Evaluation of antioxidant and ACE inhibitory activities of *Spirulina platensis* protein hydrolysates by gastrointestinal enzymes

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Abstract

This study was performed to investigate the effect of enzymatic hydrolysis of *Spirulina platensis* protein on antioxidant and angiotensin converting enzyme inhibitory activities (ACE). This research was conducted in 2018-2019 in the Iranian Research Organization for Science and Technology. *Spirulina platensis* was grown in Zarrouk culture medium. The cell wall was disrupted by freeze-thawing and ultrasonication techniques. *Spirulina platensis* enzymes were inactivated with boiling at 85^0C for 15 min. After centrifugation (9000 g, 15 min, 4^0C), the resulting supernatant (cell extract) was kept at -20°C. Enzymatic hydrolysis of *Spirulina platensis* protein with combinations of enzymes pepsin, trypsin and α-chymotrypsin was performed at 37 ^{0}C and optimal pH. Enzymatic activity was stopped by boiling at 85 °C for 15 minutes. After centrifugation of hydrolyzed proteins, supernatant was fractionated and analyzed the protein content of samples by Lowry method, degree of hydrolysis progress by ortho-phthalaldehyde (OPA), antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate) (ABTS) radical scavenging activity and ACE inhibitory activity by using N-[3-(2-furyl) acryloyl]-Phe-Gly-Gly (FAPGG) substrate. Average test outcome that performed in triplicate ± standard deviation was used to present results. Hydrolysis degree of hydrolyzed *Spirulina platensis* protein with pepsin + trypsin + chymotrypsin enzymes was 15.725 ± 1.260 %µM Leucin/mg protein, pepsin + trypsin 15.294 ± 0.290, pepsin + chymotrypsin 15.191 ± 0.653 and trypsin + chymotrypsin enzymes 11.212 ± 1.031% µM Leucine/mg protein were obtained. The fraction of <3 kDa resulted by hydrolysis with pepsin + trypsin enzymes indicated the highest antioxidant activity by scavenging of ABTS and DPPH radicals at 498.21 ±11.53 and 48.650 ± 0.00 µM TE/mg protein, respectively. Antioxidant activity of this fraction was significantly different from other fractions ($p< 0.05$). The IC50 value of ACE inhibitory activity of pepsin + trypsin, pepsin + chymotrypsin and trypsin + chymotrypsin (3.61 ± 0.04 mg/mL) hydrolysates were not significantly different ($p> 0.05$). Due to the observed antioxidant effects, biopeptides with high antioxidant activity derived from enzymatic hydrolysis of *Spirulina platensis* protein can be used as functional additives in foods.

Keywords: *Spirulina*, Degree of hydrolysis, ACE inhibitory, Antioxidant activity, ABTS and DPPH radicals.