



Foliar application is an effective method for incorporating selenium into peanut leaf proteins with antioxidant activities

Qi Gao^{a,e}, Zi-Heng Liu^a, Jia-Le Wu^a, Yi Geng^a, Qian Zhang^a, Mei Tie^c, Xue-Jun Gu^d, Masaru Tanokura^b, You-Lin Xue^{a,*}

^a College of Light Industry, Liaoning University, Shenyang 110036, PR China

^b Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

^c College of Environment, Liaoning University, Shenyang 110036, PR China

^d Institute of Rare and Scattered Elements, Liaoning University, Shenyang 110036, PR China

^e Party School of Liaoning Provincial Party Committee (Liaoning Academy of Governance, Liaoning Provincial Institute of Socialism), Shenyang 110161, PR China

ARTICLE INFO

Keywords:

Selenium
Peanut leaf protein
Protein composition
Se species
Antioxidant activity

ABSTRACT

Proteins were extracted from Se-enriched peanut leaves, an agro-byproduct, and the foliar application of sodium selenite was indicated to be an effective method to incorporate Se into leaf selenoproteins with 75–80% incorporation rates. After trypsin digestion, the most abundant proteins from Se-enriched peanut leaf (PSPL) were identified as pathogenesis-related class 10 proteins, Ara h 8 allergen and its isoforms, using LC-MS/MS. The Se species in both the low Se PSPL and high Se PSPL were determined to be selenomethionine (SeMet), methyl-selenocysteine (MeSeCys) and selenocystine (SeCys₂) with SeMet (15.6 mg/g) dominated the high Se PSPL. Their antioxidant activities were also evaluated using free radical scavenging assay, reducing power assay and ferric thiocyanate (FTC) test. As results, the PSPL exhibited potent DPPH radical (96.2%) and superoxide anion radical (98.4%) scavenging activities and showed strong reducing power in a Se-concentration-dependent manner, indicating that PSPL can be used as antioxidants and Se sources to improve health.

1. Introduction

Selenium (Se) is an essential trace element for all animals, including humans, but its distribution is uneven around the world predominately in low Se areas (Schrauzer & Surai, 2009; Zhang, Zhang, & Zhang, 2018). A dietary deficiency of Se is closely related to some diseases, such as Kaschin-Beck disease and Keshan disease (Liu, Chen, Zhao, Gu, & Yang, 2011; Zhou, Wang, Li, & Li, 2018). However, the daily dietary demand for Se cannot be obtained with normal foodstuffs due to the relatively low Se contents of these foods. To increase dietary Se intake, efforts have been made to study various Se-enriched agricultural commodities, such as rice, cabbage, pumpkin, broccoli, chive, onion, garlic, mushroom and wheat (Gong, Ai, Zhang, & Cheng, 2018; Pyrzynska, 2009). Fertilization and foliar application are usual agronomic-based strategies to deliver Se to plants, and sprouting seeds are also regarded to be a good Se-enriched media for plants (Wan, Zhang, & Adhikari, 2018). Peanut (*Arachis hypogaea* L.) is cultivated throughout the tropical and warm temperate regions of the world, with an annual output totaling approximately 39.9 million metric tons. Although peanut

originated in South America, China is the largest producer of peanut in the world (42%), followed by India (18%) and the USA (10%) (Guo, Hu, Wang, & Liu, 2018; Torres, Barros, Palacios, Chulze, & Battilani, 2014). Peanut is a great candidate for Se-enriched products due to its high protein content (25%). Gao, Luo, Hu, and Wu (2017) reported that the total Se in Se-enriched peanut (75 g m⁻² sodium selenite in soil) was 128 µg per hundred seeds and selenomethionine (SeMet) was the dominant Se species detected (65% of the total Se). Wang, Zhang, Lai, and Wu (2016) revealed that selenite significantly increased the glutathione and ascorbate concentrations in peanut seedlings by mediating the ascorbate-glutathione cycle, and the selenite-induced hydrogen peroxide may act as a second messenger in the signaling pathways. Due to the high production of peanuts in China, a large amount of peanut leaves remains after harvest, which will be ground for feed or directly discarded. Therefore, the effective use of agro-byproducts such as peanut leaves, which contain approximately 18% crude protein, with improved nutritional and functional properties are of great interest (Neucere & Godshall, 1979).

Due to the high fiber content, the utilization of green forage as a

* Corresponding author at: College of Light Industry, Liaoning University, No.66 Chongshan Middle Road, Huanggu District, Shenyang, Liaoning Province, PR China.

E-mail address: xueyoulin@lnu.edu.cn (Y.-L. Xue).

<https://doi.org/10.1016/j.foodres.2019.108617>

Received 22 December 2018; Received in revised form 6 August 2019; Accepted 11 August 2019

Available online 12 August 2019

0963-9969/ © 2019 Elsevier Ltd. All rights reserved.

feed for non-ruminants is limited, which may be overcome by the extraction of protein (Aletor & Adebayo, 2012). The amino acid profiles of leaf proteins from most species compare favorably with those of soybean, meat, fish and egg, and generally surpass the FAO essential amino acid pattern (Aletor, Oshodi, & Ipinmoroti, 2002). Growing evidence shows that Se supplementation has many health-promoting effects, such as the activation of glutathione peroxidase (Du, Zhao, Li, Zhao, & Hu, 2007; Liu, Zhao, Chen, Gu, & Bu, 2012; Zhou et al., 2018). Since Se supplementation from natural food materials is considered to be safer than directly ingesting inorganic Se, it is crucial for humans to find suitable dietary sources, for example leaf proteins (Liu et al., 2011; Wan et al., 2018).

The objectives of this study were to determine the best method for the Se enrichment of peanut leaves in Se-deficit regions, to extract, characterize, and identify the Se species of the proteins from Se-enriched peanut leaf (PSPL), and to measure the *in vitro* antioxidant activities of PSPL with different Se contents.

2. Material and methods

2.1. Chemicals

Unless otherwise stated, all chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Trypsin (V5280) was purchased from Promega (USA), alcalase (2.4 L FG) was purchased from Novozymes (Denmark) and proteinase K (P8044) was purchased from Sigma (USA).

2.2. Preparation of the Se-enriched peanut leaf

One L of sodium selenite solutions (0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/mL) was sprayed on the soils of the experimental fields ($3 \times 4 \text{ m}^2$ for each concentration with about 144 peanut plants) once while sowing the seeds (Apr. 8) or on the leaves three times (333 mL of sodium selenite solution each time) at intervals of approximately 20 d (Jul. 3, Jul. 26 and Aug. 15) during the growth of the peanuts. There were 2 rows of experimental parcels (6 for each) and the parcels were separated by ditches about 30 cm. Different peanut leaf samples were collected after harvest of the peanuts (Sep. 25). The peanut leaves were naturally dried and ground into a fine powder. The Se-enrichment method used is different from previously published work for peanut plant Se-enrichment (Gao et al., 2017; Table S1).

2.3. Extraction of the PSPL

PSPL was prepared using the alkaline extraction and acid precipitation method. Peanut leaf powder (40 g) was extracted with distilled water (20:1 solvent-to-sample ratio) and adjusted to pH 9.0. After stirring for 30 min, the slurry was centrifuged at 4425g for 20 min. After filtration, the pH of the supernatant was adjusted to pH 2.0 (the pH where the highest amount of PSPL was obtained based on our unpublished data). Then, the slurry was centrifuged at 4425g for 30 min. The pellet obtained was redissolved in distilled water by adding 1.0 M NaOH, and the pH was adjusted back to 7. The PSPL solution was then ultrafiltrated, lyophilized (about 0.8 g protein obtained) and stored in a desiccator until use.

2.4. Determination of the total Se content in leaf powder and PSPL

First, 0.25 g of peanut leaf powder or 0.1 g of PSPL powder (dry weight) was put into a PTFE microwave digestion vessel with the addition of 5 mL of nitric acids (68%, v/v) and 1 mL of hydrofluoric acid (40%, v/v). Then, the resulting solutions were digested at 700 W of microwave power, and the temperature was raised to 90 °C for 10 min followed by 130 °C for 10 min in an MD8H microwave digester (APL Instrument Co., LTD, Chengdu, China). After cooling to room

temperature, 5 mL of hydrochloric acid (0.1 M) was added and heated to 150 °C until 2–3 mL of solution remained. After cooling, the solution was diluted up to 25 mL, and the Se content was determined by an AFS-9800 atomic fluorescence spectrometer (Beijing Kechuang Haiguang Instrument Company, China). The incorporation rate was calculated by dividing the protein Se content by the total Se content of leaf power.

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and polysaccharide contents of PSPL

To simplify the experiments, only 3 PSPL samples were selected for the following studies: the control (without Se enrichment; proteins from peanut leaf: PPL), the low Se PSPL (leaf application of 0.4 mg/mL sodium selenite) and the high Se PSPL (leaf application of 2.0 mg/mL sodium selenite). SDS-PAGE was carried out according to the method of Du et al. (2007). The PSPL powder (about 50 µg) was dissolved in $2 \times$ sample buffer containing 2-mercaptoethanol. The protein was denatured by boiling the sample solution for 5 min. The sample solution was loaded on a 5% stacking gel and 15% separating gel and subjected to electrophoresis at a constant voltage (80 V). The bands were stained with 0.1% Coomassie blue R-250 for 1 h and decolorized with 5% methanol and 7.5% acetic acid. The polysaccharide contents of the 3 samples were determined using the phenol-sulfuric acid method (Balavigneswaran, Kumar, Packiaraj, Veeraraj, & Prakash, 2013).

2.6. Determination of the protein composition of PPL and low Se PSPL by LC-MS/MS

New SDS-PAGE was performed on the control (PPL) and the low Se PSPL, and the electrophoresis was stopped when the proteins entered into the separation gel (about 10 min after electrophoresis). The protein bands were removed from the polyacrylamide gel and digested with 0.2 µg of trypsin (Promega V5280) in 20 µL of a 25 mM NH_4HCO_3 solution overnight at 37 °C. The peptides obtained were fractionated on a Thermo Scientific™ UltiMate 3000 HPLC system using a C_{18} column (Acclaim PepMap RSLC C_{18} 150 mm $\times$ 75 µm i.d., 2 µm). The 65 min gradients were performed at a flow rate of 300 nL/min starting from 5% to 35% solvent B (acetonitrile with 1% formic acid) in 40 min, followed by a 5 min increase to 90% B, a 5 min maintenance period at 90% B and ultimately returning to 5% B. The peptides were subjected to positive ion nanoelectrospray ionization followed by MS/MS using a Thermo Scientific™ Q Exactive mass spectrometer. The original MS/MS data were converted to MGF formatted files using MM file conversion, and the obtained files were searched against the UniProt database using the MASCOT search engine. For protein identification, carbamidomethyl (C) was the fixed modification, and oxidation (M) was the potential variable modification with maximum missed cleavages (trypsin digestion) of 2, a peptide mass tolerance of 20 ppm and a fragment mass tolerance of 0.6 Da. The relative quantification of the identified proteins was accomplished using the exponentially modified protein abundance index (emPAI) label-free method (Ishihama et al., 2005).

2.7. Determination of the Se species in the PSPL by HPLC-MS

Approximately 0.5 g of PSPL was weighed and dissolved in 50 mL of 30 mM Tris-HCl (pH 8.0) buffer. Then, 15 µL of alcalase (Novozymes, 2.4 L FG) was added, and the mixture was incubated at 45 °C for 2 h with gentle agitation. After the addition of 5 mg of proteinase K (Sigma, P8044) and incubation at 37 °C for another 2 h, the enzymes were inactivated by heating at 100 °C for 10 min. The mixture was centrifuged at 6000g for 20 min, and the supernatant was filtered through a 0.22 µm filter membrane. Two microliters of supernatant was injected into an Agilent 1290 Infinity HPLC system (with a ZORBAX 300A SB-C18 column, 100 mm $\times$ 2.1 mm i.d., 3.5 µm) coupled to an Agilent 6460 triple quadrupole mass spectrometer. The mobile phase used was 30% A: water + 70% B: acetonitrile (both containing 0.1% formic acid)

with a flow rate of 300 $\mu\text{L}/\text{min}$. The electrospray ionization (ESI) and capillary voltage of 3500 V were applied, and nitrogen was used as the nebulizer gas and the desolvation gas.

2.8. Determination of the DPPH radical scavenging effect

The DPPH radical scavenging activity of the PSPL was measured according to a previous report with slight modifications (Zhou et al., 2009). In brief, DPPH (2 mL of 0.06 mg/mL) was added to 2 mL of various doses of PSPL. Ascorbic acid (Vc) was used as the positive control, and sodium selenite was used as the inorganic selenium control in this study. The mixture was shaken vigorously and left to stand at room temperature in the dark for 20 min. Then, the absorbance of the resulting solution was measured at 517 nm against a blank control without the sample. The DPPH radical scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the blank control (without PSPL) and A_1 is the absorbance of the test sample mixed with the reaction solution.

2.9. Determination of the hydroxyl radical scavenging effect

Hydroxyl radicals (Fenton's reaction) were produced according to a previous report with modifications (Smirnoff & Cumbes, 1989). In brief, 1 mL of sample (or positive/blank/inorganic Se control) at various concentrations was mixed with 1 mL of a salicylic acid-ethanol solution (9 mM) and 0.5 mL of a ferrous sulfate (FeSO_4) solution (9 mM). Then, 0.5 mL of a hydrogen peroxide (H_2O_2) solution (8.8 mM) was added prior to incubation at 37 °C for 20 min. Vc was used as the positive control, distilled water was used as a blank control, and sodium selenite was used as the inorganic selenium control. Finally, the absorbance of the solutions was measured at 510 nm. The hydroxyl radical scavenging activity was evaluated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_1 - A_2)/A_0] \times 100$$

where A_0 is the absorbance of the control (without PSPL), A_1 is the absorbance of the sample, and A_2 is the absorbance of the blank control (without H_2O_2).

2.10. Determination of the superoxide radical scavenging effect

The superoxide anion radical scavenging effect of the PSPL was assayed according to a previous report with slight modifications (Balavigneswaran et al., 2013). Superoxide anion radicals were produced by the autoxidation of pyrogallol under alkaline conditions. In this experiment, 1 mL of sample (PSPL, Vc or sodium selenite) at various concentrations and 4.5 mL of 0.05 M Tris-HCl buffer (pH 8.2) were mixed and incubated for 20 min at 25 °C. Then, 0.2 mL of 60 mM pyrogallol acid was added to the solution and shaken vigorously for 5 min. Subsequently, 1 mL of 8 M HCl was added to terminate the reaction, and the absorbance was measured at 325 nm. The superoxide anion radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_1 - A_2)/A_0] \times 100$$

where A_0 is the absorbance of the control (without PSPL), A_1 is the absorbance of the sample and A_2 is the absorbance of the blank control (without pyrogallol acid).

2.11. Determination of reducing power

The reducing power was determined according to a previous report (Choudhary et al., 2015). Two milliliters of the sample at different concentrations (PSPL, Vc or sodium selenite) was added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The solution was incubated at 50 °C for 20 min, then 1 mL of 10%

trichloroacetic acid was added, and the solution was centrifuged at 4425g for 10 min. Next, 2.5 mL of the upper layer was added to 0.5 mL of 0.1% ferric chloride and 2.5 mL of distilled water, and the mixture was allowed to stand for 10 min. The absorbance was measured at 700 nm.

2.12. Ferric thiocyanate (FTC) test

The FTC test was performed according to a previous report (Czyzowska, Klewicka, Pogorzelski, & Nowak, 2015). Two milliliters of the sample solution (PSPL, Vc or sodium selenite) was mixed with 2 mL of 2.5% linoleic acid anhydrous ethanol solution, 4 mL of 0.05 M phosphate buffer (pH 7.0) and 2 mL of distilled water. The mixture was incubated at 40 °C in the dark and the FTC value was measured every 24 h. Next, 0.1 mL of the mixture was added to 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. After incubation for 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl solution was added to the reaction solution, and the absorbance was measured at 500 nm. The lipid peroxidation inhibition levels were calculated from the absorbance ratio of each sample and the control without any sample.

2.13. Statistical analysis

The data were analyzed using Version 8.0 SAS (SAS Institute Inc., NC, USA). Differences among samples were assessed by analysis of variance (ANOVA). Differences with probabilities of 0.05 or lower were defined as significant.

3. Results and discussion

3.1. Total Se and protein Se in peanut leaf

Plant growth was negatively affected by a high concentration of sodium selenite (≥ 4 mg/mL), especially for the soil-applied peanut, which is consistent with previous reports on Se-enriched soybean and some sprouts (Lintschinger, Fuchs, Moser, Kuehnelt, & Goessler, 2000; Tie et al., 2015; Wan et al., 2018). Therefore, the data on Se enrichment ≤ 2.0 mg/mL were collected for further analyses. The protein yields for soil enrichment (0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/mL) were $22.9 \pm 2.0\%$, $25.2 \pm 1.8\%$, $24.4 \pm 1.3\%$, $22.8 \pm 1.6\%$, $26.5 \pm 1.6\%$ and $21.9 \pm 1.9\%$, respectively. The protein yields for leaf enrichment (0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/mL) were $22.9 \pm 2.0\%$, $21.5 \pm 1.4\%$, $22.5 \pm 1.8\%$, $21.3 \pm 1.2\%$, $22.0 \pm 2.1\%$ and $24.3 \pm 1.8\%$, respectively.

As shown in Fig. 1a and b, both enrichment methods (soil and leaf) increased the total Se content in the leaf powder in a dose-dependent manner. However, foliar application allowed a higher total Se content than soil application. For PSPL, the highest Se content of the proteins with soil enrichment was $5.3 \mu\text{g}/\text{g}$ with 1.6 mg/mL sodium selenite in the soil, while much higher Se contents ($37.8\text{--}64.8 \mu\text{g}/\text{g}$) of proteins and a dose-dependent effect were observed for foliar application of 0.4–2.0 mg/mL sodium selenite (Fig. 1b). As shown in Fig. 1c, the proportion of protein Se to total Se increased from 7.6% to 66.0% for soil enrichment and then decreased to 39.1%. A high proportion of protein Se to total Se (approximately 75–80%) was maintained with leaf enrichment at different concentrations of sodium selenite, indicating that foliar application was an effective method to incorporate Se into the selenoproteins of peanut leaves, which is similar to the situation of grape leaves (Fontanella et al., 2017). However, the situation is quite different for rice leaves, in which root irrigation is a better method for Se accumulation (Yin et al., 2019). Such difference between two studies could be due to difference in plant species, which still needs further study.

To simplify the experiments, only 3 PSPL samples were selected for the following studies: the control (without Se enrichment), the low Se PSPL (leaf enrichment with 0.4 mg/mL sodium selenite) and the high Se

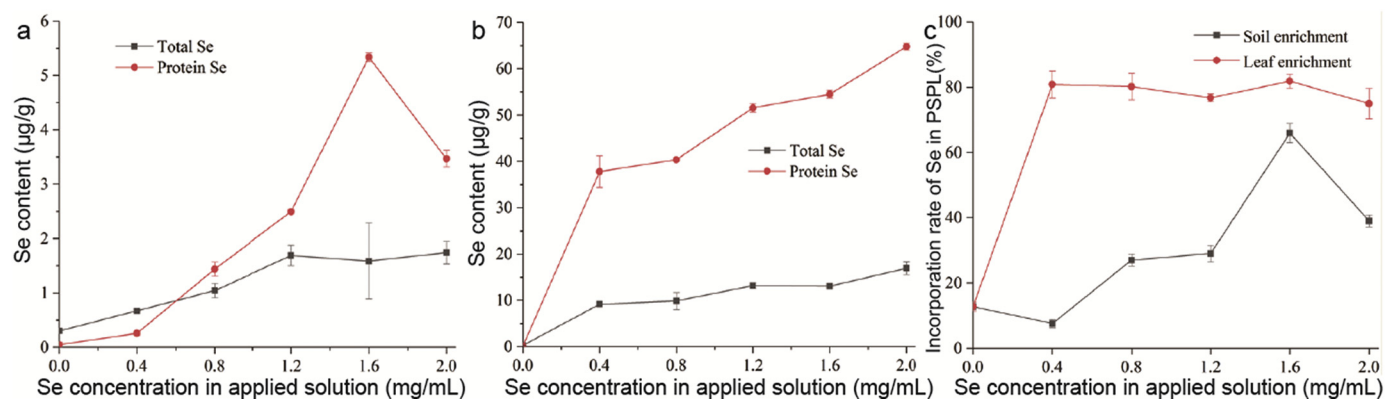


Fig. 1. Se contents of the PSPL (proteins from Se-enriched peanut leaf) and peanut leaves (dry weight) cultivated by different Se enrichment methods: Spray onto soil (a), Spray onto leaves (b); the incorporation rates (c) of both Se enrichment methods. The values shown are the mean of three replications $\pm$ SD (different samples). Total Se, Se contents of peanut leaves; Protein Se, Se contents of PSPL.

PSPL (leaf enrichment with 2.0 mg/mL sodium selenite). The polysaccharide contents of the control, low Se PSPL and high Se PSPL were $3.60 \pm 0.01\%$, $4.98 \pm 0.02\%$ and $5.39 \pm 0.02\%$, respectively. Thus, polysaccharides cannot be a major contributor for the total amount of Se in PSPL powder.

3.2. SDS-PAGE analysis of the PSPL

The SDS-PAGE of the PSPL samples (Fig. 2) revealed that protein bands at 31.5, 23.2, 20.8, 16.4 and 14.6 kDa represented the major soluble peanut leaf proteins. Clearer bands at 31.5, 23.2 and 20.8 kDa were observed for the Se-enriched samples than for the control, and only the 23.2 kDa band showed a Se concentration-dependent increase among the samples.

We tried to identify the proteins by comparing the molecular weights of the proteins in our study with those of proteins identified in peanut leaves under water stress (Katam et al., 2016). Although several proteins with similar molecular weights were obtained, such as chlorophyll *a/b*-binding protein type III (20.8 kDa), glycine-rich protein (16.0 kDa) and photosystem II oxygen-evolving complex protein 2

(14.3 kDa), it is not certain whether these proteins were expressed under Se stress and were extracted by the PSPL extraction process.

3.3. Protein composition of the PPL and low Se PSPL

The proteins in the PPL and low Se PSPL (leaf application of 0.4 mg/mL sodium selenite) were identified by LC-MS/MS and quantified using the emPAI label-free relative quantification method. The peptides for protein identification of PPL are summarized in Table S2 and the peptides for protein identification of Low Se PSPL are summarized in Table S3. The MS/MS spectrum of a representative expressed protein: pathogenesis-related class 10 protein (accession: B2ZGS2) are shown in Fig. S1. There were 161 and 190 proteins identified from PPL and low Se PSPL, respectively, and 55 proteins with emPAI values higher than 1.0 are shown in Table 1. There were 6 and 12 unique proteins with emPAI values higher than 1.0 identified from PPL and low Se PSPL, respectively. The most abundant protein was the pathogenesis-related class 10 protein, followed by Ara h 8 allergen and its isoforms, which are correlated with the 16.4 kDa band on the SDS-PAGE gel (Fig. 2). Cu/Zn-superoxide dismutase, chloroplast oxygen-evolving protein, peroxidase 2 and profilin may correlate with the 14.6 kDa band on the SDS-PAGE gel. Cytosolic ascorbate peroxidase, chitinase and mannose/glucose-binding lectin may correlate with the 31.5 kDa band on the SDS-PAGE gel. Ribulose biphosphate carboxylase small chain (detected only in low Se PSPL), peptidyl-prolyl cis-trans isomerase (detected only in low Se PSPL) and translationally controlled tumor-like protein may correlate with the 20.8 kDa band on the SDS-PAGE gel, which also explained the increased intensity of the band in the Se-enriched samples. The emPAI value of lactoylglutathione lyase changed from 0.75 (PPL) to 3.03 (low Se PSPL), which may explain the increased intensity of the 23.2 kDa band on the SDS-PAGE. Pathogenesis-related class 10 protein is induced in peanut in response to abiotic stresses such as stresses from heavy metals, salt, drought and cold temperatures (Jain et al., 2012). Ara h 8 is a minor allergen that is not thought to be responsible for fatal anaphylaxis in humans (Hurlburt et al., 2013). Chloroplast oxygen-evolving protein belongs to the PsbQ protein family, which is one of the extrinsic subunits of the photosystem II (Ifuku, 2014). Cu/Zn-superoxide dismutase and cytosolic ascorbate peroxidase are important reactive oxygen species (ROS)-scavenging enzymes that are involved in cellular defense against oxidative stress (Davletova et al., 2005). The expression of the proteins were changed by exogenous Se treatment according to their relative abundance (emPAI values), which play important roles in photosynthesis and stress resistance for plants (Ahmed, Xu, Liu, & Chen, 2018; Ning et al., 2013).

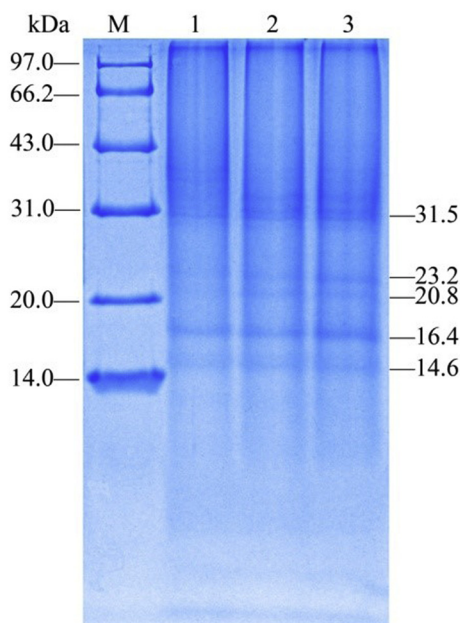


Fig. 2. SDS-PAGE analysis of the samples. M: marker, 1: PPL (proteins from peanut leaf without Se enrichment, the control), 2: low Se PSPL (proteins from Se-enriched peanut leaf) and 3: high Se PSPL.

Table 1

Proteins identified in the PPL and low Se PSPL using the UniProt database.

Accession ^a	Score	Peptides (95%)	Mr	Protein name	Organism	emPAI	
						PPL	Low Se PSPL
B2ZGS2	2660	85	16,910	Pathogenesis-related class 10 protein	<i>Arachis hypogaea</i>	17.62	79.36
Q6VT83	1910	66	16,942	Ara h 8 allergen	<i>Arachis hypogaea</i>	14.25	64.22
B0YIU5	2758	91	16,402	Ara h 8 allergen isoform	<i>Arachis hypogaea</i>	15.64	34.22
Q1HDS7	430	18	15,302	Superoxide dismutase [Cu–Zn]	<i>Arachis hypogaea</i>	0.82	12.51
A1Z1T1	1214	38	27,091	Cytosolic ascorbate peroxidase	<i>Arachis hypogaea</i>	15.23	9.2
Q5XXY3	805	15	10,360	PR protein 4A (Fragment)	<i>Arachis hypogaea</i>	6.58	6.58
U5Y4L6	839	17	16,877	Calmodulin	<i>Arachis hypogaea</i>	1.99	5.22
Q06H32	374	15	17,525	Thioredoxin fold	<i>Arachis hypogaea</i>	3.88	4.82
Q45W80	854	25	16,429	Nucleoside diphosphate kinase	<i>Arachis hypogaea</i>	4.4	4.4
Q2PXN3	132	7	8892	Disease resistance response protein	<i>Arachis hypogaea</i>	6.33	4.26
Q42515	1186	26	29,419	Chitinase (Class II)	<i>Arachis hypogaea</i>	5.19	3.48
Q06H31	285	12	19,173	Translationally controlled tumor-like protein	<i>Arachis hypogaea</i>	1.24	3.28
Q43377	1625	43	28,372	Mannose/glucose-binding lectin	<i>Arachis hypogaea</i>	0.74	3.22
B4UWA1	319	11	22,384	Lactoylglutathione lyase	<i>Arachis hypogaea</i>	0.75	3.03
B4UWE3	407	9	14,083	Peroxidase 2	<i>Arachis hypogaea</i>	1.38	2.67
E5FHZ5	242	8	17,087	Late embryogenesis abundant protein group 7 protein	<i>Arachis hypogaea</i>	0.43	2.54
A0A191UJ49	930	28	53,803	ATP synthase subunit beta, chloroplastic	<i>Arachis hypogaea</i>	1.3	2.48
A0A191UJB7	69	4	9545	Photosystem I iron-sulfur center	<i>Arachis hypogaea</i>	0.86	2.47
A0A0A6ZDP1	405	10	20,089	Glyceraldehyde-3-phosphate dehydrogenase C2	<i>Arachis hypogaea</i>	3.03	2.45
B4UWE2	263	10	20,303	Perchloric acid soluble translation inhibitor protein	<i>Arachis hypogaea</i>	1.15	2.4
Q9FZ11	1038	31	60,754	Gly1	<i>Arachis hypogaea</i>	0.88	2.03
D3K177	217	8	14,207	Profilin	<i>Arachis hypogaea</i>	0.24	1.93
A1DZF0	1005	29	60,681	Arachin 6	<i>Arachis hypogaea</i>	0.79	1.72
CS33	74	3	2416	33.0 kDa cold shock protein (Fragment)	<i>Arachis hypogaea</i>	1.61	1.61
Q1PCR4	335	7	16,062	Putative IN2-1 protein (Fragment)	<i>Arachis hypogaea</i>	0.21	1.6
PER1	718	20	34,010	Cationic peroxidase 1	<i>Arachis hypogaea</i>	1.31	1.54
B4UWD9	282	7	17,418	Threonine endopeptidase (Fragment)	<i>Arachis hypogaea</i>	0.19	1.43
Q6T2T4	1110	30	61,803	Storage protein	<i>Arachis hypogaea</i>	0.86	1.41
B4UW62	168	7	25,123	Embryo-abundant protein EMB (Fragment)	<i>Arachis hypogaea</i>	0.65	1.4
Q43375	408	15	26,140	Galactose-binding lectin (Fragment)	<i>Arachis hypogaea</i>	0.62	1.32
A7LIS6	210	10	23,078	Germin-like protein subfamily 2 member 1	<i>Arachis hypogaea</i>	0.72	1.25
B4UW50	160	5	15,990	Chitinase-like protein (Fragment)	<i>Arachis hypogaea</i>	0.78	1.16
Q2PXN7	156	6	20,227	Chloroplast thioredoxin M-type (Fragment)	<i>Arachis hypogaea</i>	0.85	1.16
B6CEX8	99	4	12,156	Non-specific lipid-transfer protein	<i>Arachis hypogaea</i>	3.42	1.1
B4UW54	167	6	21,211	GroES-like protein (Fragment)	<i>Arachis hypogaea</i>	0.55	1.09
Q45W77	61	4	17,252	Ubiquitin-conjugating enzyme 1	<i>Arachis hypogaea</i>	0.71	1.05
B4UWC8	1490	49	26,659	Mannose/glucose-binding lectin (Fragment)	<i>Arachis hypogaea</i>	0.27	1.03
<i>Proteins detected only in Low Se PSPL</i>							
B1PYZ4	2069	56	16,900	Ara h 8 allergen isoform 3	<i>Arachis hypogaea</i>	–	17.62
E3NYU3	526	16	14,801	Chloroplast oxygen-evolving protein	<i>Arachis diogeni</i>	–	13.82
Q45W78	1076	54	14,892	Ubiquitin fusion protein	<i>Arachis hypogaea</i>	–	5.38
E3NYU0	106	6	10,390	Rubisco activase (Fragment)	<i>Arachis diogeni</i>	–	4.58
A0A075M3P5	570	18	18,293	Ribulose biphosphate carboxylase small chain	<i>Arachis duranensis</i>	–	4.42
E3NYI6	431	14	18,332	Peptidyl-prolyl cis-trans isomerase	<i>Arachis diogeni</i>	–	3.58
Q5UIQ0	1625	34	17,967	Mannose glucose binding lectin	<i>Arachis hypogaea</i>	–	2.96
CONG	270	8	17,479	Conglutin	<i>Arachis hypogaea</i>	–	2.46
E3NYU2	151	7	17,542	Rubisco activase 2 (Fragment)	<i>Arachis diogeni</i>	–	2.43
Q43376	1566	55	28,185	Mannose/glucose-binding lectin (Fragment)	<i>Arachis hypogaea</i>	–	1.73
N1NKA3	998	29	60,843	Putative arachin	<i>Arachis duranensis</i>	–	1.58
E3P862	544	17	39,889	Cysteine protease	<i>Arachis diogeni</i>	–	1.05
<i>Proteins detected only in PPL</i>							
A0A0A6ZDT0	277	7	20,087	Glyceraldehyde-3-phosphate dehydrogenase C2	<i>Arachis hypogaea</i>	3.7	–
A5Z1R0	216	6	17,479	Ara h 6 allergen	<i>Arachis hypogaea</i>	3.13	–
Q06H21	878	5	17,869	Ubiquitin/ribosomal protein S27a	<i>Arachis hypogaea</i>	2.99	–
N1NG13	665	16	71,701	Seed storage protein Ara h1	<i>Arachis hypogaea</i>	1.34	–
B3IXL2	656	15	70,639	Main allergen Ara h1	<i>Arachis hypogaea</i>	1.27	–
A0A0A1EUV7	311	4	35,975	Actin (Fragment)	<i>Arachis hypogaea</i>	1.02	–

^a UniProt database accession numbers.

3.4. Se species of the PSPL

The Se species of the low Se PSPL and high Se PSPL were quantitatively analyzed using HPLC-MS with selenomethionine (SeMet), methylselenocysteine (MeSeCys), selenocystine (SeCys₂), Na₂SeO₃ and Na₂SeO₄ as standards. The chromatograms obtained for both the low and high Se PSPL samples are shown in Fig. 3a, and the corresponding contents of the Se species are shown in Fig. 3b. It is clear from the figure that inorganic Se was not detected in either PSPL sample. For the low Se PSPL, MeSeCys (6.5 mg/g) and SeMet (5.6 mg/g) were the major Se

species. However, with the increase in leaf Se enrichment (high Se PSPL), the content of SeMet (15.6 mg/g) increased greatly, but the content of MeSeCys (3.4 mg/g) decreased. In addition, the content of SeCys₂ decreased slightly after the increase in the foliar application of Se. As a result, the concentrations of Se species did not increase proportionately with Se enrichment, and SeMet was the major Se species in the high Se PSPL, which is similar to results from cereals and forage crops (such as grassland legumes and soybeans), whereas, MeSeCys is the major selenocompound in Se-enriched plants such as garlic, onions, sprouts, and wild leeks (Schrauzer, 2000; Wan et al., 2018). The high

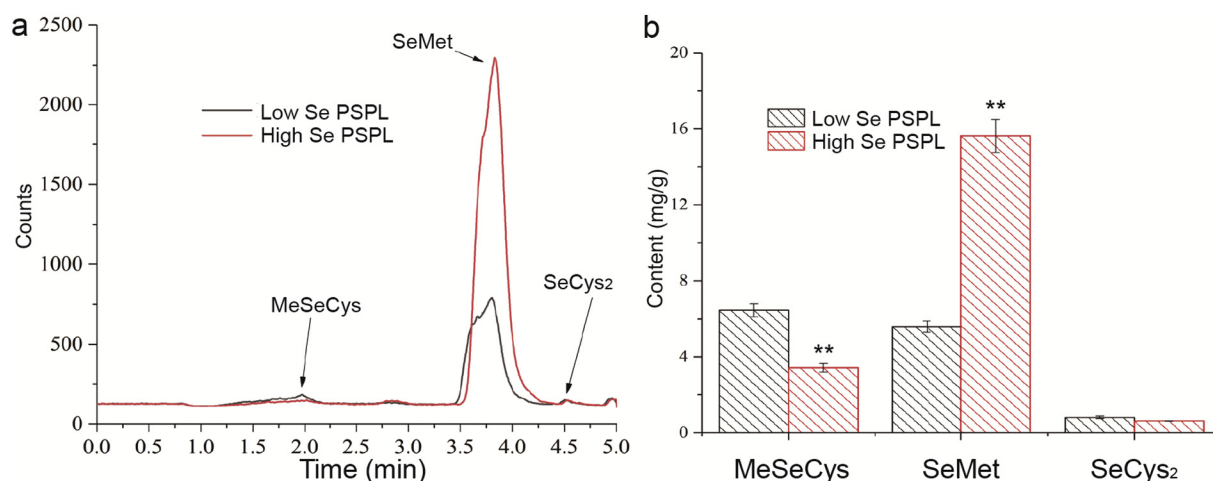


Fig. 3. HPLC-MS chromatograms of the PSPL (proteins from Se-enriched peanut leaf) samples (a) and Se species concentrations in the PSPL samples (b). The values shown are the mean of three replications $\pm$ SD (different measurements). ** $P < .01$, significantly different between samples.

accumulation of SeMet in plant proteins is achieved because tRNA^{Met} does not discriminate between Met and SeMet. However, replacement of Cys by SeCys would alter protein structure and does not occur to any significant extent (Schrauzer, 2003).

3.5. Free radical scavenging activities of the PSPL

3.5.1. DPPH radical scavenging activity

To assess the free radical scavenging activity of natural extracts, the DPPH radical scavenging test is a widely applied method. As displayed in Fig. 4a, the DPPH radical scavenging activities of the PSPL samples increased in an approximate concentration-dependent manner. The PSPL samples exhibited relatively higher DPPH radical scavenging activities than the proteins from peanut leaf (PPL) without Se enrichment. At a high concentration of 2 mg/mL, all PPL samples as well as Vc (positive control) showed high DPPH radical scavenging activities (90.4% for PPL, 94.9% for low Se PSPL, 96.2% for high Se PSPL and 97.0% for Vc).

3.5.2. Hydroxyl radical scavenging activity

Hydroxyl radicals are regarded as the most harmful radicals among the reactive oxygen species, which can affect biomacromolecules in living cells (Ahmed, Li, Li, Han, & Chen, 2018). It is therefore very important to protect living natural systems by removing hydroxyl radicals. However, all PPL samples with or without Se enrichment showed

low hydroxyl radical scavenging activities (Fig. 4b). The highest scavenging rate only reached approximately 26.4% at the protein concentration of 2 mg/mL, but interestingly, the scavenging rate of sodium selenite (inorganic Se) reached approximately 82.2% at the same concentration.

3.5.3. Superoxide anion radical scavenging activity

Redox imbalance in cells and related harmful physiological effects are induced by the accumulation of reactive oxygen species, which is initiated by the excessive generation of superoxide anion radicals (Ahmed, Xu, Liu, & Chen, 2019; Li, Chen, Wang, Tian, & Zhang, 2010). As shown in Fig. 4c, the concentration of the PPL was positively correlated with its superoxide anion radical scavenging activity. The scavenging rates of the high Se PSPL increased dramatically from 28.7% to 93.4% as the concentration was increased from 0.10 to 0.75 mg/mL. Furthermore, the PPL samples exhibited noticeably high superoxide anion radical scavenging activities in a Se-concentration-dependent manner at protein concentrations higher than 0.75 mg/mL.

According to previous reports, the proteins extracted from Se-enriched mushrooms exhibited increasing scavenging activities against superoxide anions and hydroxyl radicals with increasing Se concentrations (Du et al., 2007; Zhao et al., 2004). Similar results were obtained for proteins extracted from Se-enriched brown rice on scavenging hydroxyl, superoxide anion and DPPH radicals (Liu et al., 2012). In this study, the PPL samples exhibited potent scavenging

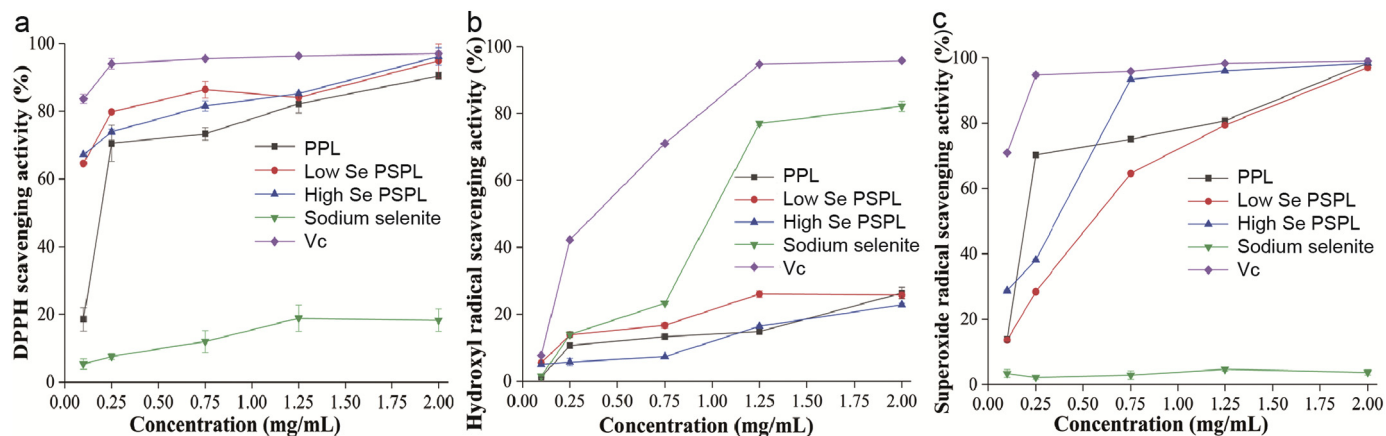


Fig. 4. Free radical scavenging activities of Vc (positive control), sodium selenite (inorganic Se), PPL (proteins from peanut leaf without Se enrichment), low Se PSPL (proteins from Se-enriched peanut leaf) and high Se PSPL against DPPH (a), hydroxyl radicals (b) and superoxide anion radicals (c) at various concentrations. The values shown are the mean of three replications $\pm$ SD (different measurements).

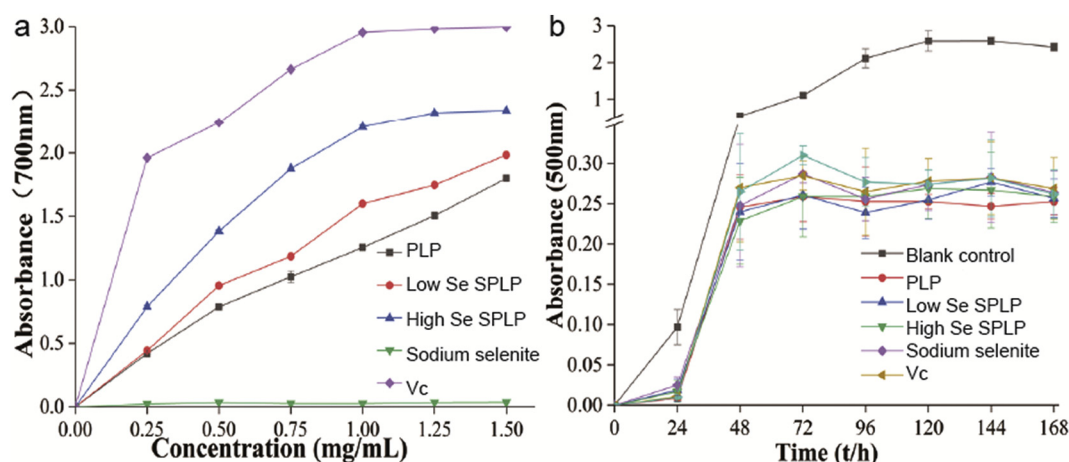


Fig. 5. Reducing power (a) and ferric thiocyanate (FTC) test (b) of Vc (positive control), sodium selenite (inorganic Se), PPL (proteins from peanut leaf without Se enrichment), low Se PSPL (proteins from Se-enriched peanut leaf) and high Se PSPL at various concentrations. The values shown are the mean of three replications $\pm$ SD (different measurements).

activities against DPPH and superoxide anion radicals but were not effective against hydroxyl radicals, which may be explained by the different free radical generation systems.

3.6. Reducing power and ferric thiocyanate (FTC) test of the PSPL

Fig. 5a shows the reducing power of the PPL samples as a function of their concentration. The reducing power of the PPL samples and Vc increased with the concentration, but sodium selenite did not show any reducing power. Furthermore, the high Se PSPL exhibited a higher reducing power than the low Se PSPL, indicating that increasing the reducing power of this protein depends on its Se content.

During linoleic acid oxidation of the FTC method, Fe^{2+} is oxidized to Fe^{3+} by the peroxides that are generated, and the Fe^{3+} ions react with SCN^- (thiocyanate) ions, showing a maximum absorbance at 500 nm (Huang, Deng, Chen, Lin, & Huang, 2014). As shown in Fig. 5b, all PSPL samples, Vc and sodium selenite, prevented lipid peroxidation. Although the high Se PSPL exhibited higher lipid peroxidation preventative effects than the low Se PSPL, the difference was not significant. The results indicated that all PPL samples have antioxidant activities but that only the reducing power is clearly Se-concentration-dependent. The antioxidative activities of proteins could be improved by hydrolysis or Maillard reaction (Xu, Han, Chen, Li, & Jin, 2018), which did not occur in our study. Thus, the Se-concentration-dependent reducing power of the PPL samples might be caused by the alteration of protein structures induced by the replacement of Met by SeMet, which needs further investigations.

4. Conclusions

Foliar application is an effective method for the incorporation of Se into selenoproteins in peanut leaves. Peanut leaf proteins were extracted, and the molecular compositions of the proteins were determined by SDS-PAGE. Pathogenesis-related class 10 protein, Ara h 8 allergen and its isoforms were identified as the most abundant proteins in the PSPL. SeMet, MeSeCys and SeCys₂ were the major Se species detected in the PSPL samples, with SeMet dominating the high Se PSPL. PSPL exhibited potent DPPH and superoxide anion radical scavenging activities and showed a strong reducing power in a Se-concentration-dependent manner, indicating that PSPL can be used as antioxidants and Se sources to improve health. However, the existence of the peanut allergen Ara h 8 should be aware.

Declaration of Competing Interest

None of the authors have a conflict of interest.

Acknowledgement

This work was supported by the Liaoning Revitalization Talents Program (XLYC1807270), the Serving Local Project of the Education Department of Liaoning Province, China (LFW201704), the Science and Technology Support Program from Shenyang City (17136800) and the College Student Innovation and Entrepreneurship Development Program of Liaoning University (x201810140252, x201810140247).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2019.108617>.

References

- Gao, L., Luo, D., Hu, X., & Wu, J. (2017). Se in Se-enriched peanut, and losses during peanut protein preparation. *International Journal of Food Science and Technology*, 52, 1–9.
- Ahmed, G. J., Li, Y., Li, X., Han, W. Y., & Chen, S. (2018). Epigallocatechin-3-gallate alleviates salinity-retarded seed germination and oxidative stress in tomato. *Journal of Plant Growth Regulation*, 37, 1349–1356.
- Ahmed, G. J., Xu, W., Liu, A., & Chen, S. (2018). COMT1 silencing aggravates heat stress-induced reduction in photosynthesis by decreasing chlorophyll content, photosystem II activity, and electron transport efficiency in tomato. *Frontiers in Plant Science*, 9, 988. <https://doi.org/10.3389/fpls.2018.00998>.
- Ahmed, G. J., Xu, W., Liu, A., & Chen, S. (2019). Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* L. *Environmental and Experimental Botany*, 161, 303–311.
- Aletor, O., & Adebayo, A. O. (2012). Nutritive and physico-chemical characteristics of the leaves and leaf protein concentrates from two edible vegetables: A comparative evaluation. *World Journal of Dairy & Food Sciences*, 7, 54–58.
- Aletor, O., Oshodi, A. A., & Ipinmoroti, K. (2002). Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. *Food Chemistry*, 78, 63–68.
- Balavigneswaran, C. K., Kumar, T. S. J., Packiaraj, R. M., Veeraj, A., & Prakash, S. (2013). Antioxidant activity of polysaccharides extracted from *Isocorys galbana* using RSM optimized conditions. *International Journal of Biological Macromolecules*, 60, 100–108.
- Choudhary, A., Kumar, R., Srivastava, R. B., Surapaneni, S. K., Tikoo, K., & Singh, I. P. (2015). Isolation and characterization of phenolic compounds from *Rhodiola imbricata*, a trans-Himalayan food crop having antioxidant and anticancer potential. *Journal of Functional Foods*, 16, 183–193.
- Czyzowska, A., Klewicka, E., Pogorzelski, E., & Nowak, A. (2015). (2015) polyphenols, vitamin C and antioxidant activity in wines from *Rosa canina* L. and *Rosa rugosa* Thunb. *Journal of Food Composition and Analysis*, 39, 62–68.
- Davletova, S., Rizhsky, L., Liang, H., Shengqiang, Z., Oliver, D. J., Coutu, J., ... Mittler, R. (2005). Cytosolic ascorbate peroxidase 1 is a central component of the reactive

- oxygen gene network of Arabidopsis. *Plant Cell*, 17, 268–281.
- Du, M., Zhao, L., Li, C., Zhao, G., & Hu, X. (2007). Purification and characterization of a novel fungi se-containing protein from Se-enriched *Ganoderma Lucidum* mushroom and its se-dependent radical scavenging activity. *European Food Research and Technology*, 224, 659–665.
- Fontanella, M. C., D'Amato, R., Regni, L., Proietti, P., Beone, G. M., & Businelli, D. (2017). Selenium speciation profiles in biofortified sangiovese wine. *Journal of Trace Elements in Medicine & Biology*, 43, 87–92.
- Gong, R., Ai, C., Zhang, B., & Cheng, X. (2018). Effect of selenite on organic selenium speciation and selenium bioaccessibility in rice grains of two Se-enriched rice cultivars. *Food Chemistry*, 264, 443–448.
- Guo, Y., Hu, H., Wang, Q., & Liu, H. (2018). A novel process for peanut tofu gel: Its texture, microstructure and protein behavioral changes affected by processing conditions. *LWT- Food Science and Technology*, 96, 140–146.
- Huang, S. S., Deng, J. S., Chen, H. J., Lin, Y. H., & Huang, G. J. (2014). Antioxidant activities of two metallothionein-like proteins from sweet potato (*Ipomoea batatas* [L.] Lam. 'Tainong 57') storage roots and their synthesized peptides. *Botanical Studies*, 55, 1–9.
- Hurlburt, B. K., Offermann, L. R., McBride, J. K., Majorek, K. A., Maleki, S. J., & Chruszcz, M. (2013). Structure and function of the peanut panallergen Ara h 8. *Journal of Biological Chemistry*, 288, 36890–36901.
- Ifuku, K. (2014). The PsbP and PsbQ family proteins in the photosynthetic machinery of chloroplasts. *Plant Physiology and Biochemistry*, 81, 108–114.
- Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., & Mann, M. (2005). Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Molecular & Cellular Proteomics*, 4, 1265–1272.
- Jain, S., Kumar, D., Jain, M., Chaudhary, P., Deswal, R., & Sarin, N. B. (2012). Ectopic overexpression of a salt stress-induced pathogenesis-related class 10 protein (PR10) gene from peanut (*Arachis hypogaea* L.) affords broad spectrum abiotic stress tolerance in transgenic tobacco. *Plant Cell Tissue and Organ Culture*, 109, 19–31.
- Katam, R., Sakata, K., Suravajhala, P., Pechan, T., Kambiranda, D. M., Naik, K. S., ... Basha, S. M. (2016). Comparative leaf proteomics of drought-tolerant and -susceptible peanut in response to water stress. *Journal of Proteomics*, 143, 209–226.
- Li, R., Chen, W. C., Wang, W. P., Tian, W. Y., & Zhang, X. G. (2010). Antioxidant activity of Astragalus polysaccharides and antitumour activity of the polysaccharides and siRNA. *Carbohydrate Polymers*, 82, 240–244.
- Lintschinger, J., Fuchs, N., Moser, J., Kuehnelt, D., & Goessler, W. (2000). Selenium-enriched sprouts. A raw material for fortified cereal-based diets. *Journal of Agricultural and Food Chemistry*, 48, 5362–5368.
- Liu, K., Chen, F., Zhao, Y., Gu, Z., & Yang, H. (2011). Selenium accumulation in protein fractions during germination of Se-enriched brown rice and molecular weights distribution of Se-containing proteins. *Food Chemistry*, 127, 1526–1531.
- Liu, K., Zhao, Y., Chen, F., Gu, Z., & Bu, G. (2012). Purification, identification, and in vitro antioxidant activities of selenium-containing proteins from selenium-enriched brown rice. *European Food Research and Technology*, 234, 61–68.
- Neucere, N. J., & Godshall, M. A. (1979). Peanut leaf extract. Chemical composition and protein characterization. *Journal of Agricultural and Food Chemistry*, 27, 1138–1140.
- Ning, C. J., Ding, N., Wu, G. L., Meng, H. J., Wang, Y. N., & Wang, Q. H. (2013). Proteomics research on the effects of applying selenium to apple leaves on photosynthesis. *Plant Physiology and Biochemistry*, 70, 1–6.
- Pyrzynska, K. (2009). Selenium speciation in enriched vegetables. *Food Chemistry*, 114, 1183–1191.
- Schrauzer, G. N. (2000). Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *Journal of Nutrition*, 130, 1653–1656.
- Schrauzer, G. N. (2003). The nutritional significance, metabolism and toxicology of selenomethionine. *Advances in Food and Nutrition Research*, 47, 73–112.
- Schrauzer, G. N., & Surai, P. F. (2009). Selenium in human and animal nutrition: Resolved and unresolved issues. A partly historical treatise in commemoration of the fiftieth anniversary of the discovery of the biological essentiality of selenium, dedicated to the memory of Klaus Schwarz (1914–1978) on the occasion of the thirtieth anniversary of his death. *Critical Reviews in Biotechnology*, 29, 2–9.
- Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, 28 (1087–1060).
- Tie, M., Li, B., Zhuang, X., Han, J., Liu, L., Hu, Y., & Li, H. (2015). Selenium speciation in soybean by high performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). *Microchemical Journal*, 123, 70–75.
- Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*, 62, 11–19.
- Wan, J., Zhang, M., & Adhikari, B. (2018). Advances in selenium-enriched foods: From the farm to the fork. *Trends in Food Science & Technology*, 76, 1–5.
- Wang, G., Zhang, H., Lai, F., & Wu, H. (2016). Germinating peanut (*Arachis hypogaea* L.) seedlings attenuated selenite-induced toxicity by activating the antioxidant enzymes and mediating the ascorbate-glutathione cycle. *Journal of Agricultural and Food Chemistry*, 64, 1298–1308.
- Xu, J., Han, D., Chen, Z., Li, M., & Jin, H. (2018). Effect of glucose glycosylation following limited enzymatic hydrolysis on functional and conformational properties of black bean protein isolate. *European Food Research and Technology*, 244, 1111–1120.
- Yin, H., Qi, Z., Li, M., Ahammed, G. J., Chu, X., & Zhou, J. (2019). Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in *Oryza sativa* L. *Ecotoxicology and Environmental Safety*, 169, 911–917.
- Zhang, S. Q., Zhang, H. B., & Zhang, Y. (2018). Quantification of selenomethionine in plasma using UPLC-MS/MS after the oral administration of selenium-enriched yeast to rats. *Food Chemistry*, 241, 1–6.
- Zhao, L., Zhao, G., Hui, B., Zhao, Z., Tong, J., & Hu, X. (2004). Effect of selenium on increasing the antioxidant activity of protein extracts from a selenium-enriched mushroom species of the *Ganoderma* genus. *Journal of Food Science*, 69, FCT184–FCT188.
- Zhou, S., Fang, Z., Lü, Y., Chen, J., Liu, D., & Ye, X. (2009). Phenolics and antioxidant properties of bayberry (*Myrica rubra* Sieb. et Zucc.) pomace. *Food Chemistry*, 112, 394–399.
- Zhou, H., Wang, T., Li, Q., & Li, D. (2018). Prevention of Keshan disease by selenium supplementation: A systematic review and meta-analysis. *Biological Trace Element Research*, 186, 98–105.

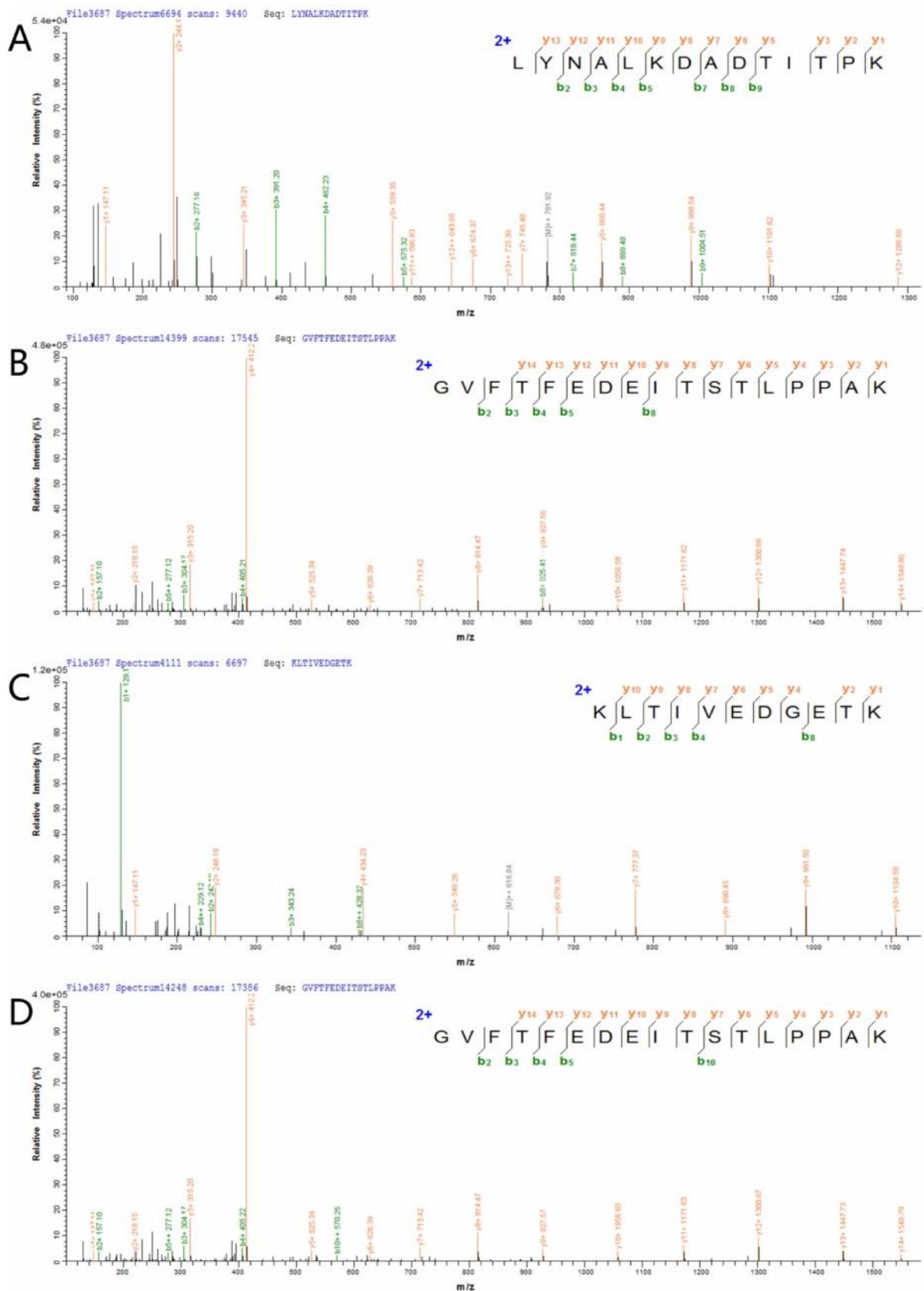


Fig. S1. MS/MS spectrum of a representative expressed protein: pathogenesis-related class 10 protein (Accession: B2ZGS2). A: MS/MS spectrum of peptide LYNALKDADTITPK (Mr: 1561.853); B: MS/MS spectrum of peptide GVFTFEDEITSTLPPAK (Mr: 1850.955); C: MS/MS spectrum of peptide KLTIVEDGETK (Mr: 1231.676); D: MS/MS spectrum of peptide GVFTFEDEITSTLPPAK (Mr: 1850.958). The orange lines represent y series ions, and the green lines represent b series ions. The fragments with the charge retained at the C-terminus were named as y1, y2, and so on, and the charge retained at the N-terminus were named as b1, b2 and so on.

Supplementary data for:

Foliar Application is an Effective Method for Incorporating Selenium into Peanut Leaf Proteins with Antioxidant Activities

Qi Gao ^{a,e}, Zi-Heng Liu ^a, Jia-Le Wu ^a, Yi Geng ^a, Qian Zhang ^a, Mei Tie ^c, Xue-Jun Gu ^d, Masaru

Tanokura ^b, You-Lin Xue ^{a,*}

^aCollege of Light Industry, Liaoning University, Shenyang 110036, P.R. China.

^bDepartment of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences,

The University of Tokyo, Tokyo 113-8657, Japan

^cCollege of Environment, Liaoning University, Shenyang 110036 P.R. China.

^dInstitute of Rare and Scattered Elements, Liaoning University, Shenyang 110036 P.R. China.

^eParty School of Liaoning Provincial Party Committee (Liaoning Academy of Governance,

Liaoning Provincial Institute of Socialism), Shenyang 110161, P. R. China.

Contents:

Table S1. Comparison of Se-enrichment methods for peanut plant

Table S2. Peptides for protein identification of PLP (Excel file)

Table S3. Peptides for protein identification of Low Se PSPL (Excel file)

Figure S1. MS/MS spectrum of a representative expressed protein: pathogenesis-related class 10 protein (Accession: B2ZGS2). A: MS/MS spectrum of peptide LYNALKDADTITPK (Mr: 1561.853); B: MS/MS spectrum of peptide GVFTFEDEITSTLPPAK (Mr: 1850.955); C: MS/MS spectrum of peptide KLTIVEDGETK (Mr: 1231.676); D: MS/MS spectrum of peptide GVFTFEDEITSTLPPAK (Mr: 1850.958). The orange lines represent y series ions, and the green lines represent b series ions. The fragments with the charge retained at the C-terminus were named as y1, y2, and so on, and the charge retained at the N-terminus were named as b1, b2 and so on.

Table S1. Comparison of Se-enrichment methods for peanut plant

	Previous work ^a	Present study
Se-enrichment methods	Sodium selenite ($75 \text{ g} \cdot \text{m}^{-2}$) was added to the soils (concentrations $< 0.4 \text{ mg} \cdot \text{kg}^{-1}$) in which the peanuts were grown.	One L of sodium selenite solutions (0.4-2.0 mg/mL) was sprayed on the leaves three times (333 mL each time) at intervals of approximately 20 d during the growth of the peanuts.
Total Se contents	128 μg Se per 100 peanuts	40.4-64.8 μg Se per g leaf powder
Se species in extracted proteins	SeMet: 10.55 μg Se per 100 peanuts	MeSeCys: 6.5 and 3.4 mg/g ^b SeMet: 5.6 and 15.6 mg/g SeCys ₂ : 0.8 and 0.6 mg/g

^a [Gao et al. \(2017\)](#)

^b Se species for Low Se PSPL and High Se PSPL