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Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation

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Abstract

Common (*Fagopyrum esculentum* Moench) and tartary (*Fagopyrum tataricum* Gaertn.) buckwheat was treated by spraying the leaves with a water solution containing 15 mg Se per litre in the form of sodium selenate in the flowering period. The selenium content in all parts of plant was found to be less than 200 ng g^{-1} in non-treated and in the range 2700–4650 ng g^{-1} in selenium treated buckwheat. Exposure to UV-B radiation lead to higher Se accumulation in flowers of both Se enriched cultivars. For speciation analysis enzymatic hydrolysis was carried out, separation and detection of selenium species was performed by high performance liquid chromatography–ultraviolet treatment–hydride generation atomic fluorescence spectrometry (HPLC–UV–HG-AFS). In flowers and leaves, on average 11% of the Se content was soluble and in the form of Se(VI), representing between 0.6% (flowers) and 3% (leaves) of the Se content. The remaining soluble non-amino acid organic Se was not detected by HPLC–UV–HG-AFS. In seeds 93% of the selenium content was found in the extracts and the main selenium species was SeMet with 93 ± 5% relative to the selenium content.

Keywords: Selenium; Speciation; HPLC; HG-AFS; Plants; Buckwheat

1. Introduction

Cultivation of plants enriched in selenium could be an effective way to improve the selenium status in man. Stadlober, Sager, and Irgolic (2001) cultivated different cereals (wheat, barley and rye) in soil supplemented using mineral fertiliser to reach the content of 30 mg Se per kg soil. SeMet was the main species in all cereals, as well as $SeCys_2$, and in some cases traces of selenate were found. In selenium-enriched ramp bulbs hydroponically grown in selenate-enriched solution, Se–methylselenocysteine and selenate were the main species, but also minor amounts of Se–cystathionine and glutamyl-Se–methylselenocysteine were found (Whanger, Ip, Polan, Uden, & Welbaum, 2000). Selenium-enriched onion bulbs were grown hydroponically in nutrient solution enriched with selenite and selenate, and Se–methylselenocysteine was found to be the predominant species in them (Wrobel et al., 2004). Zhang and Frankenberger (2001) studied selenium species in the plant *Stanleya pinnata* that was grown in sand culture with different concentrations of selenate and observed that the main part of selenium was bound as Se–amino acids, and minor parts as Se(VI) and non-amino acid organic Se. Ximenez-Embun, Alonso, Madrid-Albarran, and Camara (2004) cultivated lupin, Indian mustard and sunflower seedlings in selenate and selenite media and selenite or selenate, depending on the form of

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enrichment, were the predominant species; SeMet was also present. Plants of Indian mustard were grown hydroponically in selenate and selenite solution and in shoots and roots SeMet, Se-(methylseleno)cysteine, Se–methylselenocysteine, selenomethionine Se–oxide hydrate and selenate or selenite, depending on the added species, were found (Kahakachchi, Totoe Boakye, Uden, & Tyson, 2004). But no data on Se speciation in plants, foliar Se treated were found in the literature.

Nowadays, due to reborn interest, common buckwheat is becoming one of the most important alternative crops in Europe. Tartary buckwheat is grown in the mountainous regions of Asia, and also in some countries of central Europe (Bonafaccia, Marocchini, & Kreft, 2003; Fabjan et al., 2003). Buckwheat is used as grain (to produce flour, groats or puffed products), sprouts or herb (as a leafy vegetable, or a dried herb for herb tea) (Kim, Kim, & Park, 2004; Kreft, 1994; Norbu & Roder, 2003; Park et al., 2000). It is also known that UV-B radiation stimulates synthesis of different flavonoids and other polyphenolic compounds like tannins and lignins as a protection against harmful effects of the radiation (Kreft, Štrukelj, Gaberščik, & Kreft, 2002).

In our previous work, the ability of common buckwheat to accumulate selenium from foliar application of an aqueous solution containing 1 mg Se(VI)/L was studied. It was found that the Se content in seeds increased from 43 ng g^{-1} in untreated plants to 367 ng g^{-1} in treated ones. So buckwheat was found to be an appropriate material for selenium enrichment (Stibilj, Kreft, Smrkolj, & Osvald, 2004).

Selenium speciation in biological samples is still very problematic, because sample pretreatment should not change the chemical forms of the target analyte and the very low selenium concentrations in the environment make their identification and quantification even more difficult (Uden, Boakye, Kahakachchi, & Tyson, 2004). For separation and detection of selenium species in biological matrices the most often used method is high performance liquid chromatography (HPLC) in connection with ICP-MS (Auger, Yang, Arnault, Pannier, & Potin-Gautier, 2004; B'Hymer & Caruso, 2000; Kannamkumarath, Wrobel, Wrobel, Vonderheide, & Caruso, 2002; Stadlober et al., 2001; Whanger et al., 2000; Wrobel et al., 2004), hydride generation atomic absorption spectrometry (HG-AAS) (Zhang & Frankenberger, 2001) and hydride generation atomic fluorescence spectrometry (HG-AFS) (Bodo et al., 2003; Stefanka, Ipolyi, Dernovics, & Fodor, 2001; Vilano & Rubio, 2000).

The aim of this work was to study selenium accumulation and to identify the selenium species in buckwheat seeds, leaves and flowers enriched in selenium by foliar fertilisation and to check any possible influence of UV-B radiation on Se accumulation. The Se species were separated and detected by high performance liquid chromatography–ultraviolet treatment–hydride generation atomic fluorescence spectrometry (HPLC–UV–HG-AFS).

2. Materials and methods

2.1. Samples

Common (*Fagopyrum esculentum* Moench), cv. Darja and tartary (*Fagopyrum tataricum* Gaertn.) buckwheat, domestic variety from Luxembourg, were grown under two different radiation conditions, ambient UV-B radiation and application of supplemental UV-B radiation. A UV-B supplementary system was built as described by Björn and Teramura (1993). Elevated UV-B radiation, simulating 17% atmosphere ozone depletion, was produced using Q-Panel UV-B 313 lamps filtered with cellulose diacetate filters, which block the UV-C range (wavelengths lower than 280 nm). To correct for the effects of UV-A radiation, control plants were irradiated with Q-Panel UV-B 313 lamps filtered with Mylar foil that cuts out wavelengths lower than 318 nm. The systems were timer controlled. UV-B doses were calculated and adjusted weekly using the program published by Björn and Murphy(1985) and based on the generalized plant action spectrum of Caldwell (1968). Ambient UV-B radiation was measured using the European Light Dosimeter Network (ELDONET, Real Time Computer, Möhrendorf, Germany) measuring system which also monitors UV-A radiation and PAR (photosynthetically active radiation, 400–700 nm). Plants were treated by spraying the leaves with an aqueous solution containing 15 mg Se per litre in the form of Na_2SeO_4 35 days after sowing, at the beginning of flowering. Selenium untreated plants were grown in the same location besides the treated ones, in the same soil, and separated to prevent contamination. Seven plants of each group were sampled 90 days after sowing. Dried plants were divided into leaves, seeds and flowers (entire influorescences were applied).

2.2. Sample preparation

For analysis, dried seeds, leaves and flowers were milled and homogenised with an agate FRITSCH mill, Pulverisette 7.

2.3. Method

Se content in seeds, leaves and flowers was determined by HG-AFS (Smrkolj & Stibilj, 2004). To 0.150–0.200 g of sample 0.5 mL of conc. H_2SO_4 and 1.5 mL of HNO_3 were added and the closed tube was heated for 60 min at 130 °C in an aluminium block. Two times 2 mL of H_2O_2 was added and heated for

30 min at 115 °C. After the digestion, the solution was cooled to room temperature and 0.1 mL of V_2O_5 in H_2SO_4 was added and the tube heated at 115 °C for 20 min. Reduction of Se(VI) to Se(IV) with 2.5 mL of conc. HCl was carried out at 100 °C for 10 min. Samples were diluted and selenium was determined by HG-AFS.

For selenium speciation, 300–350 mg sample was treated with 4 mL water, or with 50 mg of the non-specific enzyme protease *Streptomyces griseus* (Protease XIV, Merck) dissolved in 4 mL of 25 mM phosphate buffer (KH_2PO_4) (pH 7.5). In both cases, the sample was stirred at 200 rpm for 24 h at 37 °C (SW 22, Julabo). After that, the sample was centrifuged at 14,000*g* for 45 min at 4 °C (5804R, Eppendorf). The supernatant was filtered through a 0.25 μm filter (Millipore) and used for selenium speciation analysis by HPLC–UV–HG-AFS.

The Se content in the supernatant was determined by digestion in HNO_3, using a method described in detail elsewhere (Stibilj, Mazej, & Falnoga, 2004). To 0.5 g of supernatant 1 mL of conc. HNO_3 was added and heated for 10 min on an electric hot plate at about 100 °C in a capped Teflon vial. Then 0.5 mL H_2O_2 was added three times and evaporated each time to 1/4 volume. Addition of 1 mL of 6 mol L^{-1} HCl was added for the reduction of Se(VI) and then selenium was measured by HG-AFS. The water and enzymatic hydrolysis extracts were analysed immediately on Se species.

Stock solutions of Se species containing about 1 mg Se g^{-1} were prepared in water and kept at 4 °C. The selenium working solutions of Se species selenite (Se(IV)), selenate (Se(VI)), Se–methionine (SeMet), Se–cystine ($SeCys_2$), Se–methylselenocysteine (SeMeSeCys) (Sigma–Aldrich) with lower Se concentration were prepared daily in water at appropriate concentrations by dilution of stock solution.

2.4. HPLC–UV–HG-AFS instrumentation

The separation system consisted of a high pressure pump (Varian Pro Star 210) and a Hamilton PRP X-100 anion exchange column (4.1 mm × 250 mm × 10 μm) and a Hamilton PRP X-200 cation exchange column (4.1 mm × 250 mm × 10 μm). The mobile phase for the anion exchange column was 40 mM $NH_4H_2PO_4$ (pH 6) with a flow rate of 0.5 mL min^{-1} and for the cation exchange column 10 mM pyridine solution (pH 1.5) with a flow rate of 1 mL min^{-1}. The injected volume of sample was 100 μL on both columns. The chromatographic system was connected on-line to a UV-HG-AFS system used for detection (Fig. 1) for which the operating conditions are described in detail elsewhere (Mazej, Falnoga, Veber, & Stibilj, 2005). The mobile phase from the column was mixed with concentrated HCl (flow rate 3 mL min^{-1}) and then passed through UV unit (PS Analytical) with 78 W lamp and 12 m coil of fluorinated ethylene propylene (FEP) around lamp. After UV unit $NaBH_4$ (1.2% m/V in 0.1 mol L^{-1} NaOH) with flow rate 3 mL min^{-1} was added for hydride formation. The purge gas was argon (0.260 L min^{-1}) and dryer gas nitrogen (3 L min^{-1}). A Se boosted discharge lamp (primary current 20 mA, boosted current 25 mA) (Super Lamp, Photron) was applied in AFS detector (Excalibur, PS Analytical).

The separation conditions described enabled the separation of the Se species (Se(IV), Se(VI), SeMet, SeMeSeCys, $SeCys_2$) on the anion and cation exchange columns (Fig. 2). Standards were prepared at concentrations of approximately 100 ng Se g^{-1} for each species, except for SeMet (approximately 400 ng Se g^{-1}). Detection limits were calculated on the basis of 3σ of the blank divided by the slope of the regression graph, and for each species were lower than 2 ng g^{-1} solution, except for SeMet on the anion (5 ng g^{-1} solution) and cation exchange column (13 ng g^{-1} solution).

3. Results and discussion

3.1. Verification of the method for SeMet determination

There are no certified reference materials available for selenium species contents in materials of plant origin. So the accuracy of selenium species determination using the developed method was checked by analysing

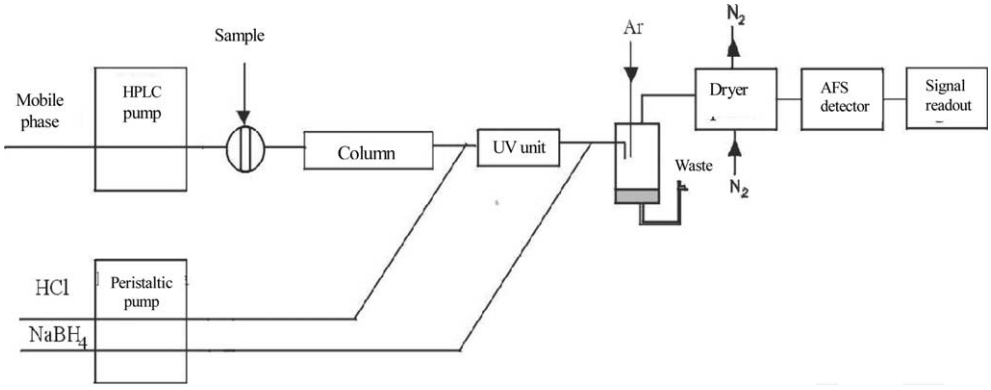

Fig. 1. Schematic diagram of Se analysis by HPLC–UV–HG-AFS.

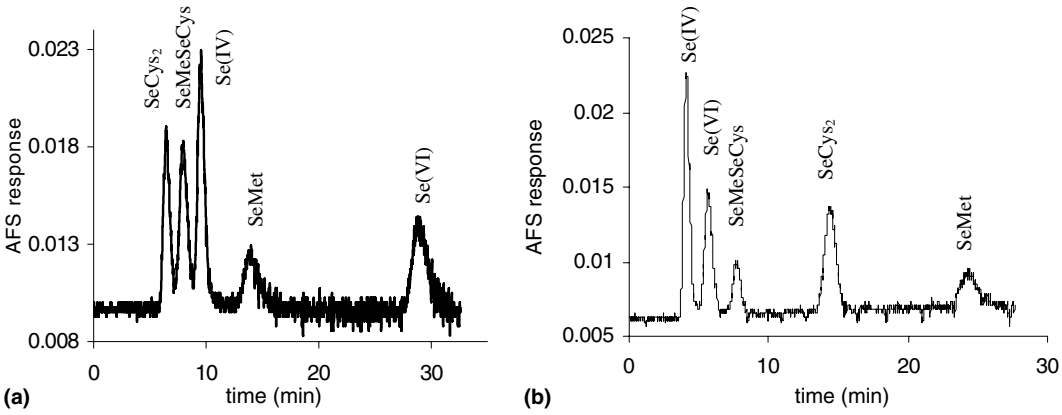

Fig. 2. Separation of Se standards species on anion (a) and cation (b) columns.

the standard reference materials Durum Wheat Flour, NIST RM 8436 and Wheat Gluten, NIST RM 8414 that are certified for the total Se content. Wolf and Goldschmidt (2004) determined SeMet in these reference materials. They used a method based on reaction of cyanogen bromide (CNBr) with SeMet that gives the reaction product methylselenocyanide which is volatile and can be determined by GC–MS. A very good agreement for SeMet content was found between our results and the values reported by Wolf and Goldschmidt (2004). In both materials Se in the form of SeMet represented between 46% and 57% according to the certified value for total Se content (Table 1).

3.2. Se content in buckwheat

Buckwheat is a moderate source of selenium, with average contents between 55 and 154 $ng\,g^{-1}$ of dried sample (Table 2). The results are given with measurement uncertainty, which was estimated as described elsewhere (Smrkolj & Stibilj, 2004). A higher selenium content was found in flowers than in leaves and seeds in Se non-treated plants regardless of UV-B conditions. The selenium content in all parts of the plant was higher in tartary than in the common buckwheat at ambient UV-B radiation (Table 2). Usually, tartary buckwheat grows higher in the mountains than common buckwheat, where UV-B radiation is stronger, and selenium could protect plants from the harmful effects of UV-B radiation (Table 2). We observed that the enhanced UV-B radiation increased the Se content in common non-treated buckwheat flowers.

Buckwheat showed a great ability to accumulate selenium (Table 3). The selenium content in the selenium treated group was between 2.7 and 4.6 $\mu g\,g^{-1}$ in all parts of the plants. In selenium treated buckwheat we can see

Table 1
Selenium content as SeMet in reference materials: comparison of results obtained by HPLC–UV-HG-AFS and the literature data

Sample	Found value ($\mu g\,Se\,g^{-1}$)[a]		Literature data ($\mu g\,Se\,g^{-1}$)[b]	Certified value for the total Se content ($\mu g\,Se\,g^{-1}$)[d]
	SeMet	$SeCys_2$	SeMet	
Durum Wheat Flour, RM 8436	0.57 ± 0.04 (46)[c]	–	0.59 ± 0.04 (48)[c]	1.23 ± 0.09
Wheat Gluten, RM 8414	1.47 ± 0.03 (57)[c]	0.19 ± 0.05	1.21 ± 0.03 (47)[c]	2.58 ± 0.19

[a] Results are given on dry weight; samples were analysed in triplicate.
[b] Wolf and Goldschmidt (2004).
[c] % of Se in the form of SeMet according to the certified value for the total Se content.
[d] Average ± measurement uncertainty.

Table 2
Selenium content in selenium untreated buckwheat samples

Plant part	Selenium content ($ng\,g^{-1}$)[a]			
	Tartary buckwheat		Common buckwheat	
	Ambient UV-B rad.	Enhanced UV-B rad.	Ambient UV-B rad.	Enhanced UV-B rad.
Seeds	113 ± 11	68 ± 10	55 ± 8	60 ± 9
Flowers	154 ± 15	147 ± 15	73 ± 11	132 ± 13
Leaves	88 ± 13	111 ± 11	57 ± 8	66 ± 10

[a] Results are given as average ± measurement uncertainty; samples were analysed at least in duplicate.

Table 3
Selenium content in buckwheat treated by foliar application of selenium

Plant part	Selenium content (ng g^{-1} dry matter)[a]			
	Tartary buckwheat		Common buckwheat	
	Ambient UV-B rad.	Enhanced UV-B rad. UV-B rad.	Ambient UV-B rad.	Enhanced UV-B rad.
Seeds	3219 ± 161	3447 ± 172	2936 ± 147	2710 ± 135
Flowers	3195 ± 159	3869 ± 193	3163 ± 158	3834 ± 191
Leaves	3328 ± 166	3824 ± 191	4649 ± 232	4525 ± 226

[a] Results are given on dry matter basis as average ± measurement uncertainty; samples were analysed at least in duplicate.

that leaves contained slightly more selenium than flowers and seeds.

It is also evident that enhanced UV-B radiation leads to higher selenium accumulation in flowers compared to ambient UV-B radiation conditions. The same tendency was observed when Se enriched pumpkin plants, after foliar application of selenium, were exposed to different light conditions. Higher selenium accumulation was found in pumpkins exposed to ambient radiation, than in the group protected by Mylar foil that does not permit UV-B rays to pass through (Smrkolj, Kreft, Kapolna, & Stibilj, 2005).

There could be a similar connection between radiation and selenium as that known for flavonoids and radiation. It is known that flavonoids, which are also antioxidants, are UV-B absorbing secondary metabolites that are synthesized in plants to protect them from this harmful effect, and tartary buckwheat contains more flavonoids than common buckwheat (Fabjan, 2003). Kreft et al. (2002) studied the influence of different levels of UV-B radiation on rutin content and established that ambient levels of UV-B radiation stimulate rutin accumulation in comparison with a reduced UV-B level.

3.3. Selenium species in buckwheat

3.3.1. Leaves and flowers in common buckwheat

After enzymatic hydrolysis of selenium-enriched buckwheat flowers and leaves we found 11 ± 2%, 13 ± 7% of selenium, respectively, to be present in soluble form. So obviously the main part of Se was insoluble. After separation of these supernatants on the HPLC anion exchange column we found only a Se(VI) peak, which was confirmed on the cation exchange column (Fig. 3). We found less than 1% and a little above 3% of Se in the form of Se(VI) in buckwheat flowers and leaves, respectively. The remaining soluble Se was not detected under the described conditions of the HPLC–UV–HG-AFS method (Table 4). This means that practically all Se(VI), foliarly added to the plant, was transformed to other Se forms in leaves and flowers.

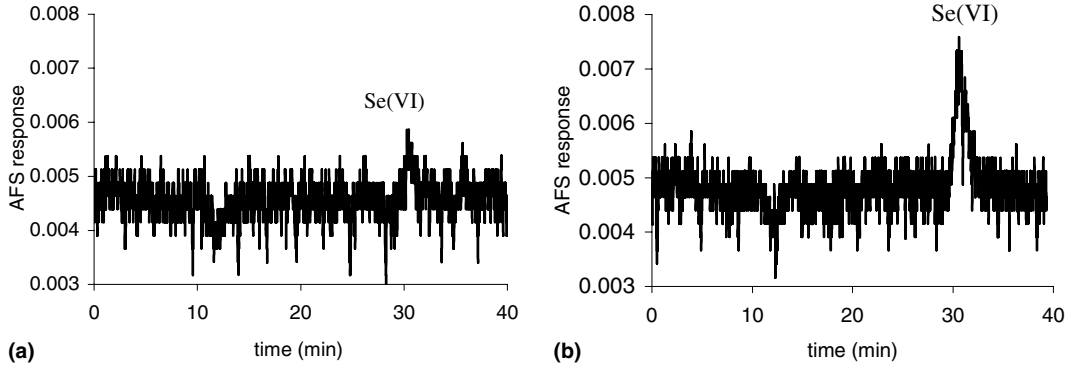

Fig. 3. Selenium species in enzyme hydrolysis extracts of flowers (a) and leaves (b) of foliarly treated buckwheat obtained by anion exchange column.

Table 4
Selenium speciation in enzyme hydrolysis extracts of foliarly treated flowers and leaves of common buckwheat

Conditions	Flowers			Leaves		
	Soluble Se (%)	Se(VI)		Soluble Se (%)	Se(VI)	
		(ng g^{-1})[a]	(% of the Se content)		(ng g^{-1})[a]	(% of the Se content)
Ambient UV-B radiation	12	22	0.7	7	146	3.1
Enhanced UV-B radiation	9	24	0.6	18	55	1.2

[a] ng Se g^{-1} of sample.

Ximenez-Embun et al. (2004) studied selenium uptake and transformation in Indian mustard, sunflower and white lupin grown hydroponically in selenite and selenate media. When selenate was added to the nutrient solution, SeO_4^{2-} was the main species in the extracts after enzymatic hydrolysis in all parts of plant and represented 34–97% according to Se content.

3.3.2. Seeds

In selenium-enriched buckwheat seeds we found 39% of the selenium to be present as water soluble selenium peptides, proteins and other compounds. Free SeMet was found only in traces. After enzymatic hydrolysis we obtained 93% of soluble selenium in common buckwheat seeds (Table 5).

On subjecting the supernatant to anion exchange we obtained a huge SeMet peak, which was also the only peak (Fig. 4). On the cation exchange column we confirmed the presence of SeMet in the samples, and additionally found traces of two other species, but were not able to identify them. For identification of peaks the standard addition method was always used.

We can conclude that in buckwheat seeds the majority of selenium was bound as SeMet in proteins, representing an average of 93 ± 5% of the Se content (Table 5). This means that most of the Se(VI) taken up after foliar application was transformed in seeds to SeMet. No difference in the form of Se species in seeds was found after different UV-B treatments of the plants.

Stadlober et al. (2001) cultivated different cereals in soil, to which fertiliser supplemented with selenate was added. In wheat, barley and rye seeds SeMet was observed as the predominant form of selenium present, also with similar percentages as we found in buckwheat seeds (Table 6). So obviously the majority of Se in cereals seeds is present as SeMet irrespective of cultivation conditions.

Table 5
Selenium in the form of SeMet in treated buckwheat seeds

	Conditions	Se content as SeMet		% of soluble Se after enzyme hydrolysis
		($ng\,g^{-1}$)[a]	(% of the Se content)	
Tartary buckwheat	Ambient UV-B radiation	3211 ± 160	99	Not analysed
	Enhanced UV-B radiation	3309 ± 165	96	Not analysed
Common buckwheat	Ambient UV-B radiation	2576 ± 129	88	93
	Enhanced UV-B radiation	2467 ± 123	91	94

[a] ng $Se\,g^{-1}$ of sample, results are given as average ± measurement uncertainty.

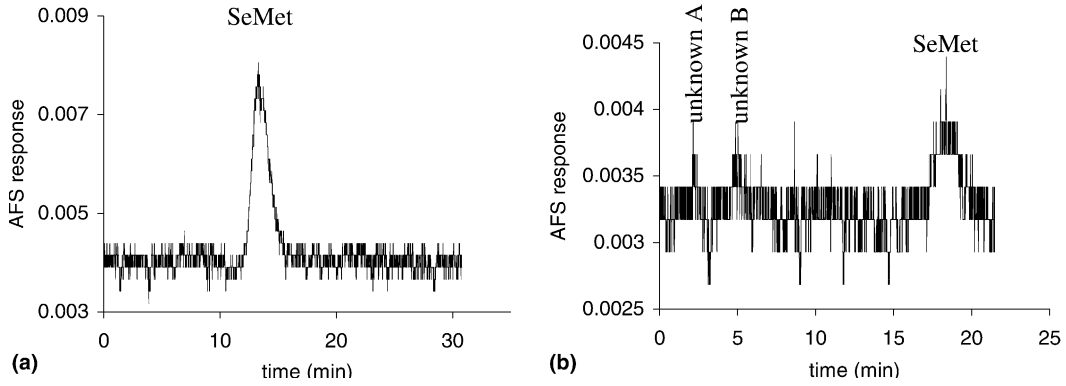

Fig. 4. Selenium species in enzyme hydrolysis extracts of seeds of foliarly treated buckwheat obtained by anion (a) and cation (b) exchange column.

Table 6
Comparison of our results with the literature data for some cereals

Seeds	Type of cultivation and Se enrichment	SeMet content (% of Se species according to the Se content)	Reference
Buckwheat	Foliar application of water solution containing 15 $mg\,L^{-1}$	93	This study
Wheat	Se supplementation in soil using	77	Stadlober et al. (2001)
Barley	mineral fertiliser to reach 30 mg Se per kg soil	70	
Rye		83	

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