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Chemical Form of Selenium in Naturally Selenium-Rich Lentils (*Lens culinaris* L.) from Saskatchewan

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Lentils (*Lens culinaris* L.) are a source of many essential dietary components and trace elements for human health. In this study we show that lentils grown in the Canadian prairies are additionally enriched in selenium, an essential micronutrient needed for general well-being, including a healthy immune system and protection against cancer. Selenium K near-edge X-ray absorption spectroscopy (XAS) has been used to examine the selenium biochemistry of two lentil cultivars grown in various locations in Saskatchewan, Canada. We observe significant variations in total selenium concentration with geographic location and cultivar; however, almost all the selenium (86−95%) in these field-grown lentils is present as organic selenium modeled as selenomethionine with a small component (5−14%) as selenate. As the toxicities of certain forms of arsenic and selenium are antagonistic, selenium-rich lentils may have a pivotal role to play in alleviating the chronic arsenic poisoning in Bangladesh.

KEYWORDS: X-ray absorption spectroscopy (XAS); selenium; selenomethionine; selenate; lentils

INTRODUCTION

Selenium is an essential micronutrient for mammals and birds with known functions in a variety of systems. It is required for both intra- and extracellular glutathione peroxidases, iodothyronine deiodinase, and thioredoxin reductase, as well as a number of selenoproteins with less well-defined physiological roles (e.g., selenoproteins P and W) (*1*). In all of these cases the selenium is present as the amino acid selenocysteine, which, where its role is understood, forms part of the catalytic active site of the enzyme. Selenium is also said to provide protection against various cancers including prostate cancer (*2*), colorectal cancer (*3*), lung cancer (*4*), and various gastrointenstinal cancers (*5*). Significantly, selenium can also protect against heavy metal poisoning by elements such as arsenic (*6*, *7*), mercury (*8*), and cadmium (*9*). With arsenic, the mechanism of this protective effect is the formation of a novel arsenic−selenium compound, the selenobis(*S*-glutathionyl)arsinium ion (**Figure 1**) (*6*), which is formed in erythrocytes (*7*) and excreted in bile (*10*). The arsenic−selenium species is thought to be a mechanism by which the body eliminates arsenic that necessarily involves the loss of one atom of essential selenium for every atom of arsenic excreted. This finding has significance for the widespread poisoning of rural communities in Bangladesh and surrounding areas. Here, tens of millions of people are affected by a chronic low-level arsenic poisoning (arsenicosis) due to exposure to toxicologically low but nutritionally high levels (up to 1−2 ppm) of arsenic in drinking water (*11*). We have previously suggested that chronic low-level arsenicosis might in fact be an arsenic-induced selenium deficiency (*6*) caused by the formation and excretion of the selenobis(*S*-glutathionyl)arsinium ion. Moreover, the diets of affected communities in Bangladesh are naturally low in selenium (*12*), and selenium supplements have been suggested as a treatment (*6*). Two independent clinical trials involving selenite (*13*) and selenomethionine (*14*) are currently under way. Both of these trials involve administering selenium as a supplement in tablet form. Because any change in daily routine tends to be opposed in rural third-world communities, augmenting dietary selenium in currently accepted foodstuffs might be a more acceptable method of providing such supplements.

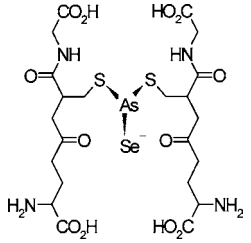

Figure 1. Schematic of the structure of the selenobis(*S*-glutathionyl)-arsinium ion.

Pulses, mainly lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.), and yellow pea (*Pisum sativum* L.), together with

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rice (*Oryza sativa* L.), are staple Bangladeshi foods. Thus, Se-enriched pulses could provide a culturally acceptable method for increasing dietary selenium intake. Soils of the lentil-growing region of western Canada (mainly south Saskatchewan) are naturally rich in selenium (>1 ppm) (*15*), and thus, Canadian pulses stand to be higher in selenium than their counterparts from other regions. Here we present data on the selenium content and chemical form of the selenium in two red lentil cultivars, CDC Redberry (*16*) and CDC Robin (*17*), from different parts of Saskatchewan, showing them to be an excellent source of selenium. As the chemical form of selenium in the lentils governs both bioavailability and projected health benefits, we use synchrotron X-ray absorption spectroscopy (XAS) to determine the chemical form of selenium in the whole lentil seeds. To our knowledge, this is the first report of Se near-edge XAS analysis in lentil seed and its chemical species at low selenium concentration (<0.29 ppm or 3.7 μmol of Se kg^{-1}). The dual objectives of this study were to measure the total selenium concentration for two red lentil cultivars (CDC Redberry, CDC Robin) from different locations in Saskatchewan and to identify the chemical species of selenium in the lentils.

MATERIALS AND METHODS

Sample Preparation. Lentil seeds were obtained from breeding trials conducted in 2005 by the Crop Development Centre (CDC), University of Saskatchewan, Canada. The selected locations were Davidson, Swift Current, Wilkie, Rouleau, and Saskatoon, covering the major lentil growing areas in Saskatchewan. Cultivars CDC Redberry and CDC Robin are widely grown commercially in Saskatchewan because of their early maturity, disease resistance, high yield, and consumer acceptance. Bangladeshi consumers tend to show a preference for red cotyledon, extrasmall seed size (30 mg or less) cultivars such as CDC Robin. CDC Redberry is preferred in countries where consumers desire larger red lentils (45 mg or greater). Dry lentil seed samples (14% moisture, 20% protein) from Davidson, Swift Current, and Wilkie were used for Se near-edge XAS measurements. Samples of between 5 and 10 g of dry lentil seeds were collected from each location for this study. Lentil seeds were collected from each location with four replicates, then the samples were homogenized, and one composite sample from each location was used for near-edge XAS measurements.

X-ray Absorption Spectroscopy. The seeds were ground into a fine powder (<0.5 mm sieve). Samples of between 0.2 and 0.4 mg of homogenized seeds were carefully packed into 2 mm path length Lucite sample holders with Mylar windows and then frozen in liquid nitrogen prior to data collection. X-ray absorption spectroscopy experiments were performed at the Stanford Synchrotron Radiation Laboratory (SSRL), with the SPEAR-3 storage ring containing 90−100 mA at 3.0 GeV, using the Structural Molecular Biology XAS beamline 9-3, which is equipped with a Si(220) double-crystal monochromator. Harmonic rejection was achieved by using the beamline upstream, vertically collimating, mirror, and downstream, refocusing mirror, both Rh-coated. The incident X-ray intensity was monitored using a N_2-filled ionization chamber, and the absorption spectrum was collected by monitoring the Se Kα fluorescence using a Canberra 30-element germanium detector equipped with arsenic filters and Soller slits (*18*). Samples were maintained between 5 and 10 K in an Oxford instruments liquid helium flow cryostat, and the X-ray energy was calibrated with reference to the lowest energy inflection point of hexagonal elemental Se, which was assumed to be 12658.0 eV. Two replicate data sets were collected, taking approximately 40 min of total time per sample. Standards (sodium selenate, sodium selenite, selenomethionine, and *Se*-methylselenocysteine) were purchased in powder form from Sigma-Aldrich and were of the highest quality available. Trimethylselenonium iodide was synthesized by treating dimethyl selenide (Strem Chemicals, Inc.) with stoichiometric methyl iodide (Sigma-Aldrich) (*19*). Standards were measured as frozen dilute aqueous solutions (5−10 mM with 30% (v/v) glycerol, in 50 mM HEPES buffer, pH 7.0).

X-ray absorption spectroscopy data reduction and analysis was performed using the EXAFSPAK suite of computer programs (http://ssrl.slac.stanford.edu/exafspak.html). Quantitative determination of the different chemical forms of selenium present was carried out by principal component analysis and least-squares fitting (*20*). Least-squares fitting methods using X-ray absorption spectroscopy are now widely accepted to be valid speciation methods for complex samples. In early work, results from these methods were found to be in excellent agreement with the results obtained from hydride generation atomic absorption spectroscopy (HG-AAS) (*20*).

Table 1. Total Se Concentrations for Field-Grown Saskatchewan Lentils[a]

	total Se concn, ppm (value ± SD)		
location	CDC Robin	CDC Redberry	mean
Saskatoon	0.72 ± 0.03	0.33 ± 0.03	0.52 a
Rouleau	0.45 ± 0.03	0.21 ± 0.01	0.33 b
Davidson	0.27 ± 0.02	0.29 ± 0.01	0.28 c
Wilkie	0.22 ± 0.01	0.16 ± 0.01	0.19 d
Swift Current	0.16 ± 0.01	0.20 ± 0.01	0.18 d
mean	0.36 a	0.24 b	

[a] Means within a column (total Se concentration for locations) or a row (mean total Se concentration for cultivars) followed by different letters are significantly different at $P < 0.05$. Four replicate samples were analyzed for each location and cultivar.

Total Se Concentration. For each replicate from each location, the total Se concentration in the lentil seeds was measured following a $HNO_3-H_2O_2$ digestion (*21*), modified as described below. All the chemicals used were of analytical grade or higher purity. Dried lentil seeds were ground into a fine powder (<0.5 mm sieve), and approximately 200 mg was placed in a digestion tube with 3 mL of concentrated HNO_3. The tubes were heated to 70−80 °C to complete the digestion, and then 0.5 mL of 30% H_2O_2 was added and the resulting solution vortexed for 2 min. After digestion, the sample solution was diluted with Millipore water to a known volume. Then all the samples were reduced with 6 M HCl at 70 °C for at least 10−15 min in a water bath. The total Se concentration was determined using an atomic absorption spectrophotometer equipped with a flow-injection hydride generator (AA220, Varian Inc., Palo Alto, CA). Measurements of total selenium levels using this modified digestion method were validated using NIST standard reference material 1573a (tomato leaves; [Se] = 0.054 ± 0.003 mg kg^{-1}). *Astragalus bisulcatus* shoots (hydroponically grown with 10 μM K_2SeO_4 solution; total [Se] = 45 mg kg^{-1}) were used as a laboratory reference material (LRM) measured periodically to ensure consistency in the methods. Additionally, the modified digestion method was compared for randomly selected samples with pressure digestion with a HNO_3/H_2O_2 mixture, and both were determined using HG-AAS, yielding identical values within the errors of the techniques. In independent measurements, ICP-MS and HG-AAS on the same biological samples gave identical results. Finally, the edge jump of the XAS data, which gives an estimate of total selenium, was compared. In all cases, the results were consistent with our total selenium analysis.

Statistical Analysis. The experiment design for total Se concentration was a randomized complete block design with four replicates, at five locations with two cultivars. The data from each location for two cultivars were combined and analyzed after error variances were tested for homogeneity. Analysis of variance was done using the general linear model procedure (Proc GLM) of SAS version 8.2 (*22*). The means were separated by Fisher's protected LSD at $P < 0.05$.

RESULTS AND DISCUSSION

Total Se Concentration. We examined the total selenium in the seeds of two lentil cultivars from five locations in Saskatchewan. Elemental analysis indicates that the total Se concentration is in the range 0.1−0.7 ppm, which is equivalent to 2−9 μmol of Se kg^{-1} (**Table 1**). Significant effects of both the cultivar and location on the total Se concentration were observed. The interaction between the cultivar and location

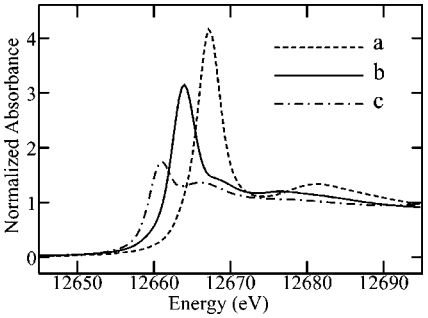

Figure 2. Se K near-edge X-ray absorption spectra of selected Se aqueous standards: (a) selenate; (b) selenite; (c) selenomethionine.

shows that most of the variation in the lentil total Se concentration may be due to available soil Se. Between the two cultivars, CDC Robin showed a significantly higher total Se concentration than CDC Redberry (**Table 1**). Lentil seeds from Saskatoon show the greatest mean total Se concentration (0.52 ppm, 6.6 μmol of Se kg^{-1}), and those from Swift Current show the lowest (0.18 ppm; 2.3 μmol of Se kg^{-1}).

Selenium Near-Edge XAS. Synchrotron XAS is unique as a tool that can provide molecular information essentially without sample pretreatment (which can change the chemical form). X-ray absorption spectroscopy provides information on the local physical and electronic structure of a particular absorbing atom (in this case selenium). The XAS spectrum can be divided into two regions, the near-edge also known as the X-ray absorption near-edge structure (XANES) and the extended X-ray absorption fine structure (EXAFS). The near-edge of selenium is a unique tool in the speciation of metals and metalloids in biological tissues including plants. The near-edge spectrum can provide a "fingerprint" of the type of chemical species present (*23*), and quantitative information can be obtained by least-squares curve fitting (*20*). Selenium shows rich variations in its near-edge spectra as a function of chemical form (*23*) and has been used to distinguish selenium chemical forms in samples such as soils (*20*), bacteria (*24*, *25*) plants (*26*−*28*), and insects (*29*). **Figure 2** shows the Se K near-edge spectra of aqueous solutions of selenomethionine, selenite, and selenate. The spectra of selenomethionine and *Se*-methylselenocysteine (data not shown) are very similar due to the close similarity of the local structure around the selenium (H_3CSeCH_2R in both cases). A variety of selenium speciation tools have been applied to investigate selenium in biological systems including food crops (see, for example, ref *30* and references therein), but essentially all of these require digestion or other preprocessing, during which it is possible that the speciation is modified or not all of the selenium is extracted. Se K-edge XAS identifies the chemical type rather than specific molecules and contrasts with conventional methods both in requiring no sample digestion and in probing all states (e.g., solid, solution, gaseous) of the selenium in the sample.

Se K near-edge spectra were collected on two lentil cultivars from four locations, and two of the spectra are shown in **Figure 3**. The low selenium concentrations (below 300 ppb) in the lentils are generally considered to be challenging concentrations for XAS measurements. Nevertheless, the Se near-edge spectrum is clearly identified in data averaged from two sweeps collected over approximately 40 min. The spectra of the lentil samples show a maximum intensity at 12662.8 eV, corresponding to organic selenium and an additional, higher energy peak whose intensity varies in different lentil samples. Principal component analysis indicated that two components are needed to fit the set of spectra from each location (data not shown). Target

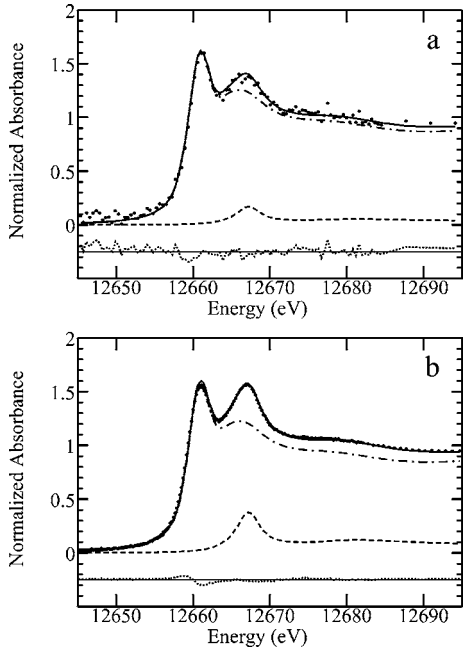

Figure 3. Se K near-edge X-ray absorption spectra of (a) CDC Redberry lentils from Davidson and (b) CDC Robin lentils from Saskatoon (Saskatchewan, Canada). Total selenium contents are (a) 290 and (b) 720 μg kg^{-1}. Compositions determined from the least-squares fits are (a) 5 ± 2% selenate and (b) 10 ± 2% selenate, with the balance modeled as selenomethionine. The figure shows background-subtracted and normalized data as filled circles, the best fit from the least-squares fit as a solid black line, and the fit residual as a dotted line offset below. Components of the fit, selenomethionine (dotted–dashed line) and selenate (dashed line), are scaled according to their contributions to the fit.

Table 2. Percentage Composition of Selenium Species in Field-Grown Saskatchewan Lentils

	CDC Robin		CDC Redberry	
	percentage of Se[a] as		percentage of Se[a] as	
location	organic selenide[b]	selenate	organic selenide[b]	selenate
Saskatoon	90 ± 2	10 ± 2	90 ± 2	10 ± 2
Davidson	94 ± 1	6 ± 1	95 ± 2	5 ± 2
Wilkie	90 ± 2	10 ± 2	90 ± 1	10 ± 1
Swift Current	95 ± 2	5 ± 2	86 ± 2	14 ± 2

[a] Selenium speciation as determined using XAS near-edge least-squares fitting. Errors are 3 times the estimated standard deviation derived from the variance–covariance matrix. [b] Organic selenide (CH_3SeCH_2–) is modeled as aqueous selenomethionine.

transformation was used to test whether a particular standard spectrum was a component of a spectrum of a mixture and showed that aqueous selenate and organic selenium modeled as selenomethionine are the major chemical forms of selenium present in field-grown lentils from Saskatchewan.

The relative content of selenium chemical forms in the seeds was determined by fitting the seed near-edge spectra to the spectra of selected selenium standard species (as **Figure 2**). Numerical results are shown in **Table 2**, and **Figure 3** shows the results of the least-squares fitting of two of the lentil samples showing different selenate contents. Our results clearly indicated that the field-grown lentils contain predominately (86−95%) organic selenium similar to selenomethionine or *Se*-methylselenocysteine with a variably minor component of selenate (5−14%); other forms such as selenite and trimethylselenonium

were not significant. While variations in the percentage of selenate are observed among the measured samples, within our limited data set these small variations do not appear to correlate with the cultivar, location, or total selenium content.

Whole-Food Solution. Selenium is an essential micronutrient which has protection against several cancers. Lentils are well-known for their health benefits in providing several essential dietary components (*31*). Our results show that lentils grown in the Canadian prairies are additionally enriched in selenium. There is some variation in the total selenium concentration of the lentil seed, probably reflecting variability in available soil selenium concentrations in Canadian prairies (**Table 1**). Saskatchewan soils are naturally rich in selenium (>1 ppm), and there is a unique potential for Se-rich pulses to be grown in Canada even without soil supplementation, although in selenium-deficient growing regions the concentration of Se in the seed can be increased by application of selenium to the growing crop or to the soil (*32*). The amount of selenium found in the whole Saskatchewan lentil seed (0.16−0.72 ppm) would be equivalent to 16−72 μg of selenium in a 100 g portion of dry lentils, providing 29−130% of the recommended daily amount (55 μg per day) for an adult (*33*). Prior to consumption, most red lentils are decorticated and are then prepared in food dishes in whole or split form. The decortication process would likely enrich the selenium content of the consumed lentils by a further ca. 10−15% by removal of the seed coat, which is mostly cellulose fiber (*34*).

Selenium intake in humans is mainly determined by the level of available selenium in the soil in which their food is grown and by dietary composition. Total selenium levels in major food classes in North America occur within the following ranges: 100−600 μg kg^{-1} (fish); 50−600 μg kg^{-1} (cereals); 50−300 μg kg^{-1} (red meat); 2−8 μg kg^{-1} (fruits and vegetables) (*35*). Our results from lentils grown in Canadian prairies (160−720 μg kg^{-1} of dry weight) show that these are an excellent source of organic selenium and are considerably richer in selenium than lentils grown in other locations such as southern India (30 μg kg^{-1} of dry weight), America (83 μg kg^{-1} of dry weight), and the Slovak Republic (28 μg kg^{-1}) (*36*−*38*). The fact that Saskatchewan lentils are rich in organic selenium means that they could provide an excellent whole-food natural source of selenium for Europe (*39*), in addition to a possible solution to the arsenic problem in Bangladesh.

Our preliminary results indicate that selenium in field-harvested lentils is partitioned between an organic form similar to selenomethionine or *Se*-methylselenocysteine and a small fraction of selenate. Selenomethionine is the major organic form of selenium found in wheat kernel, ranging from 45% to 81% of the total selenium in wheat kernels (*40*). The nutritional and toxicological aspects of selenomethionine have been comprehensively reviewed (*41*). *Se*-methylselenocysteine is the predominant form of selenium found in the selenium hyperaccumulator *Astragalus bisulcatus* (*42*, *27*), which, like lentil, is a member of the Fabaceae family. *A. bisulcatus* uptakes as much as 0.65% of its shoot dry biomass as selenium from inorganic selenium naturally present in soil (*43*). The major selenium-containing compound in the leaves is *Se*-methylselenocysteine, with smaller amounts of selenocystathione and γ-glutamyl-*Se*-methylselenocysteine localized in the seed pods (*42*). *Se*-Methylselenocysteine and its γ-glutamyl conjugate are also found in foods such as garlic (*44*) and are among compounds that have been suggested to have anticancer properties (*2*). A complete understanding of the biochemistry of selenium in lentil will require more in-depth biochemical and physiological studies. Future work will investigate the biochemistry and cellular location of the selenium chemical form in lentils by using XAS imaging techniques (*1*) as well as a determination of the exact form of organic selenium in the lentil seeds.

In summary, our results show that lentils grown in Saskatchewan fields are a naturally rich source of organic selenium. Since certain forms of selenium have an antagonistic effect on the toxicity of arsenic, and since selenium accumulation is demonstrated in lentil cultivars favored by the Bangladesh market, it is possible that Se-rich lentils could be a whole-food solution to the arsenicosis in Bangladesh.

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LITERATURE CITED

(1) Behne, D.; Kyriakopoulos, A. Mammalian selenium-containing proteins. *Annu. Rev. Nutr.* **2001**, *21*, 453−473.

(2) Clark, L. C.; Dalkin, B.; Krongrad, A.; Combs, G. F., Jr.; Turnbull, B. W.; Slate, E. H.; Witherington, R.; Herlong, J. H.; Janosko, E.; Carpenter, D.; Borosso, C.; Falk, S.; Rounder, J. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. *Br. J. Urol.* **1998**, *81*, 730−734.

(3) Kune, G.; Watson, L. Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B6, B12, C, E, selenium, and lycopene. *Nutr. Cancer* **2006**, *56*, 11−21.

(4) Della Rovere, F.; Granata, A.; Familiari, D.; Zirilli, A.; Cimino, F.; Tomaino, A. Histamine and selenium in lung cancer. *Anticancer Res.* **2006**, *26*, 2937−2942.

(5) Grau, M. V.; Rees, J. R.; Baron, J. A. Chemoprevention in gastrointestinal cancers: current status. *Basic Clin. Pharmacol. Toxicol.* **2006**, *98*, 281−287.

(6) Gailer, J.; George, G. N.; Pickering, I. J.; Prince, R. C.; Ringwald, S. C.; Pemberton, J. E.; Glass, R. S.; Younis, H. S.; DeYoung, D. W.; Aposhian, H. V. A Metabolic Link Between Arsenite and Selenite: The Seleno-bis(S-glutathionyl) Arsinium Ion. *J. Am. Chem. Soc.* **2000**, *122*, 4637−4639.

(7) Manley, S.; George, G. N.; Pickering, I. J.; Prince, R. C.; Glass, R. S.; Prenner, E. J.; Yamdagni, R.; Wu, Q.; Gailer, J.; The Seleno-bis(S-glutathionyl) Arsinium Ion is Assembled in Erythrocyte Lysate. *Chem. Res. Toxicol.* **2006**, *19*, 601−607.

(8) Gailer, J.; George, G. N.; Pickering, I. J.; Madden, S.; Prince, R. C.; Yu, E. Y.; Denton, M. B.; Younis, H. S.; Aposhian, H. V. The Structural Basis of the Antagonism between Inorganic Mercury and Selenium in Mammals. *Chem. Res. Toxicol.* **2000**, *13*, 1135−1142.

(9) Merali, Z.; Singhal, R. L. Protective effect of selenium on certain hepatotoxic and pancreotoxic manifestations of subacute cadmium administration. *J. Pharmacol. Exp. Ther.* **1975**, *195*, 58−66.

(10) Gailer, J.; George, G. N.; Pickering, I. J.; Prince, R. C.; Younis, H. S.; Winzerling, J. J. Biliary Excretion of [(GS)2AsSe]- after Intravenous Injection of Rabbits with Arsenite and Selenite. *Chem. Res. Toxicol.* **2002**, *15*, 1466−1471.

(11) Dhar, R. Kr.; Biswas, B. Kr.; Samanta, G.; Mandal, B. Kr.; Chakraborti, D.; Roy, S.; Jafar, A.; Islam, A.; Ara, G.; Kabir, S.; Wadud, Khan, A.; Akther Ahmed, S.; Abdul, Hadi, S. Groundwater arsenic calamity in Bangladesh. *Curr. Sci.* **1997**, *73*, 48−59.

(12) Spallholz, J. E.; Boylan, L. M.; Ruaman, M. M. Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Sci. Total Environ.* **2004**, *323*, 21−32.

(13) http://www.bangladesh-selenium.org/.
(14) Chen, Y.; Hall, M.; Graziano, J. H.; Slavkovich, V.; van Geen, A.; Parvez, F.; Ahsan, H. A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 207−213.
(15) Ullrey, D. E.; Brown, W. L. *Selenium in nutrition*, revised edition; National Academy Press: Washington, DC, 1983.
(16) Vandenberg, A.; Banniza, S.; Warkentin, T. D.; Ife, S.; Barlow, B.; McHale, S.; Brolley, B.; Gan, Y.; McDonald, C.; Bandara, M.; Dueck, S. CDC Redberry lentil. *Can. J. Plant Sci.* **2006**, *86*, 497−498.
(17) Vandenberg, A.; Kiehn, F. A.; Vera, C.; Gaudiel, R.; Buchwaldt, L.; Dueck, S.; Wahab, J.; Slinkard, A. E. CDC Robin lentil. *Can. J. Plant Sci.* **2002**, *82*, 111−112.
(18) Cramer, S. P.; Tench, O.; Yocum, M.; George, G. N. A 13-element Ge detector for fluorescence EXAFS. *Nucl. Instrum. Methods Phys. Res., A* **1988**, *266*, 586−591.
(19) Hope, H. The crystal structure of trimethylselenonium iodide, $(CH_3)_3SeI$. *Acta Crystallogr.* **1966**, *20*, 610−613.
(20) Pickering, I. J.; Brown, G. E., Jr.; Tokunaga, T. K. Quantitative speciation of selenium in soils using X-ray absorption spectroscopy. *Environ. Sci. Technol.* **1995**, *29*, 2456−2459.
(21) Lintschinger, J.; Fuchs, N.; Moser, J.; Kuennelt, D.; Goessler, W. Selenium-enriched sprouts. A raw material for fortified cereal-based diets. *J. Agric. Food Chem.* **2000**, *48*, 5362−5368.
(22) SAS Institute Inc. *SAS user's guide: statistics 2005*, version 9; Cary, NC, 2005.
(23) Pickering, I. J.; George, N. G.; Fleet-Stalder, V. V.; Chasteen, T. G.; Prince, R. C. X-ray absorption spectroscopy of selenium-containing amino acids. *J. Biol. Inorg. Chem.* **1999**, *4*, 791−794.
(24) Buchanan, B. B.; Bucher, J. J.; Carlson, D. E.; Edelstein, N. M.; Hudson, E. A.; Kaltsoyannis, N.; Leighton, T.; Lukens, W.; Shuh, D. K.; Nitsche, H.; Reich, T.; Roberts, K.; Torretto, P.; Woicik, J.; Yang, W.-S.; Yee, A.; Yeela, B. C. A XANES and EXAFS investigation of the speciation of selenite following bacterial metabolization. *Inorg. Chem.* **1995**, *34* (6), 1617−1619.
(25) Van Fleet-Stalder, V.; Chasteen, T. G.; Pickering, I. J.; George, G. N.; Prince, R. C. Fate of selenate and selenite metabolized by Rhodobacter sphaeroides. *Appl. Environ. Microbiol.* **2000**, *66* (11), 4849−4853.
(26) de Souza, M. P.; Pilon-Smits, E. A. H.; Lytle, C. M.; Hwang, S.; Tai, J.; Honma, T. S. U.; Yeh, L.; Terry, N. Rate-limiting steps in selenium assimilation and volatilization by Indian Mustard. *Plant Physiol.* **1998**, *117*, 1487−1494.
(27) Pickering, I. J.; Prince, R. C.; Salt, D. E.; George, G. N. Quantitative chemically-specific imaging of selenium transformation in plants. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10717−10722.
(28) Freeman, J. L.; Zhang, L. H.; Marcus, M. A.; Fakra, S.; McGrath, S. P.; Pilon-Smits, E. A. H. Spatial imaging, speciation, and quantification of selenium in the Hyperaccumulator Plants *Astragalus bisulcatus* and *Stanleya pinnata*. *Plant Physiol.* **2006**, *142*, 124−134.
(29) Vickerman, D. B.; Trumble, J. T.; George, G. N.; Pickering, I. J.; Nichol, H. Selenium biotransformations in an insect ecosystem: effects of insects on phytoremediation. *Environ. Sci. Technol.* **2004**, *38*, 3581−3586.
(30) Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium speciation from food source to metabolites: a critical review. *Anal. Bioanal. Chem.* **2006**, *385*, 1304−1323.
(31) Wang, N.; Daun, K. J. Effects of variety and crude protein content on nutrient and anti-nutrients in lentils (*Lens culinaris*). *Food Chem.* **2006**, *95*, 493−502.
(32) Doyle, P. S.; Fletche, W. K. Influence of soil parent material on the selenium content of wheat from west central Saskatchewan. *Can. J. Plant Sci.* **1977**, *3*, 859−864.
(33) Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids*; National Academy Press: Washington, DC, 2000.
(34) McDonald, M. B. In *Physiology and Determination of Crop Yield*; Boote, K. L., Bennett, J. M., Sinclair, T. R., Paulsen, G. M., Eds.; American Society of Agronomy: Madison, WI, 1995; pp 37−60.
(35) Lyons, G.; Stangoulis, J.; Graham, R. High-selenium wheat: biofortification for better health. *Nutr. Res. Rev.* **2003**, *16*, 45−60.
(36) Srikumar, T. S. The mineral and trace element composition of vegetables, pulses and cereals of southern India. *Food Chem.* **1993**, *46*, 163−167.
(37) *USDA National Nutrient Database for Standard Reference*, release 18; USDA: Washington, DC, 2006.
(38) Kadrabova, J.; Madaric, A.; Ginter, E. The selection content of selected food from Slovak Republic. *Food Chem.* **1997**, *58*, 29−32.
(39) Kellen, E.; Zeegers, M.; Buntinx, F. Selenium is inversely associated with bladder cancer risk: A report from the Belgian case-control study on bladder cancer. *Int. J. Urol.* **2006**, *13*, 1180−1184.
(40) Lyons, G. H.; Gene, Y.; Stangoulis, J. C. R.; Palmer, L. T.; Graham, R. D. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. *Biol. Trace Elem. Res.* **2005**, *103*, 155−168.
(41) Schrauzer, G. N. The nutritional significance, metabolism and toxicology of selenomethionine. *Adv. Food Nutr. Res.* **2003**, *47*, 73−112.
(42) Nigam, S. N.; McConnel, W. B. Seleno amino compounds from *Astragalus bisculcatus*. Isolation and identification of gamma-L-glutamyl-Se-methyl-seleno-L-cysteine and Se-methylseleno-L-cysteine. *Biochim. Biophys. Acta* **1969**, *192*, 185−190.
(43) Trelease, S. E.; Di Somma, A. A.; Jacobs, A. L. Seleno-amino acid found in *Astragalus bisulcatus*. *Science* **1960**, *132*, 618.
(44) Cai, X.; Block, E.; Uden, C. P.; Quimby, D. B.; Sullivan, J. J.; Allium chemistry : Identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas chromatograph with atomic emission detection. *J. Agric. Food Chem.* **1995**, *43*, 1754−1757.

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