocols provided by the manufacturer of this analyzer.

2.9. Determination of Content of Iodine Compounds

The concentrations of the following compounds were determined in lettuce leaves: SA, BeA and iodine metabolic compounds as: 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA), T3 triiodothyronine in form sodium salt of (T3-Na), triiodothyronine (T3), and iodotyrosine. The analytical procedure described in the earlier publication of Smoleń et al. [26] was used. The following extraction procedure was applied: 50 mg of plant material was placed in 7 mL polypropylene tubes, with 5 mL of 76% ethanol containing 50 ng·mL⁻¹ of deuterated salicylic acid (SA-d₄, Sigma-Aldrich). Samples were vortexed and subjected to 1-h ultrasound-assisted extraction at 50 °C. Then, samples were centrifuged (5 min, 4500 RPM) and supernatants filtered with the use of nylon 0.22 μ m syringe filters (FilterBio NY Syringe Filter). The measurements of SA, BeA, and all iodine metabolic compounds were determined by an LC-MS/MS technique (Ultimate 3000, Thermo Scientific, QTrap 4500, Sciex). The LC-MS/MS system was controlled using Analyst 1.7 with HotFix 3 software, which was also used for data processing.

2.10. Assessment of Iodine-Fortified Lettuce for Consumer Health Safety

The daily intake of I (D-I), as well as the percentage of the Recommended Daily Allowance of I (% RDA-I) from 100 g of lettuce leaves was calculated. The value of RDA-I was based on the World Health Organization (WHO) recommendation for children above 12 years old and adults, i.e., 150 μ g I [27].

The consumer safety of iodine-enriched lettuce leaves was evaluated on the basis of the HQ (hazard quotient) values that describe the risk to human health resulting from the intake of I through the consumption of fresh lettuce leaves. The calculations of HQ-I values were performed according

to the following equation: $HQ = ADD/RfD$, where ADD is the average daily dose of I (mg I per kg body weight per day) and RfD represents the recommended dietary tolerable upper intake level of I [28]. The average daily dose (ADD) was determined as: $ADD = (MI \cdot CF \cdot DI)/BW$, where MI is the iodine concentration in lettuce leaves ($mg \cdot kg^{-1}$ d.w.), CF is the fresh to dry weight conversion factor for plant samples (dry weight to fresh weight ratio; 0.162 on average), DI is the daily intake of iodine (0.1 kg), and BW is the taken body weight (70 kg). The taken RfD values for I were $1100 \mu g I \cdot day^{-1}$ [8]. The presented method of HQ calculation represents only I intake with fresh lettuce leaves.

2.11. Statistical Analysis

Statistical analysis was performed with the Statistica 13.1 PL program. All experiments were done with at least four replications. The results were analyzed using analysis of variance (ANOVA). Statistically significant differences were assessed by the post-hoc Duncan's test. p values < 0.05 were considered as statistically significant.

3. Results

3.1. Weight of Lettuce Head, Dry Matter Content, and Basic Chemical Composition

The application of iodine compounds had a significant influence on increasing the lettuce head weight, dry matter content, and chemical composition in lettuce leaves (Table 1). An average weight of lettuce head was in the range of 147.65–296.82 g. The application of 5-ISA and 3.5-diISA caused a statistically significant reduction of the lettuce head weight. An average content of dry matter in all objects ranged from 4.36% to 6.41%. There were statistically significant differences between the dry matter of lettuce cultivated in the control and 3.5-diISA combinations. The highest content of dry matter was observed in lettuce enriched with 3.5-diISA. The ash content was in the range of 18.35–22.00 g/100 g d.w (dry weight); however, differences were not statistically significant. The application of an inorganic form of iodine (KIO_3) caused a statistically significant decrease of crude fat content and an increase of protein level in comparison to the other combinations. Among the studied objects, there were no statistically significant differences in the content of dietary fiber and digestible carbohydrates.

Table 1. Weight of lettuce head, dry matter content, and the chemical composition (means for 2018–2019).

Treatment	Weight of Single Lettuce Head (g)	Dry Matter (% d.w.)	Ash (g/100 g d.w.)	Crude Fat (g/100 g d.w.)	Protein (g/100 g d.w.)	Dietary Fiber (g/100 g d.w.)	Digestible Carbohydrates (g/100 g d.w.)
Control	291.44 ± 36.78b	4.59 ± 0.12a	21.53 ± 0.08	3.26 ± 0.10a	25.75 ± 0.78b	26.72 ± 0.89	22.71 ± 1.21
KIO_3	296.82 ± 22.45b	4.36 ± 0.08a	22.00 ± 0.04	1.67 ± 0.07b	26.42 ± 1.11b	26.21 ± 0.44	24.07 ± 1.33
5-ISA	171.13 ± 7.91a	5.22 ± 0.10a	19.18 ± 0.17	2.95 ± 0.13a	24.01 ± 0.26a	28.74 ± 0.78	24.84 ± 0.71
3.5-diISA	147.65 ± 13.84a	6.41 ± 0.12b	18.35 ± 0.06	3.25 ± 0.032a	24.21 ± 0.51a	28.22 ± 0.76	26.13 ± 1.01

Results are shown as mean ± standard deviation (SD); $n = 4$; means followed by the same letter are not significantly different ($p < 0.05$); d.w. dry weight.

3.2. Fatty Acid Profile

The fatty acid composition extracted from lettuce biofortified with iodine is shown in Table 2. Twelve fatty acids were identified in the tested samples, including myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, linoleic acid, arachidic acid, α -linolenic acid, behenic acid, pentadecylic acid, and lignoceric acid. The dominant components of lettuce were polyunsaturated fatty acids (PUFAs), which constituted 71.53–73.50% of the total fatty acid content. When compared to control lettuce, the biofortified samples did not present statistically significant differences with fatty acid content, except for myristic and arachidic acids.

Table 2. Fatty acid profile (means for 2018–2019). 3,4-diISA: (4) 3,5-diiodosalicylic acid, 5-ISA: 5-iodosalicylic acid, PUFA: polyunsaturated fatty acids.

Treatment	Control	KIO ₃	5-ISA	3,5-diISA
Fatty acid in % of total fatty acid				
Myristic acid	0.27 ± 0.04a	0.25 ± 0.05a	0.29 ± 0.01ab	0.42 ± 0.09b
Palmitic acid	18.36 ± 0.26	18.42 ± 0.36	17.77 ± 0.36	18.17 ± 1.47
Palmitoleic acid	2.13 ± 0.39	1.97 ± 0.34	2.15 ± 0.44	2.16 ± 0.21
Margaric acid	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.07 ± 0.02
Stearic acid	2.31 ± 0.26	1.89 ± 0.41	2.10 ± 0.34	2.49 ± 0.17
Oleic acid	3.48 ± 0.22	2.87 ± 0.16	2.59 ± 0.14	2.42 ± 0.09
Linoleic acid	21.49 ± 1.89	22.67 ± 2.02	21.48 ± 2.38	22.08 ± 2.68
Arachidic acid	0.37 ± 0.06b	0.35 ± 0.04ab	0.35 ± 0.03ab	0.28 ± 0.02a
α-Linolenic acid	50.04 ± 2.26	50.33 ± 2.24	52.01 ± 2.36	50.66 ± 1.18
Behenic acid	0.52 ± 0.02	0.53 ± 0.05	0.44 ± 0.06	0.38 ± 0.06
SFA Saturated fatty acid	22.84 ± 0.48	22.14 ± 0.48	21.75 ± 0.74	22.66 ± 1.77
MUFAs Monounsaturated fatty acids	5.61 ± 0.18	4.85 ± 0.22	4.74 ± 0.31	4.59 ± 0.17
PUFAs Polyunsaturated fatty acids	71.53 ± 0.37	73.00 ± 0.26	73.50 ± 0.43	72.74 ± 1.89
Pentadecylic acid	0.07 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.07 ± 0.02
Lignoceric acid	0.86 ± 0.01	0.60 ± 0.04	0.69 ± 0.08	0.77 ± 0.12

Results are shown as mean ± standard deviation (SD); $n = 4$; means followed by the same letter are not significantly different ($p < 0.05$).

3.3. The Effectiveness of Lettuce Iodine Biofortification on Changes in the Content of Mineral Elements

The average amounts of the mineral elements in lettuce are shown in Table 3. The content of iodine was in the range from 3.03 to 138.40 mg I kg⁻¹ d.w. The significantly highest level was found in lettuce biofortified with 5-ISA. Fertilization with different forms of iodine resulted in a significant increase of the amount of selected macrominerals (Mg, Ca, Na) and trace elements (B, Fe, Mo, Ba, Al, V, Cd) in lettuce. The highest content of Cu was observed in control plants. Analyzed samples of lettuce did not differ in their concentration of N, P, K, and S as compared to control and other combinations. The obtained results suggest that in most instances, the level of mineral components was higher in lettuce fertilized with iodine compounds, and the differences between their content in samples were significant.

Table 3. Mineral composition (means for 2018–2019).

Treatment	N	P	Macrominerals (% d.w.)				
			K	Mg	Ca	S	Na
Control	4.37 ± 0.27	0.70 ± 0.01	8.11 ± 0.18	0.39 ± 0.01a	1.32 ± 0.06a	0.21 ± 0.01	0.09 ± 0.00a
KIO ₃	4.59 ± 0.18	0.68 ± 0.02	8.16 ± 0.21	0.37 ± 0.01a	1.29 ± 0.07a	0.20 ± 0.01	0.106 ± 0.00a
5-ISA	4.37 ± 0.58	0.66 ± 0.02	6.47 ± 0.19	0.47 ± 0.01b	1.75 ± 0.06b	0.25 ± 0.01	0.218 ± 0.01b
3,5-diISA	3.96 ± 0.04	0.61 ± 0.01	7.74 ± 0.08	0.37 ± 0.01a	1.21 ± 0.02a	0.24 ± 0.01	0.081 ± 0.00a
Treatment	I	B	Trace elements (mg kg ⁻¹ d.w.)				
			Cu	Fe	Mn	Zn	
Control	3.03 ± 0.14b	27.19 ± 0.56a	7.16 ± 0.12c	89.32 ± 3.90a	128.60 ± 2.56	46.85 ± 1.61b	
KIO ₃	18.23 ± 0.75a	25.91 ± 1.55a	6.52 ± 0.16bc	76.13 ± 3.88a	140.44 ± 5.11	44.73 ± 2.36ab	
5-ISA	138.40 ± 7.59c	39.96 ± 1.30b	4.54 ± 0.48ab	128.93 ± 8.97b	163.95 ± 7.07	44.16 ± 3.02ab	
3,5-diISA	14.83 ± 0.40a	42.58 ± 1.14b	3.62 ± 0.21a	145.65 ± 7.60b	158.02 ± 2.05	35.93 ± 2.25a	
Treatment	Mo	Sr	Li	Ba	Al	V	Cd
KIO ₃	0.64 ± 0.10a	57.63 ± 1.93	0.89 ± 0.02	2.21 ± 0.13a	15.29 ± 1.03a	1.07 ± 0.04a	0.11 ± 0.02a
5-ISA	0.57 ± 0.17a	62.22 ± 4.94	0.98 ± 0.05	4.00 ± 0.19c	47.01 ± 5.19b	1.30 ± 0.05b	0.78 ± 0.16b
3,5-diISA	1.63 ± 0.10c	49.76 ± 2.53	0.71 ± 0.01	3.21 ± 0.16b	52.46 ± 10.53b	1.16 ± 0.05ab	0.18 ± 0.04a

Results are shown as mean ± standard deviation (SD); $n = 8$; means followed by the same letter are not significantly different ($p < 0.05$); d.w. dry weight.

3.4. The Content of Sugars, Nitrogenous Compounds, and Chlorides

Treatment with the iodine compounds had a significant influence on the content of sugars in lettuce (Table 4). The plants biofortified with 3,5-diISA were characterized by the highest amount of glucose, fructose, and total sugars compared to control and other combinations. However, there was no statistically significant difference in the content of sucrose in tested plants. The average amounts of

nitrogenous compounds and chlorides in lettuce are presented in Table 5. The highest mean of free amino acids was observed in control non-enriched plants. In comparison to the control, application of the organic forms of iodine caused the increased concentration of chloride (Cl^-) in lettuce. The statistically significant lowest amount of nitrate (NO_3^-) ions was noted in lettuce after fertilization with 3.5-diISA. There was no statistically significant difference in the content of nitrite (NO_2^-) between control and iodine-fortified lettuce.

Table 4. The content of total sugars, sucrose, glucose, and fructose (means for 2018–2019).

Treatment	Sucrose (mg/100 g f.w.)	Glucose (mg/100 g f.w.)	Fructose (mg/100 g f.w.)	Total Sugars (mg/100 g f.w.)
Control	51.85 ± 8.50	97.84 ± 12.39a	104.06 ± 16.76a	253.76 ± 33.29a
KIO ₃	40.54 ± 3.95	81.18 ± 10.10a	95.08 ± 13.06a	216.82 ± 23.97a
5-ISA	38.59 ± 4.13	110.87 ± 10.45a	138.68 ± 12.21a	288.14 ± 26.18a
3.5-diISA	59.04 ± 2.83	154.70 ± 17.57b	200.52 ± 17.08b	414.28 ± 31.63b

Results are shown as mean ± standard deviation (SD); $n = 8$; means followed by the same letter are not significantly different ($p < 0.05$); f.w. fresh weight.

Table 5. The content of nitrogenous compounds and chlorides (means for 2018–2019).

Treatment	Free Amino Acids (mg N ₂ /100g f.w.)	NH ₄ ⁺ (mg/kg f.w.)	NO ₃ ⁻ (mg/kg f.w.)	NO ₂ ⁻ (mg/kg f.w.)	Cl ⁻ (mg/kg f.w.)
Control	11.58 ± 0.84b	20.49 ± 2.00bc	4639.57 ± 337.78a	0.79 ± 0.42ab	143.06 ± 16.50ab
KIO ₃	10.29 ± 1.32ab	28.18 ± 12.32c	5258.89 ± 512.81a	0.55 ± 0.06a	130.86 ± 19.58a
5-ISA	7.35 ± 0.56c	13.17 ± 2.92ab	4690.71 ± 372.58a	1.32 ± 0.49b	177.93 ± 22.15bc
3.5-diISA	9.32 ± 0.85a	11.70 ± 0.96a	2401.54 ± 259.75b	0.64 ± 0.24ab	185.82 ± 20.97c

Results are shown as mean ± standard deviation (SD); $n = 8$; Means followed by the same letter are not significantly different ($p < 0.05$); f.w. fresh weight.

3.5. The Content of Iodine Compounds

Table 6 shows the content of iodine compounds in lettuce biofortified with iodine. In comparison with the control and KIO₃ treatments, after the fertilization of 5-ISA and 3.5-diISA, the level of salicylic acid (SA), 5-iodosalicylic acid (5-ISA), and 3.5-diiodosalicylic acid (3.5-diISA) was significantly higher. The highest concentration of free T3 (triiodothyronine) was observed after KIO₃ and 3.5-diISA treatment. The application of iodine compounds had no statistically significant impact on the concentration of 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA), benzoic acid (BeA), and T3 sodium salt (T3-Na) in tested plants. However, the lettuce biofortified with KIO₃ was characterized by the highest content of iodotyrosine, which was about 10–16 times higher than in plants after 5-ISA and 3.5-diISA treatment, respectively.

Table 6. The content of iodine compounds (means for 2018–2019).

Treatment	Control	KIO ₃	5-ISA	3.5-diISA
	(mg/kg d.w.)			
SA	0.52 ± 0.05a	0.45 ± 0.02a	1.09 ± 0.09b	1.03 ± 0.07b
5-ISA	0.04 ± 0.01a	0.02 ± 0.00a	1.55 ± 0.11c	0.38 ± 0.02b
3.5-diISA	0.04 ± 0.01a	0.19 ± 0.02a	0.01 ± 0.01a	12.67 ± 0.85b
2-IBeA	0.27 ± 0.06bc	0.09 ± 0.01ab	0.02 ± 0.01a	0.36 ± 0.06c
4-IBeA	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
2,3,5-triIBeA	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
BeA	6.69 ± 0.95	7.60 ± 2.29	6.67 ± 0.95	6.22 ± 1.27
T3-Na	7.64 ± 1.36	10.67 ± 0.70	10.60 ± 0.91	23.00 ± 1.01
T3 free	0.008 ± 0.001a	0.015 ± 0.003b	0.009 ± 0.003a	0.018 ± 0.006b
Iodotyrosine	0.28 ± 0.05b	0.56 ± 0.28c	0.05 ± 0.02a	0.03 ± 0.01a

Results are shown as mean ± standard deviation (SD); $n = 8$; means followed by the same letter are not significantly different ($p < 0.05$); d.w. dry weight.

3.6. Percentage of Recommended Daily Allowance (RDA) for Iodine (RDA-I) and Hazard Quotient (HQ) for Intake of Iodine through Consumption of 50 and 100 g Portions of Fresh Lettuce Leaves by Adults 70 kg Body Weight (Means for 2018–2019)

Enrichment lettuce with iodine compounds caused an increase in the value of percentage of recommended daily allowance for I (RDA-I) and HQ for intake of iodine through the consumption of 50 and 100 g portions of fresh lettuce leaves by adults (Table 7). The value of the hazard quotient (HQ) aims to inform consumers about the safety of the consumption of a food. If the value of an HQ is higher than 1, adverse health effects are possible. The HQ value for the consumption of lettuce was ranged from 0.01–0.33 and 0.01–0.66 respectively for 50 and 100 g portions, which represent a harmless dose for consumers. The highest value of percentage RDA-I was observed in lettuce biofortified with 5-ISA, which was statistically significant compared to other combinations. The recommended daily intake of iodine for adults is 150 µg. Ingestion of the presented portion of lettuce fertilized with 5-ISA provides the highest content of iodine; however, intake of this micronutrients is higher than the tolerable upper intake level, which is 1100 µg/day.

Table 7. Percentage of Recommended Daily Allowance (RDA) for iodine (RDA-I) and hazard quotient (HQ) for intake of iodine through consumption of 50 and 100 g portions of fresh lettuce leaves by adults 70 kg body weight (means for 2018–2019).

Treatment	% RDA I (in 50 g Portion of Lettuce)	% RDA I (in 100 g Portion of Lettuce)	Daily Intake of I with 50 g of Lettuce (mg I·day ⁻¹)	Daily Intake of I with 100 g of Lettuce (mg I·day ⁻¹)	HQ for 50-g Portion of Lettuce	HQ for 100-g Portion of Lettuce
Control	4.72 ± 1.99b	9.45 ± 3.97b	0.01 ± 0.01b	0.01 ± 0.01b	0.01 ± 0.00b	0.01 ± 0.01b
KIO ₃	26.65 ± 4.92a	53.31 ± 9.84a	0.04 ± 0.01a	0.08 ± 0.01a	0.04 ± 0.01a	0.07 ± 0.01a
5-ISA	241.93 ± 35.46c	483.86 ± 70.93c	0.36 ± 0.05c	0.73 ± 0.11c	0.33 ± 0.05c	0.66 ± 0.01c
3.5-diISA	34.74 ± 1.26a	69.48 ± 2.51a	0.05 ± 0.03a	0.10 ± 0.07a	0.05 ± 0.03a	0.09 ± 0.06a

Results are shown as mean ± standard deviation (SD); *n* = 8; means followed by the same letter are not significantly different (*p* < 0.05).

4. Discussion

The main problem in the global food economy is micronutrient malnutrition, which occurs mainly in developing countries. One of the most effective ways of combating hidden hunger is fortification—an agronomic or genetic cultivation method that increases the concentration of nutrients in crops [29,30]. The results obtained in the present study indicate that the application of iodine compounds has an influence on the changes of concentration of chemical compounds and dry matter content in tested objects. We found also that fertilization with iodosalicylates led to the significant reduction of the weight of lettuce head, which indicates the need to reduce iodosalicylic acids concentration during the biofortification process. The highest concentration of dry matter was in lettuce biofortified with 3.5-diISA (6.41%). However, there were no differences between the dry matter of plants cultivated in the control and KIO₃. This result is in accordance with those obtained in the study of Signore et al. [31], in which fortified carrot was cultivated in an open field and in a greenhouse. They showed that the concentration of dry matter was not significantly influenced by biofortification with KIO₃. Similar results were also observed in the content of dry matter in biofortified tomato fruits [32]. In the study of Kiferle et al. [32], treatments with KI and KIO₃ did not significantly affect the dry matter in comparison to control plants. Therefore, it is likely that the content of dry matter in iodine-fortified plants may depend on factors such as the species of plant or technique of fertilization. This study demonstrates that the biofortification of lettuce with iodine compounds has no effect on the content of ash, dietary fiber, and digestible carbohydrates. However, the application of iodosalicylic acids caused a significant reduction in the level of protein in tested plants. Moreover, after KIO₃ treatment, the increase of crude fat content was noted as compared to control and other treatments. Other researchers who also studied the chemical composition of lettuce showed that the fertilization of iodine did not imply changes in nutrients content in plants [33,34].

The effects of the biofortification of crops on fatty acid profiles is insufficient; there are not many studies on the subject. Lettuce is a low-fat and low-calorie vegetable that contains polyunsaturated fatty acids (PUFA). These fatty acids are essential for the proper functioning of the human body. Currently, in the Western diet, it is possible to observe a high ratio of omega-6 (linoleic acid) to omega 3 (linolenic acid), which may contribute to an increased risk of obesity, cancer, cardiovascular, and neurodegenerative disease. An appropriate ratio of fatty acid composition allows us to maintain a balance of pro-inflammatory and anti-inflammatory mediators from omega 6 and omega 3, respectively [35,36]. According to the literature, the dominant fatty acids in lettuce are linolenic (C18:3) and linoleic (C18:2) acids, which are about 60% and 20% of the total fatty acids, respectively [37,38]. The results obtained in this study are comparable with the data of other authors. In tested lettuce, the main fatty acid was linolenic acid (C18:3) in the range of 50.04–52.01%. The level of linoleic acid was consistent with results reported by Kim et al. [35] in the range of 21.48–22.67%. Moreover, our research has shown that the fertilization of iodine did not affect the fatty acid profile in lettuce, except for concentrations of myristic and arachidic acids. The application of 3.5-diISA caused a significant increase in the myristic acid content when compared to control and other combinations. However, after fertilization with iodine compounds, a decrease in the percentage of arachidic acid was observed.

An adequate number of macro and microelements in the diet is essential for health [35]. The iodine fortification of crops can contribute to replenishing shortages of this micronutrient and therefore to improving human nutrition. Currently, the main technique for increasing iodine content in the diet is universal salt iodization, which involves the fortification of salt both for human and livestock consumption. One of the problems with the iodization of table salt is its possible loss during thermal treatment, transport, and long-term storage. For this reason, leafy vegetables are regarded as the best crop for micronutrient fertilization, because most often, they are consumed raw; thus, the risk of iodine loss is very limited [16]. Moreover, lettuce belongs to plants grown in a hydroponic system, which allows for the effective accumulation of iodine in plant tissues. There are numerous studies that demonstrate similar results confirming the effectiveness of the fortification of vegetables with this micronutrient [39,40].

The results of our study showed that the biofortification with iodosalicylic acids caused a significant increase in iodine in lettuce leaves in comparison to the control. The highest level of iodine was observed in plants after the application of 5-ISA, which was over 40-fold higher than in control lettuce. We also found that fertilization with KIO_3 and 3.5-diISA resulted in much lower iodine content in lettuce leaves. A review of the literature indicates that the biofortification of lettuce with iodine compounds is an efficient and cost-effective method to provide an adequate content of micronutrients. It is worth mentioning that vegetables are better accumulators of iodine than grains of cereal crops, because the accumulation of this element in the plant tissues is in large part dependent on xylem transport [41]. Additionally, as shown in the Weng et al. [42] study, in the leafy vegetables, intake of iodine may be even 70 times more than in fruits or vegetables. Jerše et al. [43] reported that fertilization with iodide (I^-) resulted in a significant increase in iodine concentration in all parts of pea plants as compared to the control. However, the application of IO_3^- caused an increased iodine content only in the roots and pod shells of pea plants [43]. Other interesting results were obtained by Lawson et al. [44], who reported a much higher iodine accumulation in vegetables after fertilization with the oxidized form of this element. Moreover, they found that the application of KIO_3 turned out to be safer than iodide (I^-) because it did not generate phytotoxic symptoms in plants. According to Smoleń et al. [45], lettuce may take up iodine in different chemical forms, not only in the inorganic form but also as 5-iodosalicylic acid, 3,5-diiodosalicylic acid, 2-iodobenzoic acid, 4-iodobenzoic acid, and 2,3,5-triiodobenzoic acid. The authors noted that the application of iodine in organic form allows for more effective absorption of this micronutrient by lettuce with respect to the mineral form, IO_3^- . Smoleń et al. [45] showed that the lower concentration of 5-ISA (8.0 μM) can result in a similar content of iodine in biofortified lettuce (without affecting the weight of heads), as did the higher application of KIO_3 (40.0 μM). The obtained results showed that fortification with 5-ISA caused a significant increase in the concentration of Mg, Ca,

Na, Ba, V, and Cd in lettuce leaves. Blasco et al. [46] also reported an improvement of Mg level after iodine application. Increasing the Mg concentration has a positive effect on the growth of plants due to its important role in essential plant processes such as protein synthesis and photosynthesis. Mg and Ca also play significant roles for the human organism by promoting bone growth and preventing its loss [47]. In our study, the highest content of Fe and Mo was observed in plants after the application of 3.5-diISA; therefore, the consumption of biofortified lettuce may effectively increase the level of these mineral elements for nutrition. This is especially important in vegetarian diets [48]. We also noticed that with the iodine treatment, there was a tendency of Cu and Zn concentration to decrease in lettuce, which is consistent with other research. Smoleń et al. [25] reported that fortification with iodine and selenium also resulted in a reduction of K, S, B, and Fe content in lettuce leaves compared to control. However, in this study, we found no negative influence of iodine on the content of these elements. The differences between results are probably due to the chemical form of iodine that was applied. The introduction of iodine into plants has the effect of changing the bioavailability of macro- and microelements. It was determined that in iodine-fortified plants, there are interactions among mineral elements that can have synergistic or antagonistic effects. The first of these is cooperation between iodine and other elements, which leads to enhancement of their transport and absorption, whereas antagonisms weaken those activities [49].

The obtained results demonstrated the positive changes in sugar concentrations after fortification with iodine compounds. The amount of total sugars was the highest after the application of 3.5-diISA; it was nearly twice as high as in control lettuce for fructose content. We also found that fertilized lettuce with another form of iodine had no influence on the concentration of sugars in the enriched plants. The increase of sugar concentration in fortified lettuce was also reported by Blasco et al. [50]. Their study showed that the concentration of glucose was higher after iodine treatments but had no effect on sucrose concentration in tested plants [50]. It is worth mentioning that Blasco et al. [46] found no effect on the amount of sucrose between IO_3^- treatment and control lettuce, which was consistent with our observations. The different results were presented by Kiferle et al. [32], who reported a decrease of sugar content after KI and KIO_3 fertilization of tomato fruits. One of the possible reasons for sugar reduction in lettuce may be participation in a detoxification mechanism. The majority of plants, as opposed to humans, do not need iodine for proper growth and development; however, there are some aquatic plants that require this micronutrient for metabolic processes [49]. Smoleń et al. [25] noted that decreasing the carbohydrate concentration in lettuce may be caused by a detoxification process that consists of the volatilization of iodine in the methylated form of CH_3I . Other research by the same authors showed similar findings to ours. In Smoleń et al. [25], treating plants with selenium and salicylic acid after an iodine treatment seemed to prevent the decrease in sugar content. This may indicate that the examined chemicals did not negatively affect the process of photosynthesis in plants.

According to WHO recommendations, 400 g of fruit and vegetables per day should be eaten to provide sufficient amounts of nutrients that are essential for good health. However, at the same time, plant products may also be contaminated with anti-nutrients, such as nitrates, pesticides, and mycotoxins [51,52]. Nearly 80% of dietary nitrate comes from vegetables, and green leafy plants are characterized by the highest concentration of nitrate [53]. To avoid harmful effects on human health, maximum limits on the nitrate amount in lettuce cultivated in greenhouses were conducted by The European Commission. The concentration of nitrates in lettuce should be no more than 5000 and 4000 mg NO_3/kg for lettuce grown under cover and in the open air, respectively. In accordance with these provisions, the limits are different depending on the time of harvest. For example, they are higher for plants harvested in winter, from October to March [54,55]. The content of nitrate in vegetables is also affected by the degree of absorption and transfer in the plants; therefore, the concentration in leaves is much higher than in root vegetables. The excessive consumption of nitrate may contribute to serious health implications. This results from the fact that in the gastrointestinal tract, the relatively harmless nitrate ion is reduced to nitrite, which is used to produce nitrosoamines. The toxic effect of nitrates is related to the incidence of methemoglobinemia, due to increased methemoglobin concentration in

the blood [53,56]. Moreover, a number of studies have shown that N-nitroso compounds may play important roles in the process of cancerogenesis. A high consumption of nitrates contributes to the risk of cancers in the gastrointestinal tract, urinary bladder, and prostate. However, the role of nitrates is not limited to negative impacts on human health. Nitric oxide may also protect against pathogenic microorganisms living in the intestine [56,57].

This research showed that lettuce fortification with 3,5-diISA was characterized by the lowest concentration of nitrates, as compared to control and other treatments. The same tendency was not observed in nitrite content. In our study, there were no observed changes in the level of NO_2^- in lettuce after iodine fertilization. Other authors found that the roots of carrot fertilized with iodate (KIO_3) was characterized by a lower content of nitrates, as compared to the control [31]. Different results were obtained by Weng et al. [58], who found an increased content of nitrate in water spinach after I^- and IO_3^- treatment [58]. Generally, the concentration of nitrate and nitrite in plants depends on the biochemical mechanism of N uptake and assimilation in which specific transporters and enzymes are involved. The most important enzymes are nitrate reductase (NR), which reduces nitrate (NO_3^-) to nitrite (NO_2^-), and nitrite reductase (NiR), which reduces nitrite to ammonium [59]. In our study, the application of iodosalicylic acids contributed to a decrease in the level of free amino acids in tested lettuce. Furthermore, the highest content of ammonium ions was observed after KIO_3 fertilization. This is in line with previous findings in the literature [60]. Blasco et al. [61], who also analyzed lettuce response to treatment with different forms of iodine, reported that the application of iodine in two forms (I^- and IO_3^-) led to a decreased accumulation of NO_3^- as well as nitrate reductase (NR) activity in tested plants. However, they do not observe any significant effect of iodine application on the concentration of NH_4^+ [61]. This is important in terms of plant breeding, because excessive amounts of NO_3^- can contribute to pathological disorders in plants and thus decrease crop quality. We also observed increased concentrations of chloride after the application of iodosalicylic acids compared to the control and KIO_3 treatments. In general, iodine is absorbed in plants through ionic channels and chloride transporters, so it is possible for there to be a distortion of transfer after fortification with iodine compounds [49,62]. Our results may suggest that the application of iodosalicylates does not interfere with the chloride transport in iodine-fertilized lettuce.

The present study confirmed the findings from previous research that showed that lettuce has the capacity to absorb iodine from nutrient solutions in different forms, such as 5-ISA, 3,5-diISA, 2-IBeA, 4-IBeA, and 2,3,5-triIBeA [45]. In our study, absorption of inorganic (KIO_3) and organic (3,5-diISA) forms of iodine by lettuce was compared. We found that the application of 5-ISA caused accumulation of a much higher content of iodine in plants than after KIO_3 and 3,5-diISA treatment. In a study conducted on tomato plants, the authors demonstrated that the absorption of iodine from the inorganic form (KI) is higher than iodine from iodosalicylates [63]. We showed that the application of iodosalicylates caused increased concentrations of these compounds in lettuce leaves. We did not observe an impact of applied iodine compounds on the content of 4-IBeA, 2,3,5-triIBe, and BeA in plants. However, these compounds were also detected in control plants, which proves that they are naturally synthesized in lettuce [26]. Our results are comparable with those that Smoleń et al. [45] obtained in which they showed that the lower dose of 5-ISA than KIO_3 should be used to obtain plants with similar concentrations of iodine. They also explained that the roots are probably responsible for the accumulation of 5-ISA in lettuce. Furthermore, the application of 5-ISA has a positive impact on the absorption, transport, and accumulation of iodine in lettuce leaves. Importantly, Palmer et al. [64] revealed that 5-ISA may act as an inducer of plant defenses; therefore, it can protect against bacterial pathogens. In the available studies, there is very little information about thyroid hormone analogs in plants.

Some researchers suggest there is a possibility of using compounds of plant origin for the treatment of symptoms of short-term hypothyroidism in patients who can not undergo continued hormonal therapy [65]. The findings presented by Smoleń et al. [26] have shown that iodosalicylates may be involved in the biosynthesis of plant-derived thyroid analogs (PDTHA). Moreover, the application 5-ISA

and 3,5-diISA resulted in an increase of triiodothyronine, 5-ISA, and 3,5-diISA in lettuce. Our results also demonstrated that fortification has the effect of enhancing the concentration of triiodothyronine. Plants fortified with KIO_3 and 3,5-diISA were characterized by the highest level of T3, compared to control and 5-ISA-treated plants. Furthermore, we observed that after fertilization with KIO_3 , the level of iodothyrosine in lettuce was the highest, which may indicate a role of iodate in the synthesis of this hormone.

In the literature, there are research studies that refer to the problem of food safety of iodine-enriched vegetables [39]. The recommended daily allowance (RDA) of iodine is 150 $\mu\text{g}/\text{day}$ for adults, depending on gender and age. During pregnancy and lactation, there are increased iodine requirements to 220 and 209 $\mu\text{g}/\text{day}$, respectively [66]. In Voogt et al. [39], the RDA for iodine (RDA-I) of a serving of lettuce was in range of 22–25% evaluated by the consumption of 50 g of fortified vegetables. Jerše et al. [43] reported a lower level of iodine with a portion of fresh seeds of pea plants treated with iodine (I^-). The consumption of a 100 g portion of pea seeds provides only about 2% of RDA-I [43]. These results showed that the leaves of 5-ISA-enriched lettuce are characterized by the highest level of iodine. We found that the consumption of 5-ISA-biofortified lettuce leaves provided from 360.89 to 725.79 $\mu\text{g I}$ from 50 and 100 g portions of edible fresh weight, respectively (data not shown). Therefore, after 5-ISA treatment, the level of iodine contained in both portions of biofortified lettuce was much higher than the recommended value of RDA-I for adults. One portion of control lettuce contained only 4.72% of the requirement for the daily intake of iodine.

According to the data of the Institute of Medicine, a tolerable upper intake level (UL) of iodine is 1100 $\mu\text{g}/\text{day}$; however, it is worth emphasizing that this level was determined for the mineral form of iodine [66]. For 50 and 100 g portions of fortified lettuce, values of HQ_{gv} were lower than 1, meaning that the consumption of these plants does not pose a risk to consumers' health and life. Other authors who studied the efficiency of consumption of iodine-biofortified vegetables on the level of iodine in the human organism showed promising results. Tonacchera et al. [16] noted that after the consumption of enriched vegetables, the urinary level of iodine is 19.6% higher than before the intake of these vegetables. The urinary iodine is regarded as a recommended and easily obtained indicator for iodine status that is measured in spot urine samples and provides information about the average iodine intake [16,67,68]. In our opinion, it is necessary to determine the bioavailability of fortified elements from lettuce in order to evaluate how enriched crops can meet the iodine requirements in the human body. Generally, iodine is an element with a relatively high bioavailability that may range up to even 99%, but there are many factors affecting the absorption and use of micronutrients. In order to determine the effect of fortified vegetables on nutrition in micronutrient-deficient populations, feeding tests should be conducted. However, today, they are frequently replaced by studies in organ systems and animal models [69]. To summarize, the consumption of iodine-fortified vegetables may be a good source of this element in the daily diet and therefore an effective strategy for preventing micronutrient deficiency in the general population and the resulting public health problems [16].

5. Conclusions

Iodine is regarded as an essential micronutrient for human organism. Its deficiency can lead to many pathologies, including physical and mental disorders. The biofortification of vegetables can be an effective strategy to reduce micronutrient malnutrition around the world. The results from this study show that the fertilization of lettuce with 5-ISA led to plants containing much higher amounts of iodine compared to the application of 3,5-ISA and KIO_3 . Moreover, fortification has positive effects on concentrations of macro- and micronutrients, such as Mg, Ca, Na, B, Zn, Mo, and the content of glucose and fructose in lettuce. On the other hand, we found that the application of iodine in both forms did not cause significant changes in fatty acid profiles, dietary fiber, and digestible carbohydrates. Another positive aspect of fortification with 3,5-diISA was decreasing the level of nitrates in plants, which is important in terms of potential consumers. Food security should be taken into consideration during the fortification of vegetables with micronutrients. In conclusion, our research suggests that further studies

are needed to determine the effect of lettuce fortified with iodosalicylic acids on human health. We also recommended the reduction of iodosalicylic acids concentration during the lettuce biofortification process in hydroponic culture because of the observed reduction in weight of lettuce heads.

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References

1. Kapil, U. Health consequences of iodine deficiency. *Sultan Qaboos Univ. Med. J.* **2007**, *7*, 267–272. [PubMed]
2. World Health Organization. Assessment of the Iodine Deficiency Disorders and Monitoring Their Elimination: A Guide for Programme Managers, 3rd ed. Available online: <https://apps.who.int/iris/handle/10665/43781> (accessed on 14 April 2020).
3. Biban, B.G.; Lichiardopol, C. Iodine deficiency, still a global problem? *Curr. Heal. Sci. J.* **2017**, *43*, 103–111. [CrossRef]
4. Heidari, Z.; Arefhosseini, S.R.; Hedayati, M.; Vaghef-Mehrabany, E.; Ebrahimi-Mameghani, M. Iodine status, and knowledge about iodine deficiency disorders in adolescent school girls aged 14–19 years. *Heal. Promot. Perspect.* **2019**, *9*, 77–84. [CrossRef]
5. Sepahvand, R.; Hatamikia, M.; Hassanzadazar, H.; Moridi, S.; Bahari, K.; Sepahvand, H.; Fatehi, R.; Bagheri, A.; Belghadr, I.; Javadi, K.; et al. Iodine concentration in iodized salts marketed in Lorestan Province, west of Iran. *J. Chem. Heal. Risks* **2016**, *6*, 99–103.
6. Goris, J.M.; Temple, V.J.; Zomerdijk, N.; Codling, K. Iodine status of children and knowledge, attitude, practice of iodised salt use in a remote community in Kerema district, Gulf province, Papua New Guinea. *PLoS ONE* **2018**, *13*, 1–16. [CrossRef] [PubMed]
7. Yadav, K.; Pandav, C.S. National iodine deficiency disorders control programme: Current status & future strategy. *Indian J. Med. Res.* **2018**, *148*, 503–510. [CrossRef]
8. Kessler, J. Are there side effects when using suprathreshold levels of iodine in treatment regimens? In *Comprehensive Handbook of Iodine—Nutritional, Biochemical, Pathological and Therapeutic Aspects*, 1st ed.; Preedy, V., Burrow, G., Watson, R., Eds.; Academic Press: Cambridge, MA, USA, 2009; Volume 1, pp. 107–118. [CrossRef]
9. Paz, S.; Rubio, C.; Gutiérrez, A.; Revert, C.; Hardisson, A. Iodine: An essential trace element. *Med. J. Clin. Trials Case Stud.* **2018**, *2*, 2–5. [CrossRef]
10. European Food Safety Authority. Scientific opinion on Dietary Reference Values for iodine. *EFSA J.* **2014**, *12*, 3660. [CrossRef]
11. World Health Organization. Iodine Deficiency in Europe: A Continuing Public Health Problem. Available online: <https://apps.who.int/iris/handle/10665/43398> (accessed on 20 March 2020).
12. Pyka, B.; Zieleń-Zynek, I.; Kowalska, J.; Ziółkowski, G.; Hudzik, B.; Gąsior, M.; Zubelewicz-Szkodzińska, B. Zalecenia dietetyczne dotyczące spożywania jodu — W poszukiwaniu konsensusu między kardiologami a endokrynologami. *Folia Cardiol.* **2019**, *14*, 156–160. [CrossRef]
13. Bouis, H.E.; Saltzman, A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob. Food Sec.* **2017**, *12*, 49–58. [CrossRef] [PubMed]
14. Krzepińko, A.; Zych-Weżyk, I.; Świącilo, A.; Molas, J.; Skwaryło-Bednarz, B. Effect of iodine biofortification of lettuce seedlings on their mineral composition and biological quality. *J. Elem.* **2016**, *21*, 1071–1080. [CrossRef]
15. Koronowicz, A.A.; Kopec, A.; Master, A.; Smoleń, S.; Piątkowska, E.; Bieżanowska-Kopeć, R.; Ledwożyw-Smoleń, I.; Skoczylas, L.; Rakoczy, R.; Leszczyńska, T.; et al. Transcriptome profiling of caco-2 cancer cell line following treatment with extracts from iodine-biofortified lettuce (*Lactuca sativa* L.). *PLoS ONE* **2016**, *11*, 1–14. [CrossRef] [PubMed]

16. Tonacchera, M.; Dimida, A.; De Servi, M.; Frigeri, M.; Ferrarini, E.; De Marco, G.; Grasso, L.; Agretti, P.; Piaggi, P.; Aghini-Lombardi, F.; et al. Iodine fortification of vegetables improves human iodine nutrition: In Vivo evidence for a new model of iodine prophylaxis. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 694–697. [[CrossRef](#)] [[PubMed](#)]
17. Hasan, M.R.; Tahsin, A.K.M.M.; Islam, M.N.; Ali, M.A.; Uddain, J. Growth and yield of lettuce (*Lactuca Sativa* L.) influenced as nitrogen fertilizer and plant spacing. *IOSR J. Agric. Vet. Sci.* **2017**, *10*, 62–71. [[CrossRef](#)]
18. Mampholo, B.M.; Maboko, M.M.; Soundy, P.; Sivakumar, D. Phytochemicals and overall quality of leafy lettuce (*Lactuca sativa* L.) varieties grown in closed hydroponic system. *J. Food Qual.* **2016**, *39*, 805–815. [[CrossRef](#)]
19. Folch, J.; Lees, M.; Sloane-Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1987**, *55*, 999–1033.
20. Morrison, W.R.; Smith, L.M. Preparation of fatty acid methyl esters and dimethylacetals from lipids. *J. Lipid Res.* **1964**, *5*, 600–608.
21. Persson, J.A.; Wennerholm, M. *Poradnik Mineralizacji Kjeldahl'a—Przegląd Metody Klasycznej z Ulepszeniami Dokonanymi Przez Firmę FOSS TECATOR*; Labconsult: Warszawa, Poland, 1999. (In Polish)
22. Kalisz, A.; Sękara, A.; Smoleń, S.; Grabowska, A.; Gil, J.; Komorowska, M.; Kunicki, E. Survey of 17 elements, including rare earth elements, in chilled and non-chilled cauliflower cultivars. *Sci. Rep.* **2019**, *9*, 1–14. [[CrossRef](#)]
23. Smoleń, S.; Kowalska, I.; Kováčik, P.; Halka, M.; Sady, W. Biofortification of six varieties of lettuce (*Lactuca sativa* L.) with iodine and selenium in combination with the application of salicylic acid. *Front. Plant Sci.* **2019**, *10*, 1–13. [[CrossRef](#)]
24. PN-EN 15111. *Foodstuffs—Determination of Trace Elements—Determination of Iodine by ICP-MS (Inductively Coupled Plasma Mass Spectrometry)*; Polish Committee of Standardization: Warsaw, Poland, 2008. (In Polish)
25. Smoleń, S.; Kowalska, I.; Czernicka, M.; Halka, M.; Kęska, K.; Sady, W. Iodine and selenium biofortification with additional application of salicylic acid affects yield, selected molecular parameters and chemical composition of lettuce plants (*Lactuca sativa* L. var. *capitata*). *Front. Plant Sci.* **2016**, *7*, 1–16. [[CrossRef](#)]
26. Smoleń, S.; Kowalska, I.; Halka, M.; Ledwożyw-Smoleń, I.; Grzanka, M.; Skoczylas, Ł.; Czernicka, M.; Pitala, J. Selected Aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. *Agronomy* **2020**, *10*, 1. [[CrossRef](#)]
27. Brantsæter, A.L.; Knutsen, H.K.; Johansen, N.C.; Nyheim, K.A.; Erlund, I.; Meltzer, H.M.; Henjum, S. Defined by age, life stage and vegetarian dietary practice in a norwegian convenience sample. *Nutrients* **2018**, *10*, 230. [[CrossRef](#)]
28. Environmental Protection Agency. *Integrated Risk Information System—Database*; Environmental Protection Agency: Washington, DC, USA, 2011.
29. De Valença, A.W.; Bake, A.; Brouwer, I.D.; Giller, K.E. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Glob. Food Sec.* **2017**, *12*, 8–14. [[CrossRef](#)]
30. Ngozi, U.F. The role of biofortification in the reduction of micronutrient food insecurity in developing countries. *African J. Biotechnol.* **2013**, *12*, 5559–5566. [[CrossRef](#)]
31. Signore, A.; Renna, M.; D'Imperio, M.; Serio, F.; Santamaria, P. Preliminary evidences of biofortification with iodine of “carota di polignano”, an Italian carrot landrace. *Front. Plant Sci.* **2018**, *9*, 1–8. [[CrossRef](#)] [[PubMed](#)]
32. Kiferle, C.; Gonzali, S.; Holwerda, H.T.; Ibaceta, R.R.; Perata, P. Tomato fruits: A good target for iodine biofortification. *Front. Plant Sci.* **2013**, *4*, 1–10. [[CrossRef](#)] [[PubMed](#)]
33. Rakoczy, R.; Kopeć, A.; Piątkowska, E.; Smoleń, S.; Skoczylas, Ł.; Leszczyńska, T.; Sady, W. The iodine content in urine, faeces and selected organs of rats fed lettuce biofortified with iodine through foliar application. *Biol. Trace Elem. Res.* **2016**, *174*, 347–355. [[CrossRef](#)]
34. Kopeć, A.; Piątkowska, E.; Biezanowska-Kopeć, R.; Pysz, M.; Koronowicz, A.; Kapusta-Duch, J.; Smoleń, S.; Rakoczy, R.; Skoczylas, Ł.; Leszczyńska, T.; et al. Effect of lettuce biofortified with iodine by soil fertilization on iodine concentration in various tissues and selected biochemical parameters in serum of Wistar rats. *J. Funct. Foods* **2015**, *14*, 479–486. [[CrossRef](#)]
35. Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; Waterland, N.L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. Food Compos. Anal.* **2016**, *49*, 19–34. [[CrossRef](#)]
36. Ristic-Medic, D.; Vucic, V.; Takic, M.; Karadžic, I.; Glibetic, M. Polyunsaturated fatty acids in health and disease. *J. Serbian Chem. Soc.* **2013**, *78*, 1269–1289. [[CrossRef](#)]

37. Dinicolantonio, J.J.; O’Keefe, J.H. Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. *Open Hear.* **2018**, *5*, 3–6. [[CrossRef](#)] [[PubMed](#)]
38. Le Guedard, M.; Schraauwers, B.; Larrieu, I.; Bessoule, J.J. Development of a biomarker for metal bioavailability: The lettuce fatty acid composition. *Environ. Toxicol. Chem.* **2008**, *27*, 1147–1151. [[CrossRef](#)] [[PubMed](#)]
39. Voogt, W.; Holwerda, H.T.; Khodabaks, R. Biofortification of lettuce (*Lactuca sativa* L.) with iodine: The effect of iodine form and concentration in the nutrient solution on growth, development and iodine uptake of lettuce grown in water culture. *J. Sci. Food Agric.* **2010**, *90*, 906–913. [[CrossRef](#)]
40. Zhu, Y.G.; Huang, Y.Z.; Hu, Y.; Liu, Y.X. Iodine uptake by spinach (*Spinacia oleracea* L.) plants grown in solution culture: Effects of iodine species and solution concentrations. *Environ. Int.* **2003**, *29*, 33–37. [[CrossRef](#)]
41. Dai, J.L.; Zhu, Y.G.; Zhang, M.; Huang, Y.Z. Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. *Biol. Trace Elem. Res.* **2004**, *101*, 265–276. [[CrossRef](#)]
42. Weng, H.X.; Hong, C.L.; Xia, T.H.; Bao, L.T.; Liu, H.P.; Li, D.W. Iodine biofortification of vegetable plants—An innovative method for iodine supplementation. *Chinese Sci. Bull.* **2013**, *58*, 2066–2072. [[CrossRef](#)]
43. Jerše, A.; Kacjan Maršič, N.; Kroflič, A.; Germ, M.; Šircelj, H.; Stibilj, V. Is foliar enrichment of pea plants with iodine and selenium appropriate for production of functional food? *Food Chem.* **2018**, *267*, 368–375. [[CrossRef](#)]
44. Lawson, P.G.; Daum, D.; Czauderna, R.; Meuser, H.; Härtling, J.W. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Front. Plant Sci.* **2015**, *6*, 1–11. [[CrossRef](#)]
45. Smoleń, S.; Ledwoźyw-Smoleń, I.; Halka, M.; Sady, W.; Kováčik, P. The absorption of iodine from 5-iodosalicylic acid by hydroponically grown lettuce. *Sci. Hortic.* **2017**, *225*, 716–725. [[CrossRef](#)]
46. Blasco, B.; Rios, J.J.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Leyva, R.; Romero, L.; Ruiz, J.M. Study of the interactions between iodine and mineral nutrients in lettuce plants. *J. Plant Nutr.* **2012**, *35*, 1958–1969. [[CrossRef](#)]
47. Lorincz, C.; Manske, S.L.; Zernicke, R. Bone health: Part 1, Nutrition. *Sports Health* **2009**, *1*, 253–260. [[CrossRef](#)]
48. Kim, M.J.; Moon, Y.; Kopsell, D.A.; Park, S.; Tou, J.C.; Waterland, N.L. Nutritional value of crisphead ‘iceberg’ and romaine lettuces (*Lactuca sativa* L.). *J. Agric. Sci.* **2016**, *8*, 734. [[CrossRef](#)]
49. Medrano-Macias, J.; Leija-Martinez, P.; González-Morales, S.; Juárez-Maldonado, A.; Benavides-Mendoza, A. Use of iodine to biofortify and promote growth and stress tolerance in crops. *Front. Plant Sci.* **2016**, *7*, 1146. [[CrossRef](#)] [[PubMed](#)]
50. Blasco, B.; Rios, J.J.; Leyva, R.; Melgarejo, R.; Constán-Aguilar, C.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Romero, L.; Ruiz, J.M. Photosynthesis and metabolism of sugars from lettuce plants (*Lactuca sativa* L. var. longifolia) subjected to biofortification with iodine. *Plant Growth Regul.* **2011**, *65*, 137–143. [[CrossRef](#)]
51. World Health Organization. Diet, nutrition and the prevention of chronic diseases. *Tech. Rep. Ser.* **2003**, *916*, 54–59.
52. Bottex, B.; Dorne, J.L.C.M.; Carlander, D.; Benford, D.; Przyrembel, H.; Heppner, C.; Kleiner, J.; Cockburn, A. Risk-benefit health assessment of food - Food fortification and nitrate in vegetables. *Trends Food Sci. Technol.* **2008**, *19*, 113–119. [[CrossRef](#)]
53. Brkić, D.; Bošnjir, J.; Bevardi, M.; Bošković, A.G.; Miloš, S.; Lasić, D.; Krivohlavek, A.; Racz, A.; Čuić, A.M.; Trstenjak, N.U. Nitrate in leafy green vegetables and estimated intake. *Afr. J. Tradit. Complement. Altern. Med.* **2017**, *14*, 31–41. [[CrossRef](#)]
54. European Union. Commission regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs. *Off. J. Eur. Union* **2011**, *320*, 15–17.
55. Burns, I.G.; Lee, A.; Escobar-Gutiérrez, A.J. Nitrate accumulation in protected lettuce. *Acta Hortic.* **2004**, *633*, 271–278. [[CrossRef](#)]
56. Ranasinghe, R.; Marapana, R. Nitrate and nitrite content of vegetables: A review. *J. Pharmacogn. Phytochem.* **2018**, *7*, 322–328.
57. Loh, Y.H.; Jakszyn, P.; Luben, R.N.; Mulligan, A.A.; Mitrou, P.N.; Khaw, K.T. N-nitroso compounds and cancer incidence: The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am. J. Clin. Nutr.* **2011**, *93*, 1053–1061. [[CrossRef](#)] [[PubMed](#)]
58. Weng, H.X.; Yan, A.L.; Hong, C.L.; Xie, L.L.; Qin, Y.C.; Cheng, C.Q. Uptake of different species of iodine by water spinach and its effect to growth. *Biol. Trace Elem. Res.* **2008**, *124*, 184–194. [[CrossRef](#)] [[PubMed](#)]

59. Krapp, A. Plant nitrogen assimilation and its regulation: A complex puzzle with missing pieces. *Curr. Opin. Plant Biol.* **2015**, *25*, 115–122. [[CrossRef](#)]
60. Strzetelski, P.; Smoleń, S.; Rozek, S.; Sady, W. The effect of diverse iodine fertilization on nitrate accumulation and content of selected compounds in radish plants (*raphanus sativus* L.). *Acta Sci. Pol. Hortorum Cultus* **2010**, *9*, 65–73.
61. Blasco, B.; Rios, J.J.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.M.; Rosales, M.A.; Ruiz, J.M.; Romero, L. Photorespiration Process and Nitrogen Metabolism in Lettuce Plants (*Lactuca sativa* L.): Induced Changes in Response to Iodine Biofortification. *J. Plant Growth Regul.* **2010**, *29*, 477–486. [[CrossRef](#)]
62. White, P.J.; Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* **2009**, *182*, 49–84. [[CrossRef](#)] [[PubMed](#)]
63. Halka, M.; Smoleń, S.; Ledwożyw-Smoleń, I.; Sady, W. Comparison of Effects of Potassium Iodide and Iodosalicylates on the Antioxidant Potential and Iodine Accumulation in Young Tomato Plants. *J. Plant Growth Regul.* **2020**, *39*, 282–295. [[CrossRef](#)]
64. Palmer, I.A.; Chen, H.; Chen, J.; Chang, M.; Li, M.; Liu, F.; Fu, Z.Q. Novel salicylic acid analogs induce a potent defense response in arabidopsis. *Int. J. Mol. Sci.* **2019**, *20*, 3356. [[CrossRef](#)]
65. Zubeldia, J.M.; Nabi, H.; Jimenez del Rio, M.; Genovese, J. Exploring new applications for *Rhodiola rosea*: Can we improve the quality of life of patients with short-term hypothyroidism induced by hormone withdrawal? *J. Med. Food* **2010**, *13*, 1287–1292. [[CrossRef](#)]
66. Trumbo, P.; Yates, A.A.; Schlicker, S.; Poos, M. Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *J. Am. Diet. Assoc.* **2001**, *101*, 294–301. [[CrossRef](#)]
67. Hussain, H.; Selamat, R.; Kuang Kuay, L.; Md Zain, F.; Yazid Jalaludin, M. Urinary Iodine: Biomarker for Population Iodine Nutrition. *Biochem. Test.-Clin. Correl. Diagnosis* **2020**, 1–16. [[CrossRef](#)]
68. World Health Organization. Urinary Iodine Concentrations for Determining Iodine Status in Populations. Available online: <https://apps.who.int/iris/handle/10665/85972> (accessed on 2 April 2020).
69. Gonzali, S.; Kiferle, C.; Perata, P. Iodine biofortification of crops: Agronomic biofortification, metabolic engineering and iodine bioavailability. *Curr. Opin. Biotechnol.* **2017**, *44*, 16–26. [[CrossRef](#)] [[PubMed](#)]



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