Selenium-Enriched Sprouts. A Raw Material for Fortified Cereal-Based Diets

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The selenium supply in almost all European countries, including Austria and Germany, is below the recommended daily intake. In these countries, selenium fortification of foods and the use of selenium supplements are quite popular to compensate for low Se intake from diets. In general, wheat (*Triticum aestivum*) is known to be a good source for bioavailable selenium, and many studies have been performed to enrich selenium in wheat by selenium fertilization of the soil. In the present work, the process of sprouting was investigated as an alternative to enrich selenium in wheat. Sprouting was chosen because it additionally improves the nutritional value of seeds, for example, by a higher vitamin content, a better quality of protein, and some other parameters. Wheat, alfalfa (*Medicago sativa*), and sunflower (*Helianthus annuus*) seeds were germinated for 5 and 7 days in solutions containing selenate. The selenium sensitivity of the sprouts was tested by measuring visible germination levels and seedling development. Uptake rates were studied by determination of total selenium using inductively coupled plasma mass spectrometry (ICP-MS). Metabolism of the absorbed selenium was analyzed by determination of selenium species in extracts of the sprouts using anion exchange HPLC coupled to ICP-MS. It was shown that sunflower sprouts were the most resistant and had the highest uptake rates (up to 900 mg/kg), but almost 100% of the selenium was extracted with water and found to be nonmetabolized selenate. Wheat and alfalfa were less resistant and enriched selenium up to concentrations of 100 and 150 mg of Se/kg of dry mass, respectively. The metabolism of the selenate was inversely related to the total uptake rates. At low Se enrichment (∼1−2 mg of Se/kg), <20% of the total selenium content within the sprouts remained as inorganic selenium, indicating a high metabolism rate. With increasing uptake the amount of selenate increased to ∼40−50%. However, with the method used it is possible to produce sprouts containing certain amounts of selenium, which might provide substantial proportions of bioavailable selenium. In combination with the generally high nutritional value of sprouts, they might serve for production of improved cereal-based diets.

Keywords: *Selenium; sprouts; bioavailability; speciation*

INTRODUCTION

The amount of selenium in our diets depends to a great extent on the selenium concentration of the soil. The large variances of soil selenium concentrations among different countries cause significant differences in the regional selenium content of the food and consequently in the daily selenium intakes, for example, 30 μg/day in Turkey, 55 μg/day in Germany, and 130 μg/day in Japan (Kumpulainen, 1993; Schelenz, 1984). The adequate physiological Se supply was estimated as 1 μg of Se/kg of body weight, representing about 57 and 80 μg/day for median female and male persons, respectively (Levander and Morris, 1984). In Austria, selenium intake ranges from 36 to 68 (mean = 49) μg/day, indicating a marginally insufficient supply (Wilplinger et al., 1998). Very low serum selenium levels were found in healthy children and adults living in Styria (area in southeastern Austria), showing that this area is one of the European areas with the lowest selenium supply (Tiran et al., 1992). Almost all other European countries also belong to low-selenium regions, especially Scandinavia. As a consequence, field treatment with selenium or direct supplementation of food and fodder is used to improve the Se nutrition of livestock and people. In Finland, commercial fertilizers have been enriched with sodium selenate since 1984 to compensate for the poor selenium content of the soil. The progress of this ongoing large scale study was recently summarized by Aro et al. (1998). Due to selenium fertilization the concentration of selenium in corn and bread increased by a factor of 20−30 (Eurola et al., 1990), and an increase of the serum selenium level of healthy young adults from 1.05 to 1.6 μmol of Se /L has been documented (Aro et al., 1998; Makela et al., 1993). Nevertheless, some problems are discussed with the selenium fertilization practice such as uncontrolled over-uptake, which might result in toxic concentrations in the corn, possible environmental problems, and high costs (Makela et al., 1995).

In addition to the total selenium concentration of our diets, the Se status of individuals is affected by the chemical form of the ingested selenium. The type of selenium species is a primary determinant of its absorption and subsequent utilization, called bioavailability (Fairweather-Tait, 1997). There are conflicting reports

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of the bioavailability of selenium, recently reviewed by other authors (Fairweather-Tait, 1997; Daniels, 1996). Looking upon individual selenium compounds, studies in humans have shown that organic selenomethionine is absorbed and retained more efficiently than selenate and selenite (Butler et al., 1991; Moser-Veillon et al., 1992; Clausen and Nielsen, 1988). Absorption ratios alone are not enough to predict bioavailability, because physiological effects include also the conversion of the absorbed species into the active compound. It was shown that although selenite and selenate are less absorbed than selenomethionine and organically bound selenium from foods, they are equally or even more effective in raising glutathione peroxidase activity, which is an indicator widely used to estimate Se bioavailability (Clausen and Nielsen, 1988; Levander et al., 1983; Alfthan et al., 1991; Thomson et al., 1993; Persson-Moschos et al., 1998). However, organic Se maintained higher postsupplementation levels than inorganic Se.

The major forms of selenium in foods are probably the amino acids selenomethionine and selenocysteine bound to proteins. Selenomethionine is presumably the prevalent form in diets from plant sources, whereas proteins from animal tissue contain both amino acids in various proportions. Inorganic selenium species are used in supplements, but it is not likely that they are present in food. According to Coombs, food sources of selenium can be characterized as follows: good sources include selenium-enriched yeast and wheat, moderate sources include most plant materials, and poor sources are most meat and fish products and soybean (Coombs, 1988). Unfortunately, in most low-selenium countries the main amount of the daily selenium intake originates from meat products (Aro et al., 1998; Coombs, 1988; Oster and Prellwitz, 1988). Hence, the poor selenium availability from these products might additionally increase the problem of low selenium intake in those countries. Apart from meat, bread and cereals are the second most abundant, but probably more effective, sources for dietary selenium. Several studies show that selenium-rich wheat products are able to significantly enhance selenium blood levels and glutathione peroxidase activity (Levander et al., 1983; Jaakkola et al., 1983; Finzel et al., 1994; Barclay and MacPherson, 1992). In Norway, serum selenium levels were found to be among the highest in Europe, despite a very low daily intake (Meltzer et al., 1990). A study by Meltzer et al. indicated that wheat Se, originating from the importation of Se-rich wheat, is the main determinant of blood Se levels in Norway (Meltzer et al., 1992). Hence, the good bioavailability of Se from wheat might be a possible explanation for the high Se blood values in Norway.

Because of the good nutritional source for selenium from wheat, the present study investigates the process of germination for its applicability to produce Se-enriched wheat sprouts containing bioavailable selenium. These sprouts might then be used directly for food or for supplementation of various diets. Wheat and, for some reasons, also alfalfa and sunflower seeds were germinated in solutions containing inorganic Se(VI). The influence of the selenium solution on sprout development and the percentage of uptake and conversion were studied in detail. Sprouting was used because it additionally improves the nutritional value of grains due to a better quality of protein, a more favorable distribution of amino acids, a higher content of polyunsaturated fatty acids, a higher content of vitamins, and a reduced content of antinutritional factors (Lintschinger et al., 1997; Harmuth-Hoene et al., 1987; Chavan and Kadam, 1989; Merx et al., 1994).

MATERIALS AND METHODS

Germination and Sample Preparation. The germination of wheat (*Triticum aestivum*), alfalfa (*Medicago sativa*), and sunflower (*Helianthus annuus*) seeds was performed with tap water and with different solutions containing between 0.78 and 800 mg of Se/L in the form of sodium selenate (analytical grade). Selenate was given preference over selenite because it is not reduced to elemental Se in the culture solution. Prior to germination, the seeds (~90 g) were soaked for 12 h in the corresponding solutions (800 mL). For sprouting, the seeds were placed into commercial germination bowls (Biosnacky), equipped with a drain and a plastic lattice at the bottom of the bowl to keep the seeds at a constant moisture without overwatering them. Germination was accomplished at ambient room temperature (19−21 °C) and under the prevailing light and dark conditions during the day and night, respectively. The total germination period (including the time for soaking) was 96 h. For some experiments the period was extended to 168 h. During germination, the sprouts were rinsed two times each day with 250 mL of the corresponding solutions. After harvesting, all sprouts were washed carefully with deionized water (3 × 800 mL) to exclude contamination of the surface of the sprouts by the culture solution. Visible germination rates and seedling development were determined by measuring the length of the bud. After that, the sprouts were put into plastic packs and stored at −18 °C in a deep freezer. The whole amount of all samples was freeze-dried (Christ Alfa 1-4; LDC-1M temperature controller). Afterward, the dry samples were ground in a contamination-free analytical mill (Retsch ZM 1000 with titanium rotor and sieve). The concentration- and time-dependent germination experiments were performed in triplicates and duplicates, respectively

Determination of Total Selenium Concentrations. The total selenium concentrations were determined from the whole sprouts including the original seed, the bud, and roots. All chemicals used were of analytical grade or higher purity. Water was purified by a cartridge deionization system (Millipore, Milli Q 18.2 MΩ cm). Mineralization of the ground samples (200 mg) was performed using an MLS, 1200 Mega (Milestone, Leutkirch, Germany) microwave digestion system with a mixture of concentrated nitric acid (3 mL), purified by sub-boiling distillation, and high-purity hydrogen peroxide (0.5 mL; 30%, Merck Suprapur). The procedure was optimized for the digestion of plant materials and included the following microwave power program: 2 min, 250 W; 0.5 min, 0 W; 10 min, 250 W; 0.5 min, 0 W; 6 min, 450 W; 0.5 min, 0 W; 7 min, 600 W; and 1 min, 500 W (Lintschinger et al., 1997). After digestion, the sample solution was transferred into a polypropylene bottle and diluted with water to a defined volume. From each sample two digestions were carried out and the total selenium concentrations were determined by using an inductively coupled plasma mass spectrometer (ICP-MS, HP 4500). For validation of the digestion and determination methods a standard reference material (NIST SRM wheat gluten) representing a similar matrix was analyzed simultaneously.

Extraction and Speciation. Extraction of the sprouts was performed in duplicates by adding separately 25 mL of H_2O, HCl (0.1 M), NaOH (0.1 M), or a solution containing nonspecific protease (Sigma P5147) to 500 mg of ground dry sample and shaking the mixture for 16 h at room temperature. The enzymatic digestion was performed at 37 °C and a pH of 7.5 (phosphate citric acid buffer). The extracts were centrifuged, and the supernatant was filtered through a 0.22 μm filter. This solution was used directly for HPLC determinations; a 2 mL aliquot of the same solution was digested, and the total selenium concentration was measured using the above procedure. The concentrations of extracted selenium species were determined using an anion exchange high-performance liquid

chromatographic system (HP 1100) coupled directly to the ICP-MS (HPLC-ICP-MS) as an element specific detector. The compounds were separated on a polymer-based anion exchange column (Hamilton PRP-X100) using an aqueous mobile phase containing 10 mM citric acid and 2% methanol (v/v) at a pH of 5.0 (adjusted with an aqueous ammonium hydroxide solution). The ion intensities at *m*/*z* 77, 78, and 82 were monitored. Identification and quantification occurred with *m*/*z* 82 by external calibration curves using standard solutions of sodium selenite, selenate, selenomethionine, and selenocysteine. *m*/*z* 77 and 78 were monitored only to prove possible interferences by using isotopic ratios. Experimental details are published elsewhere (Zheng et al., 1998).

RESULTS AND DISCUSSION

Selenium Sensitivity of Seeds. It is well-known that selenium is toxic to most plants except a few Se accumulators, such as *Astragalus* species, which are able to accumulate selenium up to several thousand milligrams per kilogram of dry mass (Brown and Shrift, 1982; Läuchli, 1993; Wu et al., 1997). Crop plants are non Se accumulators and tolerate only a few milligrams per kilogram of Se in the soil. However, it was shown that during germination, seeds are not significantly affected by high selenium levels (Levine, 1925; Spenger and Siegel, 1978; Carlson et al., 1989). Carlson et al. (1989) found that selenium in the form of selenate or selenite did not affect germination of various agronomic species at concentrations up to 32 mg of Se /L. Of the various tested species, radish and wheat were the least sensitive to selenium treatment.

In this study the selenium tolerance of various other crop seeds was screened in a preliminary experiment to learn which species were appropriate for selenium enrichment. The seeds of alfalfa, barely, millet, oat, pea, rye, sunflower, and wheat were germinated in tap water and in a solution containing 50 mg of Se as selenate/L. After 5 days of germination, it was demonstrated that sunflower seeds were the most resistant to applied selenium. There were no differences in germination capacity and lengthwise growth of buds and roots between the sprouts from the tap water and those from the selenium solution. The growth of wheat and alfalfa was slightly reduced, but viability was not influenced. All other seeds showed a significant reduction of visible germination rates and seedling development in the presence of selenium. Hence, in addition to wheat, sunflower and alfalfa were used for further dose−response experiments. They were germinated with increasing selenium concentrations for 5 days to determine highest Se tolerance. Alfalfa and wheat remained vigorous and grew for the whole period up to concentrations of 100 and 200 mg Se/L, respectively. The sunflower sprouts were still growing in solutions containing 800 mg of Se/L. Above these threshold levels the degree of viability was reduced and most sprouts died after the second or third day of sprouting.

Uptake of Selenate. The uptake rates were determined by measuring the total Se concentration in the sprouts after 5 days of germination. Surface contamination was excluded by the fact that sprouts contained the same Se concentration before and after the last washing step and no selenium was detected in the last wash solution (detection limit = 0.2 μg/L, 3σ blank).

In Figure 1 the total selenium concentrations of the sunflower, alfalfa, and wheat seeds as a function of the concentration of the applied Se solution are presented. The concentrations represent the mean values of three separate batches sprouted under the same conditions and two determinations of each batch. The relative differences between two determinations were <11% for all samples, and the relative standard deviations (RSDs) of the three batches were within these deviations. Due to their increased selenium tolerance the sunflower seeds were sprouted in much higher selenium solutions than alfalfa and wheat, which resulted in very high enrichment. The sunflower sprouts contained Se concentrations up to 900 mg of Se/kg of dry mass. Enrichment in alfalfa and wheat due to the lower applied selenium concentration was reduced. Accumulation in alfalfa was better than in wheat sprouts. Comparison of the sprouts from the same solution (100 mg of Se/L) revealed total concentrations in alfalfa and wheat sprouts of approximately 150 and 65 mg/kg of dry mass, respectively. However, as mentioned above, wheat was less sensitive to the applied selenium and could be sprouted in solutions up to 200 mg of Se/L, which resulted in an ultimate enrichment of ~100 mg Se/kg. All three species showed almost linear uptake with the Se concentrations in the culture solution, with a slight decrease at high concentrations. Hence, high enrichment is not restrained by any saturation phenomenon but limited by the lethal Se concentration.

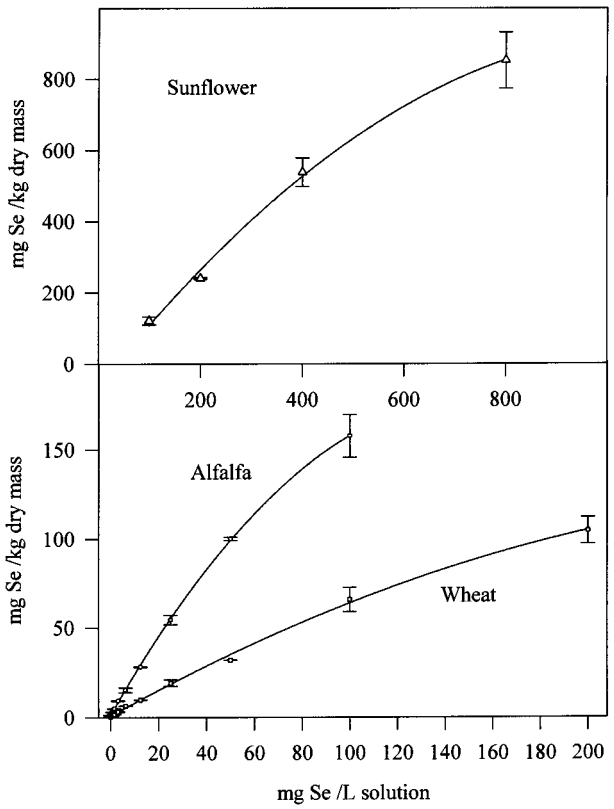

Figure 1. Total selenium concentration in wheat, alfalfa, and sunflower sprouts after 5 days of sprouting as a function of the selenium concentration in the culture solution. The concentrations represent the mean values of three separate batches sprouted under the same conditions and two determinations of each batch. The relative differences between two determinations were for all samples <11%, and the RSDs of the three batches were within these deviations.

Figure 2 shows the time-dependent Se uptake during the sprouting of alfalfa and wheat using the highest possible selenium solutions. Only small amounts (6−8%) of the total selenium were transferred into the seeds with the imbibition of water during the soaking period.

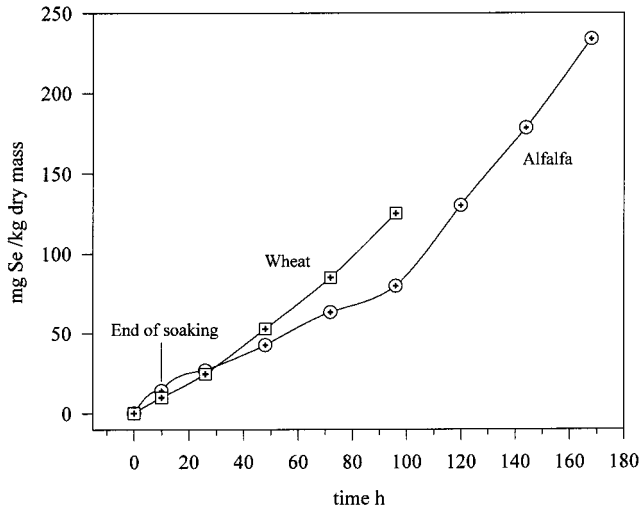

Figure 2. Time-dependent selenium uptake during sprouting of alfalfa and wheat with solutions containing 100 and 200 mg of Se/L, respectively.

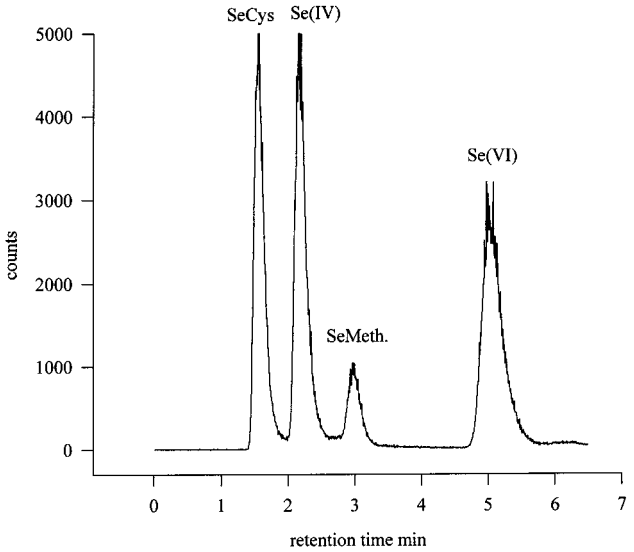

Figure 3. Chromatogram of a solution containing selenate, selenite, selenomethionine, and selenocysteine (1 mg of Se/L each) using anion exchange HPLC coupled to ICP-MS [experimental parameters: column, Hamilton PRP-X100; mobile phase, 10 mM citric acid, 2% methanol (v/v); pH of 5.0 adjusted with an aqueous ammonium hydroxide solution; flow rate, 1 mL/min].

In general, the imbibition is the essential initial step toward germination. The driving force for this uptake is the water potential gradient between the seed and its surrounding. At this stage the selenate might pass with the imbibing water through the testa and aleurone layer into the seeds and consequently also into the cells. This might happen because it is well-known that during soaking, many components from within the cells of seeds such as K, Mg, Ca, sugars, proteins, and organic acids are leaked into the environment (Bewley and Black, 1994). Hence, the selectively permeable membranes of the tonoplast and plasmalemma that normally retain solutes within the cells lose their integrity during drying and do not act as retentive barriers during the initial stage of imbibition. Because of this leakiness of the membrane, the selenate might penetrate from the interspace of the seeds into the cells of the endosperm and embryo.

When germination is finished and the radicles are developed, a different uptake process occurs. Inorganic ions are transported into the apparent free space of the apoplasts of the roots and are then incorporated into the cells by different pathways. For selenate the uptake into the cells occurs most probably through the pathways of sulfate, which was demonstrated by a competitive inhibition of sulfate uptake by selenate (Läuchli, 1993). In previous work, when solutions with lower Se concentrations (5 mg of Se/L) were used, it was shown that selenium accumulation in wheat sprouts increased proportionally with the time of sprouting (4 days observed) (Lintschinger et al., 1997). Using high-selenium solutions (100 and 200 mg of Se as selenate/L), the same tendencies in the uptake curves are observed but the exponential increase is reduced. However, unlike the uptake of other trace elements such as Fe, Zn, Cr, Cu, and Co, of which large amounts are incorporated during soaking (Lintschinger et al., 1997), selenium accumulation is more affected by root uptake than by imbibition. Hence, long germination periods and excellent root development are required for proper enrichment. Conversely, germination times $>3-4$ days result in extensive microbial growth. In the present experiments, hygienic conditions were improved by preselection of low microbiologically contaminated seeds and by rinsing all equipment with ethanol prior to use. Hence, sprouting for up to 5 days for wheat and 7 days for alflalfa was possible without significant growth of fungus, but total microbial contamination was still a serious problem that has to be solved prior to utilization of the sprouts in human food.

Availability and Characterization of Selenium Compounds. Sprouts were extracted with different solvents to obtain information about the availability and the chemical form of selenium present within the sprouts. The extracts were filtered through 0.22 μm filters, and aliquots were analyzed for their total selenium concentrations in order to estimate roughly the easily available amount. As well as the total concentrations, the extracted selenium was characterized using anion exchange HPLC-ICP-MS. For identification, standard solutions containing inorganic Se(IV) and Se(VI), selenomethionine, and selenocysteine were used. Figure 3 represents a chromatogram of a solution containing 1 mg of Se/L of each compound. The principal procedure is described elsewhere (Zheng et al., 1998) and was optimized for the special matrix of the sprout extracts.

All sprouts germinated with different selenium solutions were extracted with water. It was found that, depending on the species and the total concentration, large amounts of selenium were readily available using only water as extraction solvent. About 74 and 69% of the total selenium were extracted with water from alfalfa and wheat sprouts with highest enrichment, representing in Figures 4 and 5 the sum of Se(IV)/(VI) and "soluble not identified" selenium. With decreasing total concentrations the extraction ratios of alfalfa and wheat sprouts decreased significantly to approximately 40% (alfalfa) and 25% (wheat). Conversely, the extraction ratios for sunflower sprouts were independent of the amount of applied selenium and found to be almost 100% for all batches.

The main species in the aqueous extracts was identified as the supplied selenate and quantified by using external calibration curves (Figure 6). Besides Se(VI),

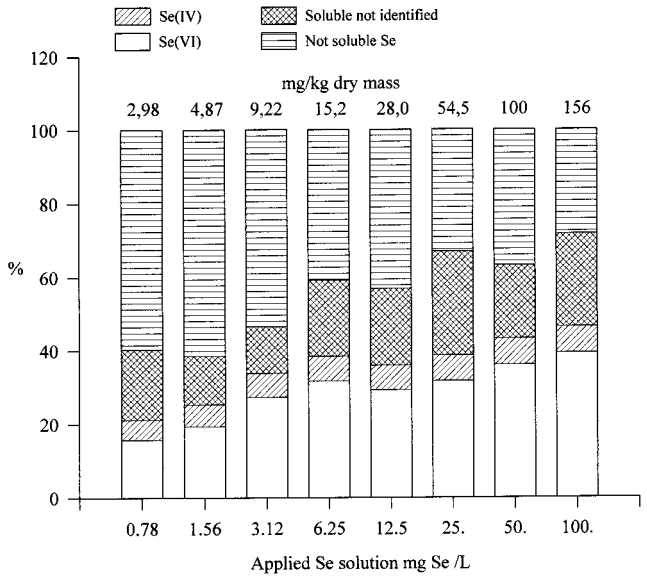

Figure 4. Distribution of selenium species in alfalfa sprouts after 5 days as a function of the concentration of selenium in the culture solution. Concentrations represent the mean values of two determinations from each sample batch; differences were <11%. Total concentrations are indicated on the tops of the bars; Se(IV) and Se(VI) were determined using HPLC-ICP-MS; "not soluble selenium" indicates the difference of the total concentration of the dry sample and water extract; "soluble but not identified" is the amount calculated from the difference of the quantitative chromatographic results and the total concentration of the extract.

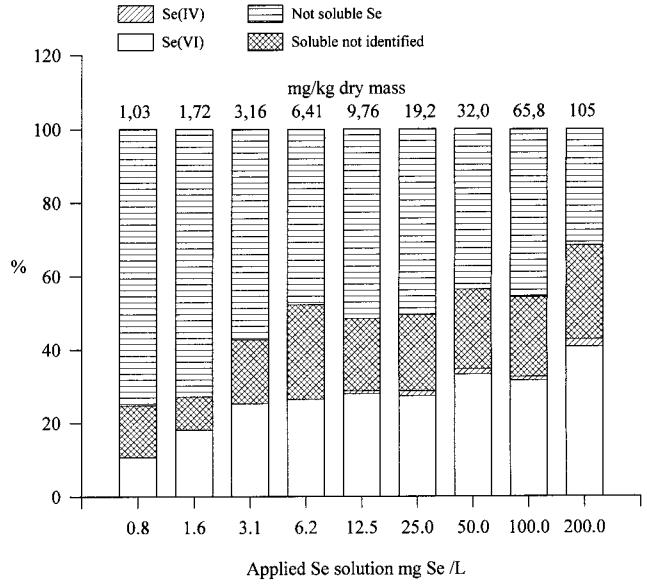

Figure 5. Distribution of selenium species in wheat sprouts after 5 days of sprouting as a function of the concentration of selenium in the culture solution. For details, see Figure 4.

small amounts of selenite were also found and quantified in all extracts. The extracts of sunflower and wheat but not alfalfa sprouts contained, additionally, traces of selenomethionine and other unidentified species. From these concentrations, together with the results of total analyses, a distribution pattern of the form of selenium within the alfalfa and wheat sprouts is drawn (Figures 4 and 5). The difference between the total concentration of the whole sprouts and the water extract was proclaimed as bound selenium. From the selenium extracted with water a certain amount could not be

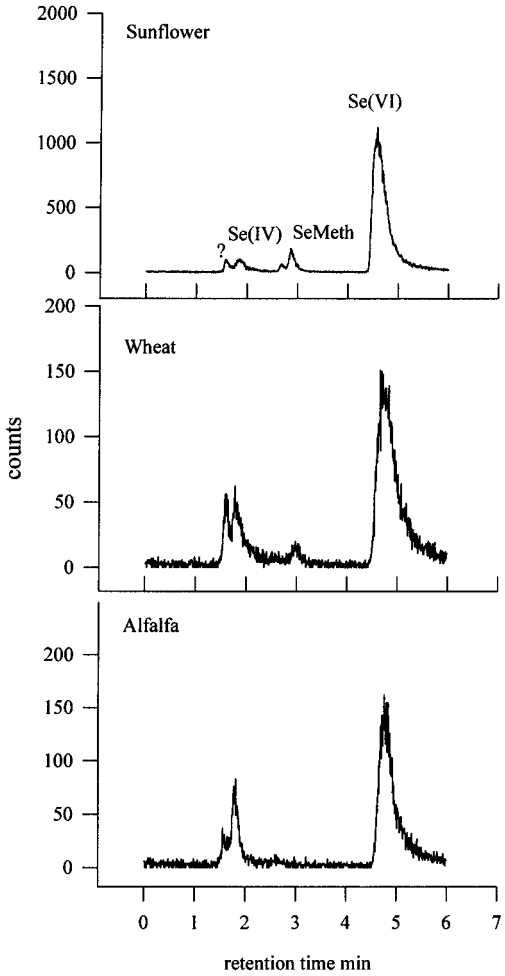

Figure 6. Chromatograms of aqueous extracts of alfalfa, wheat, and sunflower sprouts germinated in solutions containing 100, 200, and 400 mg of Se/L, respectively. For experimental parameters, see Figure 3.

identified with the used HPLC-ICP-MS system. This amount was calculated from the difference of the quantitative chromatographic results and the total concentration of the extract, which represents the "soluble but not identified" selenium. The concentration of the remaining selenate might be used as an indirect parameter for the metabolism rate (Milne, 1998).

In general, it was demonstrated that the metabolism of the selenate was inversely related to the total concentration of the sprouts. Especially in the sunflower seeds with Se concentrations up to ~1000 mg/kg, almost 100% of the selenium was extracted with water and found to be nonmetabolized selenate. Because it cannot be washed out or metabolized, it is not clear where the selenate is accumulated within these sprouts. It might be stored in vacuoles or the apparent free space of the apoplasts, or it might be moderately bound to the seed coat, where it is not available for the plant. Usually, non Se accumulators metabolize the selenate to selenocysteine and selenomethionine, which are readily incorporated into proteins instead of cysteine and methionine. This substitution causes most probably the selenium toxicity to the plants (Läuchli, 1993). Hence, the lack of metabolism is the most probable explanation for the high Se tolerance of the sunflower seeds.

Alfalfa and wheat sprouts behaved differently, and the Se metabolism rate depended on the supplied selenate concentration during sprouting. In both species,

Table 1. Total Selenium Concentrations and Extraction Ratios of Alfalfa and Wheat Sprouts after 5 Days of Germination in a Solution Containing 100 or 200 mg of Se as Selenate/L

		extraction efficiency,[a] %			
sprouts	total Se concn, mg/kg	H_2O	protease	HCl	NaOH
wheat	112	64	62	45	77
alfalfa	164	72	74	41	88

[a] Extraction solvents: H_2O, HCl (0.1 M), NaOH (0.1 M), and a solution containing a nonspecific protease (phosphate citric acid buffer at pH 7.5).

40% of the selenium was present as nonmetabolized selenate in those sprouts grown in solutions with high Se concentrations (Figures 4 and 5). Taking also the selenite concentrations into account, 53 and 58% of the inorganic Se were metabolized in alfalfa and wheat sprouts, respectively. With decreasing Se in solution, the relative amount of selenate within the sprouts decreased and the ratio of bound Se increased significantly. When a 0.78 mg of Se/L solution was used, alfalfa sprouts contained 3 mg of Se/kg of dry mass, 20% in the form of selenate and selenite and 80% in the form of other Se species. In wheat, total accumulation was lower (~1 mg/kg), but only 10% of the selenium was present as inorganic selenate.

Some alfalfa and wheat sprouts were additionally extracted with HCl (0.1 M), NaOH (0.1 M), and a solution containing nonspecific protease to assess the selenium species that were not extractable with water. Under acid conditions and with the protease solution, no increase of extraction ratio was obtained, whereas with NaOH (0.1 M) the extraction efficiency increased by ~10−15% compared to the water extract (Table 1). This increase resulted from an additional species that was not identical with any of the available standard compounds and a higher Se(IV) concentration. However, for the sprouts showing low enrichment the main amount of selenium could not be identified and proper extraction procedures have to be found for improved characterization. Additionally, in preliminary experiments, the highest selenium concentrations were found in the roots and buds and only small amounts were found in the original seeds. In future experiments different parts of the sprouts will be analyzed separately, which might provide more information about the metabolic pathways of the accumulated selenium.

Conclusions. It was shown that, in principle, significant accumulation of selenium is possible in wheat, alfalfa, and sunflower seeds during sprouting in a solution containing selenate. Depending on the type of seeds, the applied Se solution, and the time of sprouting, the selenium concentration in the sprouts, which was for all seeds <0.3 mg/kg, could be enriched to >100 mg of Se/kg of dry mass. The reproducibility of the procedure under standard conditions was 13% (calculated from five batches using the same seeds, Se solution, temperature, and light conditions, but performed on different days and by different persons). The metabolism of incorporated selenate was inversely related to the amount of uptake. At moderate enrichment (~10 mg of Se/kg), the selenate and metabolized selenium were present concurrently in approximately equal amounts. Because of the conflicting reports on the biological activity of individual selenium compounds, these sprouts, containing different selenium species, might serve as an excellent selenium food source. The residue of inorganic selenate might have an additional positive effect on glutathione peroxidase activity. In combination with the generally high nutritional value of sprouts, the Se-enriched sprouts might provide a starting material for cereal-based diets for better human nutrition and amended animal feed. However, the biological activity of enriched sprouts has to be proved by a supplementation study.

LITERATURE CITED

Alfthan, G.; Aro, A.; Arvilommi, H.; Huttunen, J. K. Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite and selenate. *Am. J. Clin. Nutr.* **1991**, *53*, 120−125.

Aro, A.; Ekholm, P.; Alfthan, G.; Varo, P. Effects of selenium supplementation of fertilizers on human nutrition and selenium status. In *Environmental Chemistry of Selenium*; Frankenberger, W. T., Engbert, R. A., Eds.; Dekker: New York, 1998; pp 81−97.

Barclay, M. N. I.; MacPherson, A. Selenium content of wheat for bread making in Scotland and the relationship between glutathine peroxidase (E.C. 1.11.1.9) levels in whole blood and bread consumption. *Br. J. Nutr.* **1992**, *68*, 261−270.

Bewley, J. D.; Black, M. *Seeds Physiology of Development and Germination*, 2nd ed.; Plenum Press: New York, 1994.

Brown, T. A.; Shrift, A. Selenium: Toxicity and tolerance in higher plants. *Biol. Rev.* **1982**, *57*, 59−84.

Butler, J. A.; Thomson, C. D.; Whange, P. D.; Robinson, M. F. Selenium distribution in blood fractions of New Zealand women taking organic or inorganic selenium. *Am. J. Clin. Nutr.* **1991**, *53*, 748−754.

Carlson, C. L.; Kaplan, D. I.; Adriano, D. C. Effects of selenium on germination and radicle elongation of selected agronomic species. *Environ. Exp. Bot.* **1989**, *29*, 493−498.

Chavan, J. K.; Kadam, S. S. Nutritional improvement of cereals by sprouting. *Crit. Rev. Food Sci.* **1989**, *28*, 401−437.

Clausen, J.; Nielsen, S. A. Comparison of whole blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenate, selenite, L-selenomethionine and high selenium yeast. *Biol. Trace Elem. Res.* **1988**, *15*, 125−138.

Coombs, G. F. Selenium in foods. In *Advances in Food Research*; Chichester, C. O., Schweiger, B. S., Eds.; Academic Press: San Diego, CA, 1988; pp 85−113.

Daniels, L. A. Selenium metabolism and bioavailability. *Biol. Trace Elem. Res.* **1996**, *54*, 185−199.

Eurola, M.; Ekholm, P.; Ylien, M.; Koivistoinen, P.; Varo, P. Effects of selenium fertilization on the selenium content of cereal grains, flour, and bread produced in Finland. *Cereal Chem.* **1990**, *67*, 334−337.

Fairweather-Tait, S. J. Bioavailability of selenium. *Eur. J. Clin. Nutr.* **1997**, *51*, S20−S23.

Finzel, S.; Müller, D.; Bayer, W.; Rückert, D.; Schmidt, K. Einfluss selenreichen Brotes auf den zellulären Immunstatus. Normalisierung im zellulären Immunstatus nach kontrolliertem Verzehr eines Brotes mit definiertem Selengehalt. *VITAMINSPUR* **1994**, *9*, 26−31.

Harmuth-Hoene, A. E.; Bognar, A. E.; Kornemann, U.; Diehl, J. F. Der Einfluss der Keimung auf den Nährwert von Weizen, Mungbohne und Kichererbsen. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 386−393.

Jaakkola, K.; Tummavuori, J.; Pirinen, A.; Kurkela, P.; Tolonen, M.; Arstila, A. U. Selenium levels in whole blood of Finnish volunteers before and during organic and inorganic selenium supplementation. *Scand. J. Clin. Lab. Invest.* **1983**, *43*, 473−476.

Kumpulainen, J. T. Selenium in foods and diets of selected countries. *J. Trace Elem. Electrolytes Health* **1993**, *7*, 107−108.

Läuchli, A. Selenium in plants: Uptake, functions and environmental toxicity. *Bot. Acta* **1993**, *106*, 455−468.

Levander, O. A.; Morris, V. C. Dietary selenium leves needed to maintain balance in North American adults self-selected diets. *Am. J. Clin. Nutr.* **1984**, *39*, 809−815.

Levander, O. A.; Alfthan, G.; Arvilommi, H.; Gref, C. G.; Huttunen, J. K.; Kataja, M.; Koivistoinen, P.; Pikkarainen, J. Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *Am. J. Clin. Nutr.* **1983**, *37*, 887−897.

Levine, V. E. The effect of selenium compounds upon growth and germination in plants. *Am. J. Bot.* **1925**, *12*, 82−90.

Lintschinger, J.; Fuchs, N.; Moser, H.; Jäger, R.; Hlebeina, T.; Markolin, G.; Gössler, W. Uptake of various trace elements during germination of wheat, buckwheat and quinoa. *Plant Food Hum. Nutr.* **1997**, *50*, 223−237.

Makela, A. L.; Nanto, V.; Makela, P.; Wang, W. The effect of nationwide selenium enrichment of fertilizers on selenium status of healthy Finnish medical students living in southwestern Finland. *Biol. Trace Elem. Res.* **1993**, *36*, 151−157.

Makela, A. L.; Wang, W. C.; Hamalainen, M.; Nanto, V.; Laihonen, P.; Kotilainen, H.; Meng, L. X.; Makela, P. Environmental effects of nationwide selenium fertilization in Finland. *Biol. Trace Elem. Res.* **1995**, *47*, 289−298.

Meltzer, H. M.; Norheim, G.; Bibow, K.; Myhre, K.; Holm, H. The form of selenium determines the response to supplementation in a selenium replete poulation. *Eur. J. Clin. Nutr.* **1990**, *44*, 435−446.

Meltzer, H. M.; Norheim, G.; Bjorge Loken, E.; Holm, H. Supplementation with wheat selenium induces a dose-dependent response in serum and urine of a Se-replete population. *Br. J. Nutr.* **1992**, *67*, 287−294.

Merx, H.; Seibel, W.; Rabe, E.; Menden, D.; Menden, E. Einfluss von Keimungsparametern auf den Vitamingehalt und die mikrobiologische Qualität von Spriesskorn (Roggen und Weizen). 1. Mitt.: Stand der Forschung. *Getreide, Mehl Brot* **1994**, *48*, 17−20.

Milne, J. B. The uptake and metabolism of inorganic selenim species. In *Environmental Chemistry of Selenium*; Frankenberger, W. T., Engbert, R. A., Eds.; Dekker: New York, 1998; pp 459−477.

Moser-Veillon, P. B.; Mangels, A. R.; Patterson, K. Y.; Veillon, C. Utilization of two different chemical forms of selenium during lactating using stable isotope tracers: an example of speciation in nutrition. *Analyst* **1992**, *117*, 559−562.

Oster, O.; Prellwitz, W. The daily dietary selenium intake of West German adults. *Biol. Trace Elem. Res.* **1988**, *20*, 1−14.

Persson-Moschos, M.; Alfthan, G.; Akesson, B. Plasma selenoprotein P levels of healthy males in different selenium status after oral supplementation with different forms of selenium. *Eur. J. Clin. Nutr.* **1998**, *52*, 363−367.

Schelenz, R. F. W. Intake of Zn, Mn, and Se by adult females. A total diet study. In *Trace Element Analytical Chemistry in Medicine and Biology*; Brätter, P., Schramel, P., Eds.; de Gruyter: Berlin, 1984; pp 73−86.

Spenger, N. E.; Siegel, S. M. Effects of sulfur and selenium oxyanions on Hg-toxicity in turnip seed germination. *Water, Air Soil Pollut.* **1978**, *9*, 423−427.

Thomson, C. D.; Robinson, M. F.; Butler, J. A.; Whanger, P. D. Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase in blood components of New Zealand women. *Br. J. Nutr.* **1993**, *69*, 577−588.

Tiran, B.; Tiran, A.; Petek, W.; Rossipal, E.; Wawschinek, O. Selenium status of healthy children and adults in Styria (Austria). *Trace Elem. Med.* **1992**, *9*, 75−79.

Wilplinger, M.; Sima, A.; Pfannhauser, W. Selengehalt in der Nahrung und dessen Zusammenhang zum Gehalt im Boden. *Lebensmittelchemie* **1998**, *52*, 93−95.

Wu, L.; Guo, X.; Banuelos, G. S. Accumulation of seleno-amino acids in legume and grass plant species grown in selenium-laden soils. *Environ. Toxicol. Chem.* **1997**, *16*, 491−497.

Zheng, J.; Goessler, W.; Kosmus, W. The chemical forms of selenium in selenium nutritional supplements: an investigation by using HPLC/ICP/MS and GF/AAS. *Trace Elem. Electrolytes* **1998**, *15*, 70−75.

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