Research article

Antioxidant Activity and Amino Acid Composition of Okara Protein Hydrolysate

Ketut Ratnayani*, Oka Ratnayani, and Indra Alexio Pane

Faculty of Mathematic and Natural Sciences, Udayana University, Jimbaran, Bali, Indonesia.

ORCID
Ketut Ratnayani https://orcid.org/0000-0003-3630-2361

Abstract.
Okara (tofu dregs) is a soybean waste product from the tofu and soymilk processing industry. To create a useful product, the remaining protein content in okara must be used. The goal of this study was to determine the enzyme-substrate (E/S) ratio capable of producing the highest antioxidant activity from okara protein hydrolysate, as well as the amino acid composition of such a protein hydrolysate (especially the amino acid potential as antioxidants). To produce protein concentrate, the protein of okara was isolated using an alkaline extraction method followed by isoelectric precipitation. The protein concentrate was then hydrolyzed for six hours with papain enzyme at 50-55^{o}C and pH 7, with E/S ratios of 3%, 4%, 5%, 6%, and 7%. Using the DPPH method, the antioxidant activity of the obtained okara protein hydrolysate was determined. The HPLC method was used to determine the amino acid composition of the protein hydrolysate. The E/S ratio of 4% had the highest antioxidant activity (98.86%); the composition of amino acids L-Phenylalanine, L-Isoleucine, L-Valine, L-Glycine, L-Lysine, L-Tyrosine, L-Proline, L-Histidine, L-Cysteine and L-Methionine potentially contributed to the antioxidant activity effect. These findings suggest that okara protein hydrolysate has the potential to be used as an antioxidant-rich food ingredient.

Keywords: antioxidant, okara, protein hydrolysate, aamino acid

Corresponding Author: Ketut Ratnayani; email: ketut_ratnayani@unud.ac.id

Published 07 June 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the PGPR 2021 Conference Committee.

1. Introduction

Protein hydrolysate is the product resulting from the decomposition of protein into peptides and free amino acids through the hydrolysis process by acids, bases, enzymes, or fermentation [1]. Protein hydrolysate has many benefits in the fields of food, health, animal husbandry, and agriculture. The benefits in the field of food and health, among others, such as a source of bioactive peptides, a continuation for the isolation of amino acids, a flavoring agent, which can improve the quality of food products and as a menu for the elderly.

Tofu dregs (internationally termed “okara”) is a soybean waste from tofu processing [2]. Although the tofu dregs is a by-product of tofu production, the nutritional content of the tofu dregs is still quite high. Dried tofu dregs contains 50% of fiber, 25% of protein

and 10% of lipid. Therefore, tofu dregs that has high nutritional value can be reused in various fields by reclaiming the important components.

Many factors can affect the antioxidant properties of a protein hydrolysate, namely the type of protein and enzyme, substrate treatment and hydrolysis degree [3]. This study aimed to determine the enzyme-substrate ratio (E/S ratio) which capable of producing okara protein hydrolysate with the highest antioxidant activity as well as to know the amino acid composition of such protein hydrolysate (especially the amino acids potential as antioxidants).

The research steps began with the isolation of tofu dregs protein so that a protein concentrate was obtained which would act as a protein substrate in the hydrolysis process. In the hydrolysis step, the papain enzyme was used and the E/S ratio was varied. Each protein hydrolysate obtained was then analyzed for its antioxidant activity using the DPPH method, while the amino acid composition analysis was carried out using the HPLC method.

2. Methodology

2.1. Isolation of Tofu Dregs Protein

At this stage, the isolation of the tofu dregs protein was carried out using alkaline extraction method. The fresh tofu dregs added with 0.7% NaOH with a ratio of 1:5 and then homogenized at 50℃ for 60 minutes in a magnetic stirrer. Then the mixture was filtered with a clean cloth and the filtrate was centrifuged at 6,000 rpm for 10 minutes. Furthermore, the isoelectric preparation stage was carried out at pH 4.5 with the addition of 2N HCl to the supernatant. After that it was centrifuged at 6000 rpm for 10 minutes, the pellets were taken and dried at 50 ℃ for 12 hours.

2.2. Hydrolysis of Tofu Dregs Protein by Papain Enzyme with Variation of Enzyme-Substrate (E/S) Ratio

A total of 2.5 g of tofu dregs protein concentrate was dissolved in 25 mL demineralized water to obtain a 10% (w/v) protein solution and then the solution was homogenized. Furthermore, papain enzyme with a certain composition was added to obtain an E/S ratio variation of 3%, 4%, 5%, 6%, and 7%. The percent unit in the E/S ratio indicated the ratio of the enzyme weight to the substrate weight (S), for example, an E/S ratio of 3% means that the ratio of enzyme weight to substrate weight is 3:100. The hydrolysis

process was carried out at a temperature of 50 ^{o}C for 6 hours. After incubation, the reaction was stopped by heating at 85 ^{o}C for 10 min. The supernatant was separated and stored in a dark glass bottle at -20 ^{o}C and called as okara protein hydrolysate.

2.3. Antioxidant Activity Assay of the Protein Hydrolysate by DPPH Method

The DPPH method was used to measure the ability of the sample as a proton (hydrogen) donor, following the method used by Bersuder et al. [4]. A total of 1 ml of the sample solution was mixed with 0.25 mL of 99.5% ethanol and 0.25 mL of 0.02% (w/v) DPPH. This mixture had been stored in the dark area at room temperature for 30 minutes before measuring its absorbance using a spectrophotometer at the wavelength of 517 nm.

The percentage of radical scavenging of tofu dregs protein hydrolyzate was calculated using the formula below:

% radical scavenging = (Control abs - Sample abs)/(Control abs) x 100%

2.4. Amino Acids Composition Analysis

The composition of 15 types of amino acids was analyzed using the HPLC instrument. The HPLC conditions were set as follows: column temperature 37 ^{o}C, column length 250-395 nm, and mobile phase flow rate 1.05 ml/min. Amino acid analysis was performed using a Waters Column C18 (3.9 x 150 mm) with sodium acetate and acetonitrile as buffer solvents (200 ml water, 0.2 ml CaEDTA).

3. Result and Discussion

3.1. Isolation of Tofu Dregs (Okara) Protein

The isolation stage of tofu dregs protein was carried out by the alkaline extraction method followed by isoelectric precipitation. The protein isolated from the tofu dregs was then analyzed for its protein content by using the Kjeldahl method and resulted in a protein content of 50.31%. This result indicated that the product of protein isolation is worthy of being called protein concentrate because it had protein content above 50%. This is in accordance with Amoo et al. [5] who stated that protein concentrate is a protein isolation product that has a protein content of at least 50-70%.

3.2. Antioxidant Activity Assay of Okara Protein Hydrolysate

The okara hydrolysates were produced by enzymatic hydrolysis reaction of tofu dregs protein concentrate (as substrate) using papain enzyme with various E/S ratio of 3%, 4%, 5%, 6%, and 7%. The antioxidant activity of the okara hydrolysates were measured by determining the free radical scavenging activity using DPPH method. The results of the free radical scavenging activities of okara protein hydrolysates can be seen in Table 1.

TABLE 1: Radical Scavenging activity of okara protein hydrolysate produced by E/S ratio variation.

E/S ratio	Radical Scavenging Activity (%)
3%	61,27
4%	9886
5%	97.69
6%	63.32
7%	71.46

Based on Table 1, it can be concluded that the okara protein hydrolysates obtained using all variations of the E/S ratio had the free radical scavenging activities above 50%, but the highest radical scavenging activity was produced when using the E/S ratio of 4%. When the amount of enzymes increased (at the same amount of substrates), the number of peptides and free amino acids produced in the hydrolysate products was expected to be higher so that the value of antioxidant activities would increase as well.

However, it can be seen from Table 1 that when using E/S ratio higher than 4%, the higher amount of enzymes was not able to raise the antioxidant activity as expected. Bordbar et al. [6] reported that the size of the peptide and its solubility, the amino acid composition, and the number of free amino acids that have antioxidant activity are the main keys that determine the DPPH radical scavenging activity of a protein hydrolysate. In the E/S ratio of 4% was thought capable to release more types of peptides and certain free amino acids that had the potential as electron donors than other ratios, so that it produced protein hydrolysate with the highest antioxidant activity. As higher ratios of E/S applied did not resulted in higher antioxidant activities, this indicated that not all peptide components and free amino acids released during enzymatic hydrolysis were peptides or amino acids that could scavenge free radicals.

3.3. Amino Acid Composition Analysis

The analysis of amino acid composition using HPLC was applied for the okara protein hydrolysate with the highest antioxidant activity (4% E/S ratio). Table 2 shows the composition of 15 types of amino acids contained in the okara protein hydrolysate.

Based on the data in Table 2, it can be seen that the highest amino acid content was glutamic acid, which was 2112.02 mg/kg, while the lowest amino acid content was tyrosine, which was 202.77 mg/kg. The data also shows that okara protein hydrolysate was rich in hydrophobic amino acids, especially leucine and proline. It is in accordance with the opinion of Polanco-Lugo et al. [7], which stated that the high content of hydrophobic amino acid residues in the protein hydrolysate of *Phaseolus lunatus* could have an impact on antioxidant activity. Waliszewski et al. [8] verified the presence of the following antioxidant amino acids in okara: cysteine (12.5 g/kg protein), methionine (10.6 g/kg protein), tyrosine (34.3 g/kg protein), phenylalanine (48.4 g/kg protein) and tryptophan (11.4 g/kg protein). Therefore, the presence of these amino acids in the hydrolysate will influence the antioxidant activity of the hydrolysate. The peptide antioxidant activity was believed to be mainly because of the presence of amino acid residues such as lysine, methionine, histidine, tryptophan, and tyrosine [9].

TABLE 2: Amino acid composition of okara protein hydrolysate (mg//kg).

Amino Acid Type	**mg/kg**	**Amino Acid Type**	**mg/kg**
Acidic Group :		**Hydrophobic Group:**	
Glutamic Acid	2112.02	Leucine	400.74
Aspartic Acid	966.33	Isoleucine	231.01
Basic Group :		Glycine	342.46
Arginine	481.19	Alanine	243.34
Histidine	188.39	Valine	316.06
Lysine	682.47	Proline	407.32
Polar Group :		Phenylalanine	242.61
Serine	395.76		
Tyrosine	202.77		
Threonine	326.37		

4. Conclusion

The treatment using E/S ratio of 4% was capable of producing okara protein hydrolysate with the highest antioxidant activity of 98.86%. The analysis results of the amino acid composition showed that the okara protein hydrolysate contained amino acid

components that were potential to have antioxidant activity, namely L-Phenylalanine, L-Isoleucine, L-Valine, L-Glycine, L-Lysine, L-Tyrosine, L-Proline, L-Histidine, L-Cysteine and L- Methionine.

References

[1] Pasupuleti VK, Demain AL. Protein hydrolysates in biotechnology. Springer Dordrecht Heidelberg London New York; 2010.

[2] Li B, Qiao M, Lu F. Composition, nutrition, and utilization of okara (soybean residue). Food Reviews International. 2012; 28:231–252. https://doi.org/10.1080/87559129.2011.595023

[3] Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and peptides. Critical Reviews in Food Science and Nutrition. 2008; 48(5):430-441.

[4] Bersuder P, Hole M, Smith G. Antioxidants from a heated histidine glucose model system I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high performance liquid chromatography. Journal of the American Oil Chemists' Society. 1998; 75:181-187.

[5] Amoo IA, Adebayo OT, Oyeleye AO. Chemical evaluation of winged beans (*Psophocarous tetragonolabus*), pitanga cherries (*Eugenia uniflora*) and orchid fruit (orchid fruit *Myristica*). African Journal of Food, Agriculture, Nutrition and Development. 2006; 2:1-12.

[6] Bordbar S, Farooq A, Nazamid S. High-value components and bioactives from sea cucumbers for functional foods: A review. Marine Drugs. 2011; 1761-1805.

[7] Polanco-Lugo E, Dávila-Drtiz G, Betancur-Ancona DA, ChelGuerrero LA. Effects of sequential enzymatic hydrolysis on structural, bioactive and functional properties of *Phaseolus lunatus* protein isolate. Food Science and Technology. 2014;34(3):441-448.

[8] Waliszewski KN, Pardio V, Carreon E. Physicochemical and sensory properties of corn tortillas made from nixtamalized corn flour fortified with spent soymilk residue (okara). Journal of Food Science. 2002; 67(8):3194-3197.

[9] Pena-Ramos EA, Xiong YL, Arteaga GE. Fractionation and characterization for antioxidant activity of hydrolyzed whey protein. Journal of the Science of Food and Agriculture. 2004;84:1908–1918.