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Physico-chemical properties, antioxidant activities and angiotensin-I converting enzyme inhibitory of protein hydrolysates from Mung bean (*Vigna radiate*)

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ABSTRACT

Mung bean Protein hydrolyses (MBPHs) have attracted a great deal of attention due to their variety of biological activities. In present study, MBPHs were fractionate according to the molecular mass into three fractions of MBPHs-I (< 3 kDa), MBPHs-II (3–10 kDa) and MBPHs-III (> 10 kDa). Their antioxidant activity and angiotensin-I converting enzyme (ACE) inhibitory of were investigated in vitro. Results showed that the alcalase-derived hydrolysate exhibited the highest degree of hydrolysis (DH) and trichloroacetic acid–nitrogen soluble index (TCA-NSI) versus those of other enzyme hydrolysates. MBPHs-I presented the best scavenge DPPH, hydroxyl radicals, superoxide radicals, Fe^{2+} chelating activities, and the best ACE inhibitory activity (IC_{50} = 4.66 μg/mL) than that of MBPHs and MBPHs-III. And MBPHs-I rich in hydrophobic and aromatic amino acids, and its secondary structure mainly contain α-helix, β-sheet and irregular coiled. Results indicated that MBPHs-I has a great potential as natural functional materials for supplement.

1. Introduction

Mung bean (*Vigna radiata* L.) is popular and important leguminous crop in Asia and other parts of the world. In China, it has been consumed as a common food for thousands of years. Nowadays, mung bean is accepted by different people all over the world for its physiological functionalities, such as antiangiotensin I-converting enzyme (ACE) inhibitory, antitumor, antioxidant, and antidiabetic (Randhir & Shetty, 2007; Soucek, Skvor, Pouckova, Matousek, & Slavík, 2005; Yao et al., 2013). It is rich in vitamins, minerals, proteins, and essential amino acids, and the mung bean protein is approximately 25–28% on a dry weight basis (Khaket, Dhanda, Jodha, & Singh, 2015). Much focus has been placed on researching the properties of mung bean starch rather than the mung bean protein (MBP) (Ahmed, 2012). The amino acid profile of mung bean protein shows that it can meets the amino acid requirements of the human body (Du et al., 2018). In most developing countries, such as China and India, mung bean protein is used in large quantities as an alternative to animal proteins in product formulations.

In addition to applied as major protein resource, beans protein can also produce biological function peptides that have biological properties, such as antioxidant and anti-inflammatory activity (Connolly, Keeffe, Piggott, Nongonierma & FitzGerald, 2015). Enzymatic hydrolysis can improve functional properties of target protein by translating it into peptides without affecting its nutritive value (Moure, Domínguez, & Parajó, 2006). Enzyme treatment has been documented to be an effective treatment to eliminate antinutritional factors (Kuhad, Gupta, & Singh, 2011). Commercial enzymes, such as alcalase, pepsine, trypsin, flavourzyme, protamex, prolyve, and promod usually used for the hydrolysis of protein.

Dietary peptides with high nutritional value from animal or plant proteins are reported to have a potential in the regulation of food intake and body weight in humans (Anderson & Moore, 2004; Hartmann & Meisel, 2007). Polypeptides may exhibit various biological activities, such as immunomodulatory, antioxidant, antihypertensive, and

Abbreviations: ACE, antiangiotensin I-converting enzyme; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DH, the degree of hydrolysis; TCA, trichloroacetic acid; FTIR, Fourier transform infrared; CD, circular dichroism; MBP, Mung bean protein; TCA-NSI, trichloroacetic acid-nitrogen soluble index; HHL, hippuryl-histidyl-leucine; ALH, alcalase hydrolysates; NTH, neutrase hydrolysate; PAH, papain hydrolysates

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antibacterial effects (Hartmann & Meisel, 2007). Studies over the past ten years have demonstrated that protein hydrolysates, such as brewers' spent grain protein (Vieira, da Silva, Carmo, & Ferreira, 2017), peanut (Jamdar et al., 2010), pea seed (Pownall, Udenigwe, & Aluko, 2010) have good antioxidant ability. *In vitro* studies also demonstrated that protein hydrolysates from other legumes, such as lupin (Boschin, Scigliuolo, Resta, & Arnoldi, 2014), soy (Chiang, Tsou, Tsai, & Tsai, 2006), *Phaseolus lunatus* and *Phaseolus vulgaris* seeds (Torruco-Ucoa, Chel-Guerrerob, Martínez-Ayalac, Dávila-Ortíza, & Betancur-Anconab, 2009) have good ACE inhibitory and antioxidant ability. However, studies on the biological activity of hydrolysates with different molecular weight from MBP are rarely reported, and their secondary structure and the composition of amino acids are still not clear. The aim of this study is to determine the antioxidant activities and ACE-inhibitory activity and to analyze the amino acid composition and secondary structure of MBPHs, MBPHs-I, MBPHs-II, and MBPHs-III obtained from MBP by ultrafiltration separation.

2. Materials and methods

2.1. Materials

Mung beans (*Vigna radiata* L.) were purchased from the Grain and Oil Processing Company (Jilin, China). Briefly, Mung beans were cleaned, dehulled and ground into flour. Alcalase, neutrase, papain, protamex were purchased from Novozymes (Denmark). 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, hippuryl-l-histidyl-l-leucine (HHL) and ACE (from rabbit lung) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals and solvents used in this study were of analytical grade.

2.2. Preparation of enzymatic hydrolysates

Protein concentrate hydrolysis from Mung bean was prepared by individual treatments with alcalase (pH 8.0, 55 °C), neutrase (pH 7.0, 45 °C), papain (pH 7.0, 45 °C), and protamex (pH 5.5–7.55, 60 °C) (Novo Nordisk, Bagsvaerd, Denmark). The mixture with enzyme/substrate ratio of 3/100 (w/v) was reacted for 2 h then heated in boiling water bath for 10 min to inactivate the enzyme. After cooling, the mixture was adjusted to pH 4.2, then centrifuged at 5000 × *g* (4 °C) for 20 min. The supernatant was recycled using decantation and then loaded into a dialysis bag (Mw, 300 Da) to desalinate for 48 h. Finally, hydrolysates were lyophilized and stored under −80 °C until next use.

2.3. Selecting the appropriate enzyme

2.3.1. Determination of DH

The extent of proteolytic degradation was by measured by means of the degree of hydrolysis (DH) according to method described by Guerard, Dufosse, De La Broise, and Binet (2001). DH values for DH is defined as the percent ratio of the number of peptide bonds broken (h) to the total number of peptide bonds in the substrate studied (h_{tot}). The values for DH were calculated as the following equation:

$$DH\,(\%) = h/h_{tot} \times 100 = (B \times N_b)/(M_P \times \alpha \times h_{tot}) \times 100\% \quad (1)$$

where B = the amount of base consumed (mL) during the reaction. N_b = the normality of the base, M_P = the mass (g) of protein (N × 6.25), α = the average degree of dissociation of the α-NH_2 released during hydrolysis, and h_{tot} is defined as 7.9 meq/g.

2.3.2. Determination of TCA-NSI value

The trichloroacetic acid–nitrogen soluble index (TCA-NSI) was estimated by measuring the nitrogen content of hydrolyzed proteins solubilized in 10% trichloroacetic acid (TCA) according to the AOAC method (Williams, 1984). A total of 10 mL of 10% TCA solution was mixed with 10 mL of enzymatic hydrolysates, and then the resulting mixture was centrifuged at 15,000 × *g* for 5 min. The supernatant was analyzed using the Kjeldahl method (Minagawa, Winter, & Kaplan, 1984) to determine nitrogen content in TCA-soluble peptides and free amino acid. TCA-NSI was calculated according to the following equation (Zhang et al., 2012):

$$TCA{-}NSI\,(\%) = (N_1/N_0) \times 100 \quad (2)$$

where N_1 is the nitrogen content (in mg) soluble in 20% TCA and N_0 is the total nitrogen content in MBP hydrolysates (in mg).

2.4. Proximate composition analysis

Lyophilized MBP and its hydrolysates were analyzed in terms of protein content using the Kjeldahl method (Minagawa et al., 1984). The fat was extracted with chloroform:methanol (2:1, v/v) with a Soxhlet extractor (Shanghai, China, then determined gravimetrically after oven-drying (80 °C) overnight. The ash contents of MBP and its hydrolysates were estimated by heating the overnight in a muffle furnace at 550 °C (AOAC, 1995), and dry matter content (oven at 105 °C). The contents were indicated in terms of dry weight (% dw).

2.5. Fractionation of the protein hydrolysates

Freeze-dried MBPHs with a concentration of 100 mg/ml was dissolved and further separated using by ultrafiltration cube with 10 and 3 kDa MWCO, resulting in three fractions, namely, MBPH-I (< 3 kDa), MBPH-II (3–10 kDa) and MBPH-III (> 10 kDa).

2.6. Physicochemical characteristics

2.6.1. UV spectrum

UV–visible spectra of samples were scanned using a spectrophotometer (TU-1900, Purkinje General Instrument Co., Beijing, China) in the range of 200–800 nm. Each of the samples was dissolved in 100 mL of distilled water.

2.6.2. Circular dichroism (CD) spectroscopy

CD spectra were obtained on a Bio-Logic MOS 450 CD spectrometer (Bio-Logic, Claix, France) in the far-UV region (190–250 nm). All CD spectra were recorded in distilled water at room temperature with a fixed concentration of MBPHs, MBPHs-I, MBPHs-II, and MBPHs-III; the distilled water signal was subtracted for baseline correction.

2.6.3. Fourier transform infrared (FTIR) spectroscopy

Proteolytic digestion samples of different molecular segments were desiccated prior to FTIR analysis. FTIR spectra of the samples were recorded using Fourier-transform infrared spectrophotometer (Thermo Electron, USA) from 400 cm^{-1} to 4000 cm^{-1} at room temperature. The measuring resolution was 4 cm^{-1} and iterations were conducted for 32 times.

2.6.4. Determination of amino acid composition

The amino acid composition of the MBPHs and the three other fractions was analyzed using a model L-8900 Amino Acid Auto-Analyzer (L-8900, HITACHI, Japan). First, the sample solution was filtrated through a 0.22 μm pore size membrane filter and then injected into the analyzer to determine the free amino acid content. Finally, the sample was determined at 110 °C for 22 h with 6 M HCl.

2.7. Screening for antioxidant activity of MBP hydrolysates

2.7.1. DPPH radical scavenging activity

The scavenging effects on DPPH free radical of MBP hydrolysates were determined by Xie et al. (2010, 2015). DPPH solution (1 mL, 1 mM in 95% ethanol) was mixed with 1 mL of samples. At 25 °C, the mixture was shaken and left for 30 min, finally the absorbance of the solution