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Preparation of bioactive peptides from lotus seed protein by enzymatic hydrolysis

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ABSTRACT

Lotus seed is the traditional health food with medicinal value in China. Many peptides with easy absorption and biological activity were prepared from protein by enzymatic hydrolysis. In this study, the extraction parameters of protein from lotus seed were optimized by using salt extraction method. The results indicated that lotus seed protein extraction rate reached 42.48% when NaCl concentration was 0.1mol/L and the ratio of solid and liquid was 1:40. Then, lotus seed protein was hydrolyzed by trypsin, and biological activities of peptides were assayed by using pyrogallol self-oxidation method. The results showed that the bioactive peptide preparation is oxidant when the ratio of enzyme and substrate is 5%. However, the bioactive peptide preparation is antioxidant when the ratio of enzyme and substrate gradually increase to 7.5%, 10%, 15% and 25%.

KEYWORDS

Lotus seed protein;
Protease;
Active peptide.

INTRODUCTION

Protein is one of the most important nutrients in our food. In recent years, some studies have demonstrated that the main form of protein utilization for human body is not amino acids, but short peptides[1]. Hence the peptides derived from protein hydrolysis have higher nutritional value than the original protein. Furthermore, the peptides originated from food proteins have all sorts of biological activity including anti-hypertensive, antioxidant, immuno-modulating, antibacterial, anti-inflammatory activities on account of the diversity of their structures[2-14]. These peptides may serve as useful ingredients in the formulation of functional foods and nutraceuticals. Therefore more and more companies and researchers are interested in the production of bioactive peptides.

Bioactive peptides can be produced via chemical synthesis, solvent extraction, protein hydrolysis and microbial fermentation. Enzymatic hydrolysis of food protein is the most commonly used method for producing bioactive peptides. M. Pokora *et al.* used a non-commercially available proteinase of fig-leaf gourd fruit for producing novel anti-hypertensive peptides[2]. Mu Gu *et al.* reported the hydrolysate of *defatted walnut* (Juglans Sigillata Dode) meal protein possess excellent antioxidant capacities[6]. The protein hydrolysate obtained from *Prosopis alba* seed flour was found to have anti-inflammatory and

antioxidant activities[7]. In a study by M. Chalamaiah *et al.*, rohu egg protein hydrolysates were confirmed to modulate immune function and exert differential influences on the immune system[11]. The study by Jiang *et al.* showed that peptide–zinc complexes prepared from silver carp protein hydrolysates exhibited high antibacterial activities[13].

Lotus seed has been regarded as a traditional Chinese drug in the Chinese Pharmacopoeia, which are obtained from members of the genus *Nelumbo,* especially *Nelumbo nucifera*[15]. The nutritional substances of the embryo of lotus seed include proteins, amino acids, vitamins, phospholipids and sugars[16]. However, unlike other food proteins, there are very few researches related to bioactive peptides derived from lotus seed proteins. The paper aims to optimize the extraction parameters of protein from lotus seed, and evaluate the biological activity of enzymatic hydrolysate of lotus seed protein.

MATERIALS AND METHODS

Materials

Lotus seeds were obtained from Guangchang, China. Trpsin was purchased from Nanning Pangbo Biotech Co., Ltd. All other chemicals were of analytical purity.

Methods

Extraction of lotus seed protein

Lotus seeds were added into 100 ml of NaCl solution and then soaked to *become soft*. The soften lotus seeds was broken and the resultant mixtures were stirred for one hours and then filtered. The filtration residue was washed two times by 100 ml of NaCl solution. The filtrate was combined.

The effects of process parameters on lotus seed protein extraction

To study the effects of NaCl concentration and solid - liquid ratios on lotus seed protein extraction, different NaCl concentrations（0.05M, 0.10M, 0.15M, 0.20M, 0.25M, 0.30M）and different solid-liquid ratios（1:8, 1:16, 1:24, 1:32, 1:40）were used in the extraction process, respectively.

Hydrolysis of lotus seed protein by trpsin

570 ml of lotus seed protein solution (1 mg/ml) was adjusted to pH 8.0. Trpsin was added into 100 ml of protein solution. Enzymatic hydrolysis was performed at 37°C. The hydrolysates were applied for the determination of biological activity.

The effects of the ratio of enzyme and substrate and hydrolysis time on enzymatic hydrolysis of lotus seed protein

To study the effects of the ratio of enzyme and substrate and hydrolysis time on enzymatic hydrolysis of lotus seed protein by trpsin, the biological activities of enzymatic hydrolysates were assayed by changing the ratio of enzyme and substrate（5%, 7.5%, 10%, 15%, 25%） and hydrolysis time（70 min, 150 min, 240 min, 330 min）, respectively.

Assay of protein concentration

The protein concentration was determined by Bradford dye method using bovine serum albumin as a standard[17].

Assay of oxidant and antioxidant activities of lotus seed protein hydrolysates

The oxidant and antioxidant activities of lotus seed protein hydrolysates were determined by using pyrogallol self-oxidation method[18]. The oxidant and antioxidant activities were calculated in the following formulas:

$$\text{oxidant activity (\%)} = (\Delta A_0 - \Delta A)/\Delta A_0 \times 100$$

$$\text{antioxidant activity (\%)} = (\Delta A - \Delta A_0)/\Delta A0 \times 100$$

where ΔA was the velocity of pyrogallol self-oxidation when adding the hydrolysates. ΔA_0 was the velocity of pyrogallol self-oxidation when not adding the hydrolysates.

RESULTS AND DISCUSSION

Optimization of process parameters of lotus seed protein extraction

The effects of NaCl concentration and solid – liquid ratio on lotus seed protein extraction rate were shown in Figure 1. The optimal NaCl concentration and solid – liquid ratio was 0.1 mol/L and 1∶40 respectively, with 42.48% of lotus seed protein

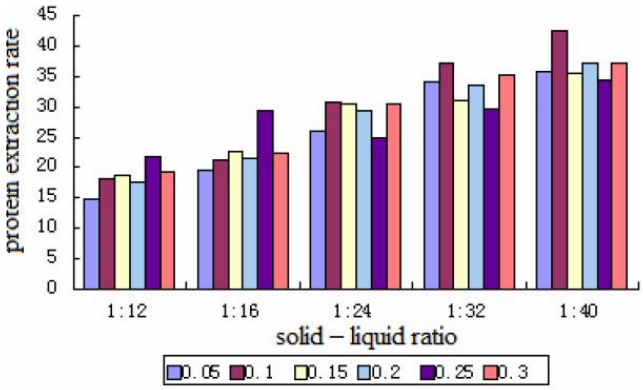

Figure 1 : the effects of NaCl concentration and the ratio of solid to liquid on protein extraction

extraction rate. In the lotus seed protein extraction procedure, the incomplete squashing of lotus seed and the reservation of protein in the filtration residue may decrease protein extraction rate.

Optimization of parameters of enzymatic hydrolysis

The effects of the ratio of enzyme and substrate and hydrolysis time on enzymatic hydrolysis of lotus seed protein were indicated in TABLE 1. The results showed that the bioactive peptide preparation is oxidant when the ratio of enzyme and substrate is 5%. However, the bioactive peptide preparation is antioxidant when the ratio of enzyme and substrate gradually increase to 7.5%, 10%, 15% and 25%. These results may suggest that the activity of lotus seed peptides is related to the length of peptide fragments. Increase of the ratio of enzyme and substrate give rise to the shortening of the length of peptide fragments. Short peptide fragments possess antioxidant activity while long counter-parts bear oxidant activity. Shen reported that if the peptide fragments were quite long, the anti-oxidative valine and leucine residues did not appear in the N-terminus and C-terminus, and hence the peptides can not bear anti-oxidative activity[19]. Furthermore, the anti-

TABLE 1 : The effects of enzymatic hydrolysis time and the ratio of enzyme and substrate on biological activity of white lotus peptides

hydrolysis time /min	ratio of enzyme and substrate /%	biological activity
70	5	30.3[a]
	7.5	8.23[b]
	10	29.87[b]
	15	59.74[b]
	25	67.01[b]
150	5	36.8[a]
	7.5	12.99[a]
	10	43.72[b]
	15	51.08[b]
	25	63.64[b]
240	5	36.8[a]
	7.5	77.92[b]
	10	80.95[b]
	15	84.85[b]
	25	84.42[b]
330	5	30.3[a]
	7.5	48.05[b]
	10	81.82[b]
	15	80.95[b]
	25	84.85[b]

Note: In TABLE 1, a means the oxidant activity of peptide fragments, whereas b means the anti-oxidant activity of peptide fragments.

FULL PAPER

oxidative activity increased with the ratio of enzyme and substrate under the same enzymatic hydrolysis time, and yet the anti-oxidative activity of the peptide fragments did not undergo obvious change when enzymatic hydrolysis time was above 150 min.

During the past years, many researches on preparation of anti-oxidant peptides have been carried out. Gu *et al.* reported that high cytoprotective activities against H_2O_2-induced oxidative damage to PC12 cells were observed, with 83% cell viability at 0.1 mg/mL of enzymatic hydrolysate of defatted walnut meal protein[6]. Ahn *et al.* found that the peptic hydrolysate of pectoralfin protein showed the highest 2,2- diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity[8]. Chen *et al.* used five different proteases to hydrolyze Soybean β2 conglycinin. Six novel anti-oxidative peptides were isolated by gel-filtration chromatography and anti-phase HPLC[20]. Nevertheless, James D. Watson postulated that diabetes, dementias, cardiovascular disease, and some cancers may be accelerated by failure of generating large numbers of reactive oxygen species recently[21]. Thus we should pay attention to the preparation of peptide with oxidative activity. To our knowledge, there have not been reports on the peptides with oxidative activity prepared from lotus seed protein until now. The present paper supplies a method for preparation of peptide with oxidative activity.

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