A Study on Angiotensin Converting Enzyme Inhibitory Activities of Bee Pupal Protein and Its Enzymatic Hydrolysate

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ABSTRACT

Bee pupa is a by-product during the royal jelly production. In Taiwan, a few amount of bee pupae are soaked in liquor for tonic use, while most are used as animal feed or discarded as waste. The bee pupa used in this research was lyophilized and extracted by various solvents, and then fractionated by ammonium sulfate to isolate the proteins. The obtained proteins were hydrolyzed with different enzymes, and the angiotensin converting enzyme (ACE) inhibitory activities of the protein hydrolysates together with the extracts were evaluated. Results showed that the ACE inhibitory activities of the ethanolic (95%)、de-ionized water and phosphate buffer (pH 7.0) extracts from bee pupa were very low, only 2.6 %. The ACE inhibitory activities of the bee pupae after different heating treatments were also very low. After fractionation by 20~100 % ammonium sulfate, the molecular weight of the main proteins obtained was around 50~35 kDa. Two commercial proteases (alcalase and flavourzyme) were tested to hydrolyze bee pupal protein. Results showed that the degree of hydrolysis (DH) of the hydrolysate by alcalase for 12 hours was 7.28 % and the ACE inhibitory activity was 29.86 %. While the DH of the hydrolysate by flavourzyme for 12 hours was 6.46 % and the ACE inhibitory activity was 28.21 %. As for the results of one-stage hydrolysis by different enzyme concentrations and hydrolysis time, the ACE inhibitory activities of protein by 1.5 % alcalase and 2.0 % flavourzyme for 10 hours was 37.85 % and 29.85 %, respectively. For the results of two-stage hydrolysis, using alcalase (1.5 %, 4hrs) followed by flavourzyme (2.0 %, 6hrs), the DH of the hydrolysate was 12.92 % and the ACE inhibitory activity was 66.71 %. The hydrolysates with high ACE inhibitory activity were further in vitro digested by gastrointestinal proteases. The digested solutions from the hydrolysates obtained by one-stage hydrolysis and two-stage hydrolysis exhibited ACE inhibitory activities of 41.87 % and 72.49 %, respectively. The digested solution from the hydrolysate obtained by one-stage hydrolysis with flavourzyme had an ACE inhibitory activity of 27.17 %, lower than the former digested product. The hydrolysates with high ACE inhibitory activity were also fractionated by different molecular weight cut-off (MWCO 5000, 3000 and 1000) membrane ultrafiltration. The ACE inhibitory activities of the filtrates of MWCO 5000~3000, 3000~1000, and < 1000 Da were found to be 69.77, 61.53 and 96.98 %, respectively, and their IC50 were 20.04、31.78 and 0.66 mg/ml, respectively. The filtrates of membrane ultrafiltration had high contents of neutral, basic and acidic amino acids. In summary, the results of this study showed that the hydrolysate of bee pupal protein exhibited high ACE inhibitory activity, and was potential ingredient in reducing blood pressure as function food.

Keywords : Bee pupa, Angiotensin converting enzyme, Protein hydrolysate, Angiotensin converting enzyme inhibitor, Enzymatic hydrolysis.


