JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Fertilizing Soil with Selenium Fertilizers: Impact on Concentration, Speciation, and Bioaccessibility of Selenium in Leek (*Allium ampeloprasum*)

Rama V. Srikanth Lavu,[†] Gijs Du Laing,*[,†] Tom Van De Wiele,[§] Varalakshmi L. Pratti,[†,§] Koen Willekens,[‡] Bart Vandecasteele,[‡] and Filip Tack[†]

[†]Laboratory of Analytical Chemistry and Applied Ecochemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

[‡]Plant Sciences Unit, Research Area Crop Husbandry and Environment, Institute for Agricultural and Fisheries Research (ILVO), Burg. van Gansberghelaan 109, B-9820 Merelbeke, Belgium

[§]Laboratory of Microbial Ecology and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

ABSTRACT: Leek was fertilized with sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) in a green house to assess the impact of selenium (Se) fertilization on Se uptake by the crop and its speciation in the crop. The bioaccessibility of Se in the Se-enriched leek was assessed using an in vitro extraction protocol mimicking the human gastrointestinal tract (stomach, small intestine, and colon). The lowest Se uptake was observed when Na_2SeO_3 was used as a fertilizer, which results in a higher risk for Se accumulation in the soil on a longer term. When soil was amended with Na_2SeO_4, 55 ± 5% of total Se in the leek occurred in an inorganic form, while only 21 ± 8% was inorganic when Na_2SeO_3 was applied. Se-methylselenocysteine and selenomethione were the major organic species in both treatments. However, concentrations of Se-methylselenocysteine and γ-glutamyl-Se-methyl-selenocysteine, which were previously reported to induce positive health effects, were lower as compared to other *Allium* species. The majority of the Se in the leek was found to be bioaccessible in the stomach (around 60%) and small intestine (around 80%). However, a significant fraction also has good chances to reach the colon, where it seems to be taken up by the microbial community and may also induce positive health effects.

KEYWORDS: *biofortification, bioavailability, leek, selenium speciation, selenate, selenite*

INTRODUCTION

Selenium (Se) is an essential trace element for humans and animals. It can occur in different inorganic and organic forms (= species), which may differ in mobility, availability, and toxicity.[1] The most important source of Se is the diet. Selenium uptake depends on the eating habits of an individual. Vegetables were found to provide more than 85% of the average daily human dietary Se intake when the vegetables were grown on Se-abundant soils (e.g., in the United States). However, a smaller proportion of Se is supplied through food crops when the crops are grown on soils poor in Se (e.g., in Europe).[2−4] Selenium supplementation is often needed due to lack of Se in soils and the crops grown on these soils, resulting in Se deficiency in humans and animals. In this context, the production and consumption of biofortified food crops such as Se-fortified *Brassica* (broccoli, rapeseed, and cabbage) and *Allium* (onion, garlic, chives, and ramps) species were previously suggested. These crops were found to be capable of accumulating higher amounts of Se during cultivation and transforming Se into appropriate chemical forms that may have positive effects on human health. The bioavailability of Se in such fortified food crops is determined by its bioaccessibility, which depends on the speciation as well as the food matrix itself.[5] The bioaccessible fraction of a compound has been defined as the fraction that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (i.e., enters the blood stream).

Vegetables such as *Brassica* and *Allium* species contain specific Se forms such as Se-methylselenocysteine and γ-glutamyl-Se-methylselenocysteine, which are known to be more effective inhibitors of tumor formation.[6,7] The formation of such Se species and Se uptake by crops depend on the form and concentration of Se available in the soil, as well as soil conditions.[8,9] Several studies described that *Allium* species are capable of transforming inorganic forms of Se applied to the soil into organic forms.[10−12] Among *Allium* species, mainly chives, onions, and garlic were studied. Not much attention was given to leek (*Allium ampeloprasum* var. *porrum*) yet, even though it is from an economic viewpoint one of the most important vegetable crops in Europe.[13] Leek is a good source of vitamin A, vitamin B6, vitamin C, vitamin K, dietary fiber, folate, calcium, iron, and magnesium with low saturated fat, sodium, and cholesterol. It is intensively cultivated in Indonesia, Turkey, France, Belgium, China, and Poland. Moreover, daily use of leek in the diet has been shown to have beneficial effects on the body, particularly on the circulatory system.[14]

Received: July 6, 2012
Revised: October 10, 2012
Accepted: October 18, 2012
Published: October 18, 2012

In the present study, the effect of type and dose of Se fertilizer on Se accumulation and speciation in leek was investigated. Therefore, leek plants were grown on soil fertilized with Na_2SeO_3 and Na_2SeO_4. The plants were harvested, and their Se contents and speciation were determined. Moreover, bioaccessibility of Se in the Se-enriched leek was assessed using an in vitro extraction protocol mimicking various stages of human digestion (stomach, small intestine, and colon).

MATERIALS AND METHODS

Cultivation and Preparation of Se-Enriched Leek Samples. For the cultivation of the Se-enriched leek, commercially available Leek (*A. ampeloprasum* var. *porrum*) plantlets of Harston variety were purchased. Recipients were filled with 25 kg of soil. The soil used for all treatments was a sandy loam soil from the upper 30 cm of an agricultural field, with pH KCl 6.15, 1.09% organic carbon, and 0.23 mg Se kg^{-1}. The required amount of Se was added to each soil, and the soil was completely homogenized in a rotating mixer. In each recipient, eight plantlets were planted in two rows and watered with deionized water. The treatments consisted of applying two sources of selenium, that is, Na_2SeO_3 and Na_2SeO_4, at four levels of selenium, that is, 0.2, 1.3, 2.6, and 3.8 mg Se kg^{-1} soil. The experiment was replicated four times. To prevent pests, pyrethra pur (Ecostyle, Belgium) was sprayed twice over a period of 3 months. After 3 months, plants were harvested and washed gently with tap water to remove surface contaminants. Washing with tap water was followed by rinsing with deionized water. The weight of each plant was recorded, and the entire plant was manually cut into pieces. The samples were shock-frozen immediately with liquid nitrogen after transferring them into polyethylene boxes. They were stored at −80 °C. Finally, they were freeze-dried by a lyophilizer (Heto PowerDry, Belgium), ground to a fine powder in a mechanical grinder (MF 10 IKA, Werke Germany), and passed through a 1 mm sieve.

Reagents and Standards. Sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), selenomethionine (SeMet), selenocystine ($SeCys_2$), and Se-methylselenocysteine (MeSeCys) were purchased from Sigma Aldrich (St. Louis, MO). γ-Glutamyl-Se-methyl-selenocysteine (γ-glut-cyst) and γ-glutamyl-Se-methylselenomethionine (γ-glut-Met) were purchased from pharmaSe (Austin, TX). For chromatographic purposes, citric acid was obtained from Sigma Aldrich, heptafluorobutryic acid was obtained from Fluka, and ammonium hydroxide was obtained from J. T. Baker (Deventer, The Netherlands). For sample preparation, protease XIV was purchased from Sigma Aldrich, while concentrated suprapure HNO_3 (65%) and H_2O_2 (37%) were purchased from Chemlab (Zedelgem, Belgium). MilliQ (MQ) water from Water Systems Ltd. (Brussels, Belgium) was used throughout the experiment. Chromatographic standards and other solutions were prepared freshly every day. To prepare gastric and small intestinal fluids, pepsin and pancreatin (porcine pancreas) were purchased from Sigma Aldrich, and dehydrated galpowder (Difco TM Oxgall) and sodium bicarbonate were from VWR (Leuven, Belgium).

Instrumentation. An inductively coupled plasma mass spectrometer (ICP-MS, PerkinElmer DRC-e, Sunnyvale, CA) was used for total Se and speciation analysis as an element-specific detector. The ICP-MS was fitted with a Babington nebulizer and a cyclonic spray chamber. For speciation analysis, the ICP-MS was coupled as detector to a liquid chromatographic system (Series 200 HPLC, PerkinElmer). It consisted of a P680 HPLC pump and an ASI-100 automated sample injector. A Hamilton PRP-X100 anion exchange column and Altima C_8 reversed phase column (250 mm × 4.6 mm i.d., 5 μm, 120 Å) were used as the stationary phase. Both columns were equipped with a guard column containing the same stationary phase material. The extracts were analyzed using the anion exchange column. However, the reversed phase column was also used to confirm the absence of the oxidized form of SeMet (SeMet oxide). HPLC-ICP-MS conditions are presented in Table 1. Extraction of Se for speciation analysis and batch incubations for bioaccessibility assessment were carried out using a shaker fitted in an incubator chamber from Sartorius (Goettingen, Germany). The samples were centrifuged on a Sigma 2-16PK centrifuge (Germany). For total Se determination, a microwave digestion apparatus from Mars (NC) was used.

Table 1. Optimized Instrumental Parameters for ICP-MS

ICP-MS Parameters	
power	1250 W
plasma Ar flow	15 L min^{-1}
isotopes monitored (mass)	76, 77, 78, 80, 82
reaction gas and flow rate	CH_4, 0.9 mL min^{-1}
dwell time for each isotope	0.1 s
Chromatographic Conditions	
anion exchange (isocratic elution)	
column	PRP-X100 (250 mm × 4.6 mm, 5 μm)
mobile phase	10 mM citric acid, 5% (v/v) methanol, pH 5.0
flow rate	1.0 mL min^{-1}
injection volume	25 μL
reversed phase (isocratic elution)	
column	Alltech Altima C8 (250 mm × 4.6 mm, 5 μm)
mobile phase	0.15% (v/v) heptaflurobutyric acid, 5% (v/v), methanol
flow rate	1.0 mL min^{-1}
injection volume	25 μL

Sample Preparation for Total Se Determination in Leek. For the determination of total Se in the leek samples, 0.2 g of sample was placed into a centrifuge tube followed by addition of 2.5 mL of concentrated HNO_3 and 2.5 mL of 30% H_2O_2. After 16 h, the tubes were capped and placed in a microwave oven.[15] In a first step, the temperature was raised to 55 °C for 10 min at 600 W and 100% power. Afterward, the temperature was raised to 75 °C for 10 min. Finally, it was maintained at 100 °C for 30 min. The clear digests were diluted to 50 mL with deionized water for ICP-MS measurement. Both standard addition and external calibration were used during ICP-MS measurement. For validation of the procedure, the certified reference plant material BCR-CRM 402 (white clover, 6.7 ± 0.27 mg Se kg^{-1}) was digested using the same procedure. The extraction recovery was found to be 97.2 ± 4.2%. Three replicates of BCR-CRM 402 were analyzed with each sample batch for quality control of total Se determination.

Sample Preparation Procedure for Speciation Analysis in Leek. For Se speciation, plant samples obtained by fertilizing with Na_2SeO_3 and Na_2SeO_4 at the dose of 3.8 mg Se kg^{-1} soil were used. A 0.2 g plant sample and 80 mg of the enzyme Protease XIV were dissolved in 5 mL of water. This mixture was shaken in a 10 mL centrifuge tube for 24 h at 37 °C and centrifuged for 30 min at 3000*g*.[16] The supernatant was separated from the residue and filtered through a 0.45 μm syringe type PVDF membrane filter. The supernatant and residue were stored at −20 °C until they were analyzed for total Se and Se speciation using (HPLC-) ICP-MS (Table 1).

Sample Preparation for Bioaccessibility Assessment. Leek grown on soil fertilized with Na_2SeO_3 and Na_2SeO_4 at a dose of 0.2 mg Se kg^{-1} soil was used for assessment of Se bioaccessibility. Two grams of powdered leek sample was boiled with 10 mL of deionized water for 3 min to mimic the food preparation process applied prior to human consumption. After it was cooled, 3 g of the obtained suspension was transferred into 100 mL amber-colored bottles. Thirty milliliters of simulated gastric juice (10 g L^{-1} pepsin adjusted to pH 2.0 with 2 M HCL) was added to the bottles, and they were capped with a rubber stopper and aluminum seal. They were placed on a mechanical shaker (80 rpm) in an incubator (37 °C) for 1 h. After 1 h, 5 mL was sampled with a syringe. This sample is considered to represent the gastric phase. Afterward, 12.5 mL of small intestine fluid was added, and the mixture was shaken in the incubator again for 2 h. The small intestine fluid was prepared by weighing 0.75 g of dehydrated bile powder, 0.5 g of pancreatin, and 1.5 g of sodium bicarbonate into 100 mL of deionized water. After 2 h, 5 mL of suspension, representing the small intestine, was sampled. Sub-

sequently, 30 mL of colon suspension, sampled from the SHIME (simulator of the human intestinal microbial ecosystem), was added to mimic colon conditions. The bottles were capped and flushed with nitrogen gas to create anaerobic conditions. They were again shaken in the incubator and sampled after 0 (T0), 2 (T2), 24 (T24), and 48 h (T48). The samples collected in each step were placed in 10 mL polypropylene tubes and centrifuged at 10000*g* for 10 min. The supernatant collected after centrifugation was filtered (0.45 μm) and stored at −80 °C. The collected filtrates and pellets (residues) were analyzed for total Se using ICP-MS. For digestion of the pellet prior to analysis, microwave digestion with concentrated HNO_3 and H_2O_2 was used. The entire procedure was repeated using pure Na_2SeO_4 spiked into the gastric solution to a concentration of 2.4 mg L^{-1} instead of the leek sample. All experiments were conducted in triplicate. The relative bioaccessibility of Se in leek and pure Se standard (Na_2SeO_4) was calculated for each digestion phase as

$$\%\ \text{bioaccessibility} = \frac{\text{Se in collected supernatant}}{\text{total Se in each suspension}} \times 100$$

RESULTS AND DISCUSSION

Total Se in the Leek. The plants differed in total Se concentrations when they were exposed to Na_2SeO_3 and Na_2SeO_4 in the treated soil (Table 2). The capability to accumulate Se was very high when the plants were grown on soil fertilized with Na_2SeO_4, with Se concentrations ranging from 102 ± 24 for the lowest Se application dose to 982 ± 159 mg Se kg^{-1} for the highest dose (Table 2). When the plants were grown on soil treated with Na_2SeO_3, only 104 ± 33 mg Se kg^{-1} accumulated in the plants even at the highest applied dose (3.8 mg Se kg^{-1}). Thus, the application of Na_2SeO_3 results in the lowest Se accumulation (Table 2). Among the vegetables, *Allium* species drew particular attention due to their potential for Se accumulation and transformation into bioactive species.[10−12] The present study confirmed also for leek that fertilization of soil with Se results in Se accumulation by the plant. However, the extent of accumulation significantly varied with the applied dose and Se form used in the fertilization, with the use of Na_2SeO_4 as fertilizer resulting in a 10-fold higher concentration in comparison to the use of Na_2SeO_3. Similar differences in Se accumulation when applying selenate versus selenite were previously also reported in literature for other *Allium* species, such as chives.[17] These differences seemed to be less pronounced when working in a hydroponic environment, which emphasizes the role of soil in retaining selenite.[12] In general, selenite is less bioavailable in comparison to selenate for plants grown on soil because the former is more strongly absorbed by iron oxides and/or hydroxides while the latter is more water soluble.[18] Moreover, the fact that total Se uptake was higher when the soil was treated with selenate may be attributed to the fact that selenate follows sulfur transporter pathways during uptake from the soil by the plant,[19] whereas selenite uptake does not depend on metabolic processes.[20]

Table 2. Effect of Se Fertilizer Type and Dose on Se Concentration (mg Se kg^{-1}) in Leek Plants (n = 3)

	fertilizer type	
Se application dose	Na_2SeO_3	Na_2SeO_4
0.2 mg Se kg^{-1} soil	24.1 ± 5.2	102 ± 24
1.3 mg Se kg^{-1} soil	49.7 ± 63.0	313 ± 29
2.6 mg Se kg^{-1} soil	71.2 ± 12.7	582 ± 72
3.8 mg Se kg^{-1} soil	103 ± 33	982 ± 159

Se Speciation in Se-Enriched Leek Grown on Two Different Se Forms. The recovery of total Se measured in the protease extracts was found to be 87.8 ± 5.4% of total Se found in the HNO_3/H_2O_2 digests, while the sum of identified species was found to be around 85% of total Se measured in the protease extracts. In the plants grown on soils treated with 3.8 mg Se kg^{-1} as Na_2SeO_4, approximately 53.6 ± 8.0% was found to be selenate, and the most abundant organic species was SeMet (12.3 ± 0.8%), followed by MeSeCys (4.3 ± 1.6%). When the soil was treated with 3.8 mg Se kg^{-1} as Na_2SeO_3, the main observed Se species in the plants were selenate (21.6 ± 7.9%), SeMet (16.7 ± 14.1%), and MeSeCys (6.8 ± 0.2%) (Table 3). The prevalence of inorganic species in leek is similar to its prevalence in chives, which was reported to vary between 21% (when applying Na_2SeO_3) and 51% (when applying Na_2SeO_4) of total recovered Se, using the same extraction method. The chromatograms of enzymatic extracts of plants treated with Na_2SeO_3 and Na_2SeO_4 have two or three unknown peaks, the largest one representing approximately 2−3% of the total Se in the leek (Figure 1). A similar number of unknown peaks was observed when enzymatic extracts of leek were analyzed using an anion exchange column or a reversed phase

Table 3. Selenium Species Concentrations in Leek Plants Grown on Soil Fertilized with 3.8 mg Se kg^{-1} as Na_2SeO_3 and Na_2SeO_4[a]

	fertilized with Na_2SeO_3		fertilized with Na_2SeO_4	
	Se (μg/g)	%[a]	Se (μg/g)	%[a]
SeCys	1.1 ± 0.6	1.4 ± 0.4	6.2 ± 2.4	0.8 ± 0.1
MeSeCys	5.6 ± 2.2	6.8 ± 0.2	35.2 ± 23	4.3 ± 1.6
SeMet	17.3 ± 7.0	16.7 ± 14.1	101.9 ± 40	12.3 ± 0.9
γ-glut-cyst	ND	ND	5.3 ± 3.0	0.7 ± 0.1
selenate	26.5 ± 4.5	21.6 ± 7.9	466 ± 186	55.4 ± 5.0
selenite	ND	ND	5.2 ± 0.6	0.9 ± 0.3

[a]Results are expressed as mean value ± standard deviation (n = 3). ND, nondetectable. [a]Recovery expressed as a percentage of the total selenium concentration.

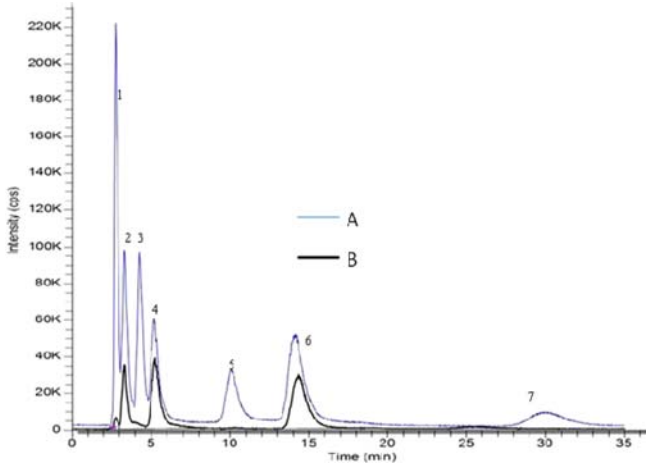

Figure 1. Overlay of chromatograms on an anion exchange column for (A) a standard solution containing seven Se species: (1) Se-cystine, (2) Se-methylselenocysteine, (3) selenite, (4) Se-methionine, (5) γ-glutamyl-methylselenocysteine, (6) selenate, and (7) γ-glutamyl-Se-methionine. (B) An enzymatic (protease) extract of Se-enriched leek fertilized by Na_2SeO_3.

Table 4. Prevalence of Selenium Species in Se-Enriched *Allium* Species[29a]

plant	Se addition	total Se[b] (μg/g)	selenium species (%)[c]					
			Se(IV)	Se(IV)	SeMet	$SeCys_2$	MeSeCys	γ-Glu-MeSeCys
garlic (*Allium sativum*)[12,27,28]	Na_2SeO_4 + myccorhiza (50 mg kg^{-1}, 4 weeks)		enzymatic/water extraction					
		969	–	–/9[d]	2/1[d]	–	3/5[d]	64/62[d]
			enzymatic/water extraction					
		296	–	2[d]	13[d]	0.5[d]	3[d]	73[d]/85[d]
	$BaSeO_{3+}$ $BaSeO_4$ (500 mg m^{-3} of each, 8 months)		water extraction					
		96	–	–	15.5[e]	6.0[d]	28.8[e]	49.7[e]
onion (*Allium cepa*) [10]			$HClO_4$–ethanol extraction					
	Na_2SeO_3	154	–	–	0.3	0.5[f]	4.0	–
	Na_2SeO_4 (15 mg kg^{-1}, 8 days)	601	–	–	0.2	0.1[f]	1.9	–
green onion (*Allium fistulosum*) [30]			enzymatic extraction/HCl hydrolysis					
	Na_2SeO_3 (15 mg kg^{-1}, 4 months)	30.3	±	–/–	±	±	±	±
ramp (*Allium tricocum*) [31]	Na_2SeO_4 (30 mg L^{-1})	252	–	42	–	–	35	1.4
shallot (*Allium ascalonicum*) [32]			water extraction					
	$BaSeO_{3+}$ $BaSeO_4$ (500 mg m^{-3} of each, 8 months)	226.8	–	28	–	–	5.4	66
			enzymatic extraction					
garlic [33]		68	–	1	18	0.5	2.5	68
		235	–	1.5	17	0.5	3	70
		1355	–	4	13	–	60	8
ramp		48	–	1	21	–	34	3
		524	–	22	5	–	44	1.5
onion		96	–	10	5	1	1	63
		140	–	33	10	–	5	35
chives (*Allium schoenpprasum*) [21]			$HClO_4$–ethanol extraction/enzymatic extraction					
	Na_2SeO_3	222	–/3	21/5	-/5	40/42	28/36	–
	Na_2SeO_4	613	–/–	81/51	–/–	5/2	3/20	–
	SeMet (10 mg L^{-1}, 14 days)	265	–/1	5/–	–/3	35/37	46/48	–

[a] +, detected but not quantified. [b] Based on dry weight. [c] Relative to total Se in the sample. [d] Relative to total chromatographed selenium. [e] Relative to total Se in the extract. [f] Se-cysteine.

column. In both treatments, selenate, SeMet, and MeSeCys were the dominant species (Table 3). Interestingly, the latter species was previously reported to exhibit potential anticarcinogenic properties.[10] Among *Allium* species reported until now, higher Se concentrations were previously reported for garlic, but these higher concentrations were also reached at higher soil Se concentrations.[12] The highest absolute amount of inorganic species was observed in plants treated with Na_2SeO_4. A similar speciation pattern was observed for the plants fertilized with 0.5 mg Se kg^{-1} (data not shown). Under these conditions, only little transformation to organic species occurred, which was previously also reported for various other food crops. Applying selenite increases the prevalence of organic Se species, which was in coherance with the observations for other crops.[21] However, this results in lower plant Se uptake and a higher risk for Se accumulation in the soil on longer term. Selenite concentrations were found to be below detection limits also in plants grown on Na_2SeO_3-treated soils. A higher prevalence of selenite was previously reported for garlic grown on a selenate-enriched medium and chives grown on a selenite-enriched medium.[17] Se-methylselenocysteine and SeMet were the major organic species in both treatments, and MeSeCys was found in slightly higher concentrations when the soils were treated with Na_2SeO_3. In other *Allium* species, MeSeCys or γ-glut-cyst were the dominant organic species, while in leek SeMet was found to be the dominant organic species (Table 4). If MeSeCys and γ-glut-cyst are considered to exhibit anticarcinogenic properties, other *Allium* species may be more suited if beneficial health effects are targeted. Although total Se accumulation in the plants is higher when Na_2SeO_4 is supplied as fertilizer, this fertilizer results in a lower transformation to organic species. When increasing the prevalence of organic Se species in leek is targeted, Na_2SeO_3 seems to be the best fertilizer. However, the lower plant uptake of Se when using Na_2SeO_3 as a fertilizer results in a higher risk for Se accumulation in the soil on longer term. Therefore, further research is needed to assess the factors affecting plant uptake and fate of selenite in the soil.

Bioaccessibility of Selenium. In the gastric phase, the bioaccessibility of Se was slightly higher when the leek was grown on selenite-enriched soil (63%), as compared to selenate-enriched soil (56%) (Figure 2), although this difference is not significant. In this phase, the bioaccessibility of pure Na_2SeO_4 was around 95%. In the small intestine, the bioaccessibility of leek grown on selenite- and selenate-enriched soil increased to 81 and 78%, respectively, whereas the bioaccessibility of the pure Na_2SeO_4 remained constant around 95%. The higher bioaccessibility in the small intestine as compared to the gastric phase should be attributed to the role of intestinal enzymes in releasing Se from the food matrix. Although our study illustrates that differences in bioaccessibility between different types of Se-enriched leek are not significant, it has previously been proven that the way Se is incorporated in different types of Se-enriched foodstuffs affects its bioaccessibility in the stomach and small intestine.[22] For example, bioaccessibility of Se in yeast-based food supplements was

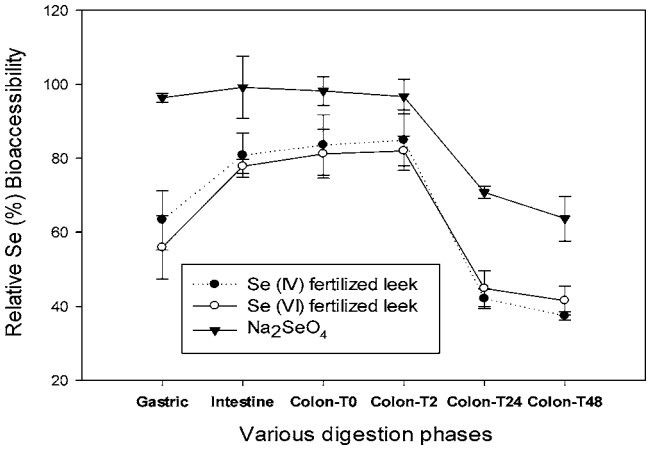

Figure 2. Relative bioaccessibility of Se from two Se-enriched leek types and pure selenate standard in different steps of an in vitro simulation of gastrointestinal digestion (T0, T2, T24, and T48 refer to 0, 2, 24, and 48 h after starting colon incubation, respectively).

reported to be 46% in the gastric phase and 89% upon intestinal digestion, whereas in fish it varied between 47 and 70% in the gastric phase and between 50 and 83% during intestinal digestion.[22,23]

The fact that Se bioaccessibility in the small intestine is not 100% for Se-enriched food crops may result in some Se not being taken up by the bloodstream in the small intestine. This Se may reach the colon, where it may play a role in inhibiting colon carcinogenesis.[24−26] In the colon, the bioaccessibility of Se from the Se-enriched leek samples started to decrease to below 50% after 24 h of incubation. Although the bioaccessibility of Se from the pure Na_2SeO_4 standard also decreased, it was still above 60% after 48 h. The decrease of Se bioaccessibility in the colon can probably be attributed to sorption of Se to organic matter or uptake by colon microbial biomass. The total Se recovery was somewhat lower in the colon (92 ± 9%) as compared to the upper intestine (102 ± 5%), which may be attributed to the formation of volatile Se compounds during colon digestion. The fact that the decrease in relative bioaccessibility when moving from small intestine to colon is more significant for Se-enriched leek as compared to pure selenate also points toward stimulation of microbial growth and/or uptake of Se by the microorganisms in presence of the leek matrix. The majority of Se is bioaccessible in the small intestine, and a significant fraction also has good chances to reach the colon, where it seems to be taken up by the microbial community and may also induce positive health effects. Further research is needed to assess whether this is actually the case.

AUTHOR INFORMATION

Corresponding Author

*Tel: +32-9-2645995. E-mail: Gijs.DuLaing@UGent.be.

Funding

This work was financially supported by FWO-Vlaanderen (project G.0194.08).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Tamas, M.; Mandoki, Z. S.; Csapo, J. The role of selenium content of wheat in the human nutrition. A literature review. *Acta Univ. Sapientiae* **2010**, *3*, 5−34.

(2) Cassens, R. G. Composition and safety of cured meats in the USA. *Food Chem.* **1997**, *59*, 561−566.

(3) Ylaranta, T. The Selenium content of some agricultural crops and soils before and after the addition of Selenium to fertilizers in Finland. *Ann. Agric. Fenn.* **1990**, *29*, 131−139.

(4) Broadley, M. R.; White, P. J.; Bryson, R. J.; Meacham, M. C.; Bowen, H. C.; Johnson, S. E.; Hawkesford, M. J.; McGrath, S. P.; Zhao, F. J.; Breward, N.; Harriman, M.; Tucker, M. Biofortification of UK food crops with selenium. *P. Nutr. Soc.* **2006**, *65*, 169−181.

(5) Thomson, C. D. Assessment of requirements for selenium and adequacy of selenium status: A review. *Eur. J. Clin. Nutr.* **2004**, *58*, 391−402.

(6) Ip, C.; Lisk, D. J. Efficacy of cancer prevention by high-selenium garlic is primarily dependent on the action of selenium. *Carcinogenesis* **1995**, *16*, 2649−2652.

(7) Finley, J. W.; Davis, C. D.; Feng, Y. Selenium from high selenium broccoli protects rats from colon cancer. *J. Nutr.* **2000**, *130*, 2384−9.

(8) Robberecht, H.; Vanden Berghe, D.; Deelstra, H.; Van Grieken, R. Selenium in the belgian soils and its uptake by rye-grass. *Sci. Total Environ.* **1982**, *25*, 61−69.

(9) Johnsson, L. Selenium uptake by plants as a function of soil type, organic-matter content and pH. *Plant Soil* **1991**, *133*, 57−64.

(10) Wróbel, K.; Wróbel, K.; Kannamkumarath, S. S.; Caruso, J. A.; Wysocka, I. A.; Bulska, E.; Światek, J.; Wierzbicka, M. HPLC-ICP-MS speciation of selenium in enriched onion leaves − a potential dietary source of Se-methylselenocysteine. *Food Chem.* **2004**, *86*, 617−623.

(11) Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium speciation from food source to metabolites: A critical review. *Anal. Bioanal. Chem.* **2006**, *385*, 1304−1323.

(12) Larsen, E.; Lobinski, R.; Burger-Meÿer, K.; Hansen, M.; Ruzik, R.; Mazurowska, L.; Rasmussen, P.; Sloth, J.; Scholten, O.; Kik, C. Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate. *Anal. Bioanal. Chem.* **2006**, *385*, 1098−1108.

(13) Benoit, F.; Ceustermans, N. Ecological growing of leeks with plastics. *Plasticulture* **1994**, *101*, 45−49.

(14) Liu, J. T.; Li, Z.; Wei, S.; Shan, W.; Wei-min, Z. Research progress of bioactive constituent and molecular biology in *Allium tuberosum*. *Food Sci. Technol. Int.* **2006**, *8*, 67−70.

(15) Williams, P. N.; Villada, A.; Deacon, C.; Raab, A.; Figuerola, J.; Green, A. J.; Feldmann, J.; Meharg, A. A. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environ. Sci. Technol.* **2007**, *41*, 6854−9.

(16) Mazeja, D.; Osvaldb, J.; Stibilja, V. Selenium species in leaves of chicory, dandelion, lamb's lettuce and parsley. *Food Chem.* **2008**, *107*, 75−83.

(17) Kapolna, E.; Fodor, P. Bioavailability of selenium from selenium-enriched green onions (*Allium fistulosum*) and chives (*Allium schoenoprasum*) after 'in vitro' gastrointestinal digestion. *Int. J. Food Sci. Nutr.* **2007**, *58*, 282−296.

(18) Gissel-Nielsen, G. Influence of pH and texture of the soil on plant uptake of added selenium. *J. Agric. Food Chem.* **1971**, *19*, 1165−1167.

(19) Li, H. F.; McGrath, S. P.; Zhao, F. J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92−102.

(20) Arvy, M. P. Selenate and Selenite uptake and translocation in bean plants (*Phaseolus-Vulgaris*). *J. Exp. Bot.* **1993**, *44*, 1083−1087.

(21) Kápolna, E.; Shah, M.; Caruso, J. A.; Fodor, P. Selenium speciation studies in Se-enriched chives (*Allium schoenoprasum*) by HPLC-ICP−MS. *Food Chem.* **2007**, *101*, 1398−1406.

(22) Reyes, L. H.; Encinar, J. R.; Marchante-Gayon, J. M.; Alonso, J. I.; Sanz-Medel, A. Selenium bioaccessibility assessment in selenized yeast after "in vitro" gastrointestinal digestion using two-dimensional

chromatography and mass spectrometry. *J. Chromatogr., A* **2006**, *1110*, 108−16.
(23) Cabanero, A. I.; Madrid, Y.; Camara, C. Mercury-selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. *Biol. Trace Elem. Res.* **2007**, *119*, 195−211.
(24) Irons, R.; Carlson, B. A.; Hatfield, D. L.; Davis, C. D. Both selenoproteins and low molecular weight selenocompounds reduce colon cancer risk in mice with genetically impaired selenoprotein expression. *J. Nutr.* **2006**, *136*, 1311−1317.
(25) Rudolf, E.; Kralova, V.; Cervinka, M. Selenium and colon cancer—From chemoprevention to new treatment modality. *Anti-cancer Agents Med. Chem.* **2008**, *8*, 598−602.
(26) Caroli, S.; Coni, E.; Alimonti, A.; Petrucci, F.; Bolis, G. B.; Cristallini, E. A pilot study on colon cancer occurrence as related to serum selenium levels. *Ann. Ist. Super Sanita* **1994**, *30*, 243−247.
(27) Ip, C.; Birringer, M.; Block, E.; Kotrebai, M.; Tyson, J. F.; Uden, P. C.; Lisk, D. J. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *J. Agric. Food Chem.* **2000**, *48*, 2062−2070.
(28) Dumont, E.; Ogra, Y.; Vanhaecke, F.; Suzuki, K.; Cornelis, R. Liquid chromatography−mass spectrometry (LC-MS): A powerful combination for selenium speciation in garlic (*Allium sativum*). *Anal. Bioanal. Chem.* **2006**, *384*, 1196−1206.
(29) Pyrzynska, K. Selenium speciation in enriched vegetables. *Food Chem.* **2009**, *114*, 1183−1191.
(30) Shah, M.; Kannamkumarath, S. S.; Wuilloud, J. C. A.; Wuilloud, R. G.; Caruso, J. A. Identification and characterization of selenium species in enriched green onion (*Allium fistulosum*) by HPLC-ICP-MS and ESI-ITMS. *J. Anal. At. Spectrom.* **2004**, *19*, 381−386.
(31) Whanger, P. D.; Ip, C.; Polan, C. E.; Uden, P. C.; Welbaum, G. Tumorigenesis, metabolism, speciation, bioavailability, and tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). *J. Agric. Food Chem.* **2000**, *48*, 5723−30.
(32) Ogra, Y.; Ishiwata, K.; Iwashita, Y.; Suzuki, K. T. Simultaneous speciation of selenium and sulfur species in selenized odorless garlic (*Allium sativum L. Shiro*) and shallot (*Allium ascalonicum*) by HPLC-inductively coupled plasma-(octopole reaction system)-mass spectrometry and electrospray ionization-tandem mass spectrometry. *J. Chromatogr., A* **2005**, *1093*, 118−125.
(33) Kotrebai, M.; Tyson, J. F.; Block, E.; Uden, P. C. High-performance liquid chromatography of selenium compounds utilizing perfluorinated carboxylic acid ion-pairing agents and inductively coupled plasma and electrospray ionization mass spectrometric detection. *J. Chromatogr., A* **2000**, *866*, 51−63.