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Effect of selenite on organic selenium speciation and selenium bioaccessibility in rice grains of two Se-enriched rice cultivars

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ABSTRACT

The accumulation of organic selenium (Se) in grains is helpful in understanding its bioaccessibility in various Se-enriched rice cultivars after soil selenite treatments. In the present study conducted with two rice cultivars, the organic Se as well as the glutelin-derived Se and the selenomethionine (SeMet) percentage in grains increased with soil Se levels. The available Se that was detected in the forms of SeMet (~40%) and selenite (~12%) accounted for more than 50% of the total Se in grains, and no significant differences were observed between the two rice cultivars ($P > 0.05$). Simulated gastrointestinal digestion showed that the digested grain SeMet increased with the soil Se treatment levels, whereas there were minimal changes in the percentages of selenite in the grain. Furthermore, approximately half of the available SeMet was derived from the grain glutelin. These results suggest that the non-glutelin-derived Se in rice should be further studied.

1. Introduction

Selenium (Se) is an essential element in human nutrition, and it plays a critical physiological role in the formation of many important enzymes, such as glutathione peroxidase (GPX) (Pezzarossa, Remorini, Gentile, & Massai, 2012). Selenium in the human body is mainly acquired through the food chain. However, the concentrations of Se in most crops produced in China are usually less than 60 μg kg^{-1} (Williams et al., 2009). Furthermore, Se bioaccessibility greatly depends on its species: selenomethionine (SeMet), selenocysteine (SeCys), selenate and selenite represent a decreasing order of bioaccessibility (Fairweather-Tait, Collings, & Hurst, 2010). Therefore, many studies have focused on improving the organic Se content in crops (Chen, Yang, Zhang, Hu, & Pan, 2002; Nothstein et al., 2016).

It is common to improve the Se status of rice or wheat grain in the agricultural practice in China, and selenite is often used as the source of Se in fertilizers on the basis of its bioavailability and immobility in soil (Hu, Chen, Xu, Zhang, & Pan, 2002; Galinha et al., 2015; Chu, Yao, Yue, Li, & Zhao, 2013). Both spraying of selenite solutions on crop leaves at certain stages of rice growth and the application of fertilizer that contains Se as selenite to the soil could enhance the Se levels in rice grains, but the grain Se content is known to vary according to cultivar differences due to differences in the Se absorption and transport in the soil-plant system (Zhou, Zhang, Wang, & Xu, 2016). Chen et al. (2017) recommended the use of 0.5 mg kg^{-1} sodium selenite in the agricultural practice. However, it is still not exactly clear whether the organic Se content of rice grains responds to the total amount of Se applied in Se-containing fertilizers.

When inorganic Se species, e.g., selenite, are applied to the soil, they can be converted into their organic forms, such as SeMet (Fofana et al., 2014), or even proteins containing Se in the agricultural products. These forms are easily absorbed and utilized by humans (Eiche et al., 2015). Protein accounts for only 8% of the total rice biomass (Haba, Ding, & Zhang, 2005), but most of the Se in the grain occurs in the form of Se-proteins (Zhang et al., 2008). In addition, 48% of the Se-proteins in polished rice can be dissolved in alkaline solutions (Zhang et al., 2008). Compared with other crops, the proteins in rice have unique hypoallergenic properties and higher nutritive qualities (e.g., they are rich in the essential amino acid lysine). For these reasons, rice is quite suitable for the production of baby food (Ju, Hettiarachchy, & Rath, 2001). The application of Se fertilizers not only improves the dietary Se intake in humans but also provides important raw materials for Se-enriched protein products (Wu, 2011). In paddies sprayed with selenite during the heading stage of growth, the glutelin protein contains the largest amount of Se, and it has been proven to be digestible by simulated gastrointestinal digestion (Fang et al., 2010). Specifically, SeMet represented 52.3% of the total Se in protein extracts. Therefore, further studies are needed to analyze Se-containing proteins such as glutelin

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and their absorption by the human body after selenite application in soil.

Bioaccessibility is usually defined as the capacity of a material or target to be transported across the organisms' biological membranes. This can be determined through in vitro simulation or animal experiments. The gastrointestinal simulation model was selected in this study to explore the species of organic Se in rice grains and their accessibility to the human body. This method presents no human ethical concerns (Moreda-Piñeiro et al., 2011), is rapid and simple to perform, is cost-effective, and represents a generic digestion process (Altundag, Albayrak, Dundar, Tuzen, & Soylak, 2015; Hur, Lim, Decker, & Mcclements, 2011).

On the basis of rice cultivar differences, the specific properties of selenite in soil, and the dose recommended for selenite supplementation in a previous study, two Se-accumulating rice cultivars were chosen for pot culture experiments. The soil was treated with selenite using an extended range from 0.5 to 5.0 mg kg^{-1} in this study. Liquid chromatography atomic fluorescence spectrometry (LC-AFS) was used to determine the Se species in the rice or in gastrointestinal hydrolysates of the rice grains. The results demonstrated the changes of the organic Se contents, Se bioaccessibility and the relationship among the different Se species in rice grains.

2. Materials and methods

2.1. Experimental design and materials

A pot experiment was conducted in this study. The pot boxes (length 16 cm, width 15 cm and height 10 cm) was made of polyvinyl chloride (PVC). Two *Oryza sativa* cultivars, a high Se-accumulating cultivar (Fengbazhan) and a low Se-accumulating cultivar (Hefengzhan), were provided by the Rice Research Institute, Guangdong Academy of Agricultural Sciences, China. The experimental soil was collected from Taishan, Guangdong, China and its general properties were as follows: pH 5.40, organic matter 3.20%, total Se 0.45 mg kg^{-1}.

After the soil was naturally dried in a clean room and sieved through 2 mm nylon mesh, it was divided into portions for the different treatments and mixed with sodium selenite (Na_2SeO_3) at 0.5, 1.0 and 5.0 mg Se per kg soil. Soil without any added Se was used as the control. The macro element fertilizers were mixed with urea, diammonium phosphate, and potassium chloride by 0.20 g·kg^{-1} (N), 0.15 g·kg^{-1} (P_2O_5), and 0.20 g·kg^{-1} (K_2O). Furthermore, a concentrated Arnon nutrient solution (× 1,000) without iron and manganese was added into the pot soil at 1.0 mL per kg soil. Only one good pregerminated seed was planted in each pot, which contained 2.30 kg soil. All of the treatments were replicated four times, and the pots were maintained in a greenhouse until harvest. When the rice matured, all the grains were collected, dried, dehusked but not polished, and then ground into powders using a pestle and agate mortar. The rice powders were kept in self-sealed plastic bags and refrigerated at 4 °C.

All the chromatographic reagents, methanol (CH_3OH), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and tetrabutyl ammonium bromide (TBAB), were purchased from the Aladdin Co., Ltd. (Pudong, Shanghai, China). Pepsin (≥1200 IU g^{-1}), trypsin (transformation capacity of casein ≥25), amylase (≥2000 IU g^{-1}) and pig bile salts (≥60%) were obtained from the Sinopharm Chemical Reagent Co., Ltd. (Caojing, Shanghai, China). The National Institute of Metrology (Chaoyang, Beijing, China) provided all the standard solutions including selenite, selenate, SeMet and $SeCys_2$. The perchloric acid ($HClO_4$), nitric acid (HNO_3), hydrochloric acid (HCI) and other chemical substances used in this experiment were all analytical grade reagents purchased from the Xilong Scientific Co., Ltd. (Jinping, Shantou, Guangdong, China).

2.2. Determination of the total and organic Se in rice grains

Each sample of rice powder (0.50 g) was put into a 50 mL digestion tube to which 1.0 mL concentrated $HClO_4$ and 5.0 mL concentrated HNO_3 were added, and the tubes were then placed on a digital hot block. The mixtures were heated at 150 °C until a residue of approximately 1.0 mL remained. Then, the temperature was adjusted to 100 °C, and 1.0 mL concentrated HCI was added to reduce the selenate into selenite. Half an hour later, the solution was transferred into a 15 mL centrifuge tube and diluted to 10 mL with Milli-Q water for the determination of the total Se.

The organic Se in the rice powder was analyzed using the dialysis bag method (flat width: 25 mm; molecular weight cutoff: 8000–14000 Da) (Chen et al., 2002; Zhou, Shi, & Yang, 2007). Another 0.50 g rice powder was weighed and put into the dialysis bag with some Milli-Q water. The bags were dialyzed against 1 L of water, which was replaced every 6 h. After 24 h, the contents of the bags were quantitatively transferred into digestion tubes and subjected to the total Se determination protocol. The organic and total Se in the rice grains were measured using an AFS analyzer (8220, Titan Instrument Co., Ltd, Chaoyang, Beijing, China) with a high performance hollow cathode lamp of Se. The lamp current and negative high voltage were 80 mA and 270 V, respectively.

2.3. Extraction of glutelin in rice powder and determination of glutelin-derived Se

Approximately 3.00 g of rice powder was weighed and transferred to a 50 mL conical flask. Then, 30 mL of sodium hydroxide (NaOH) solution (3.0 g L^{-1}) was added, and the mixture was shaken in a water bath oscillator at 40 °C for 4 h. The resulting solution was transferred to a 50 mL plastic tube and centrifuged at 4000 r/min for 30 min. The supernatant was then poured into another 50 mL plastic tube and adjusted to a pH of 5.5 with HCl. The precipitate, namely, the rice glutelin, was separated by centrifugation at 4000 r/min for 30 min. The glutelin-derived Se measurement was measured in the same manner as the determination of the total Se in the grains.

2.4. In vitro simulation and determination of Se species

The gastrointestinal simulation experiment was performed according to Bhatia et al. (2013): (1) Approximately 0.50 g rice sample was weighed and placed in a 50 mL centrifuge tube. (2) A 5 mL gastric solution (1% pepsin, w/v in 8.78 g L^{-1} NaCl, adjusted to pH 2 with 37% HCl, v/v) was added, and the tube was kept on a thermostatic water bath shaker at 37 °C for 4 h. (3) The pH was then adjusted to 7.5 with saturated sodium bicarbonate ($NaHCO_3$), and 5 mL of an intestinal solution consisting of 3% (w/v) pancreatin, 1.5% (w/v) amylase, 1% (w/v) cholate and 8.78 g L^{-1} NaCl was added. The resulting mixture was shaken for another 4 h. (4) The tubes were finally centrifuged at 3000 r/min for 30 min, and the supernatant was analyzed for the total Se and its species. The Se forms in rice glutelin were analyzed as described above.

All the solutions were filtered through a 0.45 μm membrane filter before analysis. The Se species were measured using LC-AFS equipped with a C18 column (5 μm, 4.0 × 20 mm, Xuhui, Shanghai, China). The mobile phase was composed of 3.96 g L^{-1} $(NH_4)_2HPO_4$, 0.16 g L^{-1} TBAB and 2% (v/v) CH_3OH, and the flow rate was 1.0 mL/min. The volume of the injected standard or sample solution was 300 μL, which was quantified by a specific sample loop. The linear ranges for selenite, selenate, SeMet and $SeCys_2$ were 0–53.6 μg L^{-1}, 0–51.9 μg L^{-1}, 0–49.3 μg L^{-1} and 0–55.3 μg L^{-1}, respectively, and the corresponding limits of detection (LOD) were 1.0 μg L^{-1}, 0.8 μg L^{-1}, 1.3 μg L^{-1} and 1.0 μg L^{-1}, which were calculated as the concentrations that gave a signal equal to three times the standard deviation (SD) of the blank solution.