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Accumulation and distribution of selenium in different parts and macromolecule of Se-enriched Tartary Buckwheat (*Fagopyrum tataricum* Gaertn.) during germination

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Abstract

The accumulation of selenium (Se) and distribution in the sprouts of tartary buckwheat cultivated in Na_2SeO_3 solutions with different concentrations were investigated in this study. Total Se content, organic Se content and the proportion of organic Se were determined. The results indicated that the total Se content increased gradually while the proportion of organic selenium decreased gradually with the increasing of ambient Na_2SeO_3 concentrations in the range of 10-20 μg/mL. After three days' germination, tartary buckwheat cultivated in 20 μg/mL Na_2SeO_3 solution presented the highest selenium accumulation; Se contents in different parts decreased in this order: radicle > husk > hypocotyl > cotyledon. Se contents in total protein were higher than in polysaccharides, nucleic acids and other components. Furthermore, Se contents in protein fractions decreased in the order albumin, glutelin, globulin and prolamin. Selenium in proteins was primarily present as albumin-bound Se and glutelin-bound Se.

Introduction

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is a seed-producing agricultural crop in the family Polygonaceae. Although not a cereal, it and other buckwheat species is usually grouped with cereals due to its method of cultivation and similar nutrient profile. Buckwheat is primarily grown in mountainous areas and plateaus, where soil is typically barren with less water, rare nutrition and low temperature. However, buckwheat seeds are nutrient-rich, containing proteins with high biological value and a balanced amino acid composition, relatively high crude fiber content (Farrell, 1978), and vitamins B_1, B_2, and B_6 (Bonafaccia *et al*., 2003). In addition Tartary buckwheat contains more rutin than common buckwheat (Kitabayashill *et al.*, 1995; Fabjan *et al.*, 2003). Therefore, tartary buckwheat is an important functional food that is useful in the prevention of edema and hemorrhagic diseases, and in stabilizing high blood pressure (Havsteen, 1983).

Selenium (Se) is an essential micronutrient for human beings and animals (Navarro-Alarcon and Cabrera-Vique, 2008). As the component of selenocysteine, the 21st amino acid, Se plays an essential role in the formation of some biological enzymes, such as glutathione peroxidases, thioredoxin reductase, iodothyronine deiodinases, and selenophosphate synthetase (Letavayová *et al.*, 2006). Glutathione peroxidases and thioredoxin reductase have been reported to present antioxidant activity and anticarcinogenic effects and other physiological functions (Arteel and Sies, 2001). Selenium deficiency is associated with poor health and general impairment of the immune system. Meanwhile, the bioavailability, reactivity and concentrations for toxicity of selenium are dependent on its chemical forms and concentrations (Besser *et al*., 1993). In general, the inorganic forms of selenium are more toxic than the organic forms. On the other hand, organic forms of selenium are more bioavailable to humans than inorganic selenium species (Thomson, 2004). Thus, accumulation of Se in crops is an important breeding goal, particularly with reference to organic Se.

Some research has been conducted on increasing Se contents in tartary buckwheat through foliar sprays (Smrkolj *et al.*, 2006). However, this method was associated high costs and potential environmental problems. Plant seeds have been reported to accumulate Se and transform inorganic Se to organic Se during germination (Liu and Gu, 2009). Simultaneously, the nutritional value of many types of grain seeds improves during the course of germination (Zhou *et al*., 2011).

Research on Se distribution and accumulation has been conducted in crops typically considered as good sources of selenium, such as bean, rice and mushrooms (Zhao *et al.*, 2004; Smrkolj *et al*., 2007). However, there is little information available regarding these aspects in Se-enriched tartary buckwheat sprouts. It is of interest to determine whether tartary buckwheat

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can be successfully accumulating inorganic selenium and be used as a dietary source of Se. Accordingly, the objectives of this study were to investigate the accumulation and distribution of Se in different parts of buckwheat sprouts and to determine the effect of external Se concentrations on accumulating selenium in tartary buckwheat sprouts. Additionally, the distribution of Se in nucleic acids, polysaccharides and proteins were determined as well as in different protein fractions.

Materials and Methods

Materials

Tartary buckwheat was cultivated in Datong, Shanxi, China; seeds were obtained from the Shanxi Academy of Agricultural Science (SAAS). Plump and unbroken seeds were chosen as test materials.

Preparation of Se-enriched tartary buckwheat

Four sets of tartary buckwheat seeds were weighed and surface-sterilized with 1% NaClO solution for 5 min and washed three times with 30 mL deionized water. The four sets of seeds were soaked in Na_2SeO_3 solutions of varying concentrations (10 μg/mL, 20 μg/mL, 30 μg/mL) at 30°C for 12 h, soaking in deionized water as the control. The seeds were then transferred to Petri dishes, cultivated with 10 mL of Na_2SeO_3 solutions which concentration was in the corresponding soaking concentration. The seeds were germinated at room temperature for 72 h. Additional Na_2SeO_3 solution was added to each dish daily to maintain seed wetness. The control seeds were germinated in a similar manner, except that distilled water was used instead. Germinating seeds were collected every 24 h; the sprouts were washed with distilled water and stored at -20°C for further use.

Distribution of selenium in different parts of tartary buckwheat sprouts

As shown in Figure 1, different parts of tartary buckwheat sprouts (radicle, husk, hypocotyl, and cotyledons) were cut with a scalpel and dried in an oven at 60°C. They were then ground to a powder.

Extraction of total protein from Se-enriched tartary buckwheat

Nine grams of Se-enriched tartary buckwheat powder were dissolved into 100.0 mL of 50 mmol/L Tris-HCl buffer (pH 8.5). The mixture was stirred continuously for 2 h, and then centrifuged at 3000 g for 20 min. The residue was re-extracted twice and centrifuged as indicated. To the combined supernatants, $(NH_4)_2SO_4$ was added to achieve 95% saturation. The mixture was stored overnight in a refrigerator at 4°C. The resulting precipitation was collected by centrifugation at 3000 *g* for 20 min. Selenium contents in this total protein fraction were measured after digestion to represent protein-bound Se.

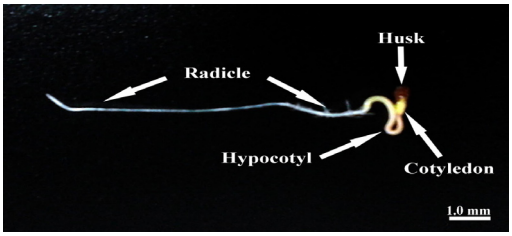

Figure 1. Different parts of tatary buckwheat sprouts. Four parts of the tartary buckwheat buds were separated, including husk, radicle, hypocotyl, cotyledon

Extraction of nucleic acid from Se-enriched tartary buckwheat

Powdered samples (15 g) were steeped in 150 mL of 2 mol/L NaCl solution. The mixture was extracted in a boiling water bath for 30 min and the supernatant was obtained by centrifugation at 3000 *g* for 20 min (Mäkelä *et al.*, 1995). The residue was re-extracted twice and centrifuged as before. The combined supernatants were mixed (5:1, v/v) with chloroform: n-butanol (4:1, v/v) to remove proteins. After adjusting the pH to 2.5 using acetic acid, the mixture solution was placed in ice-cold water overnight. Finally, precipitated nucleic acids were obtained by centrifugation at 3000 g for 20 min. Selenium contents in the samples were measured after digestion to represent the nucleic-acid-bound Se.

Extraction of polysaccharides from Se-enriched tartary buckwheat

The residue remaining after extracting nucleic acids was added to 100 mL of deionized water and extracted in a boiling water bath for 2 h. The supernatant was obtained by centrifugation at 3000 g for 20 min and the residue was re-extracted twice. The supernatants were combined and mixed (5:1 v/v) with chloroform: n-butanol (4:1, v/v) to remove proteins according to the method of Qin (Qin *et al*., 2002). Four times volume of 95% ethanol was added to the fraction. The mixture was stored in a refrigerator overnight. Precipitated polysaccharide were obtained through centrifugation at 3000 *g* for 20 min. Selenium contents were measured after digestion to represent the polysaccharide-bound selenium.

Fractionation of different protein components in Se-enriched tartary buckwheat sprouts

Protein components were extracted according to the successive extraction method described by Guo

(Guo and Yao, 2006).

Extraction of albumin: 4.5 g of the sample powder was mixed with 30 mL of distilled water and was stirred for 30 min at room temperature to extract water-soluble proteins. The supernatant was obtained by centrifugation at 5000 *g* for 10 min.

Extraction of globulin: After extraction of albumin, the residue was steeped in 30 mL of 2% NaCl solution and the mixture was continuously stirred at room temperature for 30 min. The supernatant containing globulin fraction was obtained by centrifugation at 5000 *g* for 10 min.

Extraction of prolamin: After extraction of globulin, the residue was extracted with 30 mL of 75% ethanol for 30 min, with continuous stirring at room temperature. The supernatant containing prolamin fraction was obtained by centrifugation at 5000 *g* for 10 min.

Extraction of glutelin: After prolamin extraction, the residue was steeped in 30 mL of 0.02 mol/L NaOH solution and the mixture was continuously stirred at room temperature for 30 min. The supernatant containing glutelin fraction was obtained by centrifugation at 5000 *g* for 10 min. Protein contents in each fraction were determined by the National Standard Method of China (GB-5009.5-2010) with a model VAP10 Kjeldahl nitrogen analyzer (Gerhardt, City, Germany).

Determination of total Se and organic Se contents

The contents of total Se were determined using differential pulse polarography as reported by İnam (İnam and Somer, 1998). The sample was digested in 5 mL of mixture of HNO_3 and perchloric acid (4:1, v:v) at 130°C until white smoke appeared. After cooling to room temperature, 5 mL of hydrochloric acid was added and the mixture was held at 115°C for 30 min to reduce Se (VI) to Se (IV). The clear solution obtained was diluted to a volume of 10 mL with distilled water. Total selenium in the samples was determined using polarography.

Organic selenium was detected according to the method described by Zhao and Zhang (Zhao *et al*., 2004; Zhang *et al.*, 2009). Se-enriched tartary buckwheat powder was dialyzed (8000–12000 Da, 48 h) with double-distilled water. During dialysis, the double-distilled water was changed once every 12 h until no selenium was detected in the dialysis water. Thus, Se compounds left in the sample were considered to be organic selenium.

Organic selenium proportion = (Organic selenium/ Total selenium) × 100%.

Statistical analysis

Data was analyzed using SAS software (Statistical Analysis Systems, USA). Differences were evaluated using Duncan's multiple range test. The significance analysis was established at $P \leq 0.05$.

Results and Discussion

Accumulation of Se during the germination of tartary buckwheat

As shown in Table 1, the total selenium content increased as germination time increased. Similar results have been reported previously (Zhang *et al*., 2006). In addition, the total Se content increased with increasing external selenite concentration in the range of 10-20 μg/mL. However, when 30 μg/mL of external selenite was supplied, the total Se content decreased. This is because an excess of Se inhibited the growth of sprouts during the entire germination process and reduced total selenium content (Liu and Gu, 2009). In other words, if the external Se content was less than 20 μg/mL, there was no significantly inhibitory effect on total Se content in the plant and germination proceeded normally. These results were consistent with those observed previously (Zhao *et al.*, 2004; Liu and Gu, 2009). Some studies have indicated that the inhibitory effect of selenium was due to the destruction of protein structure and function (Brown and Shrift, 1982). In addition, Se-tolerant accumulator plants differ in at least two respects from sensitive species (Brown and Shrift, 1982). For example, the thresholds for Se toxicity are 20 μg/mL and 15 μg/mL in tartary buckwheat and brown rice, respectively, both of which are tolerant of Se.

Organic selenium content increased as the germination time increased. In the presence of 20 μg/mL of Na_2SeO_3, the organic selenium content in 3 d germinating buckwheat sprouts was 8.17 times that in 1 d sprouts. This result was consistent with previous studies on this topic (Zhang *et al*., 2009). It is evident that buckwheat has the ability to convert inorganic Se to organic Se. Organic Se contents responded to variable concentrations of Na_2SeO_3 in a manner similar to that for total selenium. When 30 μg/mL of selenite was supplied in the medium during germination, the organic Se content significantly decreased with increasing selenite concentration in one-day-old sprouts. This indicated that the excess Se could inhibite the germination of buckwheat.

The proportion of organic selenium progressively decreased as the external selenite concentration increased from 10 μg/mL to 30 μg/mL. In other words, external Se may reduce the rate of synthesis of organic

Table 1. Accumulation of Total Se, Organic Se and Proportion of organic Se in tartary buckwheat

Germination time/ d	Na_2SeO_3 concent（μg/mL）								
	10			20			30		
	Organic Se content (μg/g)	Total Se content (μg/g)	Proportion of organic Se (%)	Organic Se content (μg/g)	Total Se content (μg/g)	Proportion of organic Se (%)	Organic Se content (μg/g)	Total Se content (μg/g)	Proportion of organic Se (%)
0	1.15±0.014	20.19±0.012	5.70±0.003	0.86±0.022	21.56±0.019	3.99±0.012	0.91±0.008	22.10±0.033	4.12± 0.027
1	7.17±0.023	28.45±0.016	25.20±0.032	6.96±0.017	26.84±0.026	25.93±0.005	5.86±0.033	26.34±0.001	22.25±0.016
2	47.15±0.015	69.27±0.021	68.07±0.012	46.81±0.011	73.28±0.031	63.88±0.023	30.27±0.018	53.30±0.002	56.79±0.034
3	51.60±0.020	75.60±0.003	68.25±0.008	56.86±0.014	84.29±0.022	67.46±0.046	44.43±0.043	71.03±0.011	62.55±0.040

Se-enriched buckwheat sprouts were produced by germination at 20°C for 0, 1, 2 and 3 d, using 10, 20, 30 μg /mL Na2SeO3 as medium respectively. The values shown are the means ± SD.

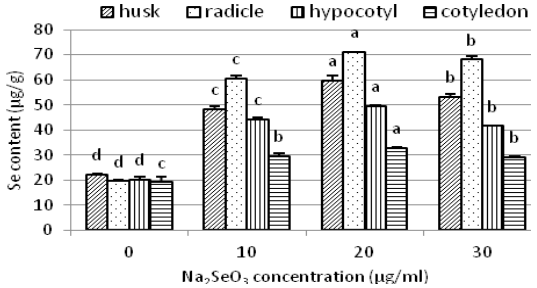

Figure 2. Distribution of Se in different parts of Se-enriched tatary buckwheat. The values shown are the means ± SD. Values followed by different letters in the same part are significantly different ($P < 0.05$). Se-enriched buckwheat sprouts were produced by germination at 20°C for 3 d with 0-30 μg/mL Na_2SeO_3 in the medium.

selenium, similar to report in a previous study (Liu and Gu, 2009). In two-day-old buckwheat sprouts treated with 30 μg/mL of Na_2SeO_3, the proportions of organic Se were 12.48% and 19.86% lower than in buckwheat sprouts treated with 20 and 10 μg/mL of Na_2SeO_3, respectively. These proportions significantly increased as germination time increased. In the presence of 20 μg/mL selenite in the culture medium, the proportion of organic selenium in germinated three-day-old buckwheat sprouts was 1.60 times that in one-day-old sprouts. Buckwheat sprouts have the ability to convert exogenous inorganic Se to organic forms of Se, but this process is regulated by the concentration of external Se. Therefore, to maximize organic Se enrichment in buckwheat sprouts, a reasonable selenium concentration in the culture medium and/or a sufficiently long germination time are recommended.

Distribution of Se in different parts of tartary buckwheat sprouts

As indicated in Figure 2, Se contents were significantly different among parts, as well as among selenite treatments. Compared with the control, Se contents were significantly higher after Se treatment. In addition, Se content was highest in the radicle and lowest in the cotyledons with a 1.97-fold difference; the content decreased in the order radicle> husk > hypocotyl> cotyledon. This parallels what has been reported in edible soy sprouts (Funes-Collado *et al.*, 2013). With increasing Na_2SeO_3 concentrations in the medium, Se contents in all parts of buckwheat sprouts increased and then decreased, with the peak Se contents observed when 20 μg/mL of Na_2SeO_3 was present in the medium. Selenate and selenite are readily absorbed by roots and translocated to other parts of the plant. Plant metabolic reactions then convert these anions into organic forms of selenium (Arvy, 1993).

Distribution of selenium bound to biological macromolecules in tartary buckwheat sprouts

The bioavailability of selenium is not only linked with its distribution in different plant parts but also its chemical forms. Therefore, it is essential to investigate the behavior of Se in relation to functional macromolecules. The proteins, nucleic acids and polysaccharides and other biological macromolecules were extracted and seperated from tartary buckwheat sprouts. Selenium contents were determined in each of these fractions (Table 2). All groups of macromolecules were observed to be bound to Se to a certain degree. Protein-bound selenium comprised 32.18% of total selenium, while nucleic-acid-bound selenium and polysaccharide-bound selenium accounted for 17.38% and 17.02% respectively. An additional 18.09% was bound to other biomolecules fraction. In addition, protein-bound Se, nucleic-acid-bound Se, polysaccharide-bound Se and other organic Se accounted for 38.70%, 20.52%, 20.11% and 21.37% of organic Se. It was evident that most of the plant Se was present in an organic form, accounting for 85.61% of total Se; the organic Se decreased in the following order: protein-bound Se > other organic Se > nucleic acid-bound Se > polysaccharide-bound Se. These results were similar to privious study (Zhao *et al*., 2004; Zhang *et al*., 2009). Another study indicated that Se incorporated into proteins exists primarily as Se-Met, taking the place of the equivalent sulfur-containing amino acids (Vogrincic *et al*., 2009). This is considered to be the underlying cause of selenium toxicity (Brown and Shrift, 1982).

Protein components from Se-enriched tartary buckwheat sprouts

In the control treatment, the most abundant protein fraction in tartary buckwheat was observed to be albumin, followed by glutelin and then prolamin, with relatively small amounts of globulin

Table 2. Distribution of organic Se in Se-enriched tartary buckwheat

Fractions	Se content (μg/g)	Percentage in orgnic Se (%)	Percentage in total Se (%)
Protein-bound Se	$27.07\pm0.048_a$	$38.70\pm0.0035_a$	$32.18\pm0.0024_a$
Necleic acid-bound Se	$14.62\pm0.01_c$	$20.52\pm0.02_c$	$17.38\pm0.015_c$
Polysaccharide-bound Se	$14.32\pm0.01_d$	$20.11\pm0.018_d$	$17.02\pm0.012_d$
Other organic Se	$15.22\pm0.018_b$	$21.37\pm0.028_b$	$18.09\pm0.018_b$

Se-enriched buckwheat sprouts were produced by germination at 20°C for 3 d with 20 μg/mL Na_2SeO_3 in the medium. The values shown are the means ± SD. Values followed by different letters in the same column are significantly different ($P < 0.05$).

Table 3. Effects of selenium concentration in protein fractions

Protein concentration (mg/g)	Na_2SeO_3 Concentration (μg/mL)			
	0	10	20	30
albumin	$16.24\pm0.66_c$	$18.29\pm1.15_a$	$18.33\pm0.59_a$	$16.89\pm0.081_b$
globulin	$8.31\pm1.19_c$	$10.50\pm0.44_{ab}$	$11.75\pm0.37_a$	$9.07\pm1.75b_c$
prolamin	$11.79\pm0.26_a$	$7.69\pm0.14_b$	$7.23\pm0.06_b$	$13.47\pm1.98_a$
glutelin	$16.14\pm0.65_a$	$16.91\pm0.86_a$	$18.28\pm0.65_a$	$17.92\pm0.61_a$

Se-enriched buckwheat sprouts were produced by germination at 20°C for 3 d with 0-30 μg/mL Na_2SeO_3 in the medium. The values shown are the means ± SD. Values followed by different letters in the same row are significantly different ($P < 0.05$).

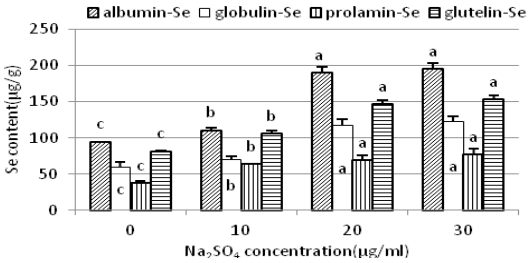

Figure 3. Distribution of Se in different proteins fraction of Se-enriched tartary buckwheat. The values shown are the means ± SD. Values followed by different letters in each protein fraction are significantly different ($P < 0.05$). Se-enriched buckwheat sprouts were produced by germination at 20°C for 3 d with 20 μg/mL Na_2SeO_3 in the medium.

(Table 3). This is consistent with what has been reported in previous studies (Wei *et al*., 2003; Guo *et al.*, 2006). Changes in the contents of protein fraction were different with respect to external selenite concentrations over the range of 0-30 μg/mL. After germination had proceeded for 3 d, Se treatment was responsible for significant increases in the contents of albumin, globulin and prolamin but not in glutelin ($P < 0.05$). In other words, the degradation of storage proteins in seeds not treated by Se would occurs after germination for 3 days and was higher than that treated by Se. This may be caused by the activities of protease. A previous research suggested that the activities of protease were affected by Se for plant exclusion from the external selenite, even when ambient selenite concentrations are low (Navarro-Alarcon and Cabrera-Vique, 2008). Though the inhibitory effects of low Se concentration were not observable from the index of total Se or of organic Se, it can theoretically be observed by examining protease activities. In the presence of 20 μg/mL of Na_2SeO_3, buckwheat sprouts that had been germinating for 3 d displayed increases of 18.71% and 41.39% in albumin and globulin, respectively ($P < 0.05$). Meanwhile, an increase of 8.1% in glutelin content was observed, but this was not significant ($P > 0.05$), compared to control sprouts.

Selenium contents of protein fractions from tartary buckwheat sprouts

Four protein fractions were extracted from Se-enriched buckwheat sprouts and their Se contents were determined (Figure 3). The results showed that the Se contents of protein fractions decreased in the following order: albumin> glutelin> globulin> prolamin; the first two fractions accounted for most of the protein-bound Se. As the concentration of selenite in the medium increased, the contents of Se bound to the four protein fractions also increased. Among them, when 20 or 30 μg/mL of Na_2SeO_3 was present in the medium, selenium contents in each protein fraction were not significantly affected by treatment. However, compared with control buckwheat sprouts, the levels of protein-bound selenium were significantly higher in Se-treated sprouts, with the highest absolute values for each fraction in the 20 μg/mL selenium treatment. Some enzymes play an important part during biosynthesis of Se-bound protein, such as cysteine synthase, which transformes selenide into selenocysteine in place of the equivalent sulphur amino acids (Ng and Anderson, 1979). The incorporation of Se into protein is considered to be the underlying cause of selenium toxicity and the selemium-toletant accounts were different in accumulator plants (Brown and Shrift, 1982). Zhao *et al.* (2004) also reported that a low concentration of Se (<100 μg/g) in substrate facilitated the synthesis of total protein, but a high concentration of Se (>150 μg/g) played a reverse role. Therefore, treatment with 20 μg/mL of Na_2SeO_3 can be considered to be appropriate for cultivating Se-enriched buckwheat sprouts at room temperature.

Conclusion

In this study, the accumulation and distribution of Se in tartary buckwheat were investigated. Tartary buckwheat exhibited the ability to convert inorganic Se to organic Se and accumulate organic Se. Total Se and organic Se contents in the sprouts increased with germination time, however, external selenite concentrations above 30 μg/mL showed significantly inhibitory effect on the enrichment of Se in the sprouts. After germination for 3 d, the Se content in various parts of buckwheat sprouts decreased in this order: radicle> husk> hypocotyl> cotyledon, while protein-bound Se, itself dominated by albumin-bound and glutelin-bound Se, was the most dominant form among macromolecule-bound Se. Further research concerning structures and functions of Se-bound

protein are needed to be carried out in the future.

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