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## ORIGINAL ARTICLE

# The extraction of different proteins in selenium enriched peanuts and their antioxidant properties



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## KEYWORDS

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**Abstract** In this study, the selenium enriched peanuts and the different solubility proteins extracted from them were investigated. The dried defatted selenium enriched peanuts (SeP) powder (0.3147 µg/g) had a 2.5-fold higher mean total selenium concentration than general peanuts (GP) powder (0.1233 µg/g). The SeP had higher concentration of selenium, manganese and zinc than that of GP, but less calcium. The rate of extraction of protein was 23.39% for peanuts and alkali soluble protein was the main component of protein in SeP, which accounted for 92.82% of total soluble protein and combined selenium was 77.33% of total selenium protein. In different forms of proteins from SeP, the WSePr due to higher concentration of selenium had higher DPPH free-radical scavenging activity, higher reducing activity and longer induction time than other proteins.

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## 1. Introduction

Selenium was an essential trace element of the human body (Davis et al., 2007). It was incorporated in a number of active selenoproteins, including the glutathione peroxidase, which acted as a cell protector against oxidative damage by free radicals (Kinsella and Melachouris, 1976). Moreover, selenium

had proved to be an inhibitor of thrombus formation, and it favorably regulated the ratio of HDL/LDL cholesterol in the blood (Okezie and Bello, 1988). According to the literature, selenium deficiency caused heart disease, muscular dystrophy and disorder in human reproduction and in that of some animal species (Naureen et al., 2015). For these reasons, it was apparent that the daily dietary intake of selenium (up to 1 mg one day) was important and necessary (Zheng et al., 2007). It was noted here that selenium was found to be toxic at higher concentrations (Abulude et al., 2006). In China, most areas were lacking in selenium except a few other areas, such as Taiwan and Hubei Shien, so it was particularly important to supplement selenium which could improve the physical quality of our people. The organic selenium mainly existed in the form of selenoprotein, selenium polysaccharide and selenium nucleic acid. It had become a popular research topic of functional

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food that strengthened selenium intake, which had very broad market prospects (Ashraf et al., 2013a,b).

Researchers generally believed that organic selenium had lower toxicity and higher absorption than inorganic (Ashraf et al., 2011). Peanut was one of the major crops of China and also a popular food for people. Therefore, supplementing the selenium element by peanut was a double benefit approach. The objective of this paper was to investigate the selenium content and different forms of SeP, and their antioxidant activity.

## 2. Experimental

Selenium enriched peanuts were provided by the Nanmu Grass Company, Ltd in Sichuan Province, while the general peanuts (GP) were purchased from local markets. Mineral contents were estimated on a Z-5000 Polarized Zeeman Atomic Absorption Spectrometer equipped with a flame atomizer. A VARIAN Cary 50 spectrophotometer equipped with computer control system was used in this study. The peanuts were crushed into powder by a multifunctional DG120 pulverizer (Factory, Chunhai medicine equipment, Ruian). The samples were processed by centrifugal separation in an Anke TD-5-A centrifuge (Factory, Anting scientific instrument, Shanghai). The induction time of samples would be obtained in a 743-type fatty acid oxidation apparatus (Company, Metrohm, Switzerland). In addition, some other frequently-used laboratory equipments were used in this study, such as digital temperature water bath, electric cooker, freeze-drying machine and so on.

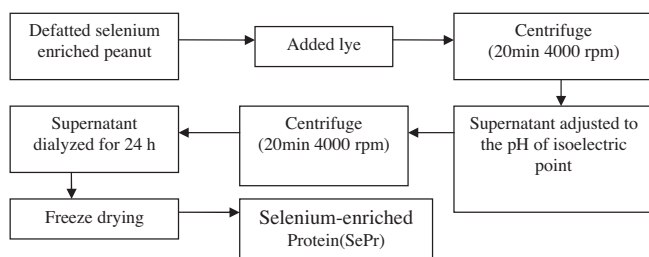
### 2.1. Determination of protein content

The content of protein in the samples was determined by the modified method described by Wang Furong<sup>7</sup>. Briefly, 10 mg of coomassie brilliant blue (Sigma) was accurately weighed and dissolved in 5 mL ethanol (95% v/v), then 10 mL phosphoric acid (85%, w/v) was added. At last, distilled water was added to the solution and the volume was made up to 100 mL.

A certain amount of NaCl (0.15 mol/L) was added to five tubes, respectively, containing 0, 0.2, 0.4, 0.6, 0.8, 1.0 mL of bovine serum albumin solution (100 µg/mL, Sigma) and the total volume was made up to 1.0 mL. Then 5 mL of the prepared coomassie brilliant blue solution was added into the tubes and placed for 3–5 min. The absorbance was measured at 565 nm, and then the standard curve was plotted.  $Y = 0.006x - 0.0125$ ,  $R^2 = 0.9985$ . The protein contents of the samples were determined by the same method (Bano et al., 2014).

### 2.2. Extraction procedure

In this study, extractions were performed by following steps.



A 5 g of SePr was accurately weighed, then added into 100 mL of distilled water, centrifuged at 4000 rpm after mixed at 60 °C for 20 min. The residue was washed with distilled water and centrifuged again. The supernatants were incorporated and dialyzed for 24 h. At last, the water soluble protein (WSePr) was obtained by freeze-drying. Three aliquots (1 g) of residues aforementioned were mixed with 100 mL of NaCl solution (0.5 mol/L), 100 mL of ethanol (75%) and 100 mL of NaOH solution (0.1 mol/L), respectively. The subsequent steps were the same as the steps of WSePr. The salt soluble protein (SSePr), the prolamin (PSePr) and the alkali soluble protein (ASePr) were obtained.

### 2.3. Se, Ca, Mg and Zn analysis

Dried peanuts of both SeP and GP were ground into fine powders and defatted with petroleum ether. The samples were digested on the electric furnace by mixed acid ( $\text{HNO}_3$ :  $\text{HClO}_4 = 4:1$ , v/v). The Ca, Mg, Zn and Se in the samples were determined by flame atomic absorption spectrometry.

### 2.4. Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

This assay detected scavenging of free radicals by the tested compound through the scavenging activity of the stable DPPH free radical. This assay was performed using a previously described method (Sumner et al., 1981) with slight modifications. Briefly, 2 mL sample solutions with different concentrations were mixed with 2 mL DPPH<sup>•</sup> solution (0.2 mg/L) in 95% ethanol, reacted for 30 min, and then the absorbance of the sample was measured at 517 nm by a spectrophotometer. The result of antiradical activity was expressed as a percentage clearance rate calculated by the following equation:

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

where  $A_0$  was the absorbance of control and  $A_1$  was the absorbance of the protein, respectively.

### 2.5. Reducing power assay

The reducing power was estimated by the following procedure proposed by Wang et al., 2006 with small modifications. 2.5 mL different concentrations of the samples were placed in five test tubes, and 2.5 mL phosphate buffer (0.2 mol/L, pH6.6), 2.5 mL  $\text{K}_3[\text{Fe}(\text{CN})_6]$  (1%) solution were added to each test tube, orderly. The test tubes were incubated in a water bath at 50 °C for exactly 20 min, and then cooled to room temperature rapidly. To this end, 10 mL distilled water and 2 mL  $\text{FeCl}_3$  (0.1%) solution were added to the five test tubes. Absorbance was monitored at 700 nm after 10 min. The reducing power of Ascorbic Acid and control was analyzed at the same conditions. Each of the experiment was run in duplicate and the experiments were performed in triplicate to confirm the results.

### 2.6. Determination of oxidative stability

It was determined by the method of accelerated oxidation test (Tzeng et al., 1988). The induction time would be obtained in a

743-type fatty acid oxidation apparatus (Voutsina et al., 1983). The oxidative stability index of lard, represented as induction time in hours, was measured with an automated Metrohm Rancimat apparatus model 743 (Metrohm, Switzerland) by using a flow of air and high temperatures to accelerate oxidation. Eight oil samples were analyzed in the equipment at the same time. For each sample, 3.00 g of lard, and then 3 mL of distilled water and sample solutions (2 mg/mL), respectively. And 3 drops of Tween 20 were added into reaction tube and mixed, respectively. The conductivity cells were filled with deionized water up to 90 mL. The air was flown through the heated oil at a temperature of  $110 \pm 0.1$  °C, with an air flow of 20 L/h. The time taken until there is a sharp increase of conductivity measured by the instrument is termed as the induction time (Ahmad et al., 2014). Oil samples without any antioxidants (control) were also analyzed under the same conditions. All tests were performed in triplicate (Noor et al., 2014).

### 3. Results and discussion

#### 3.1. The optimum parameters of protein extraction

The optimum parameters of protein extraction were obtained from SeP by the method of single factor test. The results were: liquid ratio, 1:8; extraction temperature, 60 °C; extraction time, 120 min; pH value, 9.0; extraction times, 3. The extraction rate of protein was 23.39% under these conditions. The rate was calculated using the equation:

Extraction rate of protein

$$= (\text{The quality of the extracted protein} / \text{the quality of peanut}) \times 100\%$$

**Table 1** Mean mineral content of dried defatted selenium enrich peanuts (SeP) and general peanuts (GP).

Mineral (mg/g)	Concentration	
	SeP <sup>a</sup>	GP <sup>b</sup>
Calcium	1.6289 ± 0.1022	1.8985 ± 0.2010
Manganese	1.9539 ± 0.1120	1.1427 ± 0.0762
Zinc	18.7368 ± 0.42898	13.9646 ± 0.0281
Selenium	0.3147 ± 0.0249	0.1233 ± 0.0167

<sup>a</sup> Fat contents of SeP and GP were 47.36% and 40.51%, respectively.

<sup>b</sup> Moisture contents of SeP and GP were 1.87% and 3.74%, respectively.

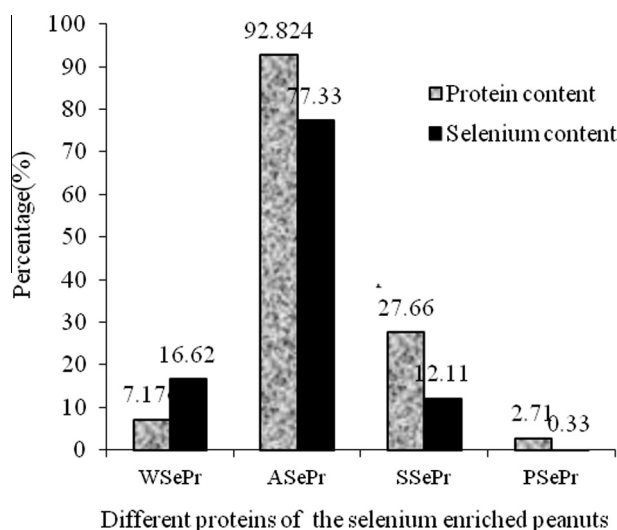
#### 3.2. The forms of selenium in SeP

The fat content of selenium-enriched peanuts was 47.36%, selenium content was 0.1653 µg/g. The major form of selenium existing in peanuts is combined with protein named as seleno-protein, which ran up to 73.39% of organic selenium and 59.89% of total selenium. ASePr was the main component of selenoprotein, which accounted 92.82% of the total soluble proteins. And the amount of the combined selenium was up to 77.33% of the total selenium content (Table 2).

As can be noticed in Fig. 1, ASePr was the main component of protein, SSePr followed, the PSePr was least, in accordance with the amount of combined selenium. This can be sorted: ASePr > WSePr > SSePr > PSePr. And the amount of combined selenium was in the following order: WSePr > ASePr > SSePr > PSePr. There was free of selenium in the peanut oil based on the tests.

#### 3.3. Contents of Se, Ca, Mg, and Zn

The powders of dried defatted SeP and GP were analyzed for the contents of Se, Ca, Mg, and Zn (Table 1). Elemental analysis indicated that the SeP had a 2.5-fold higher selenium concentration than that of GP. In addition, SeP contained higher



**Figure 1** The contents of proteins and selenium of SeP.

**Table 2** The contents of selenium of peanuts and different proteins.

Sample	Selenium	Content <sup>a</sup> (%)	Content <sup>b</sup> (%)	Content <sup>c</sup> (%)
Dried defatted SeP	0.3147 ± 0.0249	52.356	—	100
Inorganic selenium from SeP	0.0579 ± 0.0181	—	—	18.40
Organic selenium from SeP	0.2568 ± 0.0261	—	100	81.60
Protein from SeP	0.8058 ± 0.0322	23.39	73.39	59.89
Peanut oil	0	—	—	—

<sup>a</sup> The percentage of selenium content occupied defatted SeP by weight.

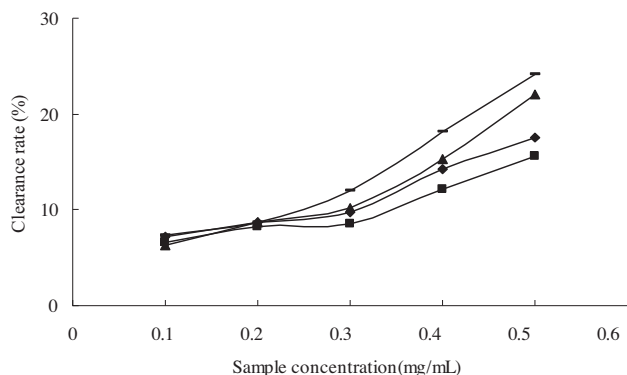
<sup>b</sup> The percentage of selenium content occupied organic selenium by weight.

<sup>c</sup> The percentage of selenium content occupied total selenium by weight.

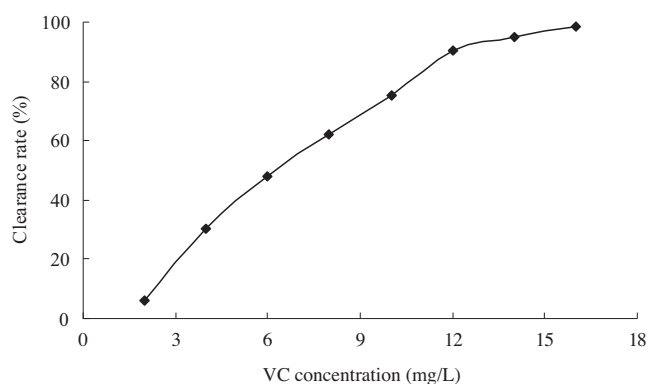
concentrations of manganese and zinc (Table 1), while GP had more calcium.

### 3.4. Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

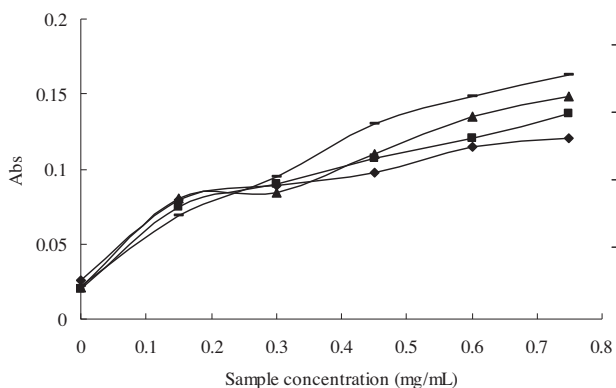
As the description of Figs. 2 and 3, Ascorbic Acid and different proteins from SeP had DPPH scavenging activity. As the



**Figure 2** The DPPH scavenging activity of different proteins from selenium enriched peanuts (SeP) compared to ascorbic acid. ■ WSePr, ◆ SSePr, ▲ ASePr, ▲ ASePr.



**Figure 3** The DPPH scavenging activity of ascorbic acid.



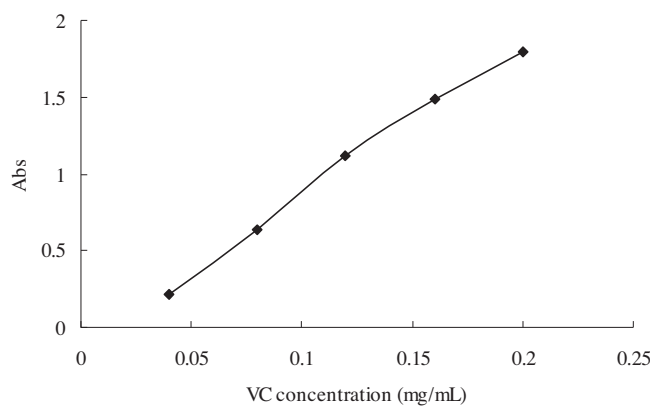
**Figure 4** The reducing power of different proteins from selenium enriched peanuts (SeP). ■ WSePr, ◆ SSePr, ▲ ASePr, ▲ ASePr.

concentration of samples increased, so did the scavenging activity. The DPPH scavenging activity of different samples was expressed as  $IC_{50}$  (the sample concentration of the radical scavenging rate was 50%). The  $IC_{50}$  values of antioxidant activity of ascorbic acid and different proteins from SeP were as follows: ascorbic acid 0.007 mg/mL, WSePr 1.141 mg/mL, ASePr 1.287 mg/mL, SSePr 1.661 mg/mL and PSePr 2.114 mg/mL.

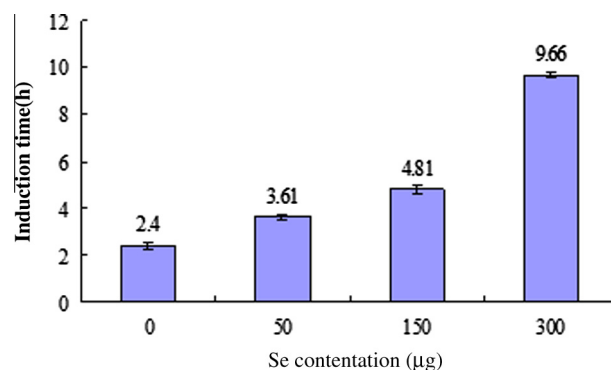
Reducing power assay: As shown in Figs. 4 and 5, as the concentrations of samples increased, the antioxidant activity ranks according to the absorbance in the following order: ascorbic acid, WSePr, ASePr, SSePr and PSePr.

### 3.5. The oxidative stability

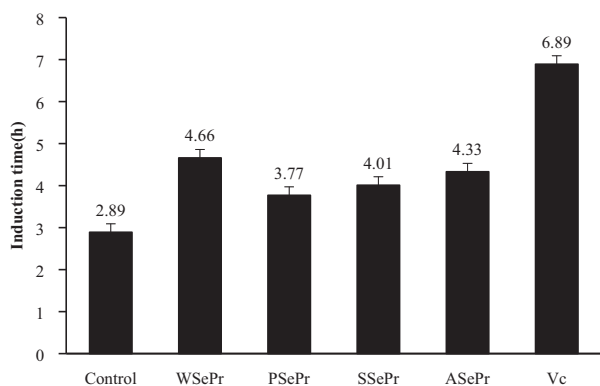
The results of the oxidative stability of fat for the different proteins were obviously different. It can be seen from Fig. 6 that the induction time increased when the selenium content was raised. The antioxidant activities of different proteins from SeP were in the following order: ascorbic acid, WSePr, ASePr, SSePr, PSePr and control (Fig. 7).



**Figure 5** The reducing power of ascorbic acid.



**Figure 6** The induction time of different concentration of inorganic selenium.



**Figure 7** The induction time of ascorbic acid and different proteins from selenium enriched peanuts (SeP).

#### 4. Conclusions

This experiment had investigated the Se, its existing forms and the antioxidant activity of selenium enriched peanuts in vitro. On the basis of the results, it was concluded that the SeP had higher concentration of selenium, manganese and zinc than that of GP, and less calcium. For different proteins from SeP, WSePr had higher DPPH free-radical scavenging activity, higher reducing activity and longer induction time than other proteins due to the higher selenium concentration. The chemical properties of the selenium of proteins remained unknown. Further studies were needed to identify the construction of selenium of different proteins of SeP.

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