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Study of selenocompounds from selenium-enriched culture of edible sprouts

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ABSTRACT

Selenium is recognised as an essential micronutrient for humans and animals. One of the main sources of selenocompounds in the human diet is vegetables. Therefore, this study deals with the Se species present in different edible sprouts grown in Se-enriched media. We grew alfalfa, lentil and soy in a hydroponic system amended with soluble salts, containing the same proportion of Se, in the form of Se(VI) and Se(IV). Total Se in the sprouts was determined by acidic digestion in a microwave system and by ICP/MS. Se speciation was carried out by enzymatic extraction (Protease XIV) and measured by LC-ICP/MS. The study shows that the Se content of plants depends on the content in the growth culture, and that part of the inorganic Se was biotransformed mainly into SeMet. These results contribute to our understanding of the uptake of inorganic Se and its biotransformation by edible plants.

1. Introduction

Selenium (Se) is an essential element for humans and higher animals, since it is present in several selenoproteins and is a constituent of the anti-oxidant enzyme glutathione peroxidase (GPX), which contributes to preventing oxidative cellular degradation (Reilly, 2006). Since Se has potential health benefits, several studies have focused on Se speciation in human nutrition (Hartikainen, 2005; Zeng & Combs, 2008).

Regarding human nutrition, Se uptake *via* the diet is mainly from vegetables. Plants can absorb the main inorganic forms of Se, such as selenate or selenite, from soil or from other culture media (Sager, 2006). The variation in soil Se contents between different countries and locations causes significant differences in the Se in edible plants and consequently in the daily intake of Se(Moreno Rodriguez, Cala Rivero, & Jiménez Ballesta, 2005; Scientific Committee on Food, 2000; Spadoni et al., 2007). Interest in the dietary content of this nutrient has increased as the effects of Se deficiency on human health, have become known (Allen, Benoist, Dary, & Hurrell, 2006). For this reason, several countries, such as Finland, have introduced Se into fertilisers and its level in crops has increased considerably (Eurola et al., 2000).

In cultivated plants, the Se content can be increased *via* different fortification processes (Li, Graef, Yee, & Yan, 2004; Lyons, Stangoulis, & Graham, 2003). Among them, a hydroponic system is an easy technique and has been widely used in several studies; the Se compound added to the solution is usually sodium selenate (Lintschinger, Fuchs, Moser, Kuehnelt, & Goessler, 2000; Tsuneyoshi, Yoshida, & Sasaoka, 2006). The present study also uses a hydroponic system applied to alfalfa, soy and lentil. These edible sprouts, used directly in various diets (in salads or soups), are sources of isoflavones, which exhibit a variety of biological activities that may influence the risk of different diseases (Lampe, 2003; Márton, Mándoki, Csapó-Kiss, & Csapó, 2010). Similar studies have been reported in the literature, in which selenite or selenate was added separately to the hydroponic media (Lintschinger et al., 2000; Thavarajah, Ruszkowski, & Vandenberg, 2008; Sugihara et al., 2004). In the present study, mixtures of sodium selenite and sodium selenate are assayed in several ratios, in order to ascertain if the simultaneous presence of both compounds influences the absorption by plants and the biotransformation to organic Se compounds. The study compares the changes in Se speciation between control sprouts (with natural Se content) and those from Se-enriched cultures. It has to be considered that the nutritional bioavailability of Se from plants depends mainly on the Se

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compounds; hence, Se speciation is assessed in the sprouts grown in all the experimental hydroponic assays studied.

2. Materials and methods

2.1. Materials

Alfalfa, soy and lentil seeds were purchased from a commercial nursery (medium diameter: 1–2 mm). The origin of the soy (INT-Salim) and alfalfa seeds (Certificated Seed R-1) was Spain, while the lentil seeds (Golden Line) were from Italy.

2.2. Plant experiment

Germination was carried out in a polypropylene container (1 L capacity and 4 cm in height) containing a mesh (pore diameter 0.3 cm). The growth solution consisted of tap water amended with selenium, in the form of Na_2SeO_3 and Na_2SeO_4, (1:1) at three concentrations: A (1 mg Se(IV) L^{-1} and 1 mg Se(VI) L^{-1}), B (2.5 mg Se(IV) L^{-1} and 2.5 mg Se(VI) L^{-1}) and C (4 mg Se(IV) L^{-1} and 4 mg Se(VI) L^{-1}). One litre of the fortified solution was added to the containers, since this volume was enough to allow the plants to grow. The containers with the growth solution were placed under a laboratory hood with constant air extraction. To control the tap water used in the hydroponic media, the main metals were determined by ICP-OES.

In the experiments, all the equipment was cleaned with water and ethanol prior to use, to avoid microbiological activity and moreover residual chlorine was guaranteed in the tap water used.

2.3. Growing conditions

Twenty grams of soy seeds and 15 g of alfalfa and lentil seeds were grown separately in their respective containers and in contact, through the mesh, with the Se-fortified aqueous solution (for three weeks). Seeds were submerged in the fortified solution for 24 h, according to the producer's recommendations. We also used a control culture for each plant, which grew in tap water without selenium salts during the same period of time.

2.4. Sample collection

Three weeks after planting the seeds, all the sprouts were harvested and their shoots and roots were separated, cleaned and dried at 40 °C. Then, the samples were milled in a glass mortar and transferred to high-density polyethylene (HDPE) containers. They were stored at room temperature until analysis (in duplicate) of each part of the plant for each culture.

2.5. Analytical methods

Inorganic Se stock solutions were prepared to a concentration of 1000 mg L^{-1} from selenite 99% Na_2SeO_3 and selenate 99% Na_2SeO_4 (both Sigma–Aldrich, St. Louis, MO). Organic Se stock solutions were also prepared to a concentration of 1000 mg L^{-1} from selenocysteine ($SeCys_2$), selenomethionine (SeMet) and selenomethylselenocysteine (SeMeSeCys) (Aldrich) with 0.5% HCl. All the standard solutions were kept at 4 °C in closed opaque HDPE bottles, since the stability of Se species depends on storage time and conditions, notably on exposure to air and elevated temperature (Amoako, Kahakachchi, Dodova, Uden, & Tyson, 2007; Liu & Bei, 2010).

Aqua regia extractable Se in seeds was determined by following the appropriate ISO standard (ISO 11466, 1995), using 1 g of seeds. A digester block (P/Selecta model, RAT 4000051) with temperature control was used. Once at room temperature, the resulting suspension was filtered (Whatman 40) and the solid residue was washed several times with 0.5 M HNO_3 (Hiperpur; Panreac, Barcelona, Spain). The resulting filtrate, together with the washings, were diluted to 20 mL with 0.5 M HNO_3, transferred to an HDPE bottle and stored at 4 °C until analysis of total Se.

For the acid microwave digestion of the sprouts, a 0.2-g sample (weighed in a Teflon vessel) was mixed with 8 mL of HNO_3 and 2 mL of 33% H_2O_2 (Prolab). The resulting mixture was digested by a closed microwave system (Milestone Ethos Touch Control, 1000 W; Milestone S.r.l., Sorisole, Italy), following the program: 10 min ramp from room temperature to 90 °C; 5 min at 90 °C; 10 min ramp from 90 °C to 120 °C; 10 min ramp from 120 °C to 190 °C; and 10 min at 190 °C. After digestion, the samples were filtered (Whatman 40) and brought up to a total volume of 20 mL with double deionised water, transferred to an HDPE bottle and stored at 4 °C until analysis. The Se contents from both *aqua regia* extraction and acid MW digestion were measured using a 7500ce Series inductively-coupled plasma mass spectrometer (ICP/MS) (Agilent Technologies, Santa Clara, CA) with an Ari Mist HP nebuliser (Burgener Research Inc., Mississauga, ON, Canada). Hydrogen was used as the reaction gas to prevent possible interferences, and Rh was used as the internal standard. The ion intensity at *m/z* 78 (^{78}Se) was monitored using time-resolved analysis software.

The total Se content of the *aqua regia* extracts from the Reference Materials was determined using an atomic fluorescence spectrometer, (PSA Excalibur) with a hydride generator module (Model 10.004; P.S. Analytical, Orpington, UK). For HGAFS measurements, a pre-reduction step ensuring the quantitative reduction of Se(VI) to Se(IV) was required. Thus an aliquot of 5 mL of extract was placed in a sand bath at 170 °C with 10 ml of 6 M HCl for 30 min. Once at room temperature, the solution was diluted to 25 mL with 6 M HCl. Hydride generation from Se (IV) was achieved with 6 M HCl, at a flow rate of 8 mL min^{-1}, and 1.5% $NaBH_4$ in 0.4% NaOH, at a flow rate of 3 mL min^{-1}.

For the enzymatic digestion of the sprouts, 0.3 g of vegetable samples and 30 mg of Protease XIV (Sigma Aldrich) were placed in a 40-mL HDPE tube with 10 mL of 25 mM $NH_4H_2PO_4$ at pH 7.5. The mixture was shaken for 16 h in a thermo-agitator water bath (Clifton NE5-28D; Nickel-Electro Ltd., Weston-super-Mare, UK) at 37 °C. The resulting solution was centrifuged for 10 min at 3000 rpm. The extracts from the enzymatic digestion were first filtered consecutively through a 0.45-μm and a 0.20-μm nylon membrane. Se species were measured immediately after extraction. The extracts were analysed by LC-ICP/MS (Agilent Technologies, 1200 Series, LC quaternary pump). The chromatographic anion exchange precolumn (20 × 2.0 mm, i.d. 10 μm) and column (250 × 4.1 mm, i.d. 10 μm) were the Hamilton PRP-X100 (Reno, NV). The chromatographic mobile phase was prepared from a buffer of $NH_4H_2PO_4$ 40 mM (PA Panreac) adjusted to pH 7.0 with 25% NH_3 (PA Panreac). The flow rate of the mobile phase was 1.5 mL min^{-1} and the injection volume was 50 μL. The detection of Se species by ICP/MS was carried out under the same conditions as those described above.

To assess the extraction efficiency and column recovery, the total Se present in the enzymatic extracts was determined by acidic microwave digestion of an aliquot of 4 mL of the extract and by ICP/MS, under the same conditions as for the Se total determination described above.

3. Results and discussion

Some aspects of the plant responses from throughout the study can be highlighted. The amount of sprout biomass grown in the dif-