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Nutrition

Selenium-biofortified corn peptides: Attenuating concanavalin A—Induced liver injury and structure characterization

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ABSTRACT

The relationship between hepatoprotective effects of selenium-biofortified corn (*Zea mays Linn)* peptides (SeCPs) and its antioxidant ability was evaluated and the structure of SeCPs was identified. SeCPs and corn peptides (CPs) both had good antioxidant ability, and the effect of SeCPs was significantly higher than CPs within a certain concentration range ($P < 0.05$). Additionally, animal experiments indicated that SeCPs (200 mg/kg) had a significantly protective effect against concanavalin A (Con A) induced hepatic lesions, as it significantly declined glutamic-pyruvic transaminase (AST), alanine transaminase (ALT) activities, tumor necrosis factor alpha (TNF-α), interferon (IFN)-γ contents in serum, and malondialdehyde (MDA) contents in liver ($P < 0.05$). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in liver were also significantly increased by SeCPs ($P < 0.05$). The amino acid composition of SeCPs with Mw < 1 kDa was mainly glutamic acid (Glu, 31.18%), leucine (Leu, 21.06%) and alanine (Ala, 13.26%). According to the retention time, the amino acid sequences of 8 selenium-biofortified corn peptides and 29 selenium-free corn peptides were identified. Our results illustrated that the mechanisms of SeCPs against Con A induced hepatic injury in mice may be related to its antioxidant ability and reduction of lipid peroxidation, inhibiting the release of immune factors, such as TNF-α and IFN-γ.

1. Introduction

Hepatitis, induced by virus infection, alcoholism and autoimmune diseases, has become an important public problem in a worldwide scale. It is also a leading cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma [1]. Currently, there are two main types of drugs for viral hepatitis therapy: nucleoside analogues (NUC) and interferon (IFN). NUC could quickly and effectively inhibit virus replication and improve the inflammation of liver tissue. However, long-term treatment of UNC could induce the mutation of virus, which then leads to the drug resistance and secondary hepatic injury [2]. It has also been reported that interferon (IFN)-γ obviously inhibited sperm motility and function [3]. Therefore, it is necessary to find more safe and effective natural products to combat liver damage. Matijin-Su, a dipeptide derivative isolated from a Chinese ethnic drug Matijin, could significantly inhibit hepatitis B virus (HBV) DNA replication and exhibit superior potential of anti-HBV activity than the positive control lamivudine [4,5]. Chromone, derivatived from Halenia elliptica, displayed a strong inhibitory ability against the HBV *in vitro* [6]. Hepatic injury induced by concanavalin A (Con A) is a widely used hepatitis animal model, and its pathogenic mechanism is similar to autoimmune hepatitis (AIH) and viral hepatitis [7,8]. Con A induces hepatocyte apoptosis and necrosis, and results in a decrease of alanine transaminase (ALT) and glutamic-pyruvic transaminase (AST) activity in serum [9]. It has been demonstrated that Con A can promote the secretion of immune factors, such as interferon (IFN)-γ and tumor necrosis factor alpha (TNF-α), which cause the development of hepatitis [10]. Some researches displayed that reactive oxygen species (ROS) was also a crucial factor in Con A induced hepatic injury [11–13].

Selenium-enriched food is regarded as the most effective way for selenium supplementation. Selenium intake afford additional health benefits, such as antioxidant ability [14], hepatoprotective effects [15], protective effects on cardiac tissue [16], and the enhancement of immunity [17]. Selenium-enriched compounds, such as selenium-enriched allophycocyanin [18] and selenium-enriched brown rice proteins [19], presented the characteristics of high antioxidant activity and effective liver protection. Foliar-spraying sodium selenite on plants increased grain selenium content [20], grain yield and antioxidant activity [21]. It has been reported that Se-treated corn (*Zea mays Linn)* exhibited a decrease of nitrate concentration and an increase of pigment contents

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(chlorophylls and carotenoids) [22]. Corn protein is the main by-product in the maize processing industry, which is used for producing coarse feed due to its amino acid composition, color, peculiar taste and inferior water-solubility [23]. Corn peptides (CPs), prepared by the hydrolysate of corn protein, are enriched in alanine (Ala), leucine (Leu), glutamine (Glu) and proline (Pro). Our previous studies indicated that CPs was a good candidate for facilitating alcohol metabolism [24], regulating blood pressure [25] and hepatoprotection effects [26–28].

Since selenium and CPs both possess the features of antioxidant activities and hepatoprotective effects, the primary aim of this study is to evaluate the antioxidant activity of selenium-biofortified corn peptides (SeCPs) and CPs *in vitro*. Secondly, the effects of SeCPs and CPs on attenuating Con A-induced liver injury is to evaluate. The third objective is to find out the existing forms of selenium in SeCPs, amino acid composition and sequence of SeCPs.

2. Materials and methods

2.1. Materials and reagents

Alcalase were purchased from Novozymes Co. (Bagsvaerd, Denmark). AST, ALT, SOD, GPx, MDA, were purchased from Jiancheng Biological Engineering Institute (Nanjing, China). Acetonitrile of HPLC-grade was purchased from Fisher Chemical (Bridgewater, NJ, USA). HPLC-grade Trifluoroacetic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were analytical grade.

2.2. Cultivation of selenium-enriched corn

Corn seeds were purchased from Henan golden camel agricultural technology Co. Ltd (Zhengzhou, China). Selenium-enriched corn was obtained by foliar-spraying selenium fertilizer (1.5 g Na_2SeO_3 and 42 g sodium alginate dissolved in 15 L water). Spraying was conducted on corn leaf surface every other day from full-bloom stage until a month before the harvest.

2.3. Preparation of SeCPs

Selenium-enriched corn protein was prepared as follows: corn flour, obtained from the maize kernels, was mixed with the alkali alcohol solution (0.1 mol/L NaOH and 95% ethanol, 1:1, v/v) in a ratio of 1:13 (w/v). The mixture was incubated at 45℃ for 2 h, then centrifuged at 4000 rpm for 10 min. The pH value of supernatant was adjusted to 6.3 and then standing for 30 min. Finally, the suspension was centrifuged at 4500 rpm for 10 min, the precipitation was collected and lyophilized as selenium-enriched corn protein.

SeCPs was prepared as follows: 2.86% selenium-enriched corn protein suspensions (w/v) were heated at 90–100℃ for 30 min and cooled to 60℃, and the pH value was adjusted to 8.0. An enzyme/substrate ratio of 0.6% (w/w) of substrate and alcalase was mixed and hydrolyzed for 4 h. After 4 h, the hydrolysates were heated at 100℃ for 10 min to inactivate the enzyme. The mixture was subsequently centrifuged at 3000 g for 10 min. Finally, the supernatant was fractionated through using a molecular weight cut-off 5 kDa (PLCC, Millipore, Billerica, MA, USA), and the < 5 kDa peptides fraction was collected and lyophilized for further study. The preparation method of CPs was the same above.

2.4. Determination of total selenium content and speciation

According to national standard GB 5009.93-2010, total selenium content was measured by hydride generation (HG)-atomic fluorescence spectrometry (AFS-8220, Jitian Instrument Co. Ltd, Beijing, China). The atomic fluorescence spectrometry conditions were set as follows: negative high voltage, 240 V; atomizer height, 8 mm; selenium lamp current, 50 mA; carrier gas flux, 300 mL/min. The forms of selenium were analyzed by atomic fluorescence morphology analyzer (LC-10AT-SA-10, Jitian Instrument Co. Ltd, Beijing, China).

The Se speciation was measured by strong anion-exchange (SAX) HPLC-hydride generation-atomic fluorescence spectrometry (HG-AFS). The model of SAX column was Hamilton PRPX100 column (250 nm × 4.1 mm i.d., 10 μm). The flowing phase was 40 mmol/L $(NH_4)_2HPO_4$ at pH 6.0, with the flow rate 0.5 mL/min. The HG-AFS system was set as the same parameters described above.

2.5. Detemination of antioxidant activity in vitro

2.5.1. Superoxide anion free radical ($O_2^{-\cdot}$) scavenging activity

The $O_2^{-\cdot}$ scavenging activity of SeCPs was determined by modified pyrogallol autoxidation method [29]. The reaction mixture was generated by mixing 4.5 mL of Tris−HCl buffer solution (pH 8.2, 0.1 mol/L), 1 mL EDTA, 1 mL samples and 2.4 mL distilled water and incubated at 25℃ for 10 min. Then, the mixture was shaken up and reacted for 3 min prior to addition of 0.1 mL pyrogallol solution (9 mmol/L, 25℃). At last, 50 μL of ascorbic acid (50 mg/mL) was added to terminate reaction. Tris−HCl buffer solution was used for control. The absorbance was measured at 325 nm (UV-102-02WF, Shimadzu, Japan). The $O_2^{-\cdot}$ radical scavenging activity of the sample was evaluated with the following equation:

$$O_2^{-\cdot} \text{ scavenging activity (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100\%$$

2.5.2. Hydroxyl radical ($^{\cdot}OH$) scavenging activity

The $^{\cdot}OH$ scavenging activity of SeCPs was measured by deoxyribose-iron system method [30]. The reaction solution was generated by mixing 0.4 mL phosphate buffer (pH 7.5, 50 mmol/L), 0.1 mL sample, 0.1 mL ethylene diamine tetraacetic acid (1.04 mmol/L), 0.1 mL H_2O_2 (10 mmol/L), 0.1 mL of deoxyribose solution (60 mmol/L), 0.1 mL ascorbic acid (2 mmol/L) and 0.1 mL $FeCl_3$ (1 mmol/L). After incubating the reaction solution at 37℃ for 1 h, 1 mL of hydrochloric acid (25%, v/v) was added to terminate reaction. Then, the solution was mixed with 1 mL 2-thiobarbituric acid (1%) followed by incubating at 100℃ for 15 min. The absorbance of sample was read immediately at 532 nm when sample has cooled down. Distilled water instead of the sample was used for control. The scavenging activity of SeCPs was evaluated by the following equation:

$$^{\cdot}OH \text{ scavenging activity (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100\%$$

2.5.3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The measurement method of DPPH radical scavenging activity was as follows [31]: DPPH was dissolved in ethanol to a final concentration of 0.1 mmol/L and stored at 4℃ for further study. 1.5 mL distilled water, 1.5 mL ethanol and 1.5 mL sample were mixed with 1.5 mL DPPH (0.1 mmol/L), respectively. The absorbance values (A_0, A_1, A_2, respectively) was recorded at 517 nm. The scavenging rate of DPPH radical of SeCPs was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

2.5.4. Reducing power

The reducing power of SeCPs was carried out according to the method of [32]. 2 mL samples were mixed with 2 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2 mL of potassium ferricyanide (1%). The mixture was incubated at 50℃ for 20 min, and then 2 mL of trichloroacetic acid (10%) was added. The mixture was centrifuged at 3000 r/min for 10 min. 2 mL supernate was collected into a tube and