National Food Institute

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SE-ENRICHMENT OF CARROT AND ONION VIA FOLIAR APPLICATION

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INTRODUCTION

The fact that selenium (Se) is a nutritionally essential element is well established. This element however, can also be toxic in larger doses [1,2]. Both the beneficial and the toxic effects of selenium are based on concentration ingested and on its chemical forms [3]. Selenium content of food varies significantly among different world regions, depending on the selenium content of the soil. Northern European countries belong to low-selenium regions, especially Scandinavia [4].

Enrichment of agricultural crops by adding Se fertilisers could be an expensive process due to high immobilisation of Se in the soil. As an alternative, a new enrichment strategy, namely the foliar application was introduced [5]. Using this method the Se containing solution is taken up to the surface of the target plant's leaves by spraying.

The aim of this work was to study the selenium accumulation in carrot and onion plants using foliar application by sodium selenite and sodium selenate. Furthermore, we aimed at identifying the Se species biosynthesised by onion and carrot plants. The results were used to prepare for production of ^{77}Se enriched plants for an ongoing human absorption study.

MATERIALS AND METHODS

Samples

A preliminary study was performed in a growth chamber to estimate the Se concentration and its species appropriate to produce the most favourable organic Se species in plants. The ^{77}Se enrichment of both plant materials was planned on the basis of this study.

Pilot study

In the pilot study both the cultivation of the carrot (*Daucus carota)* and onion (*Allium cepa*) plants and their foliar application of Se were carried out in a growth chamber under controlled temperature and humidity. Samples were cultivated from seeds in soil.

Plants were sprayed either with the solution of Na_2SeO_3 or Na_2SeO_4 at the concentration of either 10 µg Se mL^{-1} or 100 µg Se mL^{-1} once per week during a month. At every treatment 8mL Se solution was sprayed onto each sample group aiming at even distribution on the plants' leaves. Besides the treated plants, control samples were also grown under the same conditions and were separated to avoid cross contamination.

Figure 1 *Preliminary study in a growth chamber*

Plant cultivation and their enrichment with ^{77}Se

^{77}Se enrichment of the plants was carried out in a green-house under controlled temperature and humidity. Using the isotopically enriched solution where Se was in the form of sodium-selenite. Plants' leaves were sprayed at 50 µg ^{77}Se/mL two times per week for three weeks.

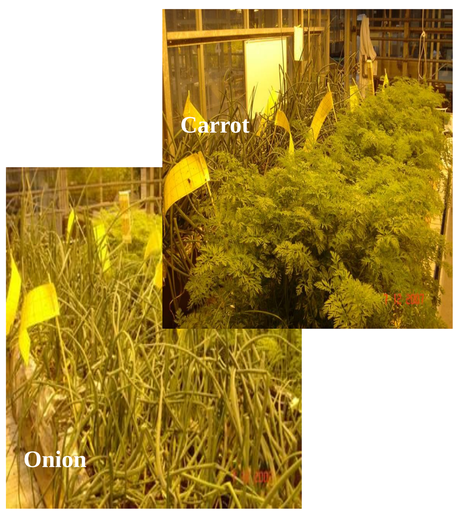

Figure 2 *Set-up of the green-house experiment*

Sample preparation

Sample preparation for total selenium

For total selenium determination, complete digestion of the plant samples was performed with a microwave digestion system in high-pressure quartz vessels. Approximately 0.25g sample (dry mass) was disgested using 2mL HNO_3.

Extraction of selenium for chromatographic speciation studies

Proteolytic sample preparation was applied to extract both the protein bound Se forms and the species present in the intracellular space of the plant sample [6].

Instrumentation

An Agilent 7500ce ICP-MS equipped with an octopole reaction cell was applied all over the study, utilizing hydrogen at optimized flow rate of 3.5 mL min^{-1}, monitoring ^{77}Se and ^{80}Se isotopes. A newly developed anion exchange chromatographic separation was applied for the study of selenium species along with a cation exchange separation for quality control purposes. The instrumental operating conditions are given in Table 1.

	LC-ICP-MS		LC- ESIMS/MS
Chromatography	Anion exchange	Cation exchange	Cation exchange
Column type	ION-120, Transgenomic (120 x 4.6 mm x 5 µm)	Chrompack IonoSpher 5C (100 x 3.0 mm x 5 µm)	Chrompack IonoSpher 5C (150x2.0mm 5 µm)
Flow rate (mL min^{-1})	1.0	1.0	0.25
Injection volume (µL)	20	20	25
Column heating (°C)	-	30	20
Mobile phase	(A): 0.1mM salicylate, 3% (v/v) MeOH, pH 8.5; (B): 20mM salicylate, 3% (v/v) MeOH, pH 8.5	(A): 0.75mM pyridinium formate, 3% (v/v) MeOH, pH 3.0; (B): 10mM pyridinium formate, 3% (v/v) MeOH, pH 3.0	(A): 1.5mM ammonium formate 5% (v/v) MeOH, pH 3.0 (B): 25mM ammonium formate 5% (v/v) MeOH, pH 3.3
Gradient program	0-4 min:98% A-2%B 4.1–12 min:50% A-50% B 12.1–16 min:98% A-2% B	0–3.5 min:100% A 3.6–5 min:95%A-5% B 5.1–7 min:80% A 20% B 7.1–16 min:100% A	0–20min:100% A 20–25min:100% A-100% B 25–30min:100% B 30–32.5 min: 100% B-100% A 32.5–45min:100%A

Table 1 *Chromatographic separations applied*

For Se species verification LC-ESI-MS/MS experiments were conducted on a Quattro Micro mass spectrometer equipped with an ESI ion source in the positive mode. The MRM transitions *m/z* 312.7 → (166.7 and 129.7), and *m/z* 197.7 → (180.7 and 151.7) were used for identification of γ-glu-MeSeCys and SeMet, respectively. Additional MRM transitions were used for the ^{77}Se-counterparts; m/z 309.7 → (163.7 and 129.7), m/z 180.3 → (119.7 and 163.7), and m/z 194.7 → (177.7 and 148.7) were used for identification of γ-Glu-Me^{77}SeCys, Me^{77}SeCys and ^{77}SeMet, respectively. Instrumental details are given in Table 2.

Auxiliary gas	N_2, 475 Lmin^{-1}
Nebulizer gas	N_2, 50 Lmin^{-1}
Collision gas	Ar_2, 8 psi
Needle voltage	3150 V
Cone temperature	120°C
Nebulizer temperature	400°C
Cone voltage	12V
Collision energy	15V

Table 2 *Instrumental operating conditions for the HPLC- ESIMS/MS set-up*

RESULTS AND DISCUSSION

The total Se content of the Se-enriched plant carrot roots and onion bulbs are presented in Table 3. However it should be noted that leaves in the case of both plant species contained much higher amount of Se than the corresponding bulbs or roots.

Treatment	Se-species applied	Plant	Total Se concentration (µg g^{-1})
10 µg Se mL^{-1}	Selenate	Carrot	0.5 ± 0.1^{a}
		Onion	2.1 ± 0.1^{b}
	Selenite	Carrot	0.4 ± 0.1^{a}
		Onion	1.1 ± 0.1^{c}
100 µg Se mL^{-1}	Selenate	Carrot	2.2 ± 0.1$^{d, b}$
		Onion	9.1 ± 0.1^{f}
	Selenite	Carrot	1.5 ± 0.1^{e}
		Onion	4.3 ± 0.1^{g}

As Table 3 shows, total Se concentrations obtained in the plant samples are strongly dependent on the Se concentration used for enrichment. Se originating from sodium selenate enrichment causes higher accumulation of Se in both plants. An upper tolerable concentration of Se was reached as enrichment with selenate at 100 µg Se mL^{-1} caused visible damages to the plant leaves.

Table 3 *Total Se content of selenised samples (average ± SD, n=3) applying 10 and 100 µg Se mL^{-1} concentration for enrichment.*

Data are presented for dry weight. Signal detected on isotope ^{80}Se was used for quantification. Values with different superscripts are significantly different (P<0.05)

After having determined the total Se content of the samples, proteolytic digestion was applied to extract the Se species and the extracts were analysed with both cation and anion exchange chromatographic systems.

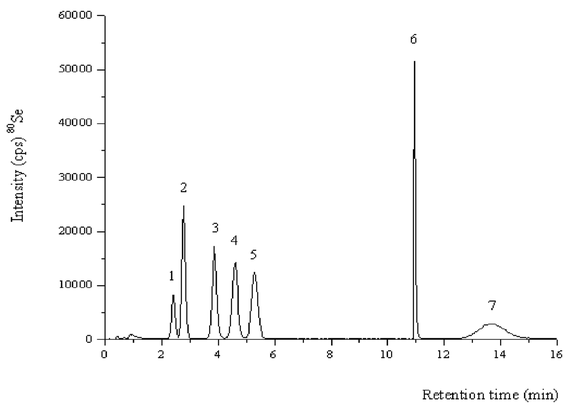

Figure 3 *Separation of a mixture of selenium standards containing 50 ng Se mL^{-1} of each species with cation exchange chromatography. Peak: 1=γ-glu-MeSeCys; 2=MeSeCys; 3=AllSeCys; 4=PrSeCys; 5=SeMet; 6=SeOMet; 7=TMSe^{+}*

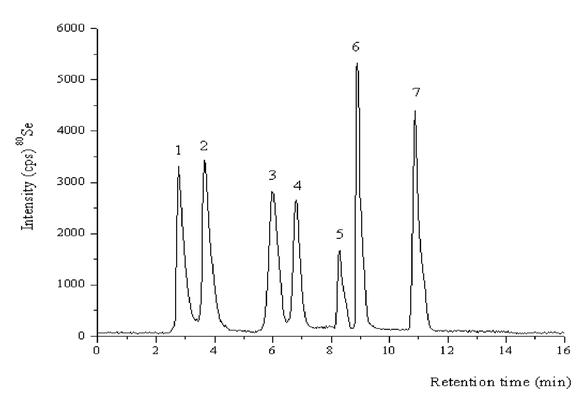

Figure 4 *Separation of a mixture of selenium standards containing 50 ng Se mL^{-1} of each species with anion-exchange chromatography. SeHoCys$_2$ is present in 100ppb concentration. Peak: 1=SeMet; 2=MeSeCys; 3=AllSeCys; 4=SeHoCys$_2$; 5=γ-glu-MeSeCys; 6=Se(IV); 7=Se(VI)*

Proteolytic digests of the samples were analysed with both chromatographic techniques. Quantitative data are only shown from the 100 µg Se mL^{-1} treatment, as identical species were detected but at lower concentration in the samples enriched with 10 µg Se mL^{-1} medium (see ***Figure 5/A*** & ***5/B***).

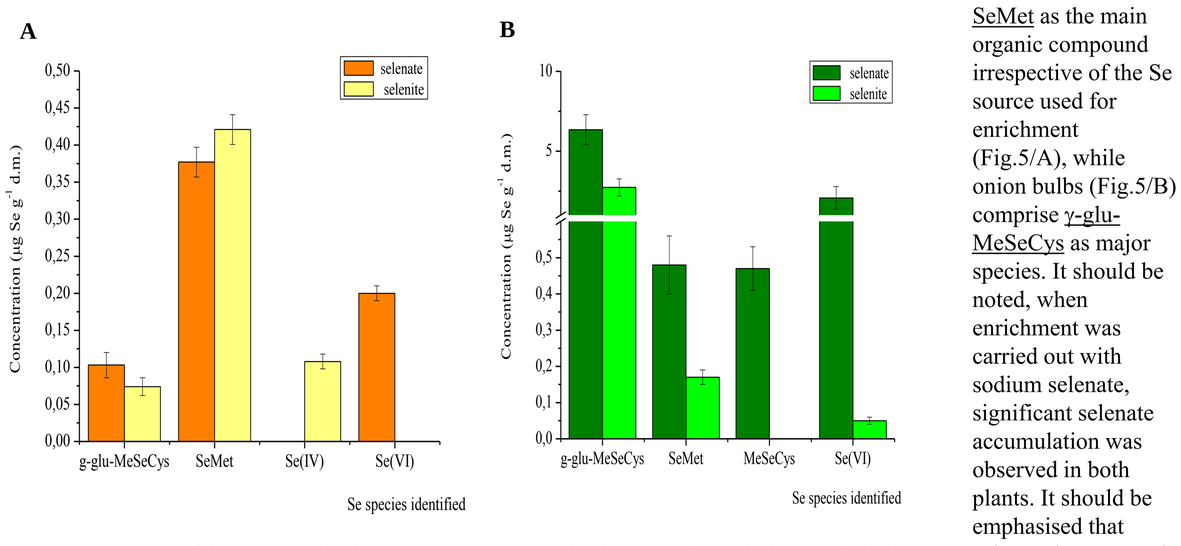

Figure 5 *Comparison of the Se species identified in the carrot roots **(A)** and in the onion bulbs **(B)** for foliar applied selenate or selenite*

Carrot roots contain SeMet as the main organic compound irrespective of the Se source used for enrichment (Fig.5/A), while onion bulbs (Fig.5/B) comprise γ-glu-MeSeCys as major species. It should be noted, when enrichment was carried out with sodium selenate, significant selenate accumulation was observed in both plants. It should be emphasised that selenate is more toxic for plants than other forms of Se [7].

Interestingly, SeMet was found in both plants' leaves at significant concentrations, namely 4 µg Se mL^{-1} and 10 µg Se mL^{-1} (d.m.) in the onion and carrot leaves, respectively.

From the pilot study, it could be concluded that lower than 100 µg Se mL^{-1} concentration should be used for the stable isotope (^{77}Se) enrichment of the two plants, in the form of sodium-selenite which is less harmful to the plants.

ug ^{77}Se/g – for dry weight	Onion bulb	Onion leaf	Carrot root	Carrot leaf
average	6,7	6,2	2,8	24,7
SD	0,4	0,6	0,06	0,3

Table 4 *Total Se content of ^{77}Se-enriched samples (average ± SD, n=3). Data are presented for dry weight and expressed in µg g^{-1}.*

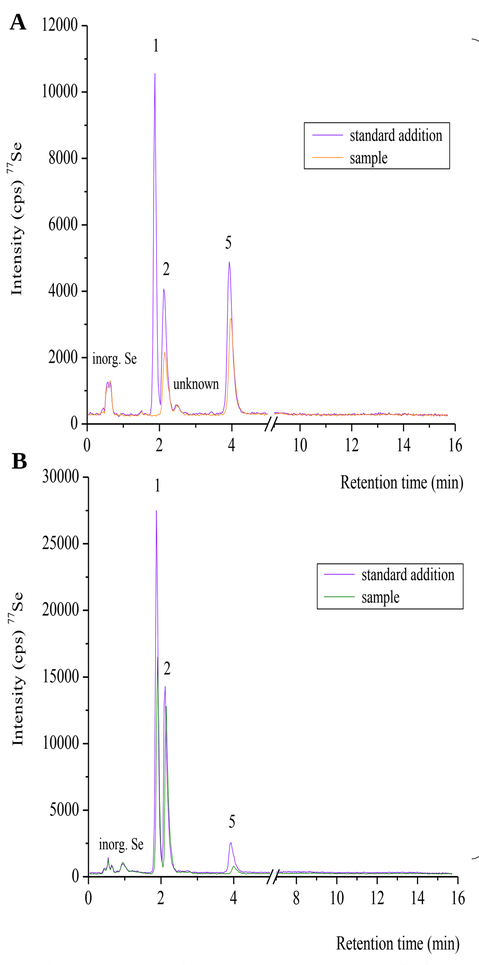

Figure 4 *Cation exchange separation of ^{77}Se enriched carrot root (A) and onion bulb (B). Peak: 1=γ-glu-MeSeCys; 2=MeSeCys; 5=SeMet*

Main species identified were MeSeCys and SeMet in carrot, while in onion γ-glu-MeSeCys, MeSeCys and also SeMet prevailed. Verification of these ^{77}Se labelled species was attempted using LC-ESI-MS/MS.

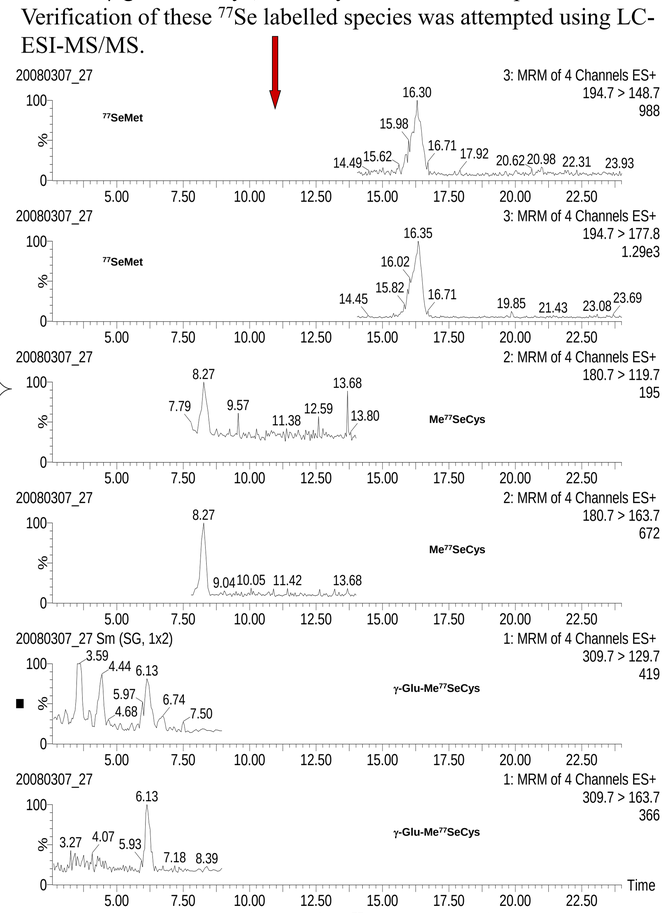

Figure 5 *Verification of the ^{77}Se species identified by HPLC-ICP-MS speciation using LC-ESI-MS/MS*

CONCLUSIONS

- A newly developed anion-exchange HPLC separation method allowed separation of seven Se compounds including amino acids and oxo-anions.
- Nutritionally favourable organic Se species in onions and carrots obtained with sodium selenite at 50 µg Se/mL *via* foliar application used in human study

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