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Research Article

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Distribution of selenium in selenite-enriched soybean

Yang Yuling[1] and Liu Yuanying[2]*
***Corresponding author: Liu Yuanying**
[1]*Department of Applied Chemistry, College of Science, Northeast Agricultural University, Harbin, China*
[2]*College of Resources and Environment, Northeast Agricultural University, Harbin, China*

ABSTRACT

Se-enriched soybean was prepared by foliar fertilization. the thesis focuses on the analysis of the distribution of selenium in soybean under different concentration of sodium selenite, which contains the contents of the total selenium, inorganic selenium, organic selenium and selenocystine. The results of experiments show that the total Se contents in soybean increased as the application of sodium selenite concentration increased. In soybean grain, selenium mainly existed in the form of organic selenium, covering 84%-91% of the total selenium contents, while inorganic selenium makes up a smaller proportion, from 8.9% to 16%. The selenium from $SeCys_2$ was approximately 7-10% of the total Se present in the selenized soybean.

Keywords: soybean, selenium, organic selenium, selenocystine($SeCys_2$).

INTRODUCTION

Selenium(Se) is an essential micronutrient and has important nutritional benefits for animals and humans[1]. Se deficiency results in serious diseases in humans such as Keshan disease and Kashin-Beck disease. however it can be toxic at high dosages[2]. Bioavailability of Se is considerably dependant on its chemical species, several studies had suggested that some organic forms of selenium could show anticarcinogenic properties against certain types of cancer [3,4]. Hence, it is necessary to study Se content and its species existing in the selenized food for evaluating their nutritional value. Selenium distribution had been accomplished in a number of plants, for example, rice and vegetables. At present, few studies dedicated to selenium distribution in soybean.

2 Test

2.1 Test materials and equipment

Materials. The experiment was conducted on May 10, 2012 in harbin city, Heilongjiang Province of P. R.China. Soybean variety is Dongnong52. Soil used in experiment was a black soil on which corn was cropped last year. The basic fertility of soils tested were listed in table 1.

Table 1 Basic fertility of soils used in experiments

Soil	O.M %	Total N %(N)	Total P %(P)	Non-exchange K mg·kg^{-1} (K)	Hydr-N mg·kg^{-1} (N)	Avail-P mg·kg^{-1} (P)	Avail-K mg·kg^{-1} (K)	Solub-Se mg·kg^{-1} (Se)	pH
Black soil	3.54	0.105	0.046	984.25	191.23	25.60	155.65	0.014	7.07

Treatments and growth conditions. Soybean plants were controlled following conventional soil culture methods during growing period. The fertilizer application rates in field experiment were as following: N 27 kg·hm^{-2}, P_2O_5 69 kg·hm^{-2}, K_2O 50 kg·hm^{-2}. Field experiment included 4 treatments: Ck as control, no Se addition; Se_1, Se_2 and Se_3 were different amount of Na_2SeO_3. The concentration of sodium selenite were 0,10 g·ha^{-1}, 20 g·ha^{-1}, 30 g·ha^{-1} (in pure selenium meter), the control was sprayed with distilled water only. There were 3 replicates in experiment arranged

randomly with the area of 300 m^2. The grains were hand harvested on September 24, 2012.

Reagents. The selenium standard solutions (1mg g^{-1}) were prepared from Na_2SeO_3 (98%, Sigma-Aldrich), dissolved in 0.1 mol l^{-1} HCl and stored at 4 °C. Working solutions (0.5, 1, 2,3 ng·g^{-1}) were prepared daily by dilution of the standard solution with a blank solution to achieve the same matrix as in the samples. All reagents were of s.p grade.The following chemicals were used: 65% HNO_3, 98% $HClO_4$, 98% HCl and NaOH. 1.2% solution of $NaBH_4$ was prepared daily in 0.1 mol l^{-1} NaOH and stored at 4 ℃.

Apparatus. A continuous flow hydride generation atomic fluorescence spectrometer (AFS-930,Jitian, China) equipped with a boosted discharge hollow cathode lamp, used as a Se radiation source. Chemical and instrumental operating conditions for determination of selenium by HG-AFS were adopted from Mazej et al.[5].SYKAM S433D automatic amino acid analyzer (Germany sykam company).

2.2 Test method

2.2.1 Extraction of total selenium

Sample (0.5000 g) was weighed in 50mL erlenmeyer flasks.10 mL thick nitric acid was added for digestion.Then 5mL mixed acid (HNO_3:$HClO_4$ = 4:1) was added.Digestion liquid was heated, remaining 40℃ until white smoke arose. 5mL 6mol·L^{-1} thick hydrochloric acid was added till white fumes was generated. Samples were diluted to 10 mL with 5% hydrochloric acid. Se was determined by HG-AFS. Standard solutions were prepared in the same acidic media as the samples.

2.2.2 Extraction of inorganic selenium

Sample (0.5000 g) was weighed in 250mL erlenmeyer flasks and was mixed with 30mL distilled water and the solution was kept micro boiling for 20 min after being ultrasonic breaking for 40min and cooled to room temperature.After being centrifuged for 15 min at 12000 r·min^{-1}, the supernatant was extracted with cyclohexane. According to the method of digestion of total selenium treated, samples were diluted to 10 mL with 5% hydrochloric acid. Se was determined by HG-AFS.

2.2.3 Pretreatment of extraction of selenocystine

Soybean sample (100mg) was weighed in ampoule bottle.Sample was mixed with10 mL 6 mol l^{-1} HCl and freozen by liquid nitrogen.Then the sample was placed in the hydrolysis tube which was vacuumized, hydrolysising in (110 ± 1)℃ drying box for 22-24h. The content of selenocystine was determined by automatic amino acid analyzer.

2.2.4 The determination conditions of selenocystine by amino acid analyzer

SYKAM LCA K06/Na 4.6×150 mm separation column was used , Sodium citrate buffer solution(pH=3.45) worked as mobile phase A and B: sodium citrate buffer solution(pH=10.85) worked as mobile phase B.The content of selenocystine in soybean sample was determined by post-column derivatization with ninhydrin.

RESULTS AND DISCUSSION

3.1 Total selenium content in Se-enriched soybean

The determination of the total selenium contents in soybean were shown in Fig 1. Contrasted with that of control group, the total selenium contents increased significantly in selenite-enriched soybean, and the total Se contents in soybean increased as the application of sodium selenite concentration increased, which was closely related to Se of foliar application. Analysis results indicated that the differences between treatments were as significant as other Se-enriched plants[6]. Eating soybean rich in Se might bring in extra nutritional benefits to human beings, No visual plant damage was observed during the Se-enriched soybean growth.

3.2 The content of Inorganic selenium and organic selenium in Se-enriched soybean

The nutritional value of selenium in the soybean also depends on the selenium speciation[7]. The contents of total selenium and inorganic selenium in soybean were determined and the content of organic selenium was obtained by the total selenium content minus inorganic selenium content. The contents of selenium in different speciation were indicated in table 2. In soybean grain, selenium mainly existed in the form of organic selenium, covering 84%-91% of the total selenium contents, while inorganic selenium makes up a smaller proportion , from 8.9% to 16%. Organic selenium contents increased as the total selenium content increased in soybean,with the reduction of inorganic selenium.

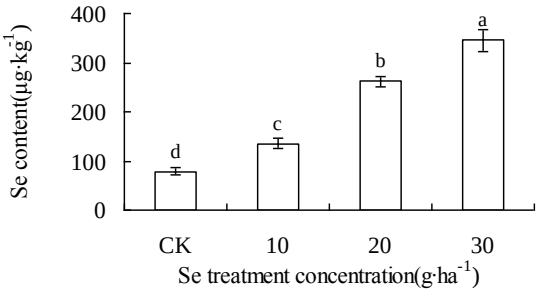

Fig. 1 Se content in soybean seeds treated by different Se concentration

Table 2 The content of inorganic selenium and organic selenium in soybean seeds

Treament	Total Se (μg·Kg^{-1})	Inorganic Se (μg·Kg^{-1})	Organic Se (μg·Kg^{-1})	Inorganic Se/Total Se (%)	Organic Se/Total Se (%)
CK	78.8909	12.6225	66.2684	16.0	84.0
Se_1	134.8121	17.9249	116.8872	13.3	86.7
Se_2	261.6306	29.5643	232.0663	11.3	88.7
Se_3	345.1218	30.7158	314.5022	8.9	91.1

3.3 Selenocystine content in Se-enriched soybean

The chromatograms of selenocystine($SeCys_2$) standard was shown in Fig.2,through which we can see that the retention time of selenocystine for 24.1min. Fig. 3 was the chromatogram of selenocystine in the soybean extract. By retention time matching, we can see that the absorption peak marked 10 is selenocystine absorption peak. The concentration of $SeCys_2$ was calculated by comparing the peak area of $SeCys_2$ between the sample and standard solutions. The content of $SeCys_2$ in selenite-enriched soybean was indicated in Table 3.Compared with the control, the content of $SeCys_2$ in selenized soybean was increased, which resulted from the increasing of the application of sodium selenite concentration. The Se from $SeCys_2$ was approximately 7-10% of the total Se present in the selenized soybean. The SeMet was not detected in the soybean extract probably because of the oxidation during acid hydrolysis. $SeCys_2$ and SeMet are the primary selenium compounds in the bean. It has been commonly reported that $SeCys_2$ found in plant is usually from oxidation of selenocysteine (SeCys)[8]. Both of SeCys and SeMet are the selenoamino acids incorporated into selenoproteins and selenium-containing proteins[9].

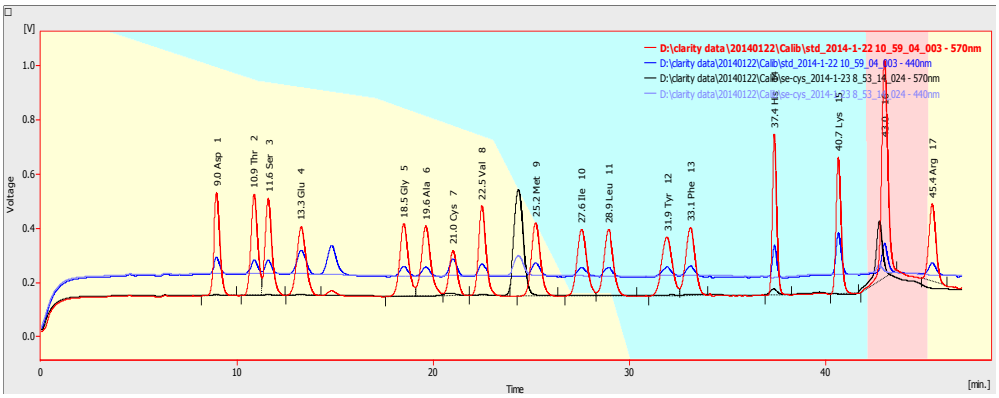

Fig.2 Chromatogram of Selenocystine standard

Table 3 Effects of different selenium concentration on the selenocystine content in soybean

Treatment	Selenocystine content (ng·g^{-1})				Mean ±SD	Significance (5%)
	I	II	III	IV		
CK	6.73	8.34	10.26	5.92	7.81±1.92	a
Se_1	23.41	28.73	31.57	30.91	28.66±3.70	b
Se_2	42.71	38.64	57.39	60.27	49.75±10.68	c
Se_3	72.83	75.54	88.53	69.42	76.58±8.35	d

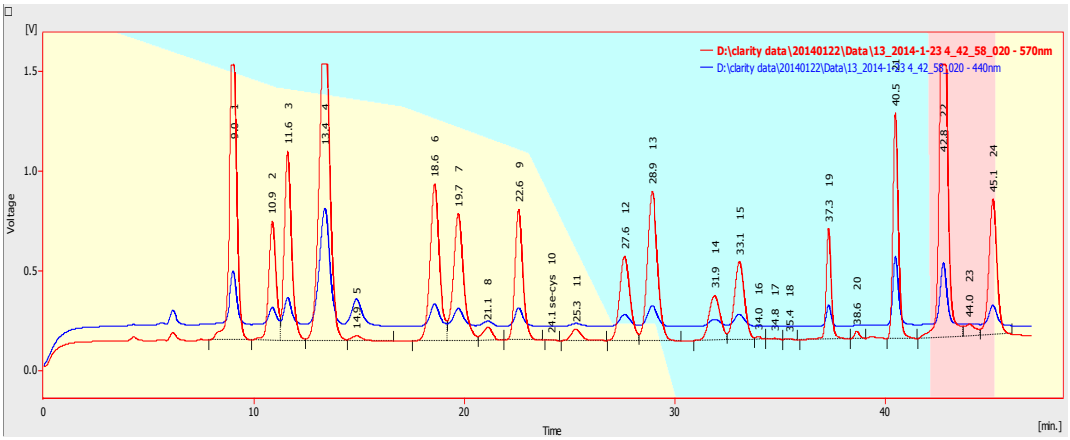

Fig. 3 Chromatogram of Selenocystine in the soybean extract

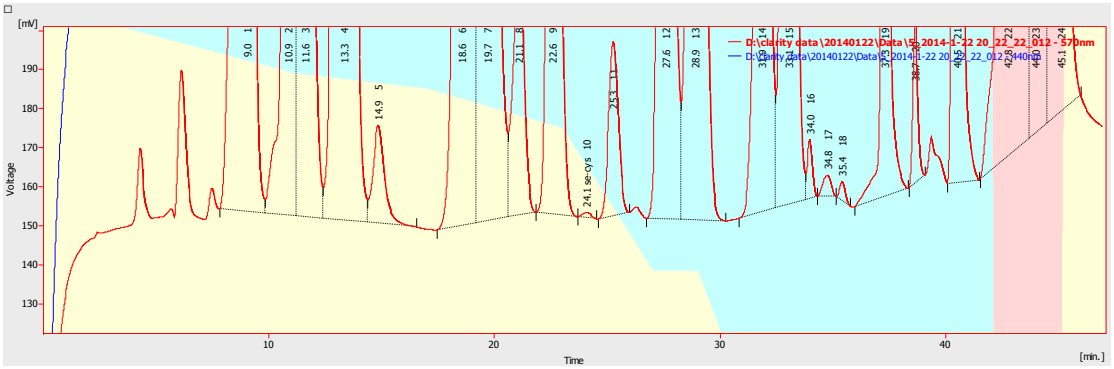

Fig. 4 Chromatogram of Selenocystine （magnifies a part）in the soybean extract

CONCLUSION

Determination of total selenium contents in soybean, compared with the control group, the total selenium content of Se-enriched soybean were significantly increased. Through the extraction of inorganic selenium in soybean seeds, using the subtraction method to get the content of organic selenium, and then analyzes the distribution of selenium in soybean seeds. The results showed that_selenium mainly existed in the form of organic selenium, covering 84%-91% of the total selenium contents, while inorganic selenium makes up a smaller proportion , from 8.9% to 16%. $SeCys_2$ was determined by amino acid analyzer. Compared with CK, the content of $SeCys_2$ in Se-riched soybean increased significantly.

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