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ORIGINAL ARTICLE

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

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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) power (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.

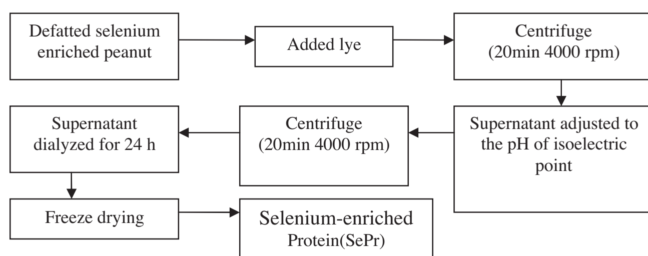
2.1. Determination of protein content

The content of protein in the samples was determined by the modified method described by Wang Furong[7]. 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 (HNO_3: $HClO_4$ = 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$^{\cdot}$ 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 $K_3[Fe(CN)_6]$ (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 $FeCl_3$ (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

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