IODINE AND SELENIUM BIOFORTIFICATION OF LETTUCE (*Lactuca sativa* L.) BY SOIL FERTILIZATION WITH VARIOUS COMPOUNDS OF THESE ELEMENTS

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**Abstract.** Relatively little is known on the interaction between iodine and selenium in plants. It may become a drawback in developing agrotechnical rules of plant biofortification with these elements. The aim of the study was to determine the influence of soil fertilization with various forms of iodine (I\(^{-}\) and I\(_2\)) and selenium (SeO\(_3^{2-}\) and SeO\(_4^{2-}\)) on yield, biofortification efficiency and selected chemical properties of lettuce plants. The study (conducted in 2012–2014) included soil fertilization of lettuce cv. ‘Valeska’ in the following combinations: control (without iodine and selenium fertilization), KI, KIO\(_3\), Na\(_2\)SeO\(_4\), Na\(_2\)SeO\(_3\), KI + Na\(_2\)SeO\(_4\), KIO\(_3\) + Na\(_2\)SeO\(_4\), KI + Na\(_2\)SeO\(_3\), KIO\(_3\) + Na\(_2\)SeO\(_3\). Iodine and selenium were applied twice: before sowing and as a top-dressing in a total dose of 5 kg I·ha\(^{-1}\) and 1 kg Se·ha\(^{-1}\). Only the application of Na\(_2\)SeO\(_4\) (individually or together with iodine) exhibited strong toxic effect on plants which was accompanied by the highest accumulation of Se, selenomethionine (SeMet) and selenocysteine (SeCys) in lettuce. The accumulation of I and Se in lettuce was respectively higher after fertilization KI than KIO\(_3\) and Na\(_2\)SeO\(_4\) than Na\(_2\)SeO\(_3\). Simultaneous application of iodine and selenium decreased the level of Se, SeMet and SeCys in lettuce – particularly in the combination with KIO\(_3\) + Na\(_2\)SeO\(_3\). Simultaneous application of KI with both forms of selenium decreased iodine content in lettuce as related to the treatment with KI alone. In the case of lettuce from the combinations with KIO\(_3\), KIO\(_3\) + Na\(_2\)SeO\(_4\) and KIO\(_3\) + Na\(_2\)SeO\(_3\), comparable results of iodine concentration were obtained.

**Key words:** biofortification, biological quality, selenomethionine, selenocysteine, cold vapor generation of iodine

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INTRODUCTION

Almost two-third of human population suffers from diseases related to insufficient supply of iodine and selenium in the diet. One of the easiest and most effective methods to counteract this problem is biofortification of crop plants with mineral nutrients [White and Broadley 2009, Przybysz et al. 2015]. Simultaneous biofortification with iodine and selenium may be however problematic as both elements are not essential mineral nutrients for plants [Kopsell and Kopsell 2007, Kabata-Pendias 2011]. Yet, selenium is included to the group of “beneficial elements” [Kopsell and Kopsell 2007].

No information is available on nationwide agrotechnical programmes of iodine biofortification of crop plants as similar to those conducted with selenium in Finland or Malawi [Eurola et al. 2003, Chilimba et al. 2012]. One of the few examples includes iodination of irrigation water using KIO$_3$ in southern Xinjiang Province of China. With low economic costs, it increased the content of available iodine in soils and consequently in the food [Ren et al. 2008].

In the recent years WHO presented the “Global Strategy on Diet, Physical Activity and Health” aimed, amongst others, at limiting the consumption of table salt with searching for alternative sources of iodine in the human diet [WHO 2004, 2014]. It seems, however, that WHO does not perceive the huge potential of iodine-enriched plants [WHO 2014], even though numerous studies on iodine biofortification of crop plants have already been conducted [i.e. Blasco et al. 2010, Voogt et al. 2010].

In lettuce cultivation in hydroponics, soilless systems or pot experiments the influence of SeO$_3^{2-}$ and SeO$_4^{2-}$ on: biomass, selenium accumulation, antioxidant activity, the content of secondary plant metabolites [Ríos et al. 2008, Ríos et al. 2010] or nitrogen metabolism of plants was described [Rios et al. 2010]. Also the effect of iodine (I$^-$ and IO$_3^-$) on basic physiological and biochemical processes in lettuce grown in hydroponics (in greenhouses or growth chambers) was tested [Blasco et al. 2010, Voogt and Jackson 2010, Voogt et al. 2010].

The problem of interaction between iodine and selenium in plants is not yet sufficiently diagnosed. Few studies on simultaneous application of iodine and selenium in spinach [Zhu et al. 2004] or lettuce cultivation [Smoleń et al. 2014] were conducted in hydroponics. With the exception of one-year studies conducted by Mao et al. [2014] with Se + Zn + I fertilization during the cultivation of wheat, maize, soybean, potato, canola and cabbage no other works focused on simultaneous application of various iodine and selenium compounds in field studies are available.


The aim of the study was to determine the influence of soil fertilization with various forms of iodine (I$^-$ and IO$_3^-$) and selenium (SeO$_3^{2-}$ and SeO$_4^{2-}$) on yield, biofortification efficiency and selected chemical properties of lettuce plants (Lactuca sativa L.).
MATERIAL AND METHODS

Plant material and treatments. In the years 2012–2014, a field study with lettuce cv. ‘Valeska’ cultivation was conducted in the Experimental Station (50°07’910 N, 19°84’764 E) of University of Agriculture in Krakow, Poland. Each year, lettuce was grown in spring on various plots of the same field.

Lettuce was cultivated on heavy soil (heavy clay: 24% sand, 23% silt and 53% loam). Chemical properties of soil in the subsequent years are presented in table 1. Lettuce was sown in the greenhouse in the first decade of March. Seedlings were planted into soil on 18, 23 and 15 April in the subsequent years in rows 30 cm apart with 30 cm spacing.

The study included soil fertilization with iodine and selenium in the following combinations: 1. Control, 2. KI, 3. KIO₃, 4. Na₂SeO₄, 5. Na₂SeO₃, 6. KI + Na₂SeO₄, 7. KIO₃ + Na₂SeO₄, 8. KI + Na₂SeO₃, 9. KIO₃ + Na₂SeO₃. Iodine and selenium were applied twice: before planting and as a top-dressing in the early stage of head formation (first decade of May; date indicated at fig. 1), each in a dose of 2.5 kg I·ha⁻¹ + 0.5 kg Se·ha⁻¹. Total amount of iodine and selenium introduced into the soil was 5 kg I·ha⁻¹ and 1 kg Se·ha⁻¹, respectively. Iodine and selenium were applied as KI and KIO₃ (Avantor Performance Materials, Poland) as well as Na₂SeO₄ and Na₂SeO₃ (Sigma-Aldrich, Germany) pure for analysis.

One day prior to lettuce planting (date indicated at fig. 1) pre-sowing fertilization with N, P and K was conducted (along with iodine and selenium application) based on the results of soil chemical analysis in order to supplement nutrient deficiencies to the level optimal for lettuce: N – 100, P – 70 and K – 200 mg·dm⁻³ of soil. Nitrogen was applied as ammonium nitrate in a dose of 50 mg N·dm⁻³ soil which is 50% N. The second dose of nitrogen was introduced with top-dressing application of iodine and selenium and followed by plant watering with approximately 15 mm of precipitation. Fertilization with other macronutrients was not applied as its content in soil covered lettuce requirements (tab. 1).

The experiment was arranged in a split-plot design. Each experimental treatment was randomized in four replications on 5 m × 1.5 m (7.5 m²) plots – 64 plants per one plot. The total area under the experiment was 270 m².

During harvest (30 May, 11 and 3 June in the subsequent years), yield was assessed by weighing sixteen lettuce heads from each plot. Eight lettuce heads were collected for further analysis as well as soil samples from 0–30 cm layer, individually for each treatment.

Plant analysis. Dry matter content in fresh lettuce samples was assayed at 105°C. After preparing ethanolic extracts of plant material, total content of phenolic compounds was determined using Folin-Ciocalteau reagent [Swain and Hillis 1959]. Additionally, lettuce leaves were dried at 70°C in a laboratory dryer with forced air circulation and ground in variable speed rotor mill Pulverisette 14, FRITSCH using 0.5 mm sieve. Thus-prepared samples were subsequently analyzed with respect to the content of iodine and selenium by ICP-OES technique (two separate procedures) as well as selenomethionine, selenocysteine, inorganic (I⁻, IO₃⁻, SeO₄²⁻, SeO₃²⁻) and organic ions (oxalates and citrates) using capillary electrophoresis (CE).

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Fig. 1. Meteorological data for lettuce cultivation in 2012 (A), 2013 (B) and 2014 (C). Dates of: lettuce planting, pre-sowing and top dressing application of I and Se as well as harvest are indicated.
Table 1. Selected chemical properties of 0–30 cm soil layer prior to the experiment in 2012–2014

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.72</td>
<td>7.45</td>
<td>7.19</td>
</tr>
<tr>
<td>EC (mS cm⁻¹)</td>
<td>0.10</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Eh (mV)</td>
<td>276.0</td>
<td>268.0</td>
<td>189.3</td>
</tr>
<tr>
<td>N-NH₄ + N-NO₃ (mg dm⁻³)</td>
<td>13.4</td>
<td>12.7</td>
<td>8.4</td>
</tr>
<tr>
<td>P (mg dm⁻³)</td>
<td>32.7</td>
<td>12.8</td>
<td>59.5</td>
</tr>
<tr>
<td>K (mg dm⁻³)</td>
<td>181.4</td>
<td>139.1</td>
<td>202.3</td>
</tr>
<tr>
<td>Ca (mg dm⁻³)</td>
<td>2 089.3</td>
<td>1 651.8</td>
<td>1 011.0</td>
</tr>
<tr>
<td>Mg (mg dm⁻³)</td>
<td>194.2</td>
<td>182.8</td>
<td>114.0</td>
</tr>
<tr>
<td>Na (mg dm⁻³)</td>
<td>8.6</td>
<td>23.5</td>
<td>2.9</td>
</tr>
<tr>
<td>S (mg kg⁻¹)</td>
<td>41.4</td>
<td>42.9</td>
<td>22.1</td>
</tr>
<tr>
<td>I (mg kg⁻¹)</td>
<td>1.87</td>
<td>1.93</td>
<td>1.80</td>
</tr>
<tr>
<td>Se (mg kg⁻¹)</td>
<td>0.90</td>
<td>0.88</td>
<td>0.92</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>2.33</td>
<td>2.56</td>
<td>2.33</td>
</tr>
<tr>
<td>Cation exchange capacity (CEC cmol kg⁻¹)</td>
<td>8.32</td>
<td>9.58</td>
<td>8.48</td>
</tr>
<tr>
<td>Saturation of the sorption complex with alkaline elements (%)</td>
<td>92.7</td>
<td>88.2</td>
<td>85.8</td>
</tr>
</tbody>
</table>

1 – units: mg dm⁻³ and mg kg⁻¹ depend on applied analytical method

**Determination of iodine and selenium content after TMAH (tetramethylammonium hydroxide) extraction.** 0.5 g of air-dried leaf samples, 10 cm³ of double-distilled water and 1 cm³ of 25% TMAH were put into 30 cm³ falcon tubes. After mixing, samples were incubated for 3 hours at 90°C. After incubation, samples were cooled to the temperature of approximately 20°C and filled to 30 cm³ with double-distilled water. After mixing, samples were centrifuged for 15 minutes at 4 500 rpm. The measurements using ICP-OES spectrometer were conducted in the supernatant (without its decanting) [PN-EN 15111, 2008].

Additionally, analyses of iodine using cold vapor I₂ generation technique (CVG) [Vtorushina et al. 2008, 2009] and selenium after sample digestion in 65% HNO₃ [Pastawski and Migaszewski 2006] were performed. For CVG: digestion of 0.5 g air-dried plant samples in the mixture of 10 cm³ 65% HNO₃ and 0.8 cm³ 70% HClO₄ was conducted in the CEM MARS-5 Xpress microwave system using teflon vessels. The process consisted of four steps with gradual increase of temperature: 60, 80, 100 (each step included 10 min of warming plus 5 min of maintaining set temperature) and 130°C (10 min + 15 min). After the digestion, solutions were transferred into the volume of 25 cm³ with redistilled water. Measurements using CVG technique required the application of the prototype gas/liquid separator for ICP-OES Prodigy spectrometer (Supplementary material Photo S1), which was constructed according to Vtorushina et al. [2008].

For selenium analysis, 0.5 g of air-dried plant samples was digested at 200°C (15 min of warming plus 15 min of maintaining set temperature) in 10 cm³ 65% super pure HNO₃ using microwave system CEM MARS-5 Xpress. Samples were then trans-
ferred to the final volume of 25 cm$^3$ using double-distilled water and analyzed with ICP-OES spectrometer.

**Determination of the content of selenomethionine (SeMet) and selenocysteine (SeCys).** In 30 cm$^3$ falcon tubes, 5 ml of solution containing 40 mg protease and 20 mg lipase in demineralized water were added to 0.25 g of air-dried leaves. Samples were incubated for 16 h at 20°C and centrifuged for 15 min at 4500 rpm [Zhao et al. 2011]. The aliquots of 0.5 cm$^3$ of supernatants were transferred into 1.5 cm$^3$ Eppendorf tubes and centrifuged for 10 min at 10 000 rpm. Measurements of SeMet and SeCys content were conducted directly in supernatants using capillary electrophoresis system Capel 105 M (Lumex, Russia) with UV detection at 254 nm. Capillaries of i.d. 75 µm, o.d. 365 µm and total length of 50 cm were used. A negative power supply of -16 kV was applied. The running buffer solution was prepared as proposed by Zhao et al. [2011] containing 30 mM NaH$_2$PO$_4$, 15 mM Na$_2$B$_4$O$_7$ and 0.2 mM CTAB (cetyltrimethylammonium bromide) (pH 8.80). Standards of L(+)-selenomethionine and L-selenocysteine (Acros Organics) were used for CE calibration.

**Analysis of the content of I$^-$, IO$_3$$^-$, SeO$_4^{2-}$, SeO$_3^{2-}$, oxalates and citrates.** 0.1 g of air-dried plant samples with 20 cm$^3$ of extraction solution containing: 4 cm$^3$ 25% TMAH and 10 cm$^3$ 0.1 M NaOH in 1 dm$^3$ of demineralized water were put into 30 cm$^3$ falcon tubes. After mixing, samples were incubated for 1 hour at 90°C, cooled to the temperature of approximately 20°C, mixed thoroughly and centrifuged for 15 min at 4 500 rpm. The supernatants were filtered through a 0.25 µm cellulose acetate membrane filters and analyzed using PA 800 Plus capillary electrophoresis system (Beckman Coulter, USA) with DAD detection. Capillaries of i.d. 75 µm, o.d. 365µm and total length of 60 cm (50 cm to detector) were used. A negative power supply of -25 kV was applied. Commercial kit Anions-2 Kit (Analis Belgium) was used for ion analysis with the following standards for CE calibration: KI, KIO$_3$, Na$_2$SeO$_3$, Na$_2$SeO$_4$ (Sigma Aldrich) as well as oxalate acid and citrate acid (POCH Poland).

The conduction of oxalates and citrates analyses resulted from the possibility of its determination along with I$^-$, IO$_3$$^-$, SeO$_4^{2-}$, SeO$_3^{2-}$ during the same separation. It reduced the costs and times of analyzing these compounds by other methods. Oxalates and citrates are the most abundant organic acid derivatives found in plant cells. Their content in plants is dependent, among others, on calcium nutrition [Wińska-Krysiak 2006]. The level of citrate synthase expression (formally the first enzyme in that cycle, responsible for the formation of citrate) is directly dependent on the growth of plants [Koyama et al. 1999]. The accumulation of citrates in plants is correlated with the H$^+$ efflux, possibly from the action of H$^+$ -ATPase on the plasma membrane [Ohno et al. 2003].

**Soil analysis.** Soil samples were dried at 70°C in a laboratory dryer with forced air circulation, ground in a mortar and sieved through 1 mm sieve. 2.5 g of soil samples were put into 30 cm$^3$ falcon tubes, then 10 cm$^3$ of double-distilled water and 1 cm$^3$ of 25% TAMH were added. Further steps were as described for I and Se determination in TMAH in lettuce leaves. The method described above is our own modified procedure [Smoleń et al. 2016] for the determination of total I [Yamada et al. 1996] and I and Se [McNally 2011] content in soil using TMAH. The modification included the application of higher temperature (90, not 70°C) and omitting sample filtration after centrifugation. Filtration of samples leads to losses of analyzed elements. The temperature of 90°C is
Iodine and selenium biofortification of lettuce (Lactuca sativa L.).

ICP-OES spectrometer settings for iodine and selenium determination.
The analysis of iodine and selenium content in lettuce and soil samples was conducted using ICP-OES Prodigy spectrometer (Teledyne Leeman Labs USA). Calibration of the instrument was performed maintaining the same matrix as for analyzed samples.

For iodine determination in lettuce samples after alkaline extraction (using TMAH) more sensitive spectral line I-206.163 was applied, while for soil – I-183.038 nm, the latter one being not affected by Cr and Zn spectral interferences. Selenium content in both lettuce and soil samples was analyzed using Se-169.090 nm line.

For iodine determination using CVG technique, the most sensitive line of iodine I-178.276 nm was chosen as for this method no interferences (including these from phosphorus) affect the reading [Vtorushina et al. 2009].

Meteorological data. Each year lettuce was cultivated from the end of April until the end of May or first days of June (43, 55 and 50 days in 2012, 2013 and 2014, respectively – fig. 1 A–C). Average daily air temperature in May was 15.6, 14.0 and 13.8°C in subsequent years. In comparison, in the years 1971–2000 average monthly air temperature was: 8.0°C in April, 13.4°C in May and 16.2°C in June [GUS 2005].

Average daily value of PAR radiation in the period from lettuce planting in field to the harvest was: 656.7, 537.8 and 562.4 µmol·m⁻²·s⁻¹, respectively. The total amount of rainfall was 31.6, 203.8 and 132.2 mm in 2012, 2013 and 2014, respectively (fig. 1 – data from HOBO Weather Station). In 2013 and 2014 heavy rainfall occurred few days before lettuce harvest. In the years 1971–2000 average monthly rainfall in the area under study was: 50 mm in April, 74 mm in May and 94 mm in June [GUS 2005].

The period of lettuce cultivation in 2012 was characterized by the most unfavorable weather conditions as compared to respective time in 2013 and 2014. In that year the lowest amount of rainfall as well as the highest values of average daily air temperature and PAR radiation were noted.

Data analysis. Obtained data were subjected to analysis of two-way variance (treatment × year of study) using ANOVA module of Statistica 10.0 PL. To determine the significance between means the Tukey’s test was used. The significance was declared at P < 0.05.

RESULTS

Yield and dry matter content. A significant influence of iodine and selenium application on total yield and dry matter content in lettuce was noted (tab. 2) – however, only the level of dry matter was affected by the treatment × year of study interaction (figs 2 A and B).

In comparison to the control and other combinations, application of Na₂SeO₄ (both alone and with KI or KIO₃) led to a decrease in yield (in each year of the study; tab. 2 and fig. 2A) as well as an increase in dry matter content in lettuce (means for 2012–2014; tab. 2). In the latter case, various yet statistically significant quantitative relations between tested combinations were found in individual years of the study. However, each year the content of dry matter was higher in lettuce grown on soil fertilized with Na₂SeO₄, KI+Na₂SeO₄ and KIO₃+Na₂SeO₄ as compared to other treatments.
Table 2. Yield and the content of dry matter, iodine and selenium (determined in TMAH, CVG and HNO$_3$) as well as SeMet and SeCys in lettuce – means from 2012–2014

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (t ha$^{-1}$)</th>
<th>D.M. (% d.w.)</th>
<th>Iodine (mg I·kg$^{-1}$ d.w.)</th>
<th>Selenium (mg Se·kg$^{-1}$ d.w.)</th>
<th>SeMet (mg SeMet·kg$^{-1}$ d.w.)</th>
<th>SeCys (mg SeCys·kg$^{-1}$ d.w.)</th>
<th>Share of Se from aminoacids (SeMet and SeCys) in the total Se content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.3 ±3.0$^{b}$</td>
<td>4.45 ±0.37$^{a}$</td>
<td>1.5 ±0.1$^{a}$</td>
<td>1.4 ±0.1$^{a}$</td>
<td>2.6 ±0.5$^{a}$</td>
<td>3.5 ±0.9$^{a}$</td>
<td>3.1 ±0.8$^{a}$</td>
</tr>
<tr>
<td>KI</td>
<td>49.6 ±1.9$^{b}$</td>
<td>4.42 ±0.32$^{a}$</td>
<td>6.8 ±0.6$^{d}$</td>
<td>5.6 ±0.5$^{e}$</td>
<td>1.7 ±0.3$^{a}$</td>
<td>1.9 ±0.1$^{a}$</td>
<td>2.3 ±0.2$^{a}$</td>
</tr>
<tr>
<td>KIO$_3$</td>
<td>48.5 ±2.1$^{b}$</td>
<td>4.45 ±0.34$^{a}$</td>
<td>3.8 ±0.3$^{b}$</td>
<td>4.0 ±0.3$^{b}$</td>
<td>2.7 ±0.6$^{a}$</td>
<td>2.7 ±0.3$^{a}$</td>
<td>3.1 ±0.3$^{a}$</td>
</tr>
<tr>
<td>Na$_2$SeO$_4$</td>
<td>30.1 ±2.8$^{a}$</td>
<td>5.18 ±0.31$^{a}$</td>
<td>1.3 ±0.3$^{a}$</td>
<td>1.5 ±0.1$^{a}$</td>
<td>142.4 ±25.0$^{e}$</td>
<td>110.6 ±25.1$^{a}$</td>
<td>155.6 ±35.5$^{f}$</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$</td>
<td>44.2 ±2.0$^{a}$</td>
<td>4.48 ±0.41$^{a}$</td>
<td>0.9 ±0.1$^{a}$</td>
<td>1.5 ±0.1$^{a}$</td>
<td>17.0 ±4.8$^{b}$</td>
<td>16.4 ±4.4$^{a}$</td>
<td>23.3 ±5.9$^{b}$</td>
</tr>
<tr>
<td>KI+Na$_2$SeO$_4$</td>
<td>29.0 ±2.7$^{c}$</td>
<td>5.09 ±0.32$^{a}$</td>
<td>5.2 ±0.8$^{c}$</td>
<td>4.6 ±0.3$^{d}$</td>
<td>116.0 ±19.4$^{e}$</td>
<td>82.7 ±14.5$^{c}$</td>
<td>107.9 ±10.6$^{d}$</td>
</tr>
<tr>
<td>KIO$_3$+Na$_2$SeO$_4$</td>
<td>30.0 ±2.9$^{a}$</td>
<td>5.23 ±0.38$^{c}$</td>
<td>3.6 ±0.3$^{a}$</td>
<td>4.2 ±0.3$^{a}$</td>
<td>118.8 ±19.2$^{c}$</td>
<td>86.7 ±15.4$^{d}$</td>
<td>147.4 ±23.1$^{a}$</td>
</tr>
<tr>
<td>KI+Na$_2$SeO$_3$</td>
<td>44.7 ±2.2$^{a}$</td>
<td>4.63 ±0.40$^{a}$</td>
<td>5.2 ±0.5$^{a}$</td>
<td>4.7 ±0.2$^{a}$</td>
<td>15.2 ±3.7$^{d}$</td>
<td>13.5 ±3.9$^{a}$</td>
<td>12.9 ±2.1$^{b}$</td>
</tr>
<tr>
<td>KIO$_3$+Na$_2$SeO$_3$</td>
<td>46.8 ±1.7$^{a}$</td>
<td>4.48 ±0.34$^{a}$</td>
<td>3.9 ±0.4$^{a}$</td>
<td>4.6 ±0.1$^{a}$</td>
<td>10.7 ±2.7$^{e}$</td>
<td>9.4 ±2.6$^{b}$</td>
<td>8.0 ±0.7$^{e}$</td>
</tr>
</tbody>
</table>

1 – CVG (cold vapor generation technique). D.M. Dry matter. Means followed by the same letters are not significantly different for $p < 0.05$; s.e. – standard error.
Efficiency of iodine and selenium biofortification. The level of iodine and selenium bioaccumulation in lettuce was significantly affected by applied fertilization with these elements as well as soil and weather conditions in each year of cultivation (significant treatment × year of study interaction). Determined values of iodine and sele-
nium concentration in lettuce depended also on applied method of sample preparation prior to the analysis (tab. 2 and fig. 2 C–F). Generally, soil fertilization with iodine and selenium increased the concentration of these elements in lettuce heads.

Analyzing the mean values from 2012–2014, it turned out that the efficiency of iodine biofortification of lettuce (determined in TMAH and by CVG) was higher after soil fertilization with KI rather than KIO₃ (tab. 2, figs 2 C and D). Simultaneous application of KI with both forms of selenium decreased iodine content in lettuce as related to the treatment with KI alone. Presented results concerning iodine biofortification of lettuce were however significantly affected by soil and weather conditions in individual years (figs 2 C and D).

In lettuce grown on soil fertilized with KI, KI + Na₂SeO₄ and KI + Na₂SeO₃, higher values of iodine content were obtained after sample extraction with TMAH than the application of CVG technique (tab. 2). This relation was mainly observed in 2012 while in other years application of CVG allowed to provide higher or comparable values of iodine to those noted for TMAH (figs 2 C and D). In the case of lettuce from the combinations with KIO₃, KIO₃ + Na₂SeO₄ and KIO₃ + Na₂SeO₃ comparable results of iodine concentration were obtained in each year irrespective of applied analytical procedure (tab. 2, figs 2 C and D).

With respect to selenium content, lettuce grown on soil fertilized with Na₂SeO₄ (alone or together with KI or KIO₃) contained several dozen-fold more Se than plants from the combinations with Na₂SeO₃ applied alone or with iodine in both forms (tab. 2, figs 2 E and F). Lettuce plants from the combinations with KI + Na₂SeO₃ and KIO₃ + Na₂SeO₄ accumulated significantly less Se than those from the combination with Na₂SeO₄ applied alone. The described relations were mainly affected by the results obtained in 2013 (fig. 2 E and F). In that year lettuce grown on soil fertilized with Na₂SeO₄ contained the highest level of selenium from all tested combinations and additional application of KI or KIO₃ significantly decreased its accumulation. On the other hand, simultaneous application of iodine and Na₂SeO₄ increased selenium concentration in lettuce in 2014 – especially when analyzed in TMAH.

For Na₂SeO₃ treatment, the additional application of KIO₃ (but no KI) decreased selenium content in lettuce (for selenium determination in TMAH and HNO₃ – tab. 2). Such relation was noted in 2013 and 2014 but not in 2012 (figs 2 E and F).

It is worth to underline that soil fertilization with Na₂SeO₄ and Na₂SeO₃ did not significantly affect iodine accumulation in lettuce when compared to the control (tab. 2). Also the application of KI and KIO₃ did not change selenium content in lettuce.

A positive value of correlation coefficient for various methods of sample preparation were noted for iodine \( r = 0.85 \) for \( I^n \text{ in } \text{TMAH} \times I^n \text{ by } \text{CVG} \) and selenium content in lettuce \( r = 0.86 \) for \( \text{Se}^{n} \text{ in } \text{TMAH} \times \text{Se}^{n} \text{ in } \text{HNO}_3 \).

It was not possible to determine the content of: \( \Gamma^-, \text{IO}_3^-, \text{SeO}_4^{2-}, \text{SeO}_3^{2-} \) in lettuce heads by capillary electrophoresis using Anions-2 Kit and DAD detection – the level of these ions were below limits of its detection by PA 800 Plus instrument. Another model of CE – Capel 105 M with UV detection was additionally tested using running buffer solutions and capillaries as chosen for the analysis of SeMet and SeCys but with lower voltage: -4 kV at 195 nm. That analytical approach allowed to separate the peaks of individual ions from interferences (in samples with added standard) yet the sensitivity of the instrument was still too low to reliably determine its level in plant material.

The content of SeMet and SeCys. Soil fertilization with Na₂SeO₄ (also together with KI or KIO₃) significantly increased the accumulation of SeMet and SeCys in lettuce plants as compared to Na₂SeO₃ applied alone or with iodine (tab. 2). Simultaneous application of iodine and selenium in all tested forms decreased the content of these two seleno amino acids in lettuce when compared to combinations treated with selenium alone (tab. 2). These relations were noted in each year of the study (figs 2 G and H). Generally, the lowest level of SeMet and SeCys in lettuce heads was noted in 2012.

Soil fertilization with KI+Na₂SeO₄ more significantly decreased the accumulation of SeMet, while KIO₃+Na₂SeO₃ treatment reduced SeCys level in lettuce as compared to the application of Na₂SeO₄ alone (tab. 2). On the other hand, additional application of iodine as KI or KIO₃ comparably reduced the level of SeMet, and to a lesser extent – of SeCys in lettuce as compared to plants from the combination with Na₂SeO₃ alone. Presented relations concerning negative impact of simultaneous application of iodine and selenium in tested forms on SeMet accumulation were observed in 2013 and 2014 but not in 2012. In the case of SeCys, this observation was valid only in 2013.

In comparison to the control, soil fertilization only with KI or KIO₃ had no significant effect on the content of SeMet and SeCys in lettuce (tab. 2, figs 2 G and H). The average share of both seleno amino acids in the total selenium content in lettuce ranged between 52.9% in the combination with KI + Na₂SeO₃ and 98.4% for KIO₃ fertilization (tab. 2).

The content of phenolic compounds, oxalates and citrates. A statistically significant influence of tested combinations as well as treatment × year of study interaction was noted with respect to the level of phenolic compounds, oxalates and citrates in lettuce (tab. 3 and figs 3 A-C). The highest content of phenolics was noted in lettuce grown on soil fertilized with Na₂SeO₄ alone or together with iodine (tab. 3) – most distinctively in 2012 (fig. 3 A). The greatest accumulation of oxalates was found in lettuce heads grown on soil treated with KI (tab. 3) – largely affected by the results obtained in 2013 (fig. 3 B). In each year of the study, soil fertilization with Na₂SeO₄, KI + Na₂SeO₃ and KIO₃ + Na₂SeO₃ significantly reduced the content of citrates in lettuce as compared to the control combination (tab. 3, fig. 3 C). Additionally, after soil fertilization with KIO₃ + Na₂SeO₃ lower accumulation of phenolic compounds was observed when compared to the control (tab. 3, fig. 3 A).

The content of iodine and selenium in soil after lettuce harvest. The content of iodine and selenium in soil after lettuce cultivation was substantially modified by applied fertilization with various compounds containing these elements (means from the three years of the study – tab. 4) as well as treatment × year of study interaction (figs 4 A and B). In combinations with iodine (KI and KIO₃) or selenium application (Na₂SeO₄ and Na₂SeO₃), soil level of selenium and iodine, respectively, was similar to the geoclimatic background represented by the control.

Significantly more iodine was determined in soil fertilized with KI than KIO₃ (means from three years of the study – tab. 4). This relation was not however noted in 2014 (fig. 4 A). No significant differences between combinations treated with iodine (both KI and KIO₃) and Na₂SeO₄ were found with respect to iodine concentration in soil. In the case of simultaneous application of iodine with Na₂SeO₄, diverse quantitative relations concerning iodine level were observed in each year of the study.
Table 3. Content of phenolic compounds, oxalates and citrates in lettuce – means from 2012–2014

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phenolic compounds (mg·100g⁻¹ f.w.)</th>
<th>Oxalates (g·kg⁻¹ d.w.)</th>
<th>Citrates (g·kg⁻¹ d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.9 ±4.7b</td>
<td>1.10 ±0.16bc</td>
<td>9.42 ±0.29c</td>
</tr>
<tr>
<td>KI</td>
<td>50.2 ±5.9c</td>
<td>1.27 ±0.21d</td>
<td>10.33 ±0.67c</td>
</tr>
<tr>
<td>KIO₃</td>
<td>47.2 ±4.8bc</td>
<td>1.00 ±0.12abc</td>
<td>8.03 ±0.95b</td>
</tr>
<tr>
<td>Na₂SeO₄</td>
<td>56.3 ±3.1bc</td>
<td>0.98 ±0.11abc</td>
<td>10.11 ±0.54c</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>38.0 ±4.3a</td>
<td>1.09 ±0.15abc</td>
<td>6.40 ±0.51c</td>
</tr>
<tr>
<td>KI + Na₂SeO₄</td>
<td>62.1 ±6.8c</td>
<td>0.94 ±0.10f</td>
<td>9.98 ±1.08c</td>
</tr>
<tr>
<td>KIO₃ + Na₂SeO₄</td>
<td>63.3 ±9.2c</td>
<td>0.95 ±0.08f</td>
<td>9.51 ±0.52c</td>
</tr>
<tr>
<td>KI + Na₂SeO₃</td>
<td>49.9 ±7.4c</td>
<td>1.03 ±0.13abc</td>
<td>7.31 ±0.54ab</td>
</tr>
<tr>
<td>KIO₃ + Na₂SeO₃</td>
<td>40.1 ±5.3c</td>
<td>1.11 ±0.14b</td>
<td>7.08 ±0.45ab</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different for p < 0.05; s.e. – standard error

Fig. 3. Content of phenolic compounds (A), oxalates (B) and citrates (C) in lettuce in each year of the study (2012–2014) depending on applied I and Se fertilization. Bars indicate standard error (n = 4)

The highest content of selenium was noted in soil fertilized with KI+Na₂SeO₄ (tab. 4). Interesting is the observation of increased concentration of this element in soil treated with Na₂SeO₄ than Na₂SeO₃ as well as with KI + Na₂SeO₄ over KIO₃ + Na₂SeO₃. It needs to be underlined, however, that obtained relations (tab. 4) were mostly affected by the results for 2012 (fig. 4 A and B) – in the other two years no such relations were observed.
Table 4. Iodine and selenium content in soil after lettuce cultivation – means from 2012–2014

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iodine (mg I·kg⁻¹ soil)</th>
<th>Selenium (mg Se·kg⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54 ±0.06</td>
<td>1.03 ±0.11</td>
</tr>
<tr>
<td>KI</td>
<td>6.47 ±0.37</td>
<td>0.95 ±0.10</td>
</tr>
<tr>
<td>KIO₃</td>
<td>4.87 ±0.72</td>
<td>0.71 ±0.04</td>
</tr>
<tr>
<td>Na₂SeO₄</td>
<td>1.31 ±0.14</td>
<td>1.79 ±0.26</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>0.98 ±0.15</td>
<td>1.26 ±0.12</td>
</tr>
<tr>
<td>KI + Na₂SeO₄</td>
<td>5.47 ±0.89</td>
<td>2.19 ±0.42</td>
</tr>
<tr>
<td>KIO₃ + Na₂SeO₄</td>
<td>5.33 ±0.93</td>
<td>1.21 ±0.07</td>
</tr>
<tr>
<td>KI + Na₂SeO₃</td>
<td>5.82 ±0.89</td>
<td>1.71 ±0.10</td>
</tr>
<tr>
<td>KIO₃ + Na₂SeO₃</td>
<td>5.46 ±1.19</td>
<td>1.54 ±0.06</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different for p < 0.05; s.e. – standard error

Fig. 4. Iodine (A) and selenium (B) content in soil after lettuce cultivation in each year of the study (2012–2014) depending on applied I and Se fertilization. Bars indicate standard error (n = 4)

DISCUSSION

Iodine and selenium uptake by lettuce plants. Iodides (I⁻) are more easily taken up by plants but exhibit higher toxicity than iodate (IO₃⁻) ions [Blasco et al. 2010, Kato et al. 2013]. It is assumed that, among selenium compounds, selenates (SeO₄²⁻) are pre-
ferred by plants over selenites (SeO$_3^{2-}$) [Kopsell and Kopsell 2007]. Selenates or selenites toxicity is influenced by plant type, form of selenium in the growth medium, and the presence of competing ions, such as sulfate and phosphate [Kopsell and Kopsell 2007, Lavu et al. 2013, Li et al. 2015]. Ríos et al. [2008, 2010] revealed greater plant uptake and less toxicity of SeO$_4^{2-}$ rather than SeO$_3^{2-}$ in lettuce cultivation at hydroponic system.

When selenium is applied foliarly, its absorption and accumulation in plants (in both forms: SeO$_3^{2-}$, SeO$_4^{2-}$) is strictly dependent on weather conditions throughout cultivation, particularly the amount and distribution of rainfall [Poblaciones et al. 2014, Rodrigo et al. 2014]. After soil fertilization, the uptake of all mineral nutrients by roots (including iodine and selenium) is strongly limited by deficient amount of water in soil. Significantly lower accumulation of iodine and selenium (including SeMet and SeCys) in lettuce noted in 2012 was therefore presumably caused by soil drought due to limited rainfall as well as substantially higher air temperature and PAR value.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TF for iodine in TMAH</th>
<th>TF for iodine in by CVG</th>
<th>TF for selenium in TMAH</th>
<th>TF for selenium in HNO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.80 ±0.04$^{a}$</td>
<td>0.72 ±0.05$^{a}$</td>
<td>2.89 ±0.50$^{a}$</td>
<td>3.88 ±0.97$^{ab}$</td>
</tr>
<tr>
<td>KI</td>
<td>2.15 ±0.19$^{c}$</td>
<td>1.78 ±0.17$^{c}$</td>
<td>1.60 ±0.37$^{c}$</td>
<td>2.12 ±0.16$^{c}$</td>
</tr>
<tr>
<td>KIO$_3$</td>
<td>1.21 ±0.10$^{a}$</td>
<td>1.28 ±0.11$^{a}$</td>
<td>2.61 ±0.63$^{a}$</td>
<td>3.07 ±0.35$^{a}$</td>
</tr>
<tr>
<td>Na$_2$SeO$_4$</td>
<td>0.72 ±0.16$^{c}$</td>
<td>0.82 ±0.08$^{c}$</td>
<td>124.22 ±22.04$^{c}$</td>
<td>92.70 ±22.33$^{c}$</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$</td>
<td>0.46 ±0.07$^{c}$</td>
<td>0.79 ±0.05$^{c}$</td>
<td>20.69 ±4.23$^{c}$</td>
<td>12.65 ±3.87$^{c}$</td>
</tr>
<tr>
<td>KI+Na$_2$SeO$_4$</td>
<td>2.18 ±0.24$^{c}$</td>
<td>1.44 ±0.09$^{c}$</td>
<td>100.70 ±16.70$^{c}$</td>
<td>72.36 ±12.95$^{c}$</td>
</tr>
<tr>
<td>KIO$_3$+Na$_2$SeO$_4$</td>
<td>1.15 ±0.09$^{a}$</td>
<td>1.33 ±0.11$^{a}$</td>
<td>103.21 ±16.53$^{a}$</td>
<td>75.91 ±13.81$^{a}$</td>
</tr>
<tr>
<td>KI+Na$_2$SeO$_3$</td>
<td>1.89 ±0.16$^{a}$</td>
<td>1.50 ±0.06$^{a}$</td>
<td>13.28 ±3.27$^{a}$</td>
<td>11.89 ±3.44$^{a}$</td>
</tr>
<tr>
<td>KIO$_3$+Na$_2$SeO$_3$</td>
<td>1.24 ±0.11$^{c}$</td>
<td>1.46 ±0.04$^{c}$</td>
<td>9.37 ±2.42$^{c}$</td>
<td>8.30 ±2.27$^{c}$</td>
</tr>
</tbody>
</table>

1 – CVG (cold vapor generation technique). Means followed by the same letters are not significantly different for $p < 0.05$; s.e. – standard error. Iodine and selenium transfer factor [TF] in soil-to-lettuce leaf system was calculated using the following formula: TF = [C$_{plant dry weight}$] / [C$_{soil dry weight}$], where C was iodine or selenium content in lettuce or soil dry weight.

The analysis of the content of discussed elements in lettuce treated only with iodine or selenium confirmed higher absorption (and TF tab. 5) of I and SeO$_4^{2-}$ by plants over other tested forms. The higher intake of SeO$_4^{2-}$ form of selenium (confirmed by the TF value) was noted also for its application together with KI and KIO$_3$. The described relations were observed in each year of the study irrespective of diverse weather conditions and chemical properties of soil. Among tested iodine and selenium compounds applied into the soil, its potentially toxic effects on plants were noted only for Na$_2$SeO$_4$ (applied alone or together with KI and KIO$_3$). In each year of the study, visual symptoms of SeO$_4^{2-}$ toxicity were observed including growth impairment and necrosis of leaf edges. In our opinion the toxic effect of selenite application directly contributed to the decrease in lettuce yield in each year of the study, but to the greatest extent in 2012. It was
accompanied by the accumulation of phenolics, intensive synthesis of which is a part of
plant response to biotic and abiotic stress, including excessive selenium application
[Ríos et al. 2008]. In our study, particularly harmful action of Na₂SeO₄ noted in 2012
was related to the above-mentioned water deficit in soil. As an effect, higher dry matter
content (lower leaf hydration) in lettuce was also noted in that year. Despite that, in
eyear of the study increased accumulation of dry matter was noted in lettuce grown
on soil fertilized with Na₂SeO₄ (alone or together with iodine), what was clearly related
to yield (biomass) reduction.

Lower efficiency of selenium biofortification of lettuce (lower values of TF –
tab. 5), including the level of SeMet and SeCys, after the application of Na₂SeO₃
(as compared to Na₂SeO₄) can be explained by the processes that ions SeO₃²⁻ and SeO₄²⁻
undergo in soil environment. Iron sesquioxides [Fe(OH)₃] may cause strong sorption of
selenium in soil [Elrashidi et al. 1989, Kopsell and Kopsell 2007]. With deficit amount
of water in soil in 2012, the above-mentioned sorption mechanism could have contrib-
uted to the lack of selenium enrichment in lettuce fertilized with Na₂SeO₃ – alone or
together with KI/KIO₃. Striking is the fact that only in 2012 selenium content in soil
after lettuce cultivation was lower after Na₂SeO₃ rather than Na₂SeO₄ application. Con-
versions of SeO₄²⁻ and SeO₃²⁻ in soil environment occur very slowly [Elrashidi et al.
1989, Kopsell and Kopsell 2007]. During the cultivation of lettuce, which is a fast
growing crop, such processes have occurred rather slowly and to a little extent, there-
fore not significantly affecting plant preference toward selenate or selenite uptake. This
assumption seems to be substantiated by the fact that the half of selenium dose was
introduced as a top-dressing fertilization. Ríos et al. [2008] found an increased content
of Cys and total SH groups in lettuce leaves after the application of SeO₄²⁻ rather than
SeO₃²⁻. Hawrylak and Szymańska [2004] noted the accumulation of non-protein SH
groups (characteristic for cysteine) in spinach and tomato roots after SeO₄²⁻ application
while SeO₃²⁻ increased its content in shoots. When taken up, SeO₃²⁻ ions are transformed
in roots into seleno organic compounds and in such form are then transported to leaves.
SeO₄²⁻, however, is mainly distributed to leaves by xylem and the formation of organic
compounds with SeO₃²⁻ after its previous reduction to SeO₃²⁻ in roots is less significant
[Zhu et al. 2009]. It can be therefore assumed that in 2012 SeO₃⁻ ions were taken up,
efficiently metabolized and accumulated in roots. Due to insufficient soil humidity the
transport of seleno organic compounds to leaves was limited when Na₂SeO₃ was intro-
duced into the soil. In our opinion it could have also been the cause of obtaining no
effect of selenium biofortification (regarding total selenium or SeMet and SeCys con-
tent) in lettuce from the combination with Na₂SeO₃ application (alone or together with
KI/KIO₃) cultivated in 2012.

Iodine and selenium interaction. Zhu et al. [2004] revealed no impact of IO₃⁻ ap-
plication on selenium content as well as of SeO₄²⁻ treatment on iodine accumulation in
spinach. Also Smoleń et al. [2014] did not note any effect of IO₃⁻ or SeO₄²⁻ present in
the nutrient solution in the concentration of 1.0 mg l⁻¹ and 0.5 mg Se on root uptake
and distribution of selenium and iodine to lettuce leaves.

Iodine uptake after the application of KI and KIO₃ with selenium compounds.
Basing on the results from 2013 and 2014, it seems substantiated to state that for the
optimal soil humidity, simultaneous fertilization with KI and Na₂SeO₃/Na₂SeO₃
(as compared to KI alone) weakens iodine uptake and accumulation in lettuce leaves. On the other hand, in the conditions of deficit amount of rainfall in 2012 combined with high air temperature (leading to soil drought) no antagonistic influence of SeO$_4^{2-}$ and SeO$_3^{2-}$ was noted with respect to iodine uptake when applied as KI. Despite that, we assume that simultaneous application of KI with selenium compounds (SeO$_4^{2-}$ and SeO$_3^{2-}$) may limit I$^-$ uptake by plants. It may be the consequence of stronger sorption of iodides in soil when introduced together with Na$_2$SeO$_4$ or Na$_2$SeO$_3$ than when applied alone. This is further supported by the observation of lower content of available iodine for plants in soil fertilized with KI + Na$_2$SeO$_4$ and KI + Na$_2$SeO$_3$ than KI alone.

In the case of KIO$_3$ application of KIO$_3$, no negative effect of selenium on iodine uptake and accumulation in lettuce was noted. This was particularly valid in the conditions of sufficient water supply in 2013 and 2014.

**Selenium uptake and the content of SeMet and SeCys after the application of Na$_2$SeO$_4$ combined with KI and KIO$_3$.** In each year of the study, diverse relations concerning total selenium content as well as SeMet and SeCys was noted in lettuce and soil from the combinations with Na$_2$SeO$_4$, KI + Na$_2$SeO$_4$ and KIO$_3$ + Na$_2$SeO$_4$ application. Therefore, antagonistic effect of iodine on SeO$_4^{2-}$ uptake cannot be named a rule as it could have been concluded from the average results from the three years of the study. This relation was strongly dependent on physicochemical properties of soil and weather conditions throughout lettuce cultivation.

**Selenium uptake and the content of SeMet and SeCys after fertilization with Na$_2$SeO$_3$ as combined with KI and KIO$_3$.** Irrespective of weather conditions in individual years of the study, a negative influence of simultaneous application of KIO$_3$ + Na$_2$SeO$_3$ (as compared to Na$_2$SeO$_3$ alone) on selenium uptake and accumulation as well as SeMet content in lettuce was revealed. It was not however reflected by selenium content in soil after lettuce cultivation. Such effect can be explained basing on the hypothesis of IO$_3^-$ reduction to I$^-$ that occurs in roots after its uptake or the incorporation of formed iodides into organic compounds within the root system [Cseh and Böszörményi 1964]. Iodate ions can also be reduced by soil microorganisms into I$_2$ or I$^-$ forming on root surface [Kato et al. 2013] directly affecting the redox potential value within the rhizosphere. Therefore, additional expense of energy for IO$_3^-$ reduction in roots may have interfered with SeO$_3^{2-}$ uptake from soil or its transformation into organic forms of Se (including SeMet and SeCys) in roots. The final consequence could be the limited distribution of organic Se into lettuce leaves.

In each year of the study in plants treated with of KIO$_3$ + Na$_2$SeO$_3$ a significant decrease in the content of phenolic compounds and citrates was noted as compared to the control. This could have been the lower concentration of citrates in lettuce – one of the key compounds in the Krebs cycle – and limited biosynthesis of secondary metabolites, including SeMet and phenolic compounds. Such negative relations were noted in the years with higher amount of rainfall (2013–2014, tab. 3 and fig. 3). There also may be the direct consequence of higher selenium uptake by plants in these years than in 2012. However, no decrease in plant biomass was noted in plants from this combination what substantiates the conclusion that simultaneous application of KIO$_3$ + Na$_2$SeO$_3$ in tested doses did not exceed tolerance levels of lettuce towards both ions (IO$_3^-$ and SeO$_3^{2-}$).

Numerous procedures differing with respect to applied mineral acids (HNO$_3$, HCl, HClO$_4$, H$_2$SO$_4$) and/or its mixtures as well as temperature and pressure parameters during digestion have been developed in order to minimize the losses of selenium and other mineral nutrients [Bahuelosa and Akohouea 1994, Zhou et al. 1997, Paslawski and Migaszewski 2006]. In the case of iodine, it is volatilized in the form of I$_2$ under acidic conditions yet the addition of AgNO$_3$ stabilizes iodine when samples are digested in HNO$_3$ [Varga 2007]. In our studies, lower selenium contents (as well as TF values) were noted after sample digestion in HNO$_3$ than after using TMAH what indicates higher Se losses when applying the former method. When analyzing iodine and selenium content after TMAH extraction, a complex matrix effect must be however taken into account. A thick, dark-brown consistency of such analyzed samples hardens aerosol formation in cyclonic spray chamber. Studies conducted by Smoleń et al. [2011] revealed that the values of correlation coefficients for iodine content in spinach after sample incubation with TMAH as compared to 2% CH$_3$COOH extraction depended not only on applied matrix but also iodine dose, method of its application (soil fertilization and/or foliar spraying) and the type of cultivation – field or pot experiment.

The applied procedure of iodine analysis using CVG is preceded by mineral digestion, not extraction as for iodine analysis in TMAH. Significantly, Vtorushina et al. [2009] – the authors of that method – proposed the optimal HNO$_3$:HClO$_4$ volume ratio to determine iodine content from its trace values to the level of approximately 150 mg I·kg$^{-1}$ d.w.

The aim of iodine and selenium biofortification from the consumer’s point of view. For the consumer, it is important that selenium occurs in the food (including vegetables) in its organic forms: SeMet, SeCys, MeSeCys as they exhibit the most positive effect on its organism [Pyrzynska 2009]. In our studies, lettuce contained selenium mostly in its organic forms. However, simultaneous application of KI/KIO$_3$ with Na$_2$SeO$_3$ strongly limited the percentage share of SeMet and SeCys in the total selenium content in plants. Positively, the presence of SeO$_4^{2-}$ and SeO$_3^{2-}$ – harmful even at low concentrations – were not detected in lettuce. The lack of free iodine ions in lettuce suggested the occurrence of this element in its organic compounds, which availability to human organism from biofortified vegetables is unlimited [Tonacchera et al. 2013].

Daily requirements of iodine and selenium (RDA) for adults is 150 µg I and 55 µg Se, respectively, while for pregnant and nursing women – 200–300 µg I and 60–70 µg Se [Food and Nutrition Board 2000, Andersson et al. 2007]. The optimal iodine to selenium weight ratio in human diet is therefore within the range of 2.7–5:1 (the optimum ratio of molar mass of both elements is within 4.4–8.8:1) and can be proposed as a necessary parameter describing the efficiency of simultaneous biofortification of plants with these elements.

The average serving of leafy vegetables is approximately 50 g f.w. [Voogt et al., 2010]. Taking this into account, it can be estimated that lettuce could be considered as
a good source of iodine and selenium for consumer (tab. 6). In further studies, however, appropriate I and Se doses must be established in order to supply the better coverage of RDA. In our study, close to the optimal I:Se weight ratio was obtained after the fertilization with KI only, with the values of 2.84–5.34:1, depending on the applied analytical method.

Table 6. Iodine and selenium content in 50g portion of fresh lettuce leaves including the percentage coverage of daily requirement for I and Se as well as I:Se ratio in lettuce calculated basing on the results of I and Se determination using various analytical procedures – means from 2012–2014

<table>
<thead>
<tr>
<th>Treatments (n = 12)</th>
<th>I and Se content in 50 g portion of fresh lettuce leaves (µg)</th>
<th>Coverage of daily requirement for I and Se by 50g portion of fresh lettuce leaves (%)</th>
<th>I : Se ratio in lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>iodine (TMAH/CVG) 3.3/2.9 selenium (TMAH/HNO₃) 6.7/9.6</td>
<td>iodine (TMAH/CVG) 2.2/1.9 selenium (TMAH/HNO₃) 12.2/17.4</td>
<td>I : Se (both in TMAH) 0.49 : 1 I by CVG : Se in HNO₃ 0.30 : 1</td>
</tr>
<tr>
<td>KI</td>
<td>14.1/11.8 2.6/4.1</td>
<td>9.4/7.8 4.8/7.5</td>
<td>5.34 : 1 2.84 : 1</td>
</tr>
<tr>
<td>KIO₃</td>
<td>8.2/8.5 4.3/5.9</td>
<td>5.5/5.7 7.8/10.8</td>
<td>1.92 : 1 1.43 : 1</td>
</tr>
<tr>
<td>Na₂SeO₄</td>
<td>3.0/4.1 328.2/246.4</td>
<td>2.0/2.7 596.8/448.0</td>
<td>0.01 : 1 0.02 : 1</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>1.9/3.5 30.4/27.1</td>
<td>1.3/2.3 55.3/49.3</td>
<td>0.06 : 1 0.13 : 1</td>
</tr>
<tr>
<td>KI+Na₂SeO₄</td>
<td>13.2/11.2 268.0/186.6</td>
<td>12.5/7.5 487.3/339.2</td>
<td>0.05 : 1 0.06 : 1</td>
</tr>
<tr>
<td>KIO₃+Na₂SeO₄</td>
<td>9.3/10.3 274.3/200.1</td>
<td>6.2/6.9 498.7/363.7</td>
<td>0.03 : 1 0.05 : 1</td>
</tr>
<tr>
<td>KI+Na₂SeO₃</td>
<td>12.4/11.1 28.7/26.9</td>
<td>9.9/7.4 52.2/48.8</td>
<td>0.43 : 1 0.41 : 1</td>
</tr>
<tr>
<td>KIO₃+Na₂SeO₃</td>
<td>8.2/10.2 20.5/19.4</td>
<td>5.5/6.8 37.2/35.3</td>
<td>0.40 : 1 0.52 : 1</td>
</tr>
</tbody>
</table>

1 – CVG (cold vapor generation technique)

At the same time, lettuce from this combination would cover adult’s requirements for iodine and selenium in 7.8–9.4 and 4.8–7.5%, respectively. Due to much easier uptake of selenium over iodine by plants, simultaneous application of these elements led to unfavorable ratio of its content in lettuce, particularly for Na₂SeO₄ application (rather than Na₂SeO₃). In combinations with Na₂SeO₃ (applied alone or together with KI/KIO₃) 50 g of lettuce would cover 35.3–55.3% of adult’s daily requirements for selenium. For Na₂SeO₄ application selenium content absorbed from 50 g portion of lettuce would substantially exceed the recommended level.

It is worth noting that we recorded improved iodine assimilability and its metabolism in rats fed with lettuce biofortified with iodine (KI) in relation to lettuce not biofortified with iodine or a diet containing synthetic KI [Kopeć et al. 2015]. Furthermore, we demonstrated the inhibition of Caco-2 cancer cell proliferation after treatment with iodine-biofortified lettuce (BFL) extract, but not potassium iodide (KI), and BFL-mediated induction of mitochondrial apoptosis and/or cell differentiation. This study showed 1326 differently expressed Caco-2 transcripts after treatment of iodine-biofortified (BFL) and non-fortified with iodine lettuce extract [Koronowicz et al. 2016].
CONCLUSION

Simultaneous application of iodine and selenium (in all tested chemical forms) limited selenium accumulation and the content of SeMet and SeCys. Particularly unfavorable, with respect to lettuce quality (but not yield), was soil fertilization with KIO$_3$ + Na$_2$SeO$_3$. Despite the decrease in selenium content, lower amounts of SeMet was accumulated in lettuce in the years with greater amount of rainfall (2013–2014) than with water deficit (2012).

Presented relations of I:Se weight ratios and the coverage of adult’s requirements towards both elements by 50 g portion of lettuce indicate the need to conduct further studies on the development of agrotechnical methods of double biofortification of plants with iodine and selenium. Such methods should also improve iodine mobility and soil-to-plant transfer. It seems advisable to determine the suitable dosage of selenium in the form of selenites and selenates as an approximate ten-fold difference in the effect of selenium biofortification of lettuce was noted. These solutions should lead to obtaining sustainable I:Se weight ratio in the yield within the range of (2.84−5.34):1 (molar mass ratio (4.4–8.8):1). In our opinion, in soils rich in selenium additional fertilization with this element should be neglected as to avoid the risk of obtaining yield with its excessive content, potentially harmful for the consumer. Additionally a disturbance in the optimal I:Se ratio in yield may be then noted even after applying relatively high iodine doses.

ACKNOWLEDGEMENTS

This work was financed by the Polish National Science Center – grant no. DEC-2011/03/D/NZ9/05560 “I and Se biofortification of selected vegetables, including the influence of these microelements on yield quality as well as evaluation of iodine absorption and selected biochemical parameters in rats fed with vegetables biofortified with iodine” planned for 2012–2015.

REFERENCES


Li, J., Liang, D., Qin, S., Feng, P., Wu, X. (2015). Effects of selenite and selenate application on growth and shoot selenium accumulation of pak choi (Brassica chinensis L.) during succes-
Iodine and selenium biofortification of lettuce (Lactuca sativa L.)...


Voogt, W., Jackson, W.A. (2010). Perchlorate, nitrate, and iodine uptake and distribution in lettuce (Lactuca sativa L.) and potential impact on background levels in humans. J. Agric. Food Chem. 58, 12192–12198. doi: 10.1021/jf101227d


Iodine and selenium biofortification of lettuce (Lactuca sativa L.)


BIOFORTYFIKACJA SAŁATY (Lactuca sativa L.) W JOD I SELEN PRZY NAWOŻENIU DOGLEBOWYM RÓŻNYMI FORMAMI CHEMICZNYMI TYCH PIERWIASTKÓW

Streszczenie. Stosunkowo niewiele wiadomo na temat interakcji pomiędzy jodem i sele- nem w roślina. Stanowi to problem w opracowaniu racjonalnych agrotechnicznych za- sad biofortyfikacji roślin w te składniki. Celem badań było określenie wpływu nawożenia do glebowego różnymi formami jodu (I⁺ i IO₃⁻) i selenu (SeO₃²⁻ i SeO₄²⁻) na plon, wydaj- ność biofortyfikacji oraz wybrane chemiczne właściwości roślin sałaty. Badania (prze- prowadzone w latach 2012–2014), obejmowały następujące kombinacje nawożenia do glebowego sałaty ‘Valeska’: kontrola (bez nawożenia jodem i selenem), KI, KIO₃, Na₂SeO₄, Na₂SeO₃, KI + Na₂SeO₄, KIO₃ + Na₂SeO₄, KI + Na₂SeO₃, KIO₃ + Na₂SeO₃. Związki jodu i selenu były aplikowane dwukrotnie: przedstawione oraz pogłębnie (każda aplikacja po 2,5 kg I·ha⁻¹ + 0,5 kg Se·ha⁻¹) – całkowita zastosowana dawka wynosiła 5 kg I·ha⁻¹ oraz 1 kg Se·ha⁻¹. Jedynie nawożenie Na₂SeO₄ (osobno i łącznie z KI i KIO₃) wywierało silnie toksyczny wpływ na rośliny. Towarzyszyło temu największe nagromadzenie selenu oraz selenometioniny (SeMet) i selenocysteiny (SeCys) w sałacie. Akumu- lacja I i Se w sałacie była odpowiednio wyższa po nawożeniu KI niż KIO₃ oraz Na₂SeO₄ niż Na₂SeO₃. Równoczesne nawożenie jodem i selenem powodowało zmniejszenie za- wartości selenu, SeMet i SeCys w salacie – zwłaszcza po nawożeniu KIO₃ + Na₂SeO₄. Równoczesne nawożenie KI z obiema formami selenu, w porównaniu z aplikowaniem samego KI, obniżało zawartość jodu w salacie. W przypadku obiektów KIO₃, KIO₃ + Na₂SeO₄ i KIO₃ + Na₂SeO₃ stwierdzono porównywalny między nimi stopień akumulacji jodu w salacie.

Słowa kluczowe: biofortyfikacja, jakość biologiczna, selenometionina, selenocysteina, generacja zimnych par jodu

Accepted for print: 23.05.2016