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

塗佳琪、張基郁

E-mail: 9417963@mail.dyu.edu.tw

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, Angiottnsin converting enzyme, Protein hydrolysate, Angiotensin converting enzyme inhibitor, Enzymatic hydrolysis.

Table of Contents

REFERENCES

1.山口庚兒。1998。 蜂子粉末的營養療效。第33-60頁。世茂出版社。台北。 2.行政院衛生署。2005。中華民國九十三年臺灣地區死因統計結果摘要。 3.江美昭。2003。酵素水解豬血漿中白蛋白以製備高血壓抑制胜?:56-69。東海大學食品科學研究所碩士論文。台中。 4.吳孟娟、涂瑞澤、吳淑姿。1998。豬肝蛋白質之水解研究。大葉學報7(1):143-152。 5.吳桂菁。2004。豬血紅蛋白之酵素水解及抗氧化活性研究:51-83。大葉大學生物產業科技學系碩士論文。彰化。 6.翁千惠。2004。利用酵素水解明膠及卵白蛋白製備抗高血壓胜?:39-85。東海大學食品科學研究所碩士論文。台中。 7.曹煒、尉亞輝。2002。蜂產品保健原理與加工技術。第278-292頁。化學工業出版社。大陸。 8.陳怡宏。2001。蛋白質酵素水解物與醫療調理營養。食品工業33(11):58-63。 9.陳培瑛。1996。使用抗高血壓藥物於老年患者之效率與危險性。藥學雜誌12:49-54。 10.陳彥霖。2000。紅麴與高血壓。食品工業月刊32(12):54-59。 11.張鴻明、陳姿利、許秀娟。1999。自明膠水解液中製備血管收縮素轉化?抑制劑之研究。中國農業化學會誌37(4):546-553。 12.許雅芳。2003。鯖柴魚水解物中胜?對血管升壓素轉換?之抑制與其純化:36-44。海洋大學食品科學系碩士論文。基隆。 13.溫惠美、李安玲、葉美吟、陳景川、林貞信、黃文瑛。1999。毛細管電泳在蜂王漿蛋白質分析上之應用。藥物食品分析 7(1):73-81。 14.鄭靜桂。1997。蛋白質水解與水解液之利用。食品工業29 (05):10-17。 15.劉毓蕙。2004。水解蛋白的特性及應用。食品工業月刊 36(2):19-24。 16.潘子明。2001。機能性發酵製品。第227-229頁。藝軒出版 社。台北。 17.潘子明。2004。機能性發酵製品-活性胜?類。生物產業15(4):305-317。 18.蔡宗堯。2004。酵素水解分離大豆蛋白以製備高血壓抑制胜?:37-61。東海大學食品科學研究所碩士論文。台中。 19.賴祥玲。2004。乳蛋白中的機能胜?。食品工業36(2):37-44。 20.Adler-Nissen, J. 1986. Enzymic Hydrolysis of Food Protein. Elsevier Applied Science Publ. Ltd., Barking, Essex, UK. 21.Amarowicz, R. and Shabidi, F. 1996. Antioxidant activity of peptide fraction of capelin protein hydrolysates. Food Chem. 58: 355-359. 22.A.O.A.C. 1984. Official methods of analysis, 14th ed. Association of Official Analytical Chemist. Washington, DC. 23.Arihara, K., Nakashima, Y., Mukai, T., Ishikawa, S. and Itoh, M. 2001. Peptides inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. Meat Science. 57:319-324. 24.Ariyoshi, Y. 1993. Angiotensin-converting enzyme inhibitors derived from food protein. Trends in Food Science and Technology. 4:139-144. 25.Astawan, M., Wahyuni, M., Yasuhara, T., Yamada, K., Tadokoro, T. and Maekaea, A. 1995. Effects of angiotensin I-converting enzyme inhibitory substances derived from Indonesian dried-salted fish on blood pressure of rats. Biosci. Biotech. Biochem. 59:425-429. 26.Bellamy, W., Takase, M., Kawase, K., Shimamura, S. and Tomita, M. 1993. Role of cell-binding in the antibacterial mechanism of Lactoferricin B. J. Appl. Bact. 75:478-484. 27.Berrocal, R., Chanton, S., Juillerat, M. A., Pavillard, B., Scherz, J. C. and Jost, R. 1989. Tryptic phosphopeptides from whole casein Ⅱ. Physicochemical properties related to the solubilization of calcium. J. Dairy Res. 56:335-341. 28.Chen, H. M., Muramoto, K. and Yamauchi, F. 1995. Structural analysis of antioxidative peptides from soybean β – conglycinin. J. Agric. Food Chem. 43:574-578. 29.Chen, H. M., Muramoto, K., Yamauchi, F. and Nokihara, K. 1996. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J. Agric. Food Chem. 44:2619-2623. 30.Chen, H. M., Muramoto, K., Yamauchi, F., Fujimoto, K. and Nokihara, K. 1998. Antioxidative properties of histidine- containing peptides designed from peptide fragments found in the digests of a soybean protein. Agr. Food Chem. 46: 49- 53. 31.Cheung, H. S., Wang, F. L., Ondetti, M.A., Sabo, E. F., and Cushman, D. W. 1980. Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. Importance of the COOH- terminal dipeptide sequence. J. Biol. Chem. 255:401-407. 32.Corvol, P., Ewilliams, T. A. and Soubrier, F. 1995. Peptidyl dipeptidase A: angiotensin I-converting enzyme. Methods Enzymol. 248:283-305. 33.Cushman, D. W. and Cheung, H. 1971. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit lung. Biochem. Pharmacol. 20:1637-1648. 34.Fahmi, A., Morimura, S., Shigematsu, T., Kida, K., Guo, H. C. and Uemura, Y. 2004. Production of angiotensin I converting enzyme inhibitory peptides from sea bream scales. Process Biochem. 39:1195-1200. 35.Ferrira, S., Bartet, D. C. and Greene, L. J. 1970. Isolation of bradykinin-potentiating peptides from Bothrops jararaca venom. Biochemistry. 9:2583-2593. 36.Fujita, H. and Yoshikawa, M. 1999. LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein. Immunopharmacology. 44:123-127. 37.Gerber, H. W. and Jost, R. 1986. Casein phosphopeptides: their effect on calcification of in vitro cultured embryonic rat bone. Calcif. Tissue Int. 38:350-357. 38.Gill, I., Lopez-Fandino, R., Jorba, X. and Vulfson, E. N. 1996. Biologically active peptides and enzymatic approaches to their production. Enzyme Microbial Technol. 18:162-183. 39.Hyun, C. K. and Shin, H. K. 2000. Utilization of bovine blood plasma proteins for the production of angiotensin I converting enzyme inhibitory peptides. Process Biochem. 36:65-71. 40.Ichimura, T., Hu, J., Aita, D. Q. and Maruyama, S. 2003. Angiotensin I-converting enzyme inhibitory activity and insulin secretion stimulative activity of fermented fish sauce. J. Biosci Bioeng. 96:496-499. 41.Kawasaki, T., Seki, E., Osajima, K., Yoshida, M., Asada, K., Matsui, T. and Osajima, Y. 2000. Antihypertensive effect of Valyl- Tyrosine, a short chain peptide derived from sardine muscle hydrolyzate, on mild hypertensive subjects. J. Human Hypertens. 14:519-523. 42.Kim, S. K., Jeon, Y. J. and Byun, H. G. 1999. Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochem. 35:471-478. 43.Kim, S. K. and Byun, H. G. 2001. Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska Pollack (Theragra chalcogramma) skin. Process

Biochem. 36:1155-1162. 44.Kinoshita, E., Yamakoshi, J. and Kikuchi, M. 1993. Purification and identification of an Angiotensin I-converting enzyme inhibitor from soy sauce. Biosci. Biotech. Biochem. 57(7):1107-1110. 45.Ko, W. C. and Hwang, J. S. 2004. Angiotensin I-converting enzyme inhibitory activity of protein hydrolysates from tuna broth. J. Food and Drug Analysis. 12(3):232-237. 46.Kunio, S. 1998. Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from Allium sativum L (garlic). J. Nutr. Biochem. 9:415-419. 47.Lahl, W. J. and Braun, S. D. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48:68-71. 48.Le, G. W., Li, G. H., Shi, Y. H. and Shrestha, S. 2004. Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. Nutrition Research. 24:469-486. 49.Lee, J. S., Lee, D. H., Kim, J. H., Park, J. S. and Choi, Y. J. 2004. Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom Tricholoma giganteum. Peptides. 25:621-627. 50.Maruyama, S. and Suzuki, H. 1982. A peptide inhibitor of angiotensin I converting enzyme in the tryptic hydrolysate of casein. Agric. Biol. Chem. 46(5):1393-1394. 51.Maruyama, S., Nakagomi, K., Tomizuka, N. and Suzuki, H. 1985. Angiotensin I-converting enzyme inhibitor derived from an enzymatic hydrolysate of casein II. Isolation and bradykinin- potentiating activity on the uterus and the ileum of rats. Agric. Biol. Chem. 49:1405-1409. 52.Maruyama, S., Miyoshi, S. and Tanaka, H. 1989. Angiotensin I-converting enzyme inhibitor derived from Ficus carica. Agric. Biol. Chem. 53(10):2763-2767. 53.Matsufuji, H., Matsui, T., Seki, E., Osajima, K., Nakashima, M. and Osajima, Y. 1994. Angiotensin I-converting enzyme inhibitory peptides in an alkaline protease hydrolysate derived from sardine muscle. Biosci. Biotech. Biochem. 58 (12): 2244 – 2245. 54.Matsui, T., Li, C. H. and Osajima, Y. 1999. Preparation and characterization of novel bioactive peptides responsible for angiotensin I-converting enzyme inhibition from wheat germ. J. Peptide Sci. 5:289-297. 55.Matsui, T., Yukiyoshi, A., Matsumoto, K., Doi, S., Sugimoto, H. and Yamada, H. 2002. Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. J. Nutr Biochem. 13:80-86. 56.Matsumura, N., Fuji, M., Takeda, Y., Sugita, K. and Shimizu, T. 1993. Angiotensin I-converting enzyme inhibitory peptides derived from bonito bowels autolysate. Biosci. Biotech. Biochem. 57:695-697. 57.Meng, Q. C. and Oparil, S. 1996. Purification and assay methods for angiotensin-converting enzyme. Journal of Chromatography A. 743:105-122. 58.Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H. and Maruyama, S. 1991. Structure and activity of angiotensin- converting enzyme inhibitors in an α – zein hydrolysate. Agric. Biol. Chem. 55:1313-1318. 59.Morimura, S., Fahmi, A., Shigematsu, T., Kida, K., Guo, C. H. and Uemura, Y. 2004. Production of angiotensin converting enzyme inhibitory peptides from sea bream scales. Process Biochem. 39:1195- 1200. 60.Nakamura, Y., Masuda, O. and Takano, T. 1996. Decrease of tissue angiotensin I-converting enzyme upon feeding sour milk in spontaneously hypertensive rats. Biosci. Biotech. Biochem. 60(3):488-489. 61.Okamoto, A., Hanagata, H., Matsumoto, E., Kawamura, Y., Koizumi, Y. and Yanagida, F. 1995. Angiotensin I-converting enzyme inhibitory activties of various fermented foods. Biosci. Biotech. Biochem. 59:1147-1149. 62.Pokorny, J. 1991. Natural antioxidants for food use. Trends in Food Science and Technology. 2: 223-227. 63.Sato, R., Noguchi, T. and Naito, H. 1986. Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. J. Nutr. Sci. Vitaminol. 32: 67-76. 64.Sato, M., Hoskawa, T., Yamaguchi, T., Nakano, T., Muramoto, K., Kahara, T., Funayama, K., Kobayashi, A. and Nakano, T. 2002. Angiotensin I-converting enzyme inhibitory peptides derived from wakame (Undaria pinnatifida) and their antihypertensive effect in spontaneously hypertensive rats. J. Agric Food Chem. 50: 6245- 6252. 65.Shahidi, F., Xiao-Qing, H. and Synowiecki, J. 1995. Production and characteristics of protein hydrolysates from capelin (Mallotus villosus). Food Chem. 53: 285-293. 66.Suetsuna, K. 1998. Isolation and characterizeation of angiotensin I-converting enzyme inhibitor dipeptides derived from Allium sativum L (garlic). J. Nutr. Biochem. 9: 415-419. 67.Suetsuna, K. and Nakano, T. 2000. Identification of an antihypertensive peptide from peptic digest of wakame (Undaria pinnatifida). J. Nutr. Biochem. 11: 450-454. 68.Suh, H. J., Choi, H. S., Cho, H. Y., Yang, H. C. and Ra, K. S. 2001. Angiotensin I-converting enzyme inhibitor from Grifola frondosa. Food Research International. 34: 177-182. 69.Suh, H. J., Whang, J. H., Kim, Y. S., Bae, S. H. and Noh, D. O. 2003. Preparation of angiotensin I converting enzyme inhibitor from corn gluten. Process Bio. 38: 1239-1244. 70.Ukeda, H., Matsuda, H., Kuroda, H., Osajima, K., Matsufuji, H. and Osajima, Y. 1991. Preparation and separation of angiotensin I converting enzyme inhibitory peptides. Nippon Nogekagaku Kaishi. 65: 1223-1228. 71.Vioque, J., Yust, M. M., Pedroche, J., Giron-Calle, J., Alaiz, M. and Millan, F. 2003. Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase. Food Chem. 81: 363-369. 72.Wu, J. and Ding, X. 2002. Characterization of inhibition and stability of soy-protein-derived angiotensin I-converting enzyme inhibitory peptides. Food Res Inter. 35: 367-375. 73.Yamamoto, N. 1997. Antihypertensive peptides derived from food proteins. Biopolymer 43: 129-134. 74.Yamauchi, F., Chen, J. R., Muramoto, K., Iwabuchi, S., Asano, M. and Suetsuna, K. 1994. Isolation and utilization of the protein from okara. Research Committee. 15: 22-27. 75.Yasuda, M., Kuba, M., Tana, C. and Tawata, S. 2005. Production of angiotensin I-converting enzyme inhibitory peptides from soybean protein with Monascus purpureus acid proteinase. Process Biochem. 40: 2191-2196. 76.Yokoyama, H., Chiba, H. and Yoshikawa, M. 1992. Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. Biosci. Biotech. Biochem. 56: 1541-1545.