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Extended Abstract

Antioxidant Activity of Faba Bean (*Vicia Faba*) Proteins Hydrolysates Produced by Alcalase and Trypsin

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Abstract

Enzymetic modification of proteins in order to break down specific peptide bonds and protein modification is widely used in the food industry. In this research, protein of faba bean seeds was hydrolyzed using alcalase and trypsin enzymes at three concentrations (1, 2 and 3%) and reaction times of 1-6 h at optimal temperature and pH of enzymes (50 and 37 °C, pH 8.5 and 7, respectively). Hydrolysis degree, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and iron chelating activity of hydrolyzed proteins were investigated. The results showed that the degree of hydrolysis increased with increasing reaction time and concentration of alcalase and trypsin enzymes. The time of hydrolysis and type of enzyme has a significant effect on the degree of hydrolysis, the antioxidant and chelating activity of the faba bean protein hydrolysates (P<0.05). The proteins hydrolyzed by alcalase at concentration of 3% and reaction time of 3 h had the highest antioxidant (75.41%) and metal chelating activity (55.95%). At the 1 and 2% concentration of trypsin, the highest DPPH radical scavenging activity was observed at 4 h which was 42.38 and 53.7 %, respectively. The most metal chelating activity in trypsin hydrolyzed treatments was observed in a reaction time of 2 h, after which the activity decreased. DPPH radical scavenging and metal chelating activity increased with increasing enzyme concentration. The results showed that alcalase have more efficiency in the production of anti-oxidant peptides compared to the trypsin.

Keywords: Antioxidant activity, Bioactive peptides, Enzymatic hydrolysis, Protein modification

Introduction

Plant proteins in the form of flour, concentrates, and isolate are used in food formulations as nutrient compounds to enhance protein content of the final product (Chardigny & Walrand, 2016). The Faba bean belonging to the (*Leguminosae*) family is rich in proteins and energy with a long history of multiple uses as food (Crépon *et al*., 2010). Legumes, including Faba bean, are combined with other plant foods to enhance the quality and quantity of protein. The enzymatic modification of proteins by proteolytic enzymes is widely used in the food industry (Mullally, O'Callaghan, FitzGerald, Donnelly, & Dalton, 1994). Peptides derived from hydrolysis have anti-oxidant (Zhao *et al*., 2012), anticancer (Shahidi & Zhong, 2008) antimicrobial (Harris, Mora-Montes, Gow, & Coote, 2009) activity and several other

physiological functions. Hydrolyzed proteins have antioxidant activity, depending on the peptide structure, such as peptide size and amino acid sequence, which is affected by the source of protein and hydrolysis condition (Shahidi & Zhong, 2008). Comparison of different enzymes showed that they had different effects on a similar substrate. Soybean protein hydrolysates which produced by pepsin, papain, chymotrypsin, and alcalase enzymes had antioxidant properties in the range of 28 to 65% (Peñta-Ramos & Xiong, 2002). Since there was no comprehensive study on enzymatic hydrolysis of faba bean protein, the aim of this study was to investigate the potential of alcalase and trypsin enzymes in the hydrolysis of faba bean protein and to study the antioxidant properties of the hydrolyzed protein.

Materials and methods

Preparation of faba bean flour

Faba bean seeds were ground using an Asan Tus mill (1000 Iranian Model) and passed through a 50 mesh sieve to obtain a fine powder. The powder was defatted with hexane in the ratio of 1:3 in 6 h, changing the solvent every two hours. The sample was dried at room temperature and then stored at -18 °C (Sogi, Arora, Garg, & Bawa, 2002).

Protein extraction

Protein extraction was done by using alkaline pH and then isoelectric point (Makri, Papalamprou, & Doxastakis, 2006).

Protein hydrolysis

The freeze-dried protein extract of crude faba bean dissolved at 4% (w/v) in phosphate buffer at pH 8.5 (Kong, Zhou, & Qian, 2007) and 7 (Chanput, Theerakulkait, & Nakai, 2009) for enzymes of alcalase and trypsin respectively. Hydrolysis was performed at optimal temperature of 50 and 37 °C for alcalase and trypsin respectively, enzyme concentrations of 1 to 3% and reaction time of 1 to 6 h in a shaker incubator (8480-VS, South Korea's) at 200 rpm. For enzyme inactivation, the protein solution was placed in a water bath at 82 °C for 15 min. After centrifugation at 1000 rpm and 20 min the supernatant freeze dried at -20 °C and 40 mbar.

Degree of hydrolysis

The degree of hydrolysis was calculated according to the following equation (Hoyle & Merrltt, 1994):

$$DH(\%)= (\text{protein content in TCA 0.44 M})/(\text{Total protein of sample})\times 100 \quad (1)$$

DPPH radical scavenging activity

1 ml of hydrolyzed protein solution (at the concentration of 10 mg/ml distilled water) was mixed with 1 ml DPPH 0.1 mM solution prepared in 96% ethanol then the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity measured by Bougatef *et al*. (2009) method.

Fe^{2+} chelating activity

4.7 mL of protein hydrolyzed solution (at the concentration of 10 mg/mL distilled water) mixed with 0.1 mL solution of 2 mM Iron (II) chloride and 0.2 mL of ferrozine 5 mM. The Fe^{2+} chelating activity measured by Nalinanon, Benjakul, Kishimura, & Shahidi (2011) method.

Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS Software, release 16. Duncan's New Multiple Range Test (DNMRT) was performed to determine the significant difference between samples at the 5% probability level.

Results and discussion

Degree of hydrolysis

The degree of hydrolysis indicates the ratio of peptide bonds broken down in the hydrolyzed protein. By increasing time of reaction, the degree of hydrolysis increased. Different time and concentration of the enzyme had a significant effect on the degree of hydrolysis by the alcalase and trypsin enzymes (P<0.05). With increasing concentration of enzymes, the degree of hydrolysis increased, the highest degree of hydrolysis was observed in the concentration of 3% alcalase and trypsin enzymes. The ability of the alcalase enzyme in the hydrolysis of faba bean protein was higher than the trypsin enzyme. Studies have shown that increasing alcalase enzyme concentration increased the degree of hydrolysis of soy and tomato seed proteins (Amiri Andi, Motamedzadegan, & Hosseini-Parvar, 2016; Hrckova, Rusnakova, & Zemanovic, 2002).

DPPH scavenging activity

DPPH scavenging activity of faba bean protein hydrolysates is shown in Fig. (1). The enzymatic hydrolysis increased the DPPH radical inhibition activity in comparison to the native protein (22.54%) (P<0.05). The effect of reaction time and enzyme concentration on DPPH radical scavenging activity was significant (P<0.05). The DPPH scavenging activity in the hydrolyzed protein produced by trypsin was lower than that produced with the alcalase enzyme, which may be due to the type of amino acids present in the resulting peptides and their sequences (Marcuse, 1962). Reducing inhibition activity by increasing the hydrolysis time can be due to the breaking of some antioxidant peptides that formed in the early stages of hydrolysis (Wu, Chen, & Shiau, 2003). In a study on the hydrolysis of gingerbread protein using two-step hydrolysis by pepsin and trypsin enzymes, similar results were obtained and the most antioxidant activity was observed at 180 min (Amza, Balla, Tounkara, Man, & Zhou, 2013).

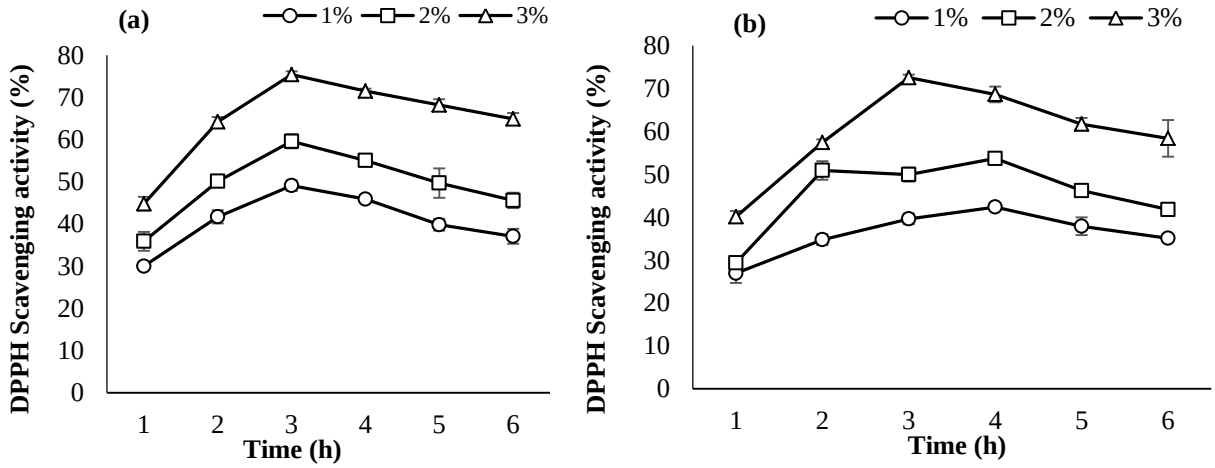

Fig. 1. DPPH radical scavenging activity at various reaction times and concentrations of alcalase and trypsin enzymes (a): Alcalase enzyme (b): Trypsin enzyme

Fe^{2+} chelating activity

The results showed that all of the hydrolyzed proteins had more chelating activity than the native faba bean protein with the Fe^{2+} chelating activity of 2.5% (P<0.05). In the treatments

produced by hydrolysis of alcalase and trypsin, increasing the enzyme concentration increased the Fe^{2+} chelating activity. The highest chelating activity was observed in concentrations of 1 and 3% of alcalase enzymes at the reaction time of 3 h which was 29.45 and 55.95% respectively. In the hydrolysates produced by the trypsin enzyme, the most Fe^{2+} chelating activity was observed at the reaction time of 2 h and enzyme concentration of 3%, after which the Fe^{2+} chelating activity decreased with increasing time and degree of hydrolysis (P<0.05). Fe^{2+} chelating activity of tea protein hydrolysates produced by alcalase showed an increase at beginning of hydrolysis time and then decreased (Li, Shen, Deng, Li, & Ding, 2014).

Conclusions

The results of this study showed that by changing the hydrolysis conditions such as enzyme type, enzyme concentration and hydrolysis time, hydrolysed proteins can be obtained with different antioxidant activity. The degree of hydrolysis increased with increasing reaction time and concentration of alcalase and trypsin enzymes. The effect of reaction time and type of enzyme had a significant effect on the antioxidant activity and Fe^{2+} chelating activity faba bean hydrolysed proteins produced by alcalase and trypsin enzymes (P<0.05).

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