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Changes in Selenium Speciation Associated with Increasing Tissue Concentrations of Selenium in Wheat Grain

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Wheat (*Triticum aestivum*) collected in the Nawanshahr-Hoshiarpur Region (Punjab, India) showed the highest selenium concentrations ever recorded in cereal grains (29−185 μg g^{-1}). There was a strong positive relationship between the selenium content in shoots and that in kernels, showing that grain selenium concentration can be predicted from that in the vegetative tissues of the plant. The identity and content of the selenocompounds in the grain samples and in wheat-based reference materials were investigated by HPLC−ICP−dynamic reaction cell−MS. Reversed-phase, cation exchange, and anion exchange HPLC were used to separate the selenium species after ultrasound-assisted enzymatic extraction with an ultrasonic probe. Selenomethionine and selenate accounted for 72−85% and 2−6% of the sum of the selenium species, respectively. The proportion of organic Se species varied with increasing Se content; namely, SeMet showed a relative reduction whereas the other organoselenium compounds increased up to 18−22% of the total chromatographed selenium. *Se*-methyl-selenocysteine was detected as a minor compound (0.2−0.5%) in high-Se wheat by both reversed-phase and cation exchange HPLC using retention time matching with the standard substance spiked to the sample extracts. Regular consumption of locally produced wheat-based food items may lead the population of the study area to an excessive intake of selenium. On the other hand, the large predominance of selenomethionine shows that local wheat can be a promising raw material for naturally enriched products to be used to supplement human and animal diets in low selenium areas.

KEYWORDS: *Triticum aestivum*; selenium; speciation; HPLC-ICP-MS; selenomethionine; selenate; *Se*-methyl-selenocysteine; food safety; nutrition

INTRODUCTION

Selenium (Se) is an essential micronutrient for humans and animals, involved as selenocysteine (SeCys) in functioning at the catalytic center of several selenoproteins such as glutathione peroxidases (GSHPx), thioredoxin reductase, and iodothyronine-deiodinases (*1*). As a constituent of selenoproteins, it plays a role in the protection of body tissues against oxidative stress, immune function, reproduction, and modulation of growth and development. Se deficiency, caused by extremely low dietary intakes, has functional consequences that result in severe disease conditions in domestic livestock and have been observed in humans as well. Apart from overt deficiency, in recent years, interest in Se as related to human health has been stimulated by the possible adverse health effects of marginal Se undernutrition (*1*) as well as the potential of Se supraphysiological intakes in cancer chemoprevention, at least in individuals with a low Se status (*2*). Increasing Se intake is possible through use of enriched food, fortified food, or Se supplements (e.g., Se-enriched yeast) (*3*).

Se enters the food-chain through plants (*2*). Accumulation of Se by plants is due to their ability to transform inorganic Se into a variety of organoselenium species, including bioactive compounds, which has important implications for human nutrition and health. The amount of Se in crops depends on a number of geological, geographical, and other factors: while the Se concentration of the soil is primarily controlled by the underlying geology (carbonatic vs silicatic), Se phytoaccessibility is dependent on soil pH, redox conditions, organic matter, competing ionic species such as sulfate, microbial activity, soil texture, compaction and mineralogy, soil temperature, level of rainfall during the growing season, irrigation, and pedoclimatic variables (temperature and rain intensity excursions) related to fluctuations of soil moisture and pH (*4*, *5*). All these factors influence Se speciation and mobility in soil, with selenite (Se(IV)) being usually more strongly adsorbed by the soil solid phase (e.g., iron oxides/hydroxides) and thus less soluble than selenate (Se(VI)) in soil solutions. Se(VI) is taken up across the root plasma membrane

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through a process of active transport mediated by a sulfate transporter and competes with sulfate for uptake (*6*). Recent evidence has been provided that Se(IV) also can be taken up through an active process likely mediated by phosphate transporters in wheat roots with an uptake rate similar to that of Se(VI) (*7*). Se(IV) is rapidly assimilated into organic forms which are retained in the roots, whereas Se(VI) is not readily converted into organoselenium compounds and is highly mobile in xylem transport. Se has no known physiological function in plants and is metabolized via the S-assimilation pathway, which involves biosynthesis of selenoamino acids, i.e., SeCys and selenomethionine (SeMet), which are nonspecifically incorporated into proteins in place of cysteine and methionine, respectively (*6*). Se-accumulators, which can contain from hundreds to several thousands of micrograms of Se per gram in their tissues, differ from nonaccumulators in that they biosynthesize primarily nonprotein selenoamino acids, such as *Se*-methyl-selenocysteine (MeSeCys) and γ-glutamyl-*Se*-methyl-selenocysteine (γ-Glu-MeSeCys), thereby preventing the damaging effects on plant functions resulting from incorporation of SeCys and SeMet in proteins.

Wheat is one of the most important Se sources for humans (*3*). The Se concentration in wheat, a nonaccumulating plant, is usually $< 0.1-1\ \mu g\ g^{-1}$, with levels $\leq 0.02\ \mu g\ g^{-1}$ in Se-deficient areas and up to $30\ \mu g\ g^{-1}$ in seleniferous regions (*8*, *4*). In wheat grain, Se is mostly protein-bound and more evenly distributed throughout the kernel than other minerals; thus, little Se is removed in the milling process and the concentration in flour is usually 80−90% of that in grain (*9*−*11*). Protein-bound SeMet is the predominant Se species in wheat grain and typically accounts for 80−90% of the sum of the selenocompounds detected in wheat grain and flour (*12*−*15*); since ≥80% of total selenium is usually extracted from wheat, this translates into approximately 70−80% of the Se present. Se(VI) is sometimes detected in both wheat grain and flour at levels of up to 5% of total Se (*12*, *15*). Selenocystine has also been tentatively reported in two studies employing HPLC-ICP-MS on the basis of retention time matching (*12*, *16*).

Because of the narrow range existing between Se deficiency and toxicity, and since plants can accumulate huge amounts of Se if present in a bioaccesible form in soil, poisoning of livestock and other animals grazing high-Se lands is well-known (*4*, *8*). Human toxicity from dietary exposure to Se is rare, and it was only reported in seleniferous areas, e.g., the Enshi District (China), where the population heavily relies upon local produce for their food (*17*).

Seleniferous areas have been identified in different states of India (*4*). The largest one (> 1000 ha) is the seleniferous belt of the Nawanshahr-Hoshiarpur Region (Punjab), where chronic selenosis in livestock feeding on Se rich fodders has been reported (*18*). Limited observations showed signs of Se toxicity also in the local population (*19*), and very high Se concentrations have recently been reported in locally grown crops (*20*).

The first aim of this study was to determine total Se and Se species in wheat grain from the seleniferous region of Nawanshahr-Hoshiarpur for risk characterization of dietary Se exposure. Another objective of the study was to investigate Se speciation in grain in relation to total Se in plant tissues in order to assess possible changes in Se metabolism with increasing Se uptake. In light of the obtained results, the possible use of wheat grown in the study area for fortification of low-Se grain batches or production of naturally enriched food and feed is discussed.

MATERIALS AND METHODS

Reagents and Standards. Deionized water obtained by a Milli-Q Element System (Millipore, Molsheim, France) was used throughout the work. Ultrapure-grade nitric acid (Carlo Erba Reagenti, Rodano, Italy) and hydrogen peroxide 31% v/v were used for microwave-assisted digestion of samples. Analytical-grade pyridine, salicylic acid, formic acid 98% (Merck KgaA, Darmstadt, Germany), and methanol (J.T. Baker, Deventer, Netherlands) were used for chromatographic separations. Calibrants and the internal standard solution (rhodium) used for total Se measurements were obtained from standard certified solutions with a content of $1\ mg\ mL^{-1}$ (High Purity Standard, Charleston, SC) by dilution with acidified (HNO_3) deionized water as necessary.

Stock solutions of $1\ mg\ mL^{-1}$, expressed as Se, were prepared by dissolving in deionized water adequate amounts of selenious acid (Se(IV)), selenic acid (Se(VI)), L-selenocystine ($SeCys_2$), seleno-L-methionine (SeMet) (all from Sigma-Aldrich, St. Louis, MO), and *Se*-methyl-seleno-L-cysteine (MeSeCys) (Fluka, Seelze, Germany). A commercially available and characterized Se-enriched garlic extract (Garliselect, SOCHIM International S.p.A., Milan, Italy) was used as the source of γ-Glu-MeSeCys. Standard stock solutions were stored at 4 °C, and the exact concentrations were ascertained by ICP-MS analysis. The purity of the standards was checked by HPLC-ICP-MS, and limited species interconversion was found only in the case of the inorganic species. Quantitative results were corrected accordingly.

For the enzymatic extraction of Se species, α-amylase from *Bacillus subtilis* (Fluka, Seelze, Germany) and bacterial protease type XIV from *Streptomyces griseus* (Sigma-Aldrich, St. Louis, MO) were used.

Samples and Sample Preparation. Samples of wheat (*Triticum aestivum*, cv. PBW343) were collected at sites near the villages of Jainpur and Barwa geographically located at 32°46′ N, 74°32′ E, in the Nawanshahr-Hoshiarpur Region, Punjab (India). The area is dominated by winter wheat, although spring wheat cultivation is also practiced and is dependent on the farmer. Cultivation is carried out according to common agricultural practices with fertilizers such as urea and diammonium phosphate (DAP) used along with pesticides. No Se toxicity symptoms were detected in the plants, and the grain yields were similar to those commonly found in the region, i.e., approximately 4.5 tons/ha. Two sampling campaigns were carried out in September (spring wheat) and February (winter wheat), respectively, during which both grain and shoots (i.e., stems and leaves) were sampled. Composite samples of about 3 kg, representative of the local production area sampled at each time, were collected.

Wheat shoots were washed with tap water and dried at 40 °C. The grain was cleaned from dust and other extraneous material, and damaged kernels were removed manually. Sample handling was carried out under clean room conditions in a laminar flow box (Spetec GmbH, Erding, Germany). Samples were ground by means of an automatic agate pestle mill (Retsch GmbH & Co., Haan, Germany) and then freeze-dried with a LyoLab 3000 system (Heto-Holten A/S, Allerød, Denmark). After freeze-drying, samples were ground again in order to obtain a fine flour and then sieved through a 125 μm mesh sieve (Retsch GmbH & Co., Haan, Germany).

Total Se Analysis. For total Se analysis, samples were submitted to oxidative digestion by means of a closed vessel system (Milestone Ethos E microwave labstation, FKV, Bergamo, Italy) equipped with an infrared temperature control system and quartz vessels. Approximately 0.15−0.25 g dry weight (d.w.), depending on the sample, was digested using 5 mL of HNO_3 and 2 mL of H_2O_2 after a 1 h premineralization. Microwave irradiation was performed with temperature control and automatic continuous adjustment of power output. The temperature profile of the irradiation process was as follows: 20 min ramp to 180 °C; 15 min at 180 °C. The digested samples were made up to 25 mL with deionized water and analyzed using an Elan DRC (dynamic reaction cell) II ICP-MS instrument (Perkin-Elmer-Sciex, Norwalk, CT) equipped with a Meinhard quartz concentric nebulizer and a quartz cyclonic spray chamber. Measurements were carried out in standard mode using ^{77}Se and ^{82}Se as analytical masses, Rh as internal standard, and the method of standard additions for quantification.

Two wheat-based certified reference materials (CRMs), NIST 1567a Wheat Flour and NIST 8436 Durum Wheat Flour, were included in each analytical batch for quality control of total Se analysis. Confirmatory analyses of high-Se wheat samples were carried out at Bhabha Atomic Research Centre by instrumental neutron activation analysis (INAA). A Compton suppressed spectrometer consisting of an HPGe-BGO detector system was used for γ-ray spectrometric measurements following the analytical procedure detailed elsewhere (*20*).

Se Speciation Analysis. For the enzymatic extraction of Se species, 0.1 g samples were added with 20 mg of α-amylase and 2 mL of degassed deionized water and then treated with an ultrasonic probe (Bandelin Electronic GNBH & Co. KG Berlin Sonopuls HD3200) for 60 s at 30 W. After adding 20 mg of protease, samples were sonicated again for 180 s at 30 W. Ultrasound-assisted enzymatic extractions were carried out at ambient temperature in a chamber purged with Ar in order to prevent species oxidation. The enzymatic extracts were centrifuged (Thermo Megafuge 11R, Thermo Fisher Scientific, Waltham, MA) at 5000*g* for 10 min, filtered through 0.45 μm PVDF syringe filters (Millipore, Molsheim, France), and then passed through 10 kDa molecular weight cutoff filters (Amicon Ultra-4 Ultracel 10k Regenerated Cellulose, Millipore, Molsheim, France) by centrifugation at 6100*g* for 20 min (4 °C). The filtrates were divided into aliquots and stored at −80 °C until analysis.

Se species were determined by means of online HPLC-ICP-MS. The HPLC apparatus was a metal-free system consisting of a Perkin-Elmer Series 200 LC binary pump, an autosampler, and a column thermostat. The outlet of the HPLC column was directly connected via PEEK capillary tubing to the nebulizer of the Elan DRC II ICP-MS instrument, which was operated in DRC mode with CH_4 as reaction gas and served as the Se-specific detector (see in **Table 1** for instrumental conditions). For sample introduction, a PFA-LC nebulizer (Elemental Scientific Inc., Omaha, NE) along with a quartz cyclonic spray chamber were used. Separations were carried out by reversed-phase, cation exchange, and anion exchange HPLC. The columns, mobile phases, and other chromatographic conditions are detailed in **Table 1**. The mobile phases were filtered (Millipore Express Plus 0.22 μm) and continuously degassed during analysis by purging with Ar to prevent species oxidation. Chromatographic data were collected, stored, and processed using the Perkin-Elmer software Chromera. Se species in extracts were identified by retention time matching with the standard substances spiked to the sample extracts. Quantitative calculations were based on peak areas using external calibration or the method of standard additions as appropriate, depending on sample dilution. Chromatograms were corrected by subtracting the signals of the selenocompounds detected in procedural blanks, i.e., SeMet and Se(VI). The occurrence of these species was likely due to impurities in the enzymes used for sample extraction (*21*) and was significant in the case of the CRMs, amounting to ∼5% and ∼40% of the endogenous content of SeMet and Se(VI), respectively.

RESULTS AND DISCUSSION

Total Se in Wheat and Estimated Human Intake. The accuracy of total Se determinations as assessed through CRM analysis was satisfactory. Found values ($n = 4$) were 1.10 ± 0.03 and 1.16 ± 0.01 μg g^{-1} d.w. for NIST 1567a and NIST 8436, respectively, which compared well with the certified values, i.e., 1.1 ± 0.2 and 1.23 ± 0.09 μg g^{-1} d.w., respectively. INAA determinations of total Se in the investigated samples, carried out as an independent quality control check, showed excellent agreement with the ICP-MS measurements discussed below.

The total Se concentrations in spring wheat grain collected during the first sampling campaign were 83.1 ± 0.1 (sample b) and 185.1 ± 1.7 μg g^{-1} d.w. (sample d), whereas 29.5 ± 0.2 (sample a) and 98.9 ± 1.3 μg g^{-1} d.w. (sample c) were found in winter wheat collected in the second sampling campaign. The difference between the two sampling campaigns is thought to be due mainly to the variable content of bioaccessible Se in soil at different sampling locations.

Se concentrations were higher in grains than in shoots. There was a positive linear relationship between the Se content in shoots and that in kernels ($y = 1.1496x + 17.791$; $R^2 = 0.9998$; $p = 0.0001$). Therefore it appears that, at least within the concentration ranges found in this study (i.e., 11−146 μg g^{-1} in shoots; 29−185 μg g^{-1} in kernels), the grain Se concentration in a given area can be predicted from that in the vegetative tissues of the plant.

Intake estimates were carried out assuming a 20% reduction in Se concentration as a result of wheat milling (*9*−*11*). Daily consumption of 100 g of wheat flour gave a Se intake of 2.0−13.0 mg day^{-1}, i.e., 7- to 43-fold the EC tolerable upper intake level (UL) for adults (300 μg Se day^{-1}).

Table 1. Instrumental Operating Conditions

ICP-MS Settings	
RF power	1.4 kW
nebulizer gas, Ar	0.85−1.0 L min^{-1}
lens voltage/V	8.25−11
DRC gas (methane) flow rate/mL min^{-1}	0.7
rejection parameter q (RPq)	0.45
analytical mass (speciation analysis)	^{78}Se, ^{80}Se

Chromatographic Conditions	
Reversed-Phase Chromatography	
column	Acclaim PepMap 100 C18 (Dionex Corporation, Sunnyvale, CA) 150 mm × 1 mm, 3 μm
temperature	22 °C
injection volume	20 μL
mobile phase	2% (v/v) MeOH in 0.1% formic acid
flow rate	0.035 mL min^{-1}
isocratic elution	0−20 min
Anion Exchange Chromatography	
column	ICSep ION-120 (Transgenomics, San Jose, CA) 120 mm × 4.6 mm, 10 μm
temperature	24 °C
injection volume	50 μL
mobile phase	3.5 mM salicylic acid in 3% (v/v) MeOH, adjusted to pH 8.5 with TRIS
flow rate	1 mL min^{-1}
isocratic elution	0−16 min
Cation Exchange Chromatography	
column	Chrompack IonoSpher-5C (Varian, Middelburg, The Netherlands) 100 mm × 3.0 mm, 5 μm
temperature	24 °C
injection volume	20 μL
mobile phase	(A) 3% (v/v) MeOH, pH 3.2; (B) 10 mM pyridinium formate in 3% (v/v) MeOH, pH 3.2; (C) 3% (v/v) MeOH, pH 3.0; (D) 10 mM pyridinium formate in 3% (v/v) MeOH, pH 3.0
flow rate	1 mL min^{-1}

Gradient Elution

Method *a*		Method *b*		Method *c*	
0−3.5 min	92.5% A, 7.5% B	0−3.5 min	92.5% A, 7.5% D	0−3.5 min	92.5% C, 7.5% D
3.5−5 min	88% A, 12% B	3.5−5 min	88% A, 12% D	3.5−11 min	72% C, 28% D
5−7 min	74% A, 26% B	5−7 min	74% A, 26% D	14−17 min	72% C, 28% D
7−16 min	92.5% A, 7.5% B	7−25 min	92.5% A, 7.5% D	15.5−25 min	92.5% C, 7.5% D

Determination of Se Species. The reversed-phase HPLC chromatogram of an enzymatic extract of the CRM NIST 1567a in **Figure 1** shows that the major compound present is SeMet, and a peak with the same retention time as $SeCys_2$ (3.9 min) appears. SeMet was the predominant species also in the extracts of the high-Se wheat from the study area, but in these samples, several other peaks were detected (**Figure 2**). The small peak with the retention time of 5.0 min coeluted with the MeSeCys standard

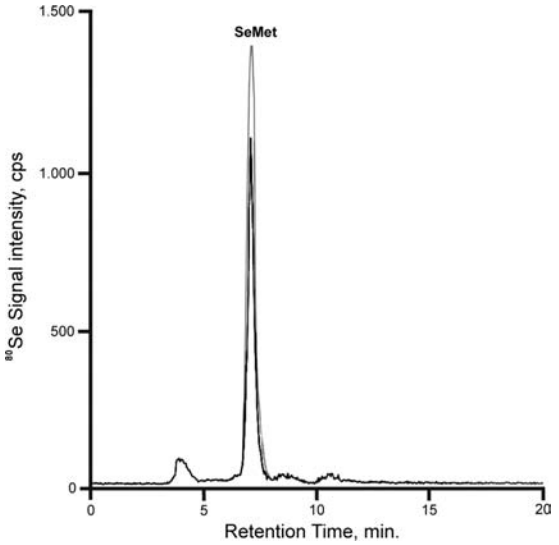

Figure 1. Reversed-phase HPLC-ICP-MS chromatogram of an extract of the reference material NIST 1567a wheat flour (see **Table 1** for conditions). Co-chromatography with a SeMet standard spiked to the extract is shown in gray.

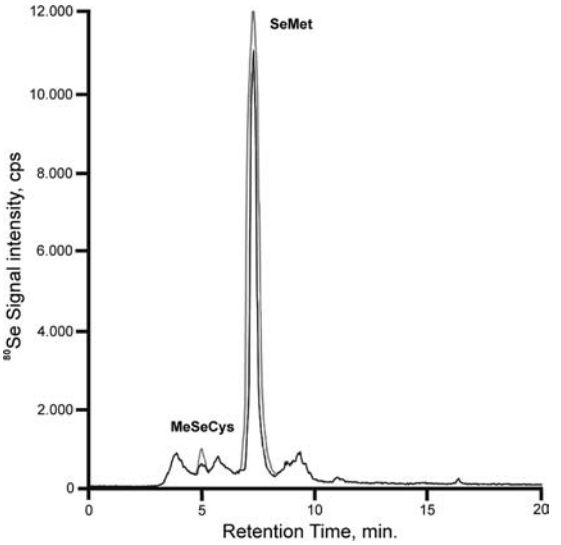

Figure 2. Reversed-phase HPLC-ICP-MS chromatogram of an extract of wheat grain (sample d, see **Table 1** for conditions). Co-chromatography with SeMet and MeSeCys standards spiked to the extract is shown in gray.

spiked into the sample extract (**Figure 2**), whereas the other peaks remained unidentified, with the exception of the first one, which again coeluted with the $SeCys_2$ spike. However, it was noticed that this peak varied strongly in intensity between replicate analyses, whereas the sum of its area and that of the SeMet was almost constant. This indicated that the peak was due, at least in part, to a conversion product of SeMet, selenomethionine-*Se*-oxide (SeOMet), which was prepared upon addition of H_2O_2 to a SeMet standard (*22*) and spiked into the sample extracts. Since attempts to separate $SeCys_2$ and SeOMet by reversed-phase chromatography were unsuccessful, gradient elution cation exchange chromatography was used to separate the two compounds and as a complementary technique for confirmation of the results obtained by reversed-phase HPLC-ICP-MS. The cation exchange HPLC chromatogram (method *a*) of a sample extract in **Figure 3a** shows the large peak of SeMet and at least eight minor peaks in addition to the anionic species eluting in the solvent front. The $SeCys_2$ standard spiked into the sample extract had the same retention time as the peak at 5.7 min and was well separated from SeOMet eluting at 8.1 min. An improved separation of the first peak (5.7 min) from the closely eluting Se species was obtained by changing the chromatographic conditions (method *b*), and coelution with the $SeCys_2$ standard was again observed (**Figure 3b**). This provided supportive evidence of the presence of $SeCys_2$ in sample extracts, which however awaits confirmation by retention time matching in a different chromatographic system or, more decisively, molecular mass spectrometry.

Further optimization of the chromatography (method *c*) was required for the separation of γ-Glu-MeSeCys and MeSeCys, which both eluted within 2.5 min. Co-chromatography of the MeSeCys standard spiked in the sample extracts (**Figure 4**) resulted in a single peak at the retention time ~2.3 min and suggested the occurrence of MeSeCys, in accordance with the qualitative and quantitative results obtained by reversed-phase chromatography.

Inorganic Se species were determined by anion exchange HPLC-ICP-MS. With the method used in this study, Se(VI) eluted at ~12 min and was well separated from the early eluting species and Se(IV), which had a retention time of ~5.7 min. Se(VI) was identified in both CRMs (**Figure 5**) and samples from the study area. Furthermore, Se(IV) was detected in trace amounts in the CRMs and two samples. However, identification of this species should be considered as tentative, owing to the presence of other closely eluting Se compounds. It has to be noted that the peak eluting at 6.4 min in anion exchange HPLC-ICP-MS did not appear when ^{78}Se was used as the analytical mass instead of ^{80}Se, and therefore, it was excluded from calculations.

Se Speciation in High-Se Wheat. The quantitative results for SeMet, MeSeCys, and Se(VI) in the CRMs and the samples from the study area are shown in **Table 2**. Since SeOMet was likely an artifact of sample preparation, the sum of SeMet and its oxide is given under SeMet in **Table 2**. Conversion of SeMet into SeOMet was observed in all hydrolysates within a relatively short time. Oxidation of SeMet was reduced by removing oxygen, as far as possible, during enzymolysis, but even fresh extracts contained the oxide to some extent. The results for MeSeCys in **Table 2** were obtained by averaging the sets of data obtained by reversed-phase and cation-exchange HPLC-ICP-MS.

On average, 83% of the Se in the samples (range 70−90%) was extracted by the ultrasound-assisted procedure used, which allowed hydrolysis to be completed in 5 min instead of up to 24 h by conventional approaches. The recovery of this Se from the chromatographic columns, calculated as the sum of the species detected, was complete. In the CRMs, which have a Se content of ~1 μg g^{-1}, SeMet accounted for 85% of the Se species detected. A similar proportion of SeMet (83%) was found in sample a (total Se 29 μg g^{-1}), whereas a lower proportion (72−78%) was found in samples b, c, and d (total Se 83−185 μg g^{-1}). Conversely, the proportion of other organic species increased with increasing total grain Se, from 12% (CRMs), to 15% (sample a), up to 18−22% (samples b−d). The concentration of Se(VI) increased along with the total Se content of the samples, whereas in relative terms this species remained within 2−6% (3% on average) of the sum of the species. MeSeCys was practically absent in the samples with a lower Se content, whereas it was found in the high-Se samples; the highest content, both in absolute and relative terms, was observed in the sample with the highest Se concentration (sample d).

The Se concentrations of the samples investigated in this study appear to be the highest ever recorded in cereal grains for human consumption. Wheat is a nonaccumulator of Se, and the Se concentration in grain rarely exceeds 30 μg g^{-1} even in seleniferous areas, such as the northern great plains of the USA.

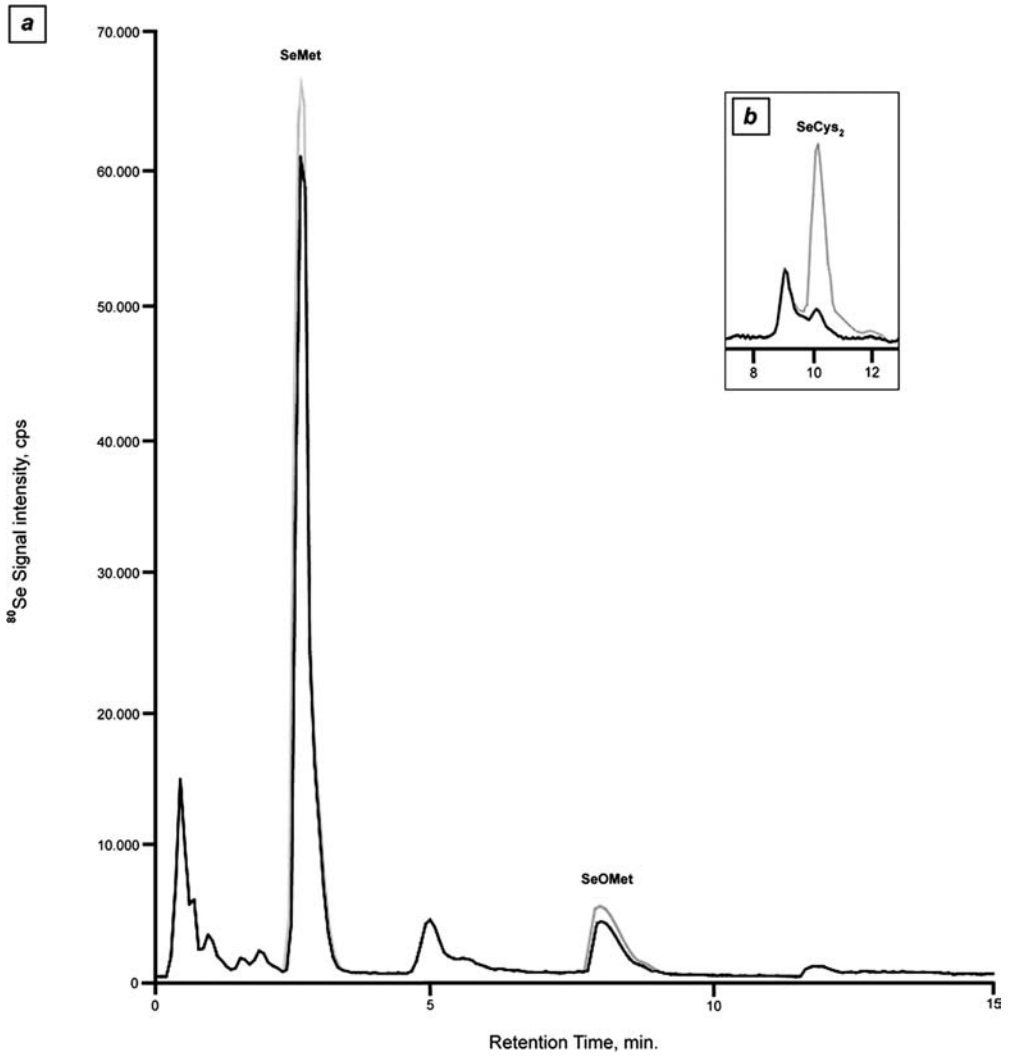

Figure 3. Cation exchange HPLC-ICP-MS chromatogram of an extract of wheat grain (sample b): (**a**) Method *a* (see **Table 1** for conditions), co-chromatography with SeMet and SeOMet standards spiked to the extract is shown in gray; (**b**) Method *b* (see **Table 1** for conditions), co-chromatography with a $SeCys_2$ standard spiked to the extract is shown in gray.

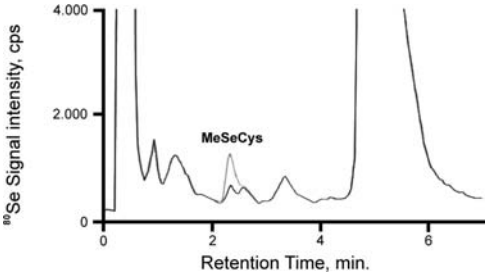

Figure 4. Cation exchange HPLC-ICP-MS chromatogram of an extract of wheat grain: detail (0−7 min, sample b, method *c*; see **Table 1** for conditions). Co-chromatography with a MeSeCys standard spiked to the extract is shown in gray.

Exceptionally high Se concentrations have been sporadically reported in wheat grain from South Dakota, e.g., from 52 μg g^{-1} (*23*) up to 63 μg g^{-1} (*24*), which however are considerably lower than the highest values observed in this study. It has been recently demonstrated that wheat exhibits a higher tolerance to Se than other crops such as tobacco, soybeans, and rice (*25*). Pot trials suggested that, for wheat grown on a sandy loam soil of pH (H_2O) 5.5 and normal mineral nutrient levels, the critical tissue level for Se toxicity is around 325 μg g^{-1} and growth inhibition of

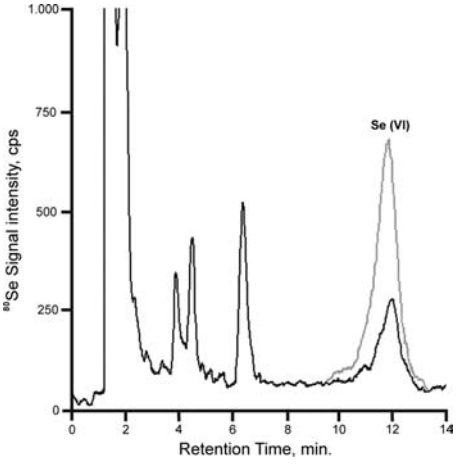

Figure 5. Anion exchange HPLC-ICP-MS chromatogram of an extract of the reference material NIST 8436 Durum wheat flour (see **Table 1** for conditions). Co-chromatography with a Se(VI) standard spiked to the extract is shown in gray.

less than 10% may commence at tissue concentrations of 200 μg Se g^{-1} (*25*). In this study it was found that wheat is able to accumulate Se at concentrations up to 146 μg g^{-1} in vegetative

Table 2. Se Species in Extracts of Wheat Grain[a]

sample no.	SeMet	MeSeCys	Se(VI)	sum of species[b]
NIST 1567a	0.821 ± 0.052	*n.d.*	0.025 ± 0.007	0.941
	(85)	(*n.a.*)	(3)	(88)
NIST 8436	0.866 ± 0.058	traces	0.027 ± 0.002	0.984
	(85)	(*n.a.*)	(3)	(82)
a	17.1 ± 0.8	0.041 ± 0.005	0.352 ± 0.041	20.5
	(83)	(0.2)	(2)	(70)
b	55.1 ± 2.8	0.303 ± 0.026	1.18 ± 0.07	70.2
	(78)	(0.4)	(2)	(84)
c	63.1 ± 2.2	0.155 ± 0.014	4.86 ± 0.28	87.6
	(72)	(0.2)	(6)	(89)
d	121.6 ± 6.7	0.744 ± 0.074	5.12 ± 0.31	155.1
	(78)	(0.5)	(3)	(84)

[a] Concentrations in $\mu g\ g^{-1}$ d.w., expressed as Se (mean ± S.D., $n = 3$, % of the sum of species is in parentheses). [b] % of total Se is in parentheses.

tissues and 185 $\mu g\ g^{-1}$ in grain without apparent detriment to the plants. It is worth noting that since tolerance to Se toxicity varies depending on several factors, notably soil properties and sulfate supply (*6*, *25*), threshold Se concentrations may not be the same in other areas with different soil conditions.

The distribution of Se in the different parts of the plant was the expected one, with a higher content in grain compared to stems and leaves (*6*). There was a strong positive linear relationship between the Se content in shoots and that in kernels. Therefore, at least within the concentration ranges found in this study, it is feasible to predict grain Se concentration from that in the vegetative tissues of the plant. This was observed in mature plants, and it would be interesting to explore whether a similar relationship exists between the Se concentration in leaves during the early vegetative stage of growth and that in grain.

The results obtained in this study showed that, even when wheat is exposed to exceedingly high concentrations of Se, the inorganic Se taken up by the roots is extensively biotransformed into a variety of organoselenium compounds, which are then found in the kernel and account for 97% of the extracted Se on average. In the samples with very high total Se concentrations in grains, the proportion of SeMet to total Se was lower (72−78%) than that in samples with less grain Se, such as the CRMs, which both have a Se content of ~1 $\mu g\ g^{-1}$, with 85% in the form of SeMet. Therefore, increased biosynthesis of organoselenium compounds different from SeMet results when uptake from soil increases considerably. These compounds can be either intermediates of the pathway converting SeCys into SeMet or products of SeMet methylation leading to Se volatilization as dimethylselenide, a known mechanism for elimination of excess Se in nonaccumulator plants (*6*). However, even though increased formation of different organoselenium compounds was apparent at higher Se uptakes, SeMet largely remained the major Se compound accounting for over 70% of the chromatographed Se even in the samples with the highest Se content.

The ratio of SeMet to total Se found in this study for the CRMs matches that of Stadlober et al. (*12*), who found 85−91% of the chromatographed Se (equal to 69−86% of total Se) to be in the form of SeMet in different wheat genotypes with a Se content of 0.17−0.24 $\mu g\ g^{-1}$ after enzymatic extraction with satisfactory yields (i.e., 80−94%). Similar ratios of SeMet to total Se have been found by other authors investigating wheat grain or flour with total Se contents up to a few micrograms of Se per gram (*13*, *14*). Lower proportions of SeMet (50−65% of total Se) were episodically reported in studies on a limited number of samples or with low or unspecified extraction efficiencies (*16*, *23*, *26*−*28*). In such studies, no attempt to detect SeOMet was made. As shown in this study, SeOMet can be a significant artifact of sample preparation and should be always determined in order to calculate the actual SeMet concentration. Besides that, SeOMet should be investigated, since it can coelute with other peaks and lead to incorrect results in speciation analysis.

This study provided strong evidence of the presence of MeSeCys in wheat grain for the first time. This compound is a known detoxification product resulting from the methylation of SeCys in Se-accumulators and plants of the *Brassica* and *Allium* families (*6*, *29*). Even though MeSeCys was more abundant in the samples which accumulated more Se, it indeed remained a very minor compound (≤0.5% of the sum of the Se species) which cannot play any role in Se detoxification. Moreover, its dipeptide derivative γ-Glu-MeSeCys was searched for but not detected in any sample. Therefore, methylation of SeCys did not appear to be a significant pathway for Se assimilation in high-Se wheat. Based on the results obtained in this study, very little, if any, MeSeCys can be found in wheat grain samples with "normal" (i.e., ≤1 $\mu g\ g^{-1}$) Se concentrations.

Some evidence of the possible presence of $SeCys_2$ was found in this study. Since identification that is only based on retention-time matching with authentic standards must be considered as tentative (*29*), further studies employing electrospray ionization MS with fragmentation of the molecular ion are ongoing to provide evidence of structural confirmation as well as try to identify some of the unknown compounds detected in the chromatograms of sample extracts.

Risk characterization of dietary Se exposure based on intake calculations using realistic consumption figures of wheat-based products showed that the population of the seleniferous belt of the Nawanshahr-Hoshiarpur Region may largely exceed the tolerable upper intake level for Se, which matches the finding of human Se toxicity previously reported in a small-scale study (*19*). It is to be noted that SeMet was the major Se species in grain and this compound has a lower chronic toxicity compared to other selenocompounds, such as $SeCys_2$ or Se(IV), in animal studies (*30*). However, the preliminary results of this study indicate that actions to limit the exposure of the population to excessive Se are urgently needed, and human biomonitoring in the severely impacted areas would be advisible. A proactive approach to tackle the problems of the local agricultural system is needed, since high Se accumulation in crops has severe repercussions on the farming community in this area. Application of sulfur from gypsum and phytoremediation, especially phytovolatilization, have been suggested as viable approaches for the management of seleniferous agricultural soils (*4*). Exploring the opportunities of using locally grown grains for fortification of low-Se grain batches or production of naturally enriched products as Se supplements for human and animal nutrition is an alternative that is worth considering. This study showed that, in wheat grain, SeMet accounts for more than 70% of the Se species detected by HPLC-ICP-MS even in samples with very high Se concentrations. This is of course a favorable circumstance, since SeMet is the main dietary form of Se and is highly bioavailable (*2*, *13*). Further studies will be carried out to investigate the bioaccessibility and speciation of Se in products derived from high-Se wheat grown in the study area.

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