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Selenium Species in Selenium-Enriched and Drought-Exposed Potatoes

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The aim of this work was to study selenium (Se) speciation in the potato (*Solanum tuberosum* L.) cultivar Desiree, enriched in Se by foliar spraying with a water solution containing 10 mg of Se/L in the form of sodium selenate. Four combinations of treatments were used: well-watered plants with and without Se foliar spraying and drought-exposed plants with and without Se foliar spraying. Water-soluble Se compounds were extracted from potato tubers by water or enzymatic hydrolysis with the enzyme protease XIV, amylase, or a combination of protease XIV and amylase. Extraction was performed using incubation at a constant temperature and stirring (37 °C at 200 rpm) or by ultrasound-assisted extraction (300 W), using different extraction times. Separation of soluble Se species ($SeCys_2$, SeMet, SeMeSeCys, selenite, and selenate) was achieved by ion-exchange chromatography, and detection was performed by inductively coupled plasma−mass spectrometry (ICP−MS). Results showed that the concentration of selenate extracted was independent of the enzymatic extraction technique (approximately 98 ng/g for drought-exposed and 308 ng/g for well-watered potato tubers), whereas the extraction yield of SeMet changed with the protocol used (10−36%). Selenate and SeMet were the main soluble Se species (representing 51−68% of total Se) in potato tubers, regardless of the growth conditions.

KEYWORDS: Potato; selenium species; HPLC−ICP−MS

INTRODUCTION

Selenium (Se) is one of the minor but biologically essential elements for animals and humans, needed for the activity of antioxidative enzymes, such as glutathione peroxidase and thioredoxin reductase. In addition, it is recognized that selenium-enriched products may provide protection against specific cancers (*1*). Therefore, it is very important that the diet is sufficiently supplied with it to maintain human health in optimum conditions. The recommended daily intakes of selenium for adults published by Germany, Austria, and Switzerland Nutrition Association (DACH) (*2*) are between 30 and 70 μg of Se per day and by the World Health Organization (WHO) (*3*) 50 μg of Se per day. While the Se content in vegetables from many areas of the world is poor, crops and food enriched with Se during cultivation could be an effective way of producing Se-rich foodstuffs. However, the range between its beneficial and toxic concentrations is quite narrow and strongly dependent upon its chemical form (*4, 5*).

The potato is one of the staple foods of modern Western civilization. In central Europe, its consumption is high. The average annual quantity of potatoes purchased in Slovenia per household member in the period 2003−2005 was 22.7 kg (*6*). The Food and Agriculture Organization (FAO) statistical yearbook for the years 2001−2003 reports consumption of 104 kcal per person per day of potato for Slovenia and for the neighboring countries 118 kcal per person per day for Austria and 71 kcal per person per day for Italy (*7*).

The yield of potatoes can be affected by global warming, which causes fluctuations in precipitation distribution and could increase the risk of the potato being exposed repeatedly to drought. Drought stress can affect growth of the potato and lower the yield (*8−10*). Deblonde et al. reported that the number of leaves per plant, stem height, and leaf length were sensitive to moderate water shortage in six potato cultivars (*11*). Also, Jefferies and MacKerron found that exposure to even a short drought period results in a yield decrease, because the development of tubers depends upon carbohydrate supply from the

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foliage and drought-stress results in a reduction in photosynthetic rate (*12*). Moreover, the protein profile of drought-stressed potato tubers is different from the protein profile of tubers from well-watered potato plants (*13, 14*).

Ferri et al. investigated the distribution of selenium in selenium-enriched potato to clarify the role of starch on selenium speciation in this matrix. For that purpose, amyloglucosidase, which promotes starch breakdown to glucose, was used. The results obtained provide evidence that the selenium content in the protein fraction is rather independent of the selenium added to the plants during their growth. On the contrary, the amount of Se in the nonprotein fraction (water and starch fraction) in the Se-enriched sample is significantly higher than in the non-enriched one, suggesting that it is the main selenium-storage site (*15*).

Turakainen et al. examined the effects of Se fertilization on potato-processing quality, possible changes in Se concentration and form in tubers during storage, and retransfer of Se from seed tubers. The next-generation tubers produced by Se-enriched seed tubers had increased Se concentrations, which showed relocation of Se from the seed tubers. In tubers, 49−65% of total Se was allocated to the protein fraction (*16*).

One of the main problems cited by different authors is directly related to the extraction methodology for quantitative recovery of selenium species in plant samples. Different approaches, such as enzymatic and basic hydrolysis, have been proposed, requiring 24 h. These methodologies are time-consuming, and the problem is not only ensuring a satisfactory recovery of Se species ("quantitative recovery" is quite difficult, at least for some matrixes) but obtaining a satisfactory recovery while preventing possible Se species interconversion. On the other hand, in studies focused on elemental speciation, when enzymes and ultrasonication are applied together for a short time, generally less than 4 min, this has been found to be a powerful methodology in recent years (*17*). These problems probably explain why selenium speciation results are hardly comparable when such methodologies were applied by different authors in intercomparison exercises (*18, 19*). For separation and determination of selenium species in plant samples, high-performance liquid chromatography (HPLC) in combination with inductively coupled plasma−mass spectrometry (ICP−MS) (*19*) or ultraviolet−hydride generation−atomic fluorescence spectroscopy (UV−HG−AFS) (*20*) are mainly used.

The purposes of this study were (a) to compare the efficiency for the solid−liquid extraction of Se (total and speciation) from potatoes using different type of enzymes and enzyme combinations. To do so, two approaches were followed, namely, enzymatic incubation at 37 °C and ultrasonic-assisted enzymatic digestion (USAED). In addition, (b) to use the best extraction procedure to study the Se distribution in potatoes cultivated with a supplement of selenium under drought and non-drought conditions.

MATERIALS AND METHODS

Samples. Potato tubers (*Solanum tuberosum* L.), cultivar Desiree, cultivated in Ljubljana, Slovenia, were planted on April 20, 2005, in plastic pots, with an inner volume 18 × 18 × 18 cm, in a mixture of soil (95%) and crushed peat (5%), one plant per pot, five pots per treatment on the experimental field of the Biotechnical Faculty, University of Ljubljana (320 m above sea level, 46° 35′ N, 14° 55′ E), Slovenia. Soil, peat, and irrigation water contained no detectable Se (i.e., soil and peat less than 0.1 mg of Se per kg and water less than 0.5 μg/L). Experiments were performed outdoors under a removable plastic roof, which was automatically positioned in rainy weather.

Four combinations of treatments were performed: well-watered plants (W+) with and without Se foliar spraying and drought-exposed plants (W−) with and without Se foliar spraying. Plants emerged on May 2. All plants were well-watered until June 20. On June 20, they were sprayed foliarly with a solution of detergent (Triton X-100, Sigma, 0.2 mg/L) without or with Se (10 mg of Se/L in the form of sodium selenate). Plants were watered daily with an amount of water corresponding to 4 L/m^2 rainfall (well-watered plants) or 1.5 L/m^2 (drought-exposed plants) until harvest on August 15 (*21*).

Reagents and Standards. The following chemicals were used: 96% H_2SO_4 (Merck, Suprapur), 65% HNO_3 (Merck, Suprapur), 30% HCl (Merck, Suprapur), 36% HCl (Merck, p.a.), 30% H_2O_2 (Merck, p.a.), V_2O_5 (Merck, p.a.), NaOH (Merck, puriss p.a.), $NaBH_4$ (Fluka, Purum p.a.), $(NH_4)_2HPO_4$ (Fluka Chemie, puriss p.a.), pyridine (Fluka Chemie, puriss p.a.), diammoniumhydrogen citrate (Fluka Chemie, puriss p.a.), citric acid (Fluka Chemie, puriss p.a.), MeOH (Primar, Fisher Scientific U.K., trace analysis grade), protease XIV from *Streptomyces griseus* (type XIV: bacterial, 4.4 units/mg of solid; Sigma-Aldrich), β-amylase from barley (type II-B: crude, 17.6 units/mg of solid; Sigma-Aldrich), β-amylase from sweet potato (type I-B, 827 units/mg of solid; Sigma-Aldrich), and α-amylase from porcine pancreas (type VI-B, 19.6 units/mg of solid; Sigma-Aldrich). For preparation of Se solutions, Na_2SeO_3 (Se^{IV}, Sigma-Aldrich, >98%), Na_2SeO_4 (Se^{VI}, Sigma-Aldrich, SigmaUltra), selenomethionine (SeMet, Fluka Chemie, >99%), selenocystine ($SeCys_2$, Fluka Chemie, >98%), and selenomethylselenocysteine (SeMeSeCys, Fluka Chemie, >98%) were used. Stock solutions of Se species containing about 1 mg of Se/g in water were prepared and kept at 4 °C. For preparation of solutions and sample treatment, ultrapure water (Milli Q, Millipore Corporation, Bedford, MA) was used.

Procedures. *Sample Preparation.* For analysis, potato tubers were lyophilized at −50 °C and 0.050 mbar (CHRIST ALPHA 1-4, LOC-1, freeze-dryer), milled, and homogenized in a planetary micro mill (FRITSCH, Pulverisette 7, Idar-Oberstein, Germany; 2600 rpm, time of 6 min).

Extraction of Se Species. For selenium speciation, samples were prepared as follows: 60 mg of each enzyme was separately mixed with 600 mg of lyophilized potato tubers and made up to 8 mL with water. Samples containing no enzyme or 60 mg of amylase (α-amylase or β-amylase) plus 60 mg of protease XIV were also prepared. All of the samples were stirred at 200 rpm at 37 °C for different times (2, 4, 6, 12, and 24 h) (SW 22, Julabo). As an alternative to stirring, an ultrasound probe (Cole-Parmer, 300 W) was used at various sonication amplitudes (50, 70, and 95%) and sonication times (1 and 3 min). After extraction, each sample was centrifuged at 11 000 rpm for 60 min at 4 °C (5804R, Eppendorf). The supernatant was filtered through 0.45 and 0.22 μm Millex GV filters (Millipore Corporation) and used for selenium speciation analysis by HPLC−ICP−MS. Supernatants and sediments were stored at −20 °C until analysis for total Se by HG−AFS was carried out.

Determination of Se. Potato Tubers. Total Se was determined in homogenized freeze-dried samples, composed of tubers of two plants from each combination, using HG−AFS as described in more detail by Smrkolj et al. (*22*). Mineralization was carried out on 0.2 g of sample. This was weighed in a Teflon tube, and mineralization was performed using concentrated HNO_3 (1.5 mL) and concentrated H_2SO_4 (0.5 mL) by heating the closed tube in an aluminum block, kept at 80 °C overnight and then for 1 h at 130 °C. After cooling, 2 mL of hydrogen peroxide was added and the tubes were heated for 15 min at 115 °C. This step was repeated. After the solution had cooled to room temperature, 0.1 mL of V_2O_5 in H_2SO_4 was added and the tube was reheated at 115 °C until the solution became blue in color. To reduce selenate to selenite, 2.5 mL of concentrated HCl was added to the solution and heated at 100 °C for 10 min. Samples were diluted with Milli Q water. Sensitive detection was achieved by HG−AFS with the chemical and instrumental operating conditions according to Smrkolj et al. (*22*). Working standard solutions of Se^{IV} were prepared daily by dilution of a stock standard solution with a solution containing appropriate amounts of H_2SO_4 and HCl to obtain the same acid media as in the samples. The accuracy and precision of the method were tested by using the reference material Durum Wheat Flour (NIST 8436), and good agreement was obtained in 12 replicates between the certified (1.23 ± 0.09 mg/kg) and obtained (1.10 ± 0.11 mg/kg) results.

Table 1. Optimal HPLC−ICP−MS Operating Conditions for Se Species Determination

parameter	value
	HPLC
anion-exchange chromatography	
Hamilton PRP-X 100 column	4.1 mm × 250 mm × 10 μm
mobile phase A	3 mM citrate buffer in 2% MeOH (pH 4.8)
mobile phase B	10 mM citrate buffer in 2% MeOH (pH 4.8)
gradient	14 min gradient from 100% A to 50% A, 1 min gradient to 100% B, isocratic to 25 min, gradient for 2 min to 100% A, isocratic to 32 min
flow rate (mL/min)	0.5
injected volume (μL)	50
cation-exchange chromatography	
Zorbax 300-SCX column	4.6 mm × 250 mm × 5 μm
mobile phase	3 mM pyridine solution in 2% MeOH (pH 2.1)
flow rate (mL/min)	0.5
injected volume (μL)	50
	ICP−MS
instrument	Agilent 7500ce, Tokyo, Japan
nebulizer	Micro Mist
plasma	
RF power (W)	1500
outer gas flow rate (L/min)	15.0
carrier gas flow rate (L/min)	0.80
makeup gas flow rate (L/min)	0.17
octopole reaction cell	
H_2 gas flow rate (mL/min)	4.0
measuring parameters	
m/*z* monitored	^{77}Se, ^{78}Se
integration time (s)	0.3

Sediments and Supernatants. Selenium in the sediment was determined by the same procedure as that for total selenium in tubers described above. The Se content in the supernatant was determined by digestion in HNO_3. To 0.5 g of supernatant 1 mL of concentrated HNO_3 was added and heated for 30 min at 80 °C and then for 15 min at 160 °C. A total of 0.5 mL of H_2O_2 was added 3 times, and the solution evaporated to 0.5−0.6 g of sample. A total of 0.5 mL of concentrated HCl was added for reduction of Se^{VI} to Se^{IV}, which was determined by HG−AFS. Working standard solutions of Se^{IV} were prepared daily by dilution of a stock standard with 0.5 M HCl (*22*).

Separation and Detection of Se Species. *HPLC−ICP−MS.* For Se species determination, a Hamilton PRP-X 100 anion-exchange column (4.1 mm × 250 mm × 10 μm) and a Zorbax 300-SCX cation-exchange column (4.6 mm × 250 mm × 5 μm) were used. Anion-exchange chromatography was used for Se^{IV}, Se^{VI}, SeMeSeCys, and SeMet determination, and cation-exchange chromatography was used for SeMet, SeMeSeCys, and $SeCys_2$ determination. Citrate buffer was selected as the mobile phase for anion-exchange chromatography, and pyridine solution was used as an eluent for cation-exchange chromatography. To increase the sensitivity of the selenium signal, methanol was added to the mobile phase. The flow rate was 0.5 mL/min, and the volume of the sample injected was 50 μL.

The chromatographic system consisted of a high-performance liquid chromatography pump, Series 1100, from Agilent (Waldbronn, Germany), equipped with a Rheodyne (Cotati, CA) Model 7725i injector using a 50 μL loop. The outlet of the column was directly connected to the concentric nebulizer and the Scott-type spray chamber of the ICP−MS (Agilent 7500ce, Tokyo, Japan). Treatment of data was performed with Agilent ChemStation software. Data processing was based on the peak area. HPLC−ICP−MS operating conditions for determination of Se species are listed in **Table 1**.

The separation conditions described enabled separation of the Se species (Se^{IV}, Se^{VI}, SeMet, SeMeSeCys, and $SeCys_2$) using both columns. Standards were prepared at concentrations of approximately 100 ng of Se/g for each species in supernatants of the control group of potatoes, to check for the different retention times of Se species caused by matrix interactions in the system of measurement. Moreover, the Se species in the supernatant were confirmed by the standard addition method.

Table 2. Optimal HPLC−UV−HG−AFS Operating Conditions for Se Species Determination

parameter	value
	HPLC
anion-exchange chromatography	
Hamilton PRP-X 100 column	4.1 mm × 250 mm × 10 μm
mobile phase	40 mM $(NH_4)_2HPO_4$ (pH 6.0)
flow rate (mL/min)	0.5
injected volume (μL)	100
cation-exchange chromatography	
Zorbax 300-SCX column	4.6 mm × 250 mm × 5 μm
mobile phase	3 mM pyridine solution (pH 2.4)
flow rate (mL/min)	0.5
injected volume (μL)	100
	UV−HG−AFS
carrier flow rate (mL/min)	1
Ar flow rate (L/min)	0.26
N_2 flow rate (L/min)	3
reduction	concentrated HCl/UV
reductive solution	1.4% (w/v) $NaBH_4$, dissolved in 0.4% (w/v) NaOH solution
primary and boosted current (mA)	20 and 25

HPLC−UV−HG−AFS. To check the accuracy of the HPLC−ICP−MS method, Se species in the supernatants were also determined by HPLC−UV−HG−AFS. The separation system consisted of a high pressure pump (Varian ProStar 210), a Rheodyne 7725i injector, and ion (anion, Hamilton PRP X 100; cation, Zorbax SCX-300) exchange columns. The chromatographic system was connected to a UV−HG−AFS system used for online detection, for which the operating conditions are shown in **Table 2** and described in detail elsewhere (*20*). The standards were prepared in the same way as for ICP−MS detection.

RESULTS AND DISCUSSION

Optimization of Chromatographic Separation of Se Species. Because of the ionic properties of selenoamino acids and inorganic forms over a wide pH range, ion-exchange chromatography was used for their separation. Both anionic and cationic exchange columns were used. For anion-exchange chromatography, a citrate buffer was selected as the mobile phase (*23*). The concentration and pH of the citrate buffer were optimized. Optimization of the pH of citrate buffer was made in the range of 3.0−5.0, and the concentration of citrate was between 3 and 10 mM. The best separation of five Se species was obtained using gradient elution with a 3 mM citrate buffer (A) and 10 mM citrate buffer (B) at pH 4.8. Because ionization of Se in the plasma is low, we used methanol (1, 2, and 3%) and acetone (0.5, 1, 1.5, and 2%) to obtain a better ionization efficiency (*24*). The best results were obtained using 2% MeOH in both mobile phases, A and B (**Figure 1a**).

Because $SeCys_2$ was eluted in the void volume of the anion-exchange column, separation on the cation-exchange column was also used. For the cation-exchange column, a pyridine solution was selected as the mobile phase. The concentration and pH of the pyridine solution and organic solvent addition (MeOH and acetone) were optimized. Optimization of the pH of pyridine was made in the range of 2.1−2.4, and the concentration of pyridine solution was between 2 and 7 mM. The best separation of Se species was obtained using isocratic elution with 3 mM pyridine in 2% MeOH at pH 2.1 (**Figure 1b**).

Column Recovery. Column recovery was tested on both columns. Peak areas obtained for separated Se species (100 ng of Se/g) after column separation were compared to peak areas obtained without a column. Ratios between peak areas were expressed as column recovery, taking peak areas obtained without a column as 100% intensity. Each species was analyzed

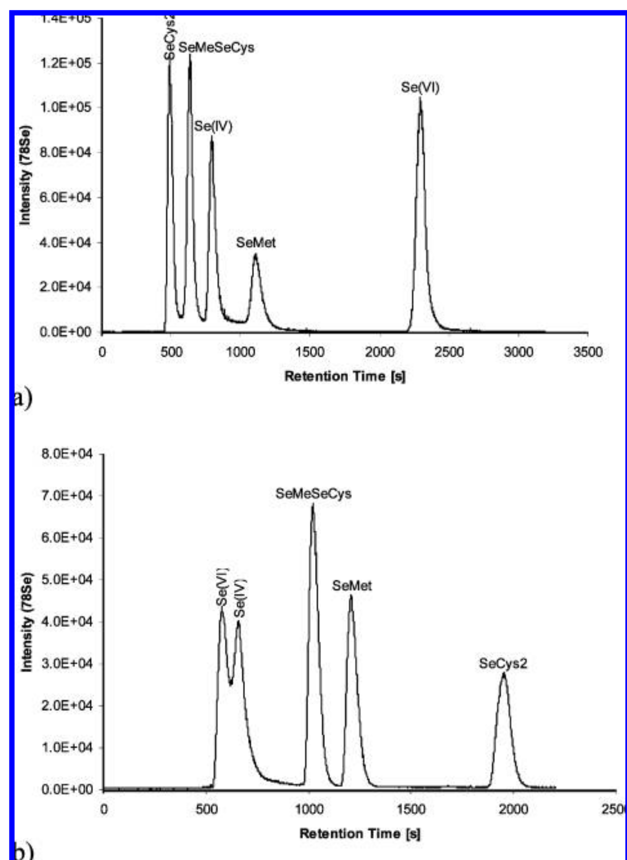

Figure 1. Chromatogram of a mixture of Se species with mass fractions of Se around 100 ng/g solution each on (a) anionic and (b) cationic exchange columns.

Table 3. Column Recovery Obtained by Ion-Exchange Chromatography in Conjunction with ICP−MS

Se species	column recovery	
	anionic exchange (%)	cationic exchange (%)
Se^{VI}	93.5 ± 1.2	86.6 ± 1.1
Se^{IV}	81.2 ± 0.4	80.9 ± 1.9
SeMet	63.6 ± 0.3	102.5 ± 1.3
SeMeSeCys	62.5 ± 0.9	95.3 ± 0.7
$SeCys_2$	85.2 ± 0.6	91.6 ± 1.0

in triplicate, and results obtained are shown in **Table 3**. Mazej et al. (*20*) studied column recovery on an anion-exchange column coupled to a UV−HG−AFS system. They took peak areas for Se^{IV} obtained after column separation as 100% intensity. All data were normalized to this value. Results reported for Se^{IV}, Se^{VI}, and SeMet were approximately 20% lower than the ones reported in this study.

Extraction of Se Species from Se-Enriched Potato. The total Se content was found to be 16 ± 10 ng/g in drought-exposed and 117 ± 4 ng/g in well-watered potato tubers, 347 ± 17 ng/g in drought-exposed Se-enriched potato, and 1101 ± 46 ng/g in well-watered Se-treated potato. The results are expressed on a dry matter basis. The water content was on average 85% for drought-exposed and 87% for well-watered potato tubers. Results for total selenium content in control and Se-enriched samples show that the selenium content in well-watered potato foliarly treated with Se^{VI} solution was higher than in those exposed to drought.

Extraction efficiency depends upon the nature of the sample and the extraction conditions. However, 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 modes/procedures of extraction were used for Se species from potato tubers using (a) water or (b) enzyme protease XIV: incubation at constant temperature and stirring (37 °C at 200 rpm) using different times of shaking (2−24 h) or an ultrasound probe (300 W) using different extraction times (1 or 3 min) and amplitude of sonication (50, 70, and 95%, 300 W).

Table 4. Concentration and Efficiency of Soluble Se Obtained for Potato Samples after Different Extraction Procedures (Incubation and Ultrasound-Assisted Extraction) without and with Enzyme Protease XIV[a]

extraction procedure	soluble Se (ng/g)	percent of soluble Se according to the total Se
incubation for 2 h (W+; protease XIV)	804.0	73.0
incubation for 24 h (W+; protease XIV)	778.0	70.7
incubation for 2 h (W−; protease XIV)	277.1	79.9
incubation for 24 h (W−; protease XIV)	292.0	84.1
ultrasound probe for 1 min, 50% (W−; protease XIV)	268.1	77.3
ultrasound probe for 1 min, 50% (W−; water)	245.1	70.6
ultrasound probe for 3 min, 50% (W−; protease XIV)	276.0	79.5
ultrasound probe for 3 min, 50% (W−; water)	236.3	68.1

[a] W−, potatoes grown in drought; W+, well-watered potato.

The results showed that the time of incubation had almost no influence on the extraction efficiency of Se from potato samples (differences were less than 4%). We presumed that ultrasound would fragment plant cells and other structures and Se would be released more efficiently from cells and their components than by incubation. The differences between the extraction efficiency of soluble Se obtained for potato samples after different extraction procedures (incubation and ultrasound-assisted extraction) were below 10% and lower in the case of ultrasound-assisted extraction. However, comparable results were observed using ultrasound assisted using various amplitudes of sonication (50, 70, and 95%, 300 W) and times of extraction (1 or 3 min). The most important results are presented in **Table 4**. Because of these results, we decided to use 24 h incubation on 37 °C and also to obtain comparable results to literature data.

In potato, enzyme hydrolysis with amylase was used to clarify the role of starch in selenium speciation. Several amylases of different origin and classification were used. α-Amylase acts at random locations along the starch chain (glucose unit) and therefore tends to be faster acting with a higher efficiency than *β*-amylase, which works from the nonreducing end, catalyzing the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. To hydrolyze peptide bonds in proteins, protease XIV was used (**Table 5**). The extraction efficiency, after 24 h incubation, using different enzymes was between 59 and 91%, the lowest when using only α-amylase (62% for W− and 59% for W+) and the highest when using protease XIV and *β*-amylase from sweet potato together (91% for W− and 77% for W+), although little difference was obtained when using protease XIV in combination with α-amylase or *β*-amylase or alone. The extraction efficiency was calculated in comparison to the total concentration of Se in the sample. A little higher extraction efficiency was found in the case of drought-exposed potato. In water extracts, we found about 20% lower results (69% for W− and 44% for W+); therefore, obviously that part of Se was

Table 5. Se Content in Supernatant and Sediment, Extraction Efficiency, and Se^{VI} and SeMet Content Obtained after 24 h Incubation of Se-Enriched Potato Using Different Types of Enzyme[a]

extraction	growing condition	ng of Se/g of sample		extraction efficiency (%)	Se^{VI} (% Se^{VI} according to total Se) ng/g of sample	SeMet[b] (% SeMet according to total Se) ng/g of sample
		supernatant	sediment			
water	W−	241 ± 20	128 ± 17	69	89 ± 4 (26)	<LOD
	W+	488 ± 42	473 ± 55	44	321 ± 15(29)	<LOD
protease XIV	W−	292 ± 28	87 ± 9	84	80 ± 4 (23)	124 ± 5 (36)
	W+	768 ± 58	202 ± 20	70	288 ± 13 (26)	313 ± 15 (28)
β-amylase from barley	W−	275 ± 21	130 ± 14	79	87 ± 5 (25)	73 ± 4 (21)
	W+	737 ± 40	293 ± 22	67	313 ± 15 (28)	113 ± 5 (10)
protease XIV plus β-amylase from barley	W−	273 ± 20	139 ± 14	79	100 ± 5 (29)	115 ± 5 (33)
	W+	710 ± 40	317 ± 30	64	321 ± 16 (29)	351 ± 14 (32)
β-amylase from sweet potato	W−	255 ± 20	150 ± 10	73	98 ± 5 (28)	44 ± 4 (13)
	W+	680 ± 20	524 ± 50	62	286 ± 14 (26)	<LOD
protease XIV plus β-amylase from sweet potato	W−	315 ± 11	119 ± 12	91	103 ± 5 (30)	100 ± 5 (29)
	W+	848 ± 30	248 ± 11	77	278 ± 14 (25)	284 ± 15 (26)
α-amylase	W−	227 ± 15	139 ± 11	62	110 ± 6 (32)	34 ± 4 (10)
	W+	694 ± 40	474 ± 47	59	325 ± 15 (30)	<LOD
protease XIV plus α-amylase	W−	316 ± 30	100 ± 7	76	115 ± 6 (33)	122 ± 6 (35)
	W+	932 ± 24	261 ± 22	78	332 ± 15 (30)	285 ± 14 (26)

[a] Results are given as the average of three measurements ± standard deviation. W−, potatoes grown in drought (347 ng/g, on dry matter basis); W+, well-watered potato (1101 ng/g, on dry matter basis); LOD, limit of detection ($LOD_{Se^{VI}}$ = 1 ng/g sample; LOD_{SeMet} = 10 ng/g sample). [b] Se as SeMet.

incorporated into protein and starch structures. The mass balance for drought-exposed and well-watered plants was in good agreement with the total Se content.

Montes-Bayon et al. studied several sample extraction techniques to obtain the highest Se extraction efficiency from the green part of two types of selenium-enriched plants, garlic (*Allium sativum*) and Indian mustard (*Brassica juncea*). The action of ultrasound (3 min, 80%) allowed for reduction of the extraction time while maintaining Se recoveries between 75 and 120% (*19*). In our study, the content of soluble Se was around 70%, regarding the total Se in the samples.

Se Species in Se-Enriched Potato. After separation of Se species on the HPLC anion-exchange column, we found Se^{VI} and SeMet, which were confirmed on the cation-exchange column (**Figure 2**). In chromatograms obtained for potato supernatants (**Figure 2**), some Se species at trace level were observed and were not identified.

We can see (**Table 5**) that concentrations of Se^{VI} are independent of the extraction used. In drought-exposed potato, the values are around 98 ng/g, and in water-treated samples, the values are around 308 ng/g. If we look at SeMet concentrations, we can see that protease XIV released around 30% of SeMet according to the total Se content of the sample, regardless of the growing conditions. Also, amylase released some SeMet, but the values were lower. When both enzymes were combined, we expected around 40% of SeMet to be released, but the results obtained were similar to those using only protease XIV (30%). Therefore, protease XIV was chosen as the optimal enzyme for extraction of selenium from potato tubers. Inorganic selenium (selenate) applied as a foliar spray was converted in the plant to selenomethionine. On average, 30% of the Se content was in the form of SeMet, regardless of the growing conditions (drought or watered potato) and the enzyme used for extraction (**Table 5**). While only about 80% of soluble Se was determined, column recovery was tested in the considered matrix. First, supernatant was injected through the column and, second, without the column. The ratio obtained was approximately 80%; therefore, about 20% of the soluble selenium remained on the column.

As already mentioned, we compared two extraction procedures. Speciation analyses of supernatants obtained after 2 and 24 h of incubation and those obtained with ultrasound-assisted extraction (3 min, 50%) using protease XIV as an enzyme were made, but no differences in Se species present were observed.

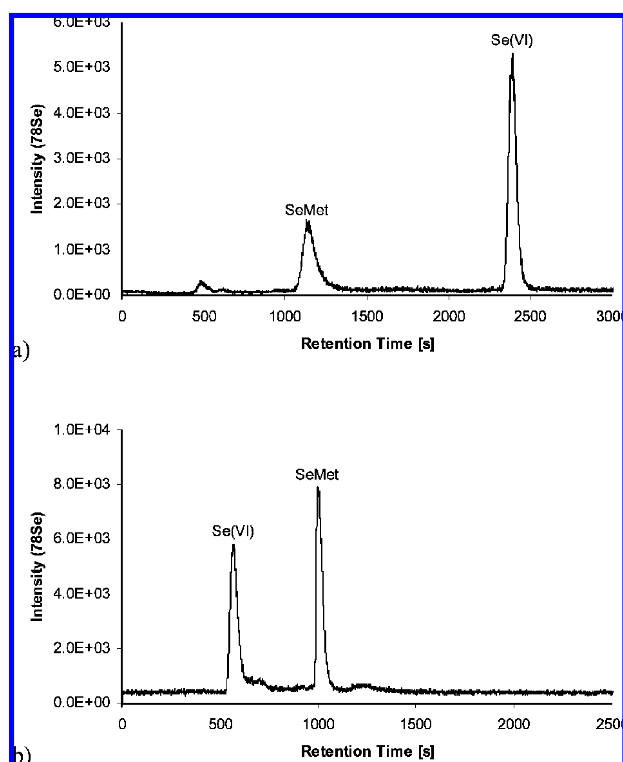

Figure 2. Se species from a potato sample foliarly treated with selenate solution and exposed to drought obtained after extraction with protease XIV using HPLC−ICP−MS on (a) anion- and (b) cation-exchange columns.

The same observations were made by Montes-Bayon et al. (*19*). They found no differences in the chromatographic profiles in speciation analysis of the green part of two types of selenium-enriched plants (garlic and Indian mustard) in extracts obtained by ultrasound or incubation (20 h, 37 °C). On the other hand, Mazej obtained different results when using incubation (24 h, 37 °C) or an ultrasound probe (1 min, 70%) for leaves of three plant species (lamb's lettuce,

parsley, and chicory) and comparable results for samples of yeast and dandelion leaves (*25*).

Because there are no certified reference materials available for the concentration of selenium species in materials of plant origin, the accuracy of selenium species determination using the method developed (HPLC–ICP–MS) was checked by HPLC–UV–HG–AFS. For this purpose, supernatants obtained after extraction with (a) protease XIV and (b) a combination of protease XIV and β-amylase from sweet potato were analyzed. Results for selenate and SeMet in supernatants obtained by both techniques were within 10%.

Literature data on Se species in potato are very scarce, and therefore, a comparison to our results is very difficult. Ferri et al. reported that, in potatoes, selenium is mainly stored in the nonprotein fraction, probably as inorganic Se, even if organic forms cannot be excluded (*15*). Our results showed that the sum of the different forms of Se species represents about 80% of the soluble selenium, regardless of which type of amylase in combination with protease XIV was used. Approximately half of it is present in the protein fraction (as SeMet), and the other half is in the nonprotein fraction (as selenate).

Turakainen et al. reported that 49–65% of total Se was present in the protein fraction in potato tubers (*Solanum tuberosum* L. cv. Satu) (*16*). In our study, the same Se form (selenate) was used to obtain selenium-enriched potato (cv. Darja), but only about 30% of the total Se in tubers was found to be present in the protein fraction. This difference could be due to the different potato cultivar used.

To conclude, no differences were observed using an ultrasound probe or incubation for the extraction of potato tubers. The extraction efficiency was the highest when using protease XIV and amylase (α-amylase or β-amylase) together, although little difference was obtained when using protease XIV in combination with amylase or alone. Well-watered potatoes foliarly sprayed with Se^{VI} solution took up more Se than drought-exposed ones. The soluble Se species detected in Se-enriched potato were SeMet and Se^{VI}, and about 30% of Se in the tuber was present as SeMet, regardless of the growing conditions. The accuracy of determination of Se species by ICP–MS was checked, and the agreement between the results obtained for Se^{VI} and SeMet by HPLC–ICP–MS and HPLC–UV–HG–AFS was good.

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