Innovative Food Science and Emerging Technologies 43 (2017) 92–101

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Identification and characterization of antioxidant peptides from sweet potato protein hydrolysates by Alcalase under high hydrostatic pressure

Miao Zhang, Tai-Hua Mu*

Laboratory of Food Chemistry and Nutrition Science, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences; Key Laboratory of Agro-Products Processing, Ministry of Agriculture, No. 2 Yuan Ming Yuan West Road, Haidian District, P.O. Box 5109, Beijing 100193, China

A R T I C L E I N F O

Keywords:
Sweet potato protein hydrolysates
Antioxidant peptides
Amino acid sequence
Alcalase
High hydrostatic pressure

A B S T R A C T

Sweet potato protein hydrolysates (SPPH) were generated by Alcalase under high hydrostatic pressure (HHP, 100, 200 and 300 MPa). HHP significantly improved the degree of hydrolysis (DH) and antioxidant activity, and increased the < 3 kDa fraction content of SPPH ($P < 0.05$). SPPH by Alcalase at 300 MPa for 60 min exhibited the highest DH and antioxidant activity and was separated into three fractions by ultrafiltration. The most active fraction FIII (< 3 kDa) was further separated into fifty four fractions by semi-preparative RP-HPLC and measured using the ORAC assay. In addition, more active fractions were examined by LC–MS/MS, and diverse peptides were identified, matching sequences of Sporamins A and B. To evaluate the structure–activity dependences, twenty sequences were synthesized, of which the antioxidant activity was assessed. Five peptides showed good activity: HDSASGQY ≥ YYMVSA ≥ HDSESGQY ~ YYIVS ~ RYYDPL, with the contribution of His and Tyr.

Industrial relevance: This study will give a novel technique for using industrial waste slurry, a byproduct in the process of sweet potato starch manufacturing, which contains various bioactive components (such as protein, minerals, etc.) since most of them are normally discarded. The present study is focused on assessing the effects of enzymatic hydrolysis by Alcalase under high hydrostatic pressure (HHP) on the release of antioxidant peptides from sweet potato protein (SPP). The results of this work provide a potential application of enzymatic hydrolysis assisted by HHP on the development of ingredients from SPP in functional foods.

1. Introduction

Diverse peptides, which can be obtained by enzymatic hydrolysis via commercial protease, gastrointestinal digestion and food processing, have displayed potential health beneficial, one of most researched being antioxidant activity (Mazorra-Manzano, Ramírez-Suarez, & Yada, 2017; Singh, Vij, & Hati, 2014). Antioxidant peptides can act as a free radical scavenger, transition metal ion chelation agent and lipid peroxidation inhibitor, protect cells from damage by reactive oxygen species, and also show good nutritional and functional properties (Erdmann, Cheung, & Schröder, 2008; Xie, Huang, Xu, & Jin, 2008). At present, antioxidant peptides have been obtained from many food proteins by enzymatic hydrolysis, such as rice protein (Wattanasiritham, Theerakulkait, Wickramasekara, Maier, & Stevens, 2016), corn protein (Tang et al., 2010), potato protein (Udenigwe, Udechukwu, Yiridoe, Gibson, & Gong, 2016), amaranth protein (Orsini Delgado et al., 2016), peach kernel protein (Vásquez-Villanueva, Marina, & García, 2016) and egg protein (Memarpoor-Yazdi, Asoodeh, & Chamani, 2012).

High hydrostatic pressure (HHP) technology has made significant development over the last twenty years being utilized realistically in food industry, which offers the applications in the innovation of novel textures and tastes of foods (Norton & Sun, 2008). HHP technology was designed to help improve enzymatic hydrolysis and digestibility of protein, and enhanced the bioactivities of protein hydrolysates. HHP could also make the protein conformations change and protein molecular chain extension, which were beneficial for the enzymatic reaction due to the exposed new restriction sites (Bonomi et al., 2003), and might also produce some novel peptides with special physiological function. HHP has been reported to increase the surface hydrophobicity of ovalbumin, and promoted the proteolysis and bioactive peptides release (Quirós, Chichón, Recio, & López-Fandiño, 2007). In addition,

Abbreviations: SPP, sweet potato protein; SPPH, sweet potato protein hydrolysates; HHP, high hydrostatic pressure; ORAC, Oxygen radical absorbance capacity; MW, molecular weight; MWCO, molecule weight cut-off; RP-HPLC, reverse-phase high-performance liquid chromatography; TFA, trifluoroacetic acid; ACN, acetonitrile; LC-MS/MS, liquid chromatography - tandem mass spectrometry

* Corresponding author.
E-mail address: mutaihuacaas@126.com (T.-H. Mu).

http://dx.doi.org/10.1016/j.ifset.2017.08.001
Received 9 July 2017; Received in revised form 3 August 2017; Accepted 4 August 2017
Available online 05 August 2017
1466-8564/ © 2017 Elsevier Ltd. All rights reserved.

the enzymatic hydrolysis of soybean protein with trypsin, chymotrypsin and pepsin was significantly increased when the soybean protein was treated by HHP before or during the reaction (Peñasa, Préstamoa, & Gomez, 2004). HHP also improved the enzymatic hydrolysis and production of peptides from lentil proteins with antioxidant activity and angiotensin I-converting enzyme inhibitory potential (Garcia-Mora, Peñas, Frias, Gomez, & Martinez-Villaluenga, 2015).

In China, sweet potato ranks the fifth greatest food crop, possesses 80% of The world's total output, and contains approximately 1.73%–9.14% of protein on a dry weight basis (FAOSTAT, 2016; Mu, Tan, & Xue, 2009). Sweet potato protein (SPP) riches in essential amino acids, exhibiting higher nutritive value compared to most other plant proteins (FAO, 1990), but is normally discarded as industrial waste in the process of sweet potato starch manufacturing. SPP is mainly composed of Sporamins, and its monomeric forms Sporamins A and B have similar composition of amino acids, peptide map and characteristics (Maeshima, Sasaki, & Asahi, 1985). In our previous study, it was found that SPP hydrolysates (SPPH) generated by enzymatic hydrolysis with Alcalase presented noteworthy antioxidant activities and great protection effect on oxidative DNA damage (Zhang, Mu, & Sun, 2012; Zhang, Mu, & Sun, 2014). However, no study on the preparation and identification of antioxidant peptide from SPPH by Alcalase under HHP is currently available.

In this study, the effect of HHP on the degree of hydrolysis (DH), antioxidant capacity and molecular weight (MW) distribution of SPPH by Alcalase was investigated. Then the antioxidant peptides from SPPH was separated and subsequently identified, and the structure–activity dependences were explored, thus to provide potential utilization ways of SPPH in the food system.

2. Materials and methods

2.1. Materials

Sweet potatoes with variety Shangshu 19 were provided by Shangqiu Academy of Agriculture and Forestry Sciences, Henan, China. Alcalase with declared activity of 2.4 Au/g was purchased from Novozymes (Bagsvaerd, Denmark). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,20-Azobis (2-amidino propane) dihydrochloride (AAPH), Trifluoroacetic acid (TFA) and acetonitrile (ACN) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). All other reagents and chemicals were of analytical grade.

2.2. SPP preparation

SPP was produced followed the method of Mu et al. (2009). Twenty kilograms of sweet potatoes were sufficiently washed, peeled, cut, soaked in 20 L of 0.1% sodium bisulphite solution, ground, filtered to remove the residues, and centrifuged at 10,000*g* at 25 °C for 20 min to obtain and collect supernatant. The pH value of the supernatant was set to approximately 4.0 employing 1.0 M of HCl with continuously stirring. Then, the suspension obtained was centrifuged at 5000*g* at 25 °C for 15 min to get the precipitated substance, which was resolubilized in distilled water at pH 7.0–8.0, ultrafiltrated and lyophilized to obtain the SPP powder with purity of 85.79% by the Kjeldahl method (N × 6.25).

2.3. Preparation of SPPH by Alcalase under HHP

The HHP treatment was performed using HHP.M1-600/10 high-pressure equipment (Tianjin Huatai Senmiao Engineering and Technique Co. Ltd., Tianjin, China). HHP equipment has a 10 L of hydraulic-type cell and a water jacket for temperature controlling. Briefly, SPP powder was solubilized in 50 mM Tris-HCl buffer (pH 8.0) to get a final concentration of 3% (w/v), stirred with a magnetic stirrer (Changzhou Guohua Electrical Equipment Co., Ltd., Jiangsu, China) and equilibrated at 57 °C. Each SPP solution was mixed with Alcalase (4%, w/w), packed under vacuum in polyethylene bags, put into the HHP equipment immediately, and hydrolyzed at 57 °C for 30 and 60 min at 100, 200 and 300 MPa, respectively. The objective pressure attained at a rate of ~10 MPa/s and decreased to atmospheric pressure at ~10 MPa/s. The hydrolysis experiment for the control was done at 0.1 MPa (atmospheric pressure) at 57 °C for 30 and 60 min, separately. After hydrolysis, the SPPH solution was treated in boiling water for 10 min, and centrifuged at 10,000*g* at 10 °C for 20 min. The supernatant was lyophilized for subsequent analysis.

2.4. Degree of hydrolysis (DH)

The DH of SPPH was evaluated based on the o-phthalaldehyde (OPA) reaction (Nielsen, Petersen, & Dambmann, 2001), and calculated as follows:

$$h = (\text{serine NH}_2 - \beta)/\alpha \text{ milliequivalent/g protein},$$

where h represents amino groups number of hydrolyzed bonds of the peptide being expressed as the milliequivalent serine NH_2, while α and β are considered to be 1.00 and 0.40, respectively.

$$\text{DH}\ (\%) = h/h_{\text{tot}} \times 100,$$

where h_{tot} represents total peptide bonds number of each protein molecule, being 8 g equivalents/kg protein with the average MW of amino acids approximately 125 g/mol.

2.5. ·OH scavenging activity assay

·OH scavenging activity was determined based on the method of Ren et al. (2008). Briefly, SPPH was solubilized in deionized water to generate the concentration of 1.0 mg/mL. The reaction solution comprised 0.2 mL of SPPH solution, 0.9 mL of 0.1 M sodium phosphate buffer (pH 7.4), 0.1 mL of 10 mM $FeSO_4$, 0.1 mL of 10 mM ethylenediaminetetraacetic acid (EDTA), and 0.5 mL of 10 mM α-deoxyribose. After mixed sufficiently, 0.2 mL of 10 mM H_2O_2 was used to initiate the reaction, and kept in darkness at 37 °C for 1 h. Then, 1.0 mL of 2.8% trichloroacetic acid (TCA) was used to terminate it. Finally, 1.0 mL of 1.0% 2-thiobarbituric acid (TBA) was added and placed in heated water for 20 min. After cooled, the absorbance was evaluated at 532 nm. Deionized water was given as control. ·OH scavenging activity was calculated as follows:

$$\text{OH scavenging activity}\ (\%) = [(\text{Absorbance of control} - \text{Absorbance of SPPH}) / \text{Absorbance of control}] \times 100.$$

2.6. Fe^{2+}-chelating ability assay

Fe^{2+}-chelating ability assay was performed in accordance with the report of Jeong, De Lumen, and Jeong (2010). SPPH was solubilized in deionized water to generate 1.0 mg/mL of sample solution. The reaction solution comprised 1815 μL of distilled water, 450 μL of SPPH solution and 45 μL of 2 mM $FeSO_4$, being shaken energetically and maintained for 30 min at 25 °C. Then, 90 μL of 5 mM ferrozine were added and mixed well with the absorbance evaluated at 562 nm. Deionized water was given as control. The Fe^{2+}-chelating ability was calculated using the following formula:

$$\text{Fe}^{2+} - \text{chelating ability}\ (\%) = [(\text{Absorbance of control} - \text{Absorbance of SPPH}) / \text{Absorbance of control}] \times 100.$$

Download English Version:

https://daneshyari.com/en/article/5521692

Download Persian Version:

https://daneshyari.com/article/5521692

Daneshyari.com