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Selenium species in leaves of chicory, dandelion, lamb's lettuce and parsley

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Abstract

Lamb's lettuce, dandelion, parsley and four cultivars of chicory were cultivated aeroponically for 41 days with nutrient solution containing 7 mg Se/L in the form of Na_2SeO_4. Se compounds were determined by high performance liquid chromatography–ultraviolet treatment–hydride generation atomic fluorescence spectrometry (HPLC–UV–HG-AFS) in the green parts of the selected plants. Se species were extracted by water and by enzymatic hydrolysis with Protease XIV. Separation of Se^{IV}, Se^{VI}, SeMet, SeMeSeCys and $SeCys_2$ was made by a combination of anion and cation exchange chromatography in which the columns were connected on-line to a UV–HG-AFS detection system. Se accumulated efficiently in plant leaves up to 480 μg/g dry mass, mostly as Se^{VI}, i.e. the form of Se in the nutrient solution. Beside inorganic Se, selenomethionine (6–21%), selenomethylselenocysteine (0.5–4.4%) and selenocistine (<DL-0.8%) were determined in the extracts after enzymatic hydrolysis. Some unidentified peaks were also observed in the chromatograms.

Keywords: Plants; Se compounds; HPLC–UV–HG-AFS

1. Introduction

Selenium is an essential element for human nutrition, generally exerting its biological effects through selenoproteins. There are over 30 known selenoproteins in mammalian systems including glutathione peroxidases, iodothyronine deiodinases, thioredoxin reductases and selenophosphate synthetase. Deficiency of Se in human nutrition is associated with impairments in antioxidant protection, redox regulation and energy production as a consequence of suboptimal expression of one or more selenoproteins (Combs, 2001). Most of the recent interest in Se nutrition, however, is not directed towards restoring adequacy in deficient individuals. Rather, it is directed toward over-supplementation in amounts 3–6-fold beyond the Recommended Dietary Allowance of 55 μg/d (DRI, 2000), because there is evidence that such intakes are protective against cancer (Combs, 2001; Finley, 2005). In response to a clinical trial report about reduced risk of cancers of the prostate, lung and colon after Se supplementation of 200 μg/d, additional intervention trials have been initiated to assess the effectiveness of Se as a cancer chemo-preventive agent (Burk, Norsworthy, Hill, Motley, & Byrne, 2006).

The most important source of selenium for man is food. Daily intake is low in many European countries, including Slovenia at 30–80 μg/d (Pokorn, Stibilj, Gregorič, Dermelj, & Štupar, 1998; Smrkolj, Pograjc, Hlastan-Ribič, & Stibilj, 2005). Vegetables are a poor source of selenium since most of them contain low amounts, less than 100 μg Se/kg dry mass (DRI, 2000). But some plants can assimilate Se when grown on seleniferous soils. The degree of Se uptake is species-dependent. Primary Se-accumulators reach Se contents from hundreds to thousands of μg Se/kg dry weight. The tolerance of these plants to Se is attributed to their ability to convert Se into compounds that are not incorporated into plant proteins and which, therefore, do not interfere with plant growth and metabolism. But despite numerous

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investigations Se has not yet been proven to be essential for the growth of plants themselves (Terry, Zayed, de Souza, & Tarun, 2000).

There are different techniques available to enhance the Se content in plants, such as Se addition to the soil, foliar treatment of plants with Se solution, soaking seeds in Se solution and hydroponic or aeroponic cultivation in a nutrient solution containing Se (Smrkolj, Osvald, Osvald, & Stibilj, 2007). Selection of an appropriate method must be done with care due to possible harmful effects on the environment. The use of Se fertilizers caused run-off of the element in the USA resulting in its accumulation in aquatic biota. Therefore a closed system is more suitable for producing selenium-rich vegetables (Combs, 2001). The chemical form of added selenium affects the distribution of selenium species and also the translocation of essential elements from roots to aerial parts, being more noticeable in plants exposed to selenite (Pedrero, Madrid, & Cámara, 2006).

Much research has been directed towards determining the various forms of selenium in plants. The combination of separation by high performance liquid chromatography (HPLC) and detection by inductively coupled plasma mass spectrometry (ICP-MS) was mainly used (Dumont, De Pauw, Vanhaecke, & Cornelis, 2006; Dumont, Vanhaecke, & Cornelis, 2006; Kotrebai, Birringer, Tyson, Block, & Uden, 2000; Montes-Bayón, Molet, González, & Sanz-Medel, 2006; Mounicou, Vonderheide, Shann, & Caruso, 2006; Pedrero et al., 2006; Slekovec & Goessler, 2005). If selenate were added during cultivation of plants Se was mainly accumulated in the same form in leaves (Pedrero et al., 2006; Slekovec & Goessler, 2005; Wróbel et al., 2004; Ximénez-Embún, Alonso, Madrid-Albarrán, & Cámara, 2004). In the case of selenite addition during plant growth, Se was metabolised mostly to selenomethionine (SeMet), selenomethylselenocysteine (SeMeSeCys) and γ-glutamyl-selenomethylselenocysteine (Montes-Bayón et al., 2006; Sugihara et al., 2004; Wróbel et al., 2004; Ximénez-Embún et al., 2004).

In our study different cultivars of chicory (*Cichorium intybus* L.), lamb's lettuce (*Valerianella locusta* L.), dandelion (*Taraxacum officinale* Waggers) and parsley (*Petroselinum crispum* Mill.) were chosen due to their abundant growth and high consumption in Slovenia and also in other parts of Southern Europe. For example chicory is the second most cultivated leafy salad vegetable in Slovenia with a production of 3400 tons in 2004 (Statistical Yearbook of the Republic of Slovenia, 2005). We used the aeroponic system for cultivation because of its many advantages. Aeroponics is a form of hydroponic plant cultivation in which the plant roots are suspended in a closed chamber and misted with a complete nutrient solution. Aeroponics requires no solid or aggregate growing medium and allows easy access to the roots. The chamber and misting system provide complete control of the root zone environment, including temperature, nutrient level, pH, humidity, misting frequency and duration, and oxygen availability. Another important advantage is that there is no influence on the surrounding environment when enriched solutions are used. In our case selenium in the form of Na_2SeO_4 was added to the nutrient solution.

The purpose of our work was to study the uptake and distribution of selenium in the selected plants after 41 days of aeroponic cultivation with elevated concentrations of Se. Water extraction and enzymatic hydrolysis of lyophilised samples were performed. High performance chromatography connected on-line with ultraviolet treatment–hydride generation atomic fluorescence spectrometry (HPLC–UV–HG-AFS) was used for determination of Se species in water extracts and in extracts after enzymatic treatment.

2. Experimental

2.1. Samples

Four cultivars of chicory (*Cichorium intybus* L. cultivars 'Anivip', 'Monivip', 'Tržaški solatnik' and 'Goriški'), lamb's lettuce (*Valerianella locusta* L.), dandelion (*Taraxacum officinale* Waggers) and parsley (*Petroselnium crispum* Mill.) were chosen for the experiment. Seeds were cultivated in peat and after 60 days 84 plants of each species or cultivar were transferred to the aeroponic system, where plants were placed in small pots, fixed with peat, but the roots left free in the air. The roots were sprinkled every 15 min with Resh nutrient solution containing the macro- and microelements essential for growth of leafy vegetables. 100 L of Resh nutrient solution contained Fe-EDTA (4.17 g), $CuSO_4 \cdot 5H_2O$ (0.0393 g), MoO_3 (0.0075 g), $MnSO_4$ (0.203 g), $ZnSO_4 \cdot 7H_2O$ (0.0044 g), H_3BO_3 (0.286 g), Na_2SO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (40.5 g), KH_2PO_4 (22.0 g), K_2SO_4 (32.8 g), $Ca(NO_3)_2$ (82.1 g), NH_4NO_3 (14.2 g). After 90 days, plants were divided into two groups, 42 plants in the control and 42 plants in the exposed group of each species or cultivar. For the treated group Se was added to the nutrient solution in the form of Na_2SeO_4 at a concentration of 7 μg Se/mL for the next 41 days. At the time of Se addition to the nutrient solution, the plants had approximately six leaves. The average temperature in the greenhouse during winter was maintained around 10 °C. At the end of the experiment, each plant in the control or in the treatment group had approximately 10–12 leaves. six plants of each cultivar were taken for analysis. Leaves were lyophilised at −50 °C and 0.050 mbar (Freeze-dryer CHRIST ALPHA 1–4, LOC-1) and milled in a planetary micro mill (FRITSCH, Pulverisette 7; speed 6, time 6 min). The average content of dry matter in leaves was 20.0% for chicory cv. 'Monivip', 14.7% for 'Tržaški solatnik', 11.8% for 'Goriški', 13.2% for lamb's lettuce, 19.2% for dandelion and 23.4% for parsley.

2.2. Chemicals

MilliQ water (Millipore system) was used in the whole process and the following chemicals: 65% HNO_3 (Merck,

suprapur), 96% H_2SO_4 (Merck, suprapur), 30% HCl (Merck, suprapur), 36% HCl (Merck, p.a.), 30% H_2O_2 (Merck, p.a.), V_2O_5 (Merck, p.a.), 40% HF (Merck, suprapur), NaOH (Merck, puriss p.a.), $NaBH_4$ (Fluka, purum p.a.), $(NH_4)_2HPO_4$ (Fluka, puriss p.a), $NH_4H_2PO_4$ (Fluka, puriss p.a.), pyridine (Fluka, puriss p.a.), Protease XIV (Sigma–Aldrich, 4 U/mg).

For preparation of stock Se solutions with a concentration around 1 mg/L Na_2SeO_3 (Sigma, >98%), Na_2SeO_4 (Sigma, SigmaUltra), selenomethionine (SeMet, Fluka, >99%), selenocystine ($SeCys_2$, Fluka, >98%) and selenomethylselenocisteine (SeMeSeCys, Fluka, >98%) were used.

2.3. Instrumentation

The HPLC system consisted of a Varian ProStar 210 pump, a Rheodyne 7725i injector and Hamilton PRP X100 and X200 columns. The HG-AFS system was constructed of a peristaltic pump (Ismatec, MCP 380), a gas liquid separator (A-type, PS Analytical), a gas dryer (Nafion dryer, Perma Pure Products) and an AFS detector (Excalibur, PS Analytical) with a Se boosted discharge lamp (Super Lamp, Photron). A UV unit (PS Analytical) with a 12 m coil of FEP around a 78 W lamp was also used.

3. Procedures

3.1. Extraction

Water extraction: 10 g of water was added to 0.2 g dry sample in a 15 mL centrifuge tube and the mixture was shaken for 5 min at room temperature.

Enzyme hydrolysis: 80 mg of the enzyme Protease XIV was dissolved in 10 g of water and added to 0.2 g sample in a 15 mL centrifuge tube and the mixture was shaken for 24 h at 37 °C.

After extraction the mixture was centrifuged for 30 min at 14,000 rpm/min (Eppendorf 5804R). The supernatant was separated from sediment and filtered through a 0.22 μm Millex GV filter. Supernatants and sediments were stored at −20 °C until analyses for total Se and Se speciation were carried out.

3.2. Total Se determination

For the determination of total Se in lyophilised plant samples and sediments after extraction, digestion with H_2SO_4, HNO_3, H_2O_2, HF and V_2O_5 in H_2SO_4 was used, followed by reduction with HCl and detection with HG-AFS. The whole procedure is described in detail elsewhere (Smrkolj & Stibilj, 2004).

For the determination of total selenium in supernatants and chromatographic fractions, digestion with HNO_3 and H_2O_2 was applied. The next steps were reduction with HCl and detection with HG-AFS. A detailed description is given in Stibilj, Mazej, and Falnoga (2003).

3.3. Determination of Se compounds

An HPLC–UV–HG-AFS system was used for the determination of Se compounds. The scheme of the system used has been published in Mazej, Falnoga, Veber, and Stibilj (2006). HPLC conditions were: for anion exchange: Hamilton PRP X100 column (250 mm × 4.1 mm × 10 μm), mobile phase 40 mmol/L phosphate buffer (pH 6.0; 0.5 mL/min) and for cation exchange: Hamilton PRP X200 column (250 mm × 4.1 mm × 10 μm), mobile phase 10 mmol/L pyridine (pH 1.5; 1 mL/min). Injected volume was 100 μL. The eluent from the column was mixed with concentrated HCl (flow rate 3 mL/min) and then passed through the UV unit. 1.2% $NaBH_4$ in 0.1 mol/L NaOH (flow rate 3 mL/min) was added after the UV unit. Argon as carrier gas (260 mL/min) transferred H_2Se from the gas liquid separator through the dryer into the AFS detector. The dryer gas was nitrogen with a flow rate of 3 L/min. The AFS unit was equipped with a Se boosted discharge lamp (primary current 20 mA, boosted current 25 mA). Identification of peaks was made on the basis of comparison of the elution times of standards and sample and by the standard addition method. Fig. 1 shows the separation of five Se compounds ($SeCys_2$, SeMet, SeMeSeCys, Se^{IV}, Se^{VI}) on anion and cation exchange columns and UV–HG-AFS as a detection system using optimised conditions. A detailed description is given in Mazej et al., 2006. The analytical parameters reported for the determination of Se species were: (1) linearity up to 200 ng/g for all species; (2) repeatability between 9% and 15%; (3) detection limits between 2 and 9 ng Se/g solution; (4) accuracy checked by comparison with literature data for SeMet for a yeast candidate reference material from the SeAs project and Durum Wheat Flour RM 8436.

4. Results and discussion

4.1. Accumulation

All exposed plants (four cultivars of chicory, lamb's lettuce, dandelion, parsley) were aeroponically cultivated with a nutrient solution with added selenium (7 μg Se/mL) in the form of Na_2SeO_4 for 41 days. When cultivated under the usual conditions these plants contained low amounts of selenium irrespective of species, 0.034–0.057 μg/g on a dry matter basis. Accumulation of Se in 41 days was very high in all cases, from 49 to 480 μg/g on a dry matter basis (Table 1), in spite of the fact that they belong to different plant families. The lowest accumulation, only one tenth of the highest value, was observed for dandelion. This could be assigned to its poorly developed root system in comparison to chicory, the relatively related species. Despite the high Se content there was no sign of toxicity such as a garlic smell, red spots on the roots, black spots on the leaves and drying of the leaves (Kabata-Pendias, 2001; Terry et al., 2000). Sugihara et al. (2004) observed no abnormalities in shape or colour but a little slower

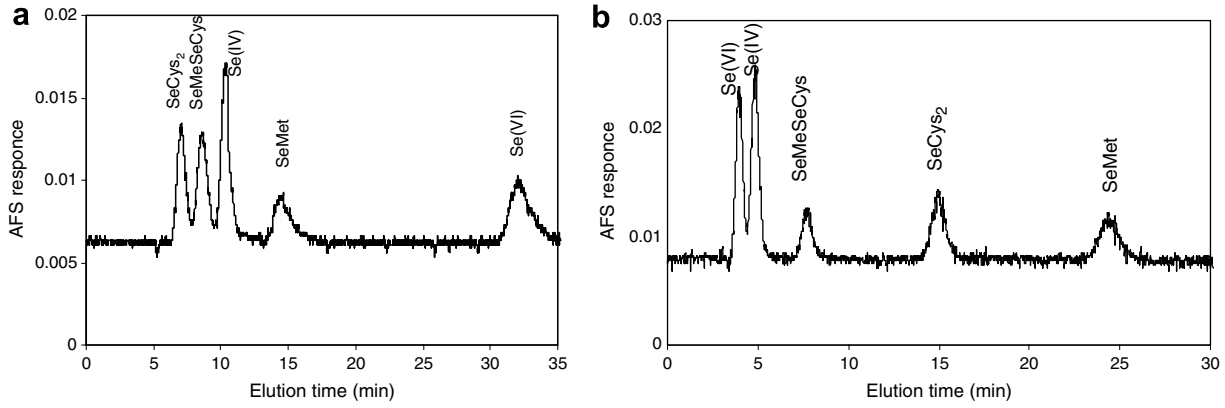

Fig. 1. Chromatograms of a mixture of Se species ($SeCys_2$, SeMet, SeMeSeCys, Se^{IV}, Se^{VI}) with mass fractions around 100 ng Se/g for each species on anion (a) and cation (b) exchange columns.

Table 1
Total Se content in lyophilised green parts of exposed plants (expressed as average ± SD ($n = 3$), on a dry matter basis) and distribution of Se between the water soluble extract and solid residue after water extraction and enzymatic hydrolysis of lyophilised green parts of exposed plants (expressed as average ± SD ($n = 3$))

Plant (cultivar)	Total Se content (μg/g)	Water extraction	Enzymatic hydrolysis		
		Supernatant (%)	Supernatant (%)	Sediment (%)	Sum (%)
Chicory cv. 'Anivip'	480 ± 10	64 ± 2	75 ± 2	NA	
Chicory cv. 'Monivip'	460 ± 10	70 ± 2	89 ± 9	14 ± 1	103 ± 9
Chicory cv. 'Tržaški solatnik'	456 ± 10	82 ± 1	88 ± 2	19 ± 1	107 ± 2
Chicory cv. 'Goriški'	167 ± 1	85 ± 8	86 ± 7	19 ± 3	105 ± 8
Lamb's lettuce	455 ± 3	53 ± 2	91 ± 3	24 ± 4	115 ± 5
Dandelion	49 ± 1	45 ± 1	88 ± 3	21 ± 4	109 ± 5
Parsley	290 ± 6	63 ± 2	79 ± 10	19 ± 4	98 ± 11

NA, not analysed.

growth in sprouts exposed hydroponically to 10 μg/mL of selenium for 5–8 days.

5. Extraction

Extraction of Se species from solid samples into a suitable solvent should be done before separation on chromatographic columns. Beside the maximum efficiency, the stability of Se species during the procedure should be taken into account when choosing the extraction conditions. In our study two different types of extraction of Se species from exposed plant samples were used. The first was simple water extraction for water soluble Se species, and the second was enzymatic hydrolysis to release Se species bound to proteins. The extraction efficiency was calculated according to the total concentration of Se in the sample. The water soluble fraction of Se in the exposed samples was between 45% and 85%, while the percentage of selenium released in enzymatic hydrolysis, as expected, was higher i.e. between 75% and 91% (Table 1). Around 20% of selenium remained in the solid residue after the action of the unspecific enzyme Protease XIV. Slekovec and Goessler (2005) reported extraction efficiencies of 20–30% for methanol/water solution (9:1) from leaves of four plants. Sugihara et al. (2004) used extraction into 0.2 M HCl and the extractable amount of Se accumulated in sprouts of several young plants was 69–98%. Montes-Bayón et al. (2006) studied three extraction solutions, one of them containing protease, and obtained extraction recoveries between 75% and 127% for two plant species enriched with selenium, garlic and Indian mustard. Roberge, Borgerding, and Finley (2003) reported of about 30% losses of Se by volatilisation during buffered extractions of broccoli samples. But in our case the mass balance indicated that there were no losses during extraction since the sum of the Se in the supernatant and solid residue was 96 ± 4% for water extraction, the mass balance was made only for chicory, cultivar Anivip, and between 98% and 115% for enzymatic hydrolysis, when the mass balance was made for all selected plants (Table 1).

5.1. Speciation studies

In our previous work the distribution of Se in the water soluble part of chicory, cultivar 'Anivip', according to molecular mass was studied by size exclusion chromatography on Superdex 75 and Superdex Peptide columns. The results showed that the majority of the water soluble Se,

more than 95%, eluted in the low molecular weight fraction, Mr under 1000 (Mazej et al., 2006). In the present work the identification of Se species in water extracts was made by ion exchange columns coupled to a UV–HG-AFS detection system. Se^{VI} represented >95% of all Se in the water extracts of chicory cv. 'Anivip'. Similar results were obtained in this study with the other plants studied. Therefore we can conclude that the main form accumulated was Se^{VI}, the same as the form of Se added. Traces of Se^{IV} were observed in all extracts, but traces SeMet only in extracts of chicory cv. 'Tržaški solatnik' and 'Anivip'. An unknown peak (A) was also noticed in the void volume on the anion exchange column. The number of peaks observed and the proportion between them in our study are comparable to the results obtained by Slekovec and Goessler (2005) for methanol/water (9:1) extraction of Se compounds from the green parts of four selenate-supplemented vegetables (cabbage, radish, onion, garlic). They reported that 90–100% of extracted Se was present as selenate, traces of selenite were found, while SeMet was identified only in the extract of garlic, and peaks of unknown Se compounds eluting in the void volume were observed in some cases. The main difference between their results and ours is the percentage of Se compounds identified in the samples, their's being considerably lower. They identified 50–100% of the Se in extracts, but the extraction efficiency was 20–30%, which together lead to 10–30% of identified Se in the samples. We obtained 45–85% Se in water extracts, of which more than 95% was determined as selenate and hence the percentage of identified Se compounds was between 43% and 80%.

For the determination of Se species bound to proteins enzymatic hydrolysis was chosen. Anion and cation exchange chromatograms of three selected plant leaf extracts are shown in Fig. 2. Beside five known compounds, one or more unknown peaks were observed on

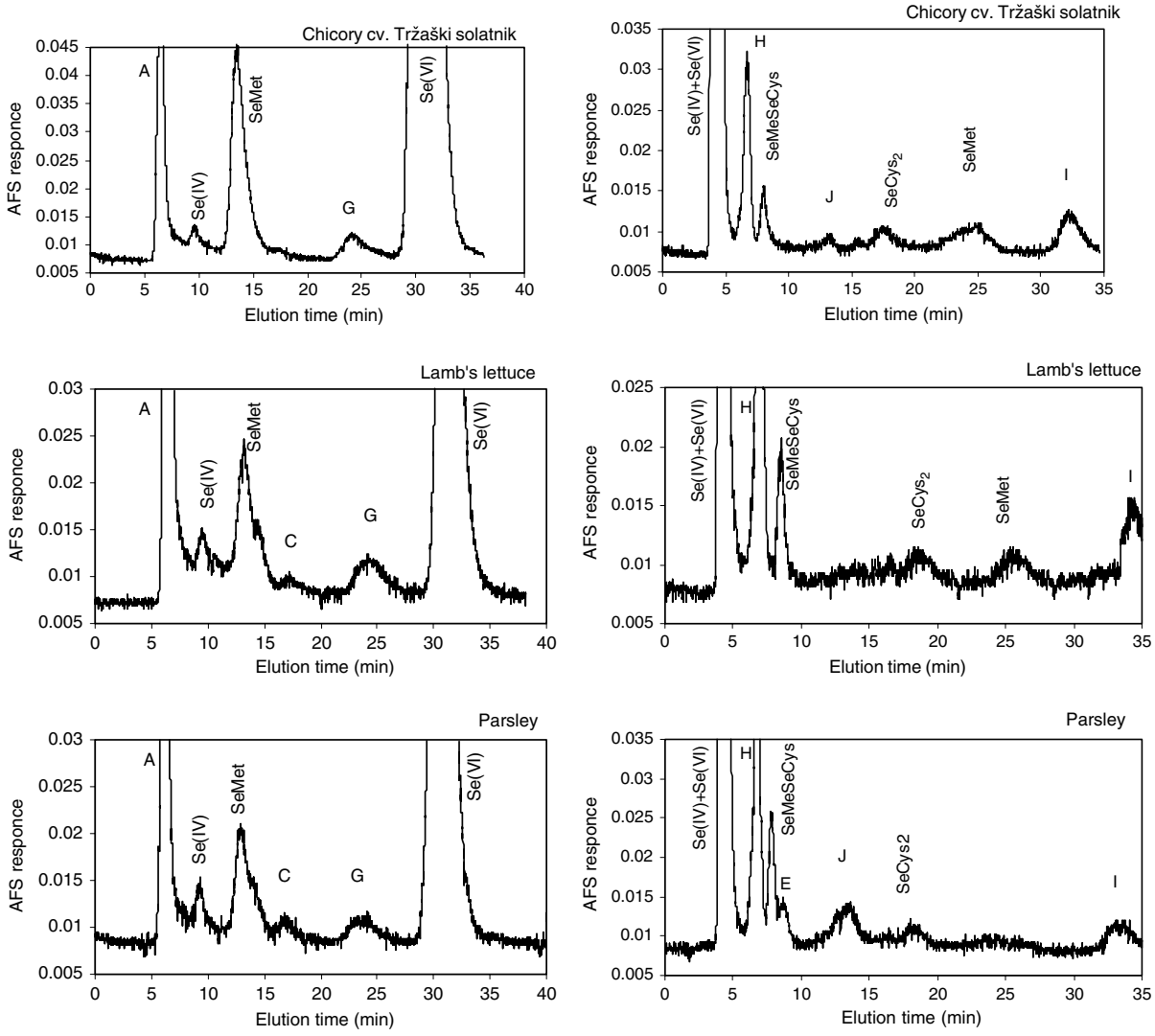

Fig. 2. Anion (left column) and cation (right column) exchange chromatograms of plant leaf extracts after enzymatic hydrolysis (A, G, H, I, J – unknown species).

each column. As the unknown peaks did not elute at the same elution time as products of oxidation or reaction between the enzyme and SeMet (Mazej et al., 2006), we extended this study under the same experimental conditions as for SeMet to compounds $SeCys_2$ and SeMeSeCys. Ten grams of a solution (10 g) of $SeCys_2$ and SeMeSeCys with a mass fraction of Se around 1 μg/g was: (a) oxidised in a test experiment with a 2% solution of H_2O_2 overnight at room temperature and (b) with 80 mg of Protease XIV added to the solution, incubated for 24 h at 37 °C and stored at −20 °C until analysis. However, the elution times of the new reaction products were not the same as any of the unknown peaks of the sample extracts (Fig. 3).

The results of the determination of Se species in enzymatic hydrolysis extracts of the selected plants are summarised in Table 2. In comparison with water extracts, an increase of the SeMet peak is most obvious. To sum up, the ranges of organic Se species detected after enzymatic hydrolysis, expressed as a percentage of the total Se in the samples, were: SeMet 6–21%, SeMeSeCys 0.5–4.4% and $SeCys_2$ up to 0.8%. It is interesting that SeMeSeCys was found only in extracts after enzymatic hydrolysis and not in water extracts, as would be expected according to the fact that SeMeSeCys is a non-protein amino acid. SeMeSeCys was most probably connected with another molecule forming a dipeptide, for example γ-glutamyl-SeMeSeCys which hydrolyses in the presence of an enzyme.

Of the seven observed unknown peaks, the largest one was peak A representing approximately 2–7% of the total Se in different chicory cultivar leaves and 9–18% in the other plants. The percentage was estimated with respect to $SeCys_2$, which eluted at the same retention time. The other interesting peak was that before SeMeSeCys at t_e 7 min (H), which was also quite high in all plant extracts. The proportion of Se in this peak was estimated as the response of the detection system for the unknown species

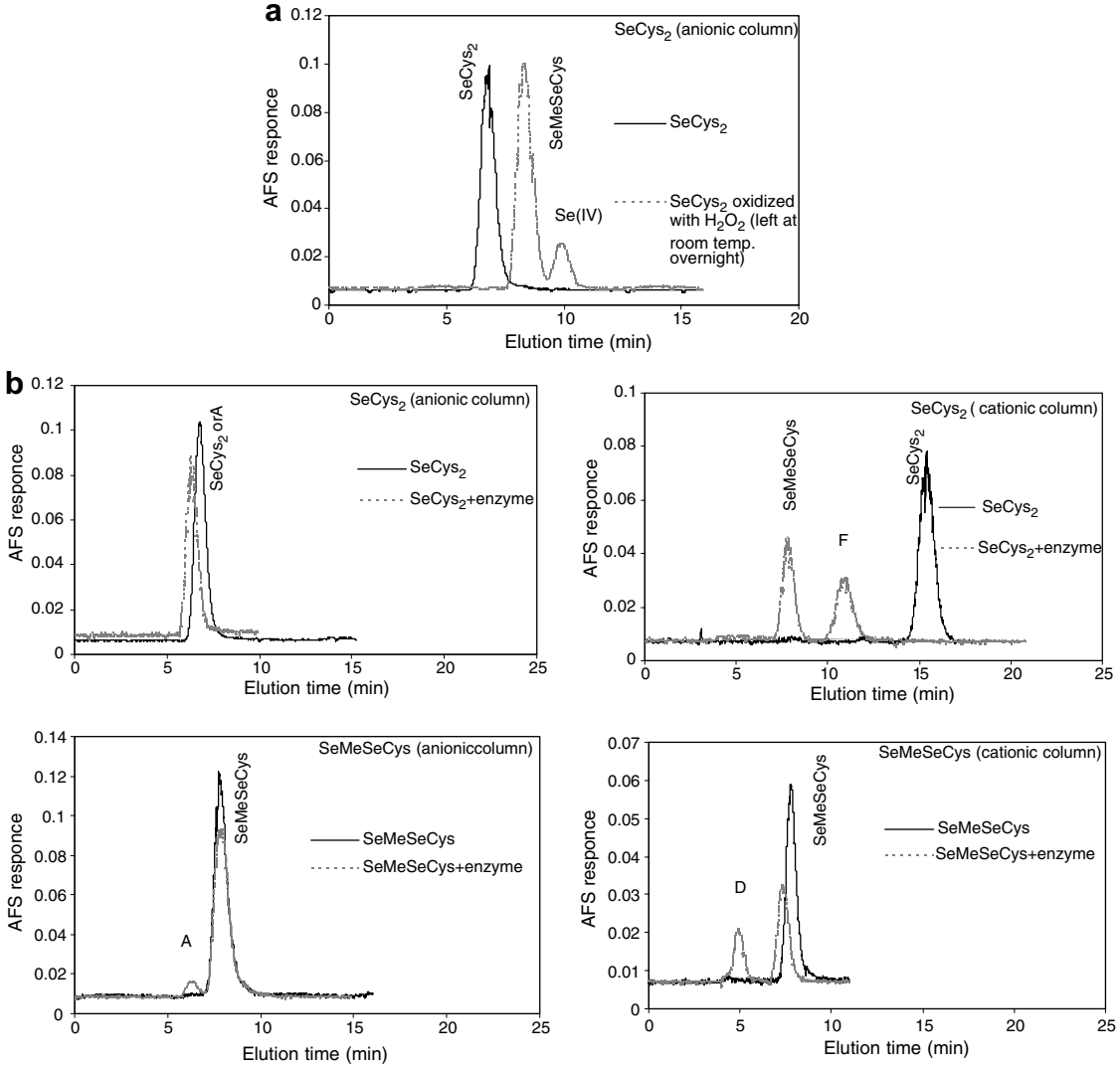

Fig. 3. Chromatograms of: (a) oxidation products of a solution of $SeCys_2$ and (b) transformation products after reaction between enzyme and a solution of $SeCys_2$ and of SeMeSeCys.

Table 2
Se species in extracts after enzymatic hydrolysis (μg Se/g sample and % of Se with respect to the total content in the sample, average of at least two determinations)

Plant (cultivar)	SeMet		$SeCys_2$		SeMeSeCys		Se^{IV}		Se^{VI}		Unknown species[a]	Sum (%)	Total Se in extracts (%)
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%			
Chicory cv. 'Anivip'	40	8.4	<DL		3.4	0.7	0.9	0.2	313	63	A, G, H, I	73	75
Chicory cv. 'Monivip'	28	6.1	0.9	0.2	2.3	0.5	1.0	0.2	390	85	A, G, H, I, J	92	89
Chicory cv. 'Tržaški solatnik'	68	15	3.7	0.8	7.4	1.6	1.8	0.4	264	58	A, G, H, I, J	76	88
Chicory cv. 'Goriški'	20	11.9	<DL		2.7	1.7	0.2	0.1	113	68	A, H, I	82	86
Lamb's lettuce	32	6.9	3.5	0.7	13	3.0	3.1	0.6	182	40	A, C, G, H, I	51	91
Dandelion	10	21	<DL		1.0	2.0	0.1	0.7	27	55	A, H, I	79	88
Parsley	25	8.5	2.3	0.8	12.8	4.4	2.0	0.6	175	60	A, C, E, G, H, I, J	74	79

<DL, under detection limit.
[a] Unknown Se species with t_e: A, C and G with 6, 18 and 25 min on anionic column; E, H, I and J with 9, 7, 33 and 14 min on cationic column.

H supposing it was the same as for Se^{IV}. The percentages obtained were 0.3–2% for chicory samples and around 4% for other plant samples. Other unknown peaks were estimated in the same way as H. The two peaks with longer elution times (G and I) represented about 0.5–2% of Se, and in the other peaks (J, C and E) the proportion of selenium was below 0.5%.

The sum of Se in the identified peaks in the extracts was between 51% and 92% (Table 2). A comparison of the sum of Se in the identified peaks with the total Se content in the extracts showed only a small difference in the case of three samples, i.e. parsley and the chicory cultivars 'Monivip' and 'Goriški', which also means that the proportion of Se in the unknown peaks was very low in these samples. The contribution of unknown species was evident and not negligible for the other three samples, chicory cv. 'Tržaški solatnik', lamb's lettuce and dandelion. This was especially noticeable in the lamb's lettuce extract where the difference between the sum and the total Se content was around 40%, which was even comparable with the percentage of Se in the identified peaks. For further identification of these unknown Se species other detection techniques should be used such as ES-MS.

Some interesting data in Table 2 such as the highest number of peaks observed in the parsley extract, the highest amount of SeMeSeCys observed in lamb's lettuce and parsley, and the highest percentage of unidentified Se species in lamb's lettuce extract could be explained by differences in metabolism of the selected plants belonging to

Table 3
Comparison of results of this work with literature data for SeMeSeCys in plants exposed to selenium

Plant	Se addition	Total Se in leaves (μg/g dry weight)	SeMeSeCys (%)[a]	Literature
Chicorv (leaves) (*Cichorium intybus* L.)	Na_2SeO_4 7 mg/L 41 days	167–480	0.5–1.7	This study
Lamb's lettuce (leaves) (*Valerianella locusta* L.)	aeroponically	455	3.0	
Dandelion (leaves) (*Taraxacum officinale* Waggers)		49	2.0	
Parsley (leaves) (*Petroselinum crispum* Mill.)		290	4.4	
Onion (leaves) (*Allium cepa*)	Na_2SeO_4 5 mg/L 8 days hydroponically	154–601	2–4	Wróbel et al. (2004)
Onion (sprouts) (*Allium cepa*)	Na_2SeO_3 10 mg/L 8 days	17[b]	100	Sugihara et al. (2004)
Parsley (sprouts) (*Petroselinum crispum*)	hydroponically	15[b]	100	
Red-tip leaf lettuce (sprouts) (*Lactuca sativa*)		27[b]	90	
Radish (*Raphanus sativus*)	Na_2SeO_3 1 mg/L 40 days hydroponically	112[b]	74	Pedrero et al. (2006)
	Na_2SeO_4 1 mg/L 40 days hydroponically	120[b]	5.8	
Onion (bulb) (*Allium cepa*)	?	96–140	1–5	Kotrebai et al. (2000)
Bean (seeds) (*Phaseolus vulgaris* L.)	Na_2SeO_4 10 mg/L 10 days	2	30	Smrkolj et al. (2007)

?, not reported.
[a] % SeMeSeCys relative to total Se in sample.
[b] μg/g wet weight.

different families. Chicory and dandelion are members of the same *Asteraceae* family, lamb's lettuce of the *Valerianaceae* family, and parsley of the umbellate plants, the *Apiaceae* family.

The chemical form of selenium present in the culture medium influences the speciation of Se in the plant. In leaves of plants grown in selenite supplemented media, independently of the mode of Se addition, practically all of the selenium content was identified as selenoaminoacids, of which most was found to be SeMeSeCys. But a low transformation into organic forms was observed in leaves of plants grown in selenate supplemented media, with Se(VI) being the major species identified. Selenate uses the same transporters as sulphate in crossing plant membranes. The reduction of selenate to selenite is the rate-limiting step in selenate transformation to selenoaminoacids. It therefore remains mainly as selenate in various plant tissues (Sugihara et al., 2004; Terry et al., 2000; Wróbel et al., 2004; Ximénez-Embún et al., 2004). Exceptions are seeds, grains and nuts where SeMet is the predominant species (Dumont et al., 2006; Smrkolj, Stibilj, Kreft, & Kapolna, 2005; Smrkolj et al., 2007; Stadlober, Sager, & Irgolic, 2001). In our case no toxic effects on plants were observed despite the high Se concentrations. There were probably some protective mechanisms operating: (a) one possibility is storage of Se^{VI} in vacuoles as this is the main form found in extracts; (b) because SeMeSeCys was identified in the extracts, the other possibility is the formation of the non-protein aminoacid SeMeSeCys and the dipeptide γ-glutamyl-SeMeSeCys (Terry et al., 2000). The fraction of SeMeSeCys depends on many factors such as plant species, plant part and Se addition. Literature data (Kotrebai et al., 2000; Pedrero et al., 2006; Smrkolj et al., 2007; Sugihara et al., 2004; Wróbel et al., 2004) on the amount of SeMeSeCys in the leaves of plants exposed to selenium and a comparison with our results are shown in Table 3.

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References

Burk, R. F., Norsworthy, B. K., Hill, K. E., Motley, A. K., & Byrne, D. W. (2006). Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. *Cancer Epidemiology, Biomarkers & Prevention, 15*(4), 804–810.

Combs, G. F. (2001). Selenium in global food systems. *British Journal of Nutrition, 85*, 517–547.

Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids (DRI) (2000). National Research Council, Washington, DC: National Academy Press (pp. 284–319).

Dumont, E., De Pauw, L., Vanhaecke, F., & Cornelis, R. (2006). Speciation of Se in Bertholletia excelsa (Brazil nut): A hard nut to crack?. *Food Chemistry 95*, 684–692.

Dumont, E., Vanhaecke, F., & Cornelis, R. (2006). Selenium speciation from food source to metabolites: A critical review. *Analytical and Bioanalytical Chemistry, 385*, 1304–1323.

Finley, J. W. (2005). Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. *Annales of Botany, 95*, 1075–1096.

Kabata-Pendias, A. (2001). *Trace elements in soils and plants* (3rd ed.). Boca Raton, FL: CRC Press.

Kotrebai, M., Birringer, M., Tyson, J. F., Block, E., & Uden, P. C. (2000). Selenium speciation in enriched and natural samples by HPLC–ICP-MS and HPLC–ESI-MS with perfluorinated carboxylic acid ion-pairing agents. *Analyst, 125*, 71–78.

Mazej, D., Falnoga, I., Veber, M., & Stibilj, V. (2006). Determination of selenium species in plant leaves by HPLC–UV–HG-AFS. *Talanta, 68*, 558–568.

Montes-Bayón, M., Molet, M. J. D., González, E. B., & Sanz-Medel, A. (2006). Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum* and *Brassica juncea*) and Se speciation by HPLC–ICP-MS. *Talanta, 68*, 1287–1293.

Mounicou, S., Vonderheide, A. P., Shann, J. R., & Caruso, J. A. (2006). Comparing a selenium accumulator plant (*Brassica juncea*) to a nonaccumulator plant (*Helianthus annuus*) to investigate selenium containing proteins. *Analytical and Bioanalytical Chemistry, 386*(5), 1367–1378.

Pedrero, Z., Madrid, Y., & Cámara, C. (2006). Selenium species bioaccessibility in enriched radish (*Raphanus sativus*): A potential dietary source of selenium. *Journal of Agricultural and Food Chemistry, 54*(6), 2412–2417.

Pokorn, D., Stibilj, V., Gregorič, B., Dermelj, M., & Štupar, J. (1998). Elemental composition (Ca, Mg, Mn, Cu, Cr, Zn, Se and I) of daily diet samples from some old people's home in Slovenia. *Journal of Food Composition and Analysis, 11*, 47–53.

Roberge, M. T., Borgerding, A. J., & Finley, J. W. (2003). Speciation of selenium compounds from high selenium broccoli is affected by the extracting solution. *Journal of Agricultural and Food Chemistry, 51*, 4191–4197.

Slekovec, M., & Goessler, W. (2005). Accumulation of selenium in natural plants and selenium supplemented vegetable and selenium speciation by HPLC–ICPMS. *Chemical Speciation and Bioavailability, 17*(2), 63–73.

Smrkolj, P., Osvald, M., Osvald, J., & Stibilj, V. (2007). Selenium uptake and species distribution in selenium-enriched bean (*Phasolus vulgaris* L.) seeds obtained by two different cultivations. *European Food Research and Technology, 225*, 233–237.

Smrkolj, P., Pograjc, L., Hlastan-Ribič, C., & Stibilj, V. (2005). Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium. *Food Chemistry, 90*, 691–697.

Smrkolj, P., & Stibilj, V. (2004). Determination of selenium in vegetables by hydride generation atomic fluorescence spectrometry. *Analytica Chimica Acta, 512*, 11–17.

Smrkolj, P., Stibilj, V., Kreft, I., & Kapolna, E. (2005). Selenium species determination in selenium enriched pumpkin (*Cucurbita pepo* L.) seeds by HPLC–UV–HG-AFS. *Analytical Sciences, 21*, 1501–1504.

Stadlober, M., Sager, M., & Irgolic, K. J. (2001). Effects of selenate supplemented fertilisation on the selenium level of cereals-identification and quantification of selenium compounds by HPLC–ICP-MS. *Food Chemistry, 73*, 357–366.

Statistical Yearbook of the Republic of Slovenia (2005). Statistical Office of the Republic of Slovenia (pp. 298–299).

Stibilj, V., Mazej, D., & Falnoga, I. (2003). A study of low level selenium determination by hydride generation atomic fluorescence spectrometry in water soluble protein and peptide fractions. *Analytical and Bioanalytical Chemistry, 377*, 1175–1183.

Sugihara, S., Kondô, M., Chihara, Y., Yûji, M., Hattori, H., & Yoshida, M. (2004). Preparation of selenium enriched sprouts and identification of their selenium species by high-performance liquid chromatography–

inductively coupled plasma mass spectrometry. *Bioscience Biotechnology and Biochemistry, 68*(1), 193–199.

Terry, N., Zayed, A. M., de Souza, M. P., & Tarun, A. S. (2000). Selenium in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 51*, 401–432.

Wróbel, K., Wróbel, Kz., Kannamkumarath, S. S., Caruso, J. A., Wysocka, I. A., Bulska, E., et al. (2004). HPLC–ICP-MS speciation of selenium in enriched onion leaves – a potential dietary source of Se-methylselenocysteine. *Food Chemistry, 86*, 617–623.

Ximénez-Embún, P., Alonso, I., Madrid-Albarrán, Y., & Cámara, C. (2004). Establishment of selenium uptake and species distribution in lupine, indian mustard, and sunflower plants. *Journal of Agricultural and Food Chemistry, 52*, 832–838.