Studies on the Angiotensin Converting Enzyme Inhibitory Activity of Bee Pupal Protein Hydrolysates

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ABSTRACT

Bee pupa, a by-product during the royal jelly production, was used as materials in this study. The bee pupa was lyophilized and fractionated by ammonium suflate to isolate the proteins. The obtained proteins were hydrolyzed with two commercial proteases (alcalase and flavourzyme) under various enzyme/substrate (E/S) ratios, and the angiotensin converting enzyme (ACE) inhibitory activities of the protein hydrolysates were evaluated.

The results were as follows:

1.After fractionation by 20-100 % ammonium sulfate, the result showed that the molecular weight of the main proteins obtained was around 50-35 kDa.

2.Two commercial proteases (alcalase and flavourzyme) were tested to hydrolyze bee pupal protein in this study. The degree of hydrolysis (DH) of the hydrolysate by 1.5 % alcalase for 10 hours was 9.31 %, the ACE inhibitory activity was 40.85 % ; The DH of the hydrolysate by 2.0 % flavourzyme for 18 hours was 7.98 %, the ACE inhibitory activity was 34.65 %; for the result of two-stage hydrolysis, using alcalase (1.5 %, 10 hrs) followed by flavourzyme (2.0 %, 4 hrs), the DH of hydrolysate was 9.68 %, the ACE inhibitory activity was 73.29 %.

3.The ACE inhibitory activities of the 5-1 kDa MWCO filtrates of the hydrolysates, obtained by alcalase, flavourzyme and two-stage enzymatic hydrolysis, were 65.39, 56.42 and 82.01 %, respectively, and their IC50 were 24.07, 29.94 and 12.01 %, respectively.

4.The hydrolysates and filtrates with high ACE inhibitory activity were further in vitro digested by gastrointestinal proteases. The digested solutions from the hydrolysates obtained by one-stage hydrolysis with alcalase and the filtrates obtained by different molecular weight cut-off (MWCO 5000 and 1000) membrane separation exhibited ACE inhibitory activities of 50.01 % (hydrolysate), 74.16 % (5-1 kDa filtrate) and 72.54 % (lower than 1 kDa filtrate), respectively. The digested solutions from the hydrolysates obtained by two-stage hydrolysis and the filtrates obtained by different molecular weight cut-off membrane separation exhibited ACE inhibitory activities of 79.53 % (hydrolysate), 84.17 % (greater than 5 kDa filtrate), 89.34 % (5-1 kDa filtrate) and 88.19 % (lower than 1 kDa filtrate), respectively. The digested solutions from the hydrolysates obtained by one-stage hydrolysis with flavourzyme and and the filtrates obtained by different molecular weight cut-off membrane separation demonstrated ACE inhibitory activities of 27.89 % (hydrolysate), 24.88 % (greater than 5 kDa filtrate), 53.33 % (lower than 1 kDa filtrate), lower than the former digested product.

5.The gel filtration chromatograms of the 5-1 kDa MWCO filtrate of the pupal protein hydrolysate, obtained through two-stage enzymatic hydrolysis and fractionated by membranes with 5 and 1 kDa cut-off, had three groups of peaks, Peaks I (tube no. 34-46), Peaks II (tube no. 47-59), and Peaks III (tube no. 60-80). The ACE inhibitory activity of Peaks II had the highest value of 86.03 %.

Keywords : Bee pupa、Angiotensin converting enzyme、Protein hydrolysate、Angiotensin converting enzyme inhibitor 、Enzymatic hydrolysis、Gel filtration chromatography、Ultrafiltration

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