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Analytical Methods

Ultra-high-pressure processing improves proteolysis and release of bioactive peptides with activation activities on alcohol metabolic enzymes in vitro from mushroom foot protein

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ABSTRACT

This study was to find an effective process to extract bioactive peptides from mushroom foot and determine their effects on activation of alcohol metabolic enzymes in vitro. The optimum extraction assisted by ultra-high-pressure processing of mushroom foot peptides was obtained with a pressure of 400 MPa and a processing time of 10 min. After ultrafiltration, peptides with molecular weight of 0–3 kDa had the highest activity to activate alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) by 70.79% and 71.35%, respectively. Following dextran gel chromatography, two peaks (p-I and p-II) appeared and the activation activities on ADH and ALDH of p-I were 72.00% and 73.43%, both higher than p-II. Nine peptides were found in p-I as determined by LC–MS/MS, and two of them (IPLH and IPIVLL) were synthesized. IPLH activated ADH and ALDH by 42.7% and 29.2% respectively, which were higher than IPIVLL.

1. Introduction

Mushrooms, which are used as both medicines and foods, are popular worldwide for their rich nutrients and flavors (Liu et al., 2012; Manzi, Aguzzi, & Pizzoferrato, 2001; Ouzouni, Petridis, Koller, & Riganakos, 2009). They are well-known healthy foods that contain large quantities of proteins, vitamins, carbohydrates, and useful nutraceuticals, such as ascorbic acid and phenols (Liu et al., 2012; Oboh & Shodehinde, 2009; Ouzouni et al., 2009). However, a by-product of mushroom products, mushroom foot, constitute approximately 25% of the weight of fresh mushrooms, and are usually discarded as waste, probably because of their high degree of fibrosis, tough texture, and poor palatability (Chou, Sheih, & Fang, 2013). Compared with common vegetables such as potatoes and carrots, mushrooms are excellent sources of protein and almost all essential amino acids, especially threonine, tyrosine, and arginine (Mattila, Salo-Väänänen, Könkö, Aro, & Jalava, 2002). Thus, extracting bioactive peptides from mushroom foot not only improves the environment, but also makes full use of the mushroom, which enhances their value.

Currently, alcohol abuse and alcohol dependence have become increasingly serious public health problems. The liver is the main metabolic site of alcohol absorption, and it is also the main target organ of alcohol toxicity (Xu, Wu, Fei, Zhang, & Zhang, 2003). Alcoholic liver disease (ALD) is a serious disease that is mainly caused by excessive alcohol consumption (Palipoch et al., 2016). There are several pathways of alcohol metabolism in the human liver, and the system of catalysis initiated by alcohol dehydrogenase (ADH) is the most critical one. In this system, after the reaction of two important enzymes -ADH and aldehyde dehydrogenase (ALDH)- with NAD^+, about 90% alcohol is transformed into acetaldehyde and acetic acid which are less toxic step by step, and most of the acetic acid is released into the blood and transported to extrahepatic tissues (Han et al., 2013; Xu et al., 2003; Yu, Li, He, Huang, & Zhang, 2013). Therefore, the activations of ADH and ALDH are important indicators to determine the degradation of alcohol in vivo.

Ultra-high-pressure processing (UHP), also known as high-pressure processing (HPP) or high-hydrostatic-pressure processing (HHP), is a rapidly emerging technology in the food industry (Norton & Sun, 2007). Compared with the traditional technology of heat processing, UHP can ensure food quality, including not only the texture, shape, and color, but also the flavor and nutritional characteristics of fresh foods (Norton & Sun, 2007; Zhou et al., 2016). As a new technology, UHP is used to treat protein solution to increase the exposure of peptides to enzymatic digestion sites,

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which is beneficial for subsequent hydrolysis (Garcia-Mora, Penas, Frias, Gomez, & Martinez-Villaluenga, 2015; Kim & Han, 2012; Zhou et al., 2016). Garcia-Mora et al. (2015) reported that when using UHP to treat a hyacinth bean protein, pressurization (100–300 MPa) enhanced the hydrolytic efficiency of Protamex, Savinase and Corolase 7089 compared with Alcalase (Garcia-Mora et al., 2015). Enzyme hydrolysates can also promote the digestive absorption of proteins after UHP (Quirós, Chichón, Recio, & López-Fandiño, 2007). Furthermore, during UHP, the degree of hydrolysis is mainly dependent on the protein system, types of proteins, pressure intensity and pressurizing time (Belloque, Chicón, & Lópezfandiño, 2007; Chichón, Belloque, Recio, & Lopez-Fandino, 2006; Lópezexpósito et al., 2008; Peñas, Snel, Floris, Préstamo, & Gomez, 2006).

The aim of our study was to explore whether UHP enhances the degree of peptide hydrolysis by single-factor experiment with different pressure intensities and pressurizing times, and to evaluate the abilities of bioactive peptides of different molecular weights to increase the activities of ADH and ALDH from mushroom foot in vitro.

2. Materials and methods

2.1. Materials and reagents

Mushrooms were provided by Beijing Baiao Biotechnology Co., Ltd. (Beijing, China), disintegrated to 0.423-mm pieces by a disintegrator, and stored in sealed plastic bags at 20.0 ± 1 °C. The pressure equipment was manufactured by the Chinese Academy of Agricultural Mechanization Sciences (Beijing, China). An ultrafiltration machine and membranes were purchased from Beijing Kebangxingye Technology Co., Ltd. (Beijing, China). ADH and ALDH test kits were purchased from Beijing BioDee Biotechnology Co., Ltd. (Beijing, China). Alcalase and other analytical grade chemicals, including hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and sodium hydroxide (NaOH), were all supplied by Sigma-Aldrich (St. Louis, Mo, China).

2.2. Extraction of mushroom foot protein

Mushroom foot protein was prepared by an alkali-solution and acid-isolation method. A 200-ml Pyrex beaker containing a mixture of 10.0 g mushroom foot powder suspended in water and NaOH (150 ml, pH 10, based on a preliminarily experiment) was placed in the ultrasonic equipment and subjected to 20 min of ultrasonication at 100 W. Then, the proteins were kept at 4 °C for 2 h. Next, the supernatant was obtained by centrifugation at 4000 rpm for 10 min. Subsequently, the pH of the supernatant was adjusted to 4.0 based on a preliminarily experiment, and proteins were precipitated by centrifugation at 4000 rpm for 10 min. The precipitate was dried in a vacuum freeze dryer until it reached a constant weight.

2.3. UHP-assisted proteolysis

Freeze-dried mushroom foot protein concentrates were suspended in deionized water (2%, w/v). One group of vacuum-packed encapsulated samples was introduced into the pressure unit filled with water, and then treated at pressures of 100, 200, 300, 400, and 500 MPa, respectively. The pressure was increased at a rate of 500 MPa/min, and it was maintained at the desired pressure for a holding time of 10 min. The other groups of samples were treated at a pressure of 200 MPa, with holding times of 2, 6, 10, 14, and 18 min; the decompression time was less than 4 s. The temperature of the pressure unit vessel was thermostatically controlled at 18 °C by a computer program throughout all of the treatments, and it was constantly monitored and recorded during the process. The treated samples were freeze-dried and stored at −20 °C until use.

After UHP treatment, the mushroom foot proteins were suspended in deionized water (5%, w/v) containing alkaline proteases (enzyme: substrate = 1:20, based on a preliminary experiment), and the pH was maintained at 8.5 with 0.1 M NaOH at 55 °C for 2 h (Zheng, Shen, Bu, & Luo, 2008). The enzymatic reactions were stopped by heating at 90 °C for 10 min to denature the enzymes. Then, the pH of the solution was adjusted to pH 7 with 0.1 M HCl to maintain the peptide activity for subsequent experiments (Yu et al., 2013). Subsequently, the supernatant was collected via centrifugation at 4000 rpm for 10 min, and the supernatant was freeze-dried and stored at −20 °C for further analysis.

2.4. Determination of the degree of hydrolysis and the peptide extraction rate

The soluble protein content in unpressurized mushroom foot protein concentrates were measured by the Folin protein assay using bovine serum albumin (BSA) as standard, and it was about 61.71 ± 1.02%. After removing macromolecular proteins with trichloroacetic acid, the soluble protein contents in pressurized mushroom foot protein concentrates were also measured by the same method. Then, the degree of hydrolysis was calculated by the following formula.

$$\text{The degree of hydrolysis} = (C_1/C_0) \times 100\%.$$

where C_1 is the content of soluble protein in pressurized mushroom protein concentrates, and C_0 is the content of soluble protein in unpressurized mushroom protein concentrates.

The peptide content was determined by UV spectrophotometry. The sample of freeze-dried mushroom foot protein concentrates was prepared into a solution with a certain concentration. The peptide content of the sample can be obtained by measuring the absorbance of the solution at a wavelength of 238 nm on an ultraviolet spectrophotometer and comparing with the standard curve of BSA. Then, the peptide extraction rate can be calculated by the following formula.

$$\text{The peptide extraction rate} = (C_2/C_0) \times 100\%$$

where C_2 is the peptide content of the sample, and C_0 is the content of soluble protein in unpressurized mushroom protein concentrates.

2.5. Ultrafiltration and peptide fractionation via Sephadex chromatography

Protein hydrolysates were subjected to ultra-filtration through membranes of 3 kDa, 5 kDa, and 10 kDa pore size, respectively. The three fractions of peptides with different molecular weights were collected individually to assess their bioactivities to activate ADH and ALDH compared with *Pueraria* isoflavone.

The peptides (0–3 kDa) obtained after ultra-filtration were fractionated by Sephadex G-25 size-exclusion chromatography (Muhialdin, Hassan, Bakar, & Saari, 2016). The dry resin powder was swollen in boiling water for 24 h and sterilized for 2 h by heating at 90 °C. Then, the resin was washed with separation buffer. The resin, which had been washed with 1 M Tris-HCl and degassed, was poured into the chromatography column (size 1.6 cm × 60 cm) and buffered with boiling water. The flow rate was adjusted to 1 ml/min. The concentration of the samples was adjusted to 50 mg/ml by dissolving the freeze-dried samples in deionized water.