Antioxidative activities of bee pupal protein and their hydrolysates

王智群、張基郁

E-mail: 324890@mail.dyu.edu.tw

ABSTRACT

Bee pupae, the larva of honeybee that contains amino acids, minerals and vitamins, is a good source of animal proteins. This work aims to study the effect of antioxidative activities of bee pupae proteins and their protein hydrolysates. The bee pupae used in this research was heated in 90 ℃ for 30 minutes and extracted by phosphate buffer, and then fractionated by ammonium sulfate to isolate the proteins. The results showed that the ferrous ion chelating ability of the heated bee pupae extracts were higher than that of unheated extracts, and the heated extracts after fractionation by 40~70 % ammonium sulfate were the highest in ferrous ion chelating ability. The ferrous ion chelating ability of hydrolysates obtained by Alcalase and Flavourzyme (E/S = 1.5% for Alcalase; 2.0% for Flavourzyme) for 24 and 36 hours were 14.55 and 14.29 %, respectively. The DPPH free radical scavenging activities were 27.22 and 29.99 %, respectively. The degree of hydrolysis (DH) increased with hydrolysis time by using Flavourzyme, the DH for 1 and 36 hours were 27.15% and 81.45%, respectively; the DH of the hydrolysates by Alacalase for 1 to 24 hours was 7.09%. The results showed that the antioxidative activities were significantly decreased after ammonium sulfate precipitation and hydrolysis.

Keywords : antioxidant、ammonium sulfate、protein、Alcalase、Flavourzyme

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REFERENCES

1. 山口庚兒。1998。蜂子粉末的營養療效。第33-60頁。世貿出版社。台北。 2. 王正仁、陳孟伶、林畢修平、陳啟祥。1999。水解酵素在工業上的利用。生物產業10: 1-11。 3. 王念慈。2004。新鮮與老化葉菜類蔬菜之總抗氧化力之測定與變異:1- 2。台灣大學園藝學研究所碩士論文。台北。 4. 王鏵葦。2008。牛初乳乳清水解物對LDL及細胞DNA氧化性傷害之抑制研究。大葉大學生物產業科技學系碩士論文。彰化。 5. 安奎、何鎧光、陳裕文 編著。2004。養蜂學。第363-364頁。華香園出版社。台北，臺灣。 6. 吳登楨。2002。台灣蜂業研究之回顧與展望。科學農業，50（3,4）:189-193。 7. 李錦楓。2002。蜂產品的保健功能(五)蜜蜂子(蜂蛹)。健康世界 199（319）:56-58。 8. 李綾晏。2009。蜂王蛹蛋白及其酵素水解物對血管收縮素轉換酵素之抑制活性研究。私立大葉大學生物產業科技研究所碩士論文。彰化。 9. 李佳珍，張文昌，吳思敬。2009。複方保健茶之抗致突變性及抗氧化性。台灣農業化學與食品科學。47（6）:277-284。 10. 林建豪。2008。牛初乳對LDL及細胞DNA氧化性傷害之抑制研究。大葉大學生物產業科系學系碩士論文。彰化。 11.林天送。1995。氧

自由基:促使細胞的老化與死亡。健康世界。111: 9-14。 12.林天送。1995。?基自由基:毒性極高的破壞分子。健康世界。112: 6-10。 13.林玫欣。1999。鯖魚肉與內臟水解物之抗氧化性研究。國立台灣海洋大學食品科學系碩士論文，基隆。 14.郭智宏。2001。腸道吸收-胺基酸與胜?。食品工業。33: 15-25。 15.陳麗婷、程竹青、華傑 編著。2004。食品胜?技術發展路程圖(Roadmap)。第63頁。食品工業發展研究所。新竹，台灣。 16.陳志瑋。2007。微膠囊化牛初乳蛋白質水解物之抗氧化安定性。私立大葉大學生物產業科技研究所碩士論文。彰化。 17.塗佳琪。2006。蜂王蛹蛋白及其酵素水解物對血管收縮素轉換酵素之抑制活性研究。私立大葉大學生物產業科技研究所碩士論文。彰化。 18.許惠惇。2000。黃豆蛋白質酵素水解物中生理活性胜?之篩檢研究。國立陽明大學生物化學研究所碩士論文，台北。 19.廖恬瑤。2009。蜂王蛹蛋白及水解物對細胞DNA氧化性傷害及LDL氧化之抑制研究。私立大葉大學生物產業科技研究所碩士論文。彰化。 20.劉毓蕙。2004。水解蛋白的特性及運用。食品工業。36: 19-24。 21.鄭宇哲，莊榮輝，廖大修。2003。蛋白質體學。教育部顧問室生物技術科技教育改進計畫醫藥基因生物技術教學資源中心。台北，台灣。 22.鄭名凡。1999。蛋白質水解物的功能與應用。食品資訊。160: 49-54。 23.鄭靜桂。1997。蛋白質水解與水解液之利用。食品工業，29(5): 10-17。 24.Adler-Nissen, J. 1986. Some fundamental aspects of food protein hydrolysis. In ”Enzymatic Hydrolysis of Food Protein”. pp. 20-21. Elesevier Science Publishing Co., Inc. New York, USA. 25.Afans, E. B., Dcrozhko, A. I., and Brodskii, A. 1989. Chelating and free radical scavenging mechanisms of inhibitory action of ruin and quercetin in lipid peroxidation. Biochem. Pharmacol. 38(11): 1763 – 1989. 26.Agarwal, S. and Sohal, R. S. 1994. DNA oxidative damage and life expectancy in houseflies. P. Natl. Acad. Sci. U. S. A. 91: 12332-12335. 27.Ames, B. N., Shigenaga, M. K., and Hagen, T. M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. P. Natl. Acad. Sci. U. S. A. 90: 7915-7922. 28.Arnao, B. M., Cano, A., Hernandez-Ruiz, J., Garcia-Canovas, F., and Acosta, M. 1996. 2, 2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of food. Ana. Biol. Chem. 236: 255-261. 29.Ames, B. N. 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Sci. 211: 1256-1264. 30.Anderson, D. and Phillips, B. J. 1999. Comparative in vitro and in vivo effects of antioxdants. Food Chem. Toxicol. 37: 1015-1025. 31.Ariyoshi Y. 1993. Angiotensin-converting enzyme inhibitors derived from food proteins. Trends Food Sci. Technol. 4: 139-144. 32.AOAC. 1995. Offical method of Analysis, 14th ed. Association of Offical American Chemists, Washington, D.C., U.S.A. 33.Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical, Nature. 181: 1199-1200. 34.Chang, C. Y., Wu, K. C. and Chiang, S. H. 2007. Antioxidant properties and protein compositions of porcine haemoglobin Hydrolysates. Food Chem. 100: 1537-1543. 35.Chen, H. M., Muramoto, K. and Yamauchi, F. 1995. Structural analysis of antioxidative peptides from soybean β-conglycinin. J. Agric. Food Chem. 43(3): 574-578. 36.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(9): 2619-2622. 37.Clement, A. 2000.Enzymatic protein hydrolysates in human nutriation. Trends Food Sci. Technol. 11: 254-262. 38.Clement, A. and Chambers, S. J. 2000. Development and production of hypoallergenic protein hydrolysates for use in infant formulas. Food Allergy Int. 1: 175-190. 39.Conner, E. M. and Grisham, M. B. 1996. Inflammation, free radicals, and antioxidants. Nutrition 12: 274-277. 40.Decker, E. A. and Welch, B 1990. Role of ferritin as a lipid oxidants. J. Chem. Soc. Faraday Trans. 94: 1971- 1978. 41.Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidants. J. Chem. Soc. Faraday Trans. 94: 1971- 1978. 42.Dorman, H. J. D., Peltoketo, A., Hiltunen, R., and Tikkanen, M. J. 2003. Characterization of the antioxidant properties of deodorised aqueous extracts from selected Lamiaceae Herbs. Food Chem. 83(2): 255 – 262. 43.Fagbenro, O. and Tauncery, K. 1993. Chemical and nutriational quality of raw, cooked and salted fish silage. Food Chem. 48: 331-335. 44.Freitas O. D., Padovan, G. J., Vilela, L., Santos, J. E. D., Oliveria, J. E. and Green, L. J. 1993. Characterization of protein hydrolysate prepared for enteral nutrition. J. Agric. Food Chem. 41: 1432?-1438. 45.Gildberg, A. 1993. Enzymatic processing of marine raw materials. Process Biochem. 28: 1-15. 46.Gordon, M. H. 1990. The mechanism of the antioxidant action in vitro. Food Antioxidants. New York: Elsevier. pp. 1 – 18. 47.Halliwell, B. and Gutteridge, J. M. C. 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Methods in Enzymology. 48.Harman, D. 1995. Role of antioxidant nutrients in aging: overview. Age 18: 51-62. 49.Kagawa, K., Matsutaka, H., Fukuhama, C., Watanabe, Y. and Fujino, H. 1996. Globin digest, acidic protease hydrolysate, inhibits dietary hypertriglyceridemia and Val-Val-Tyr-Pro, one of its constituents, possesses most superior effect. Life Sci. 58(20): 1745-1755. 50.Kim, H. H. Y., and Jimenez-Flores. R. 1994. Comparison of milk proteins using preparative isoelectric focusing followed by polyacrylamide gel electrophoresis. J Dairy. Sci. 77: 2177-2129. 51.Koner, B. C., Banerjee, B. D. and Ray, A. 1997. Effects of in-vivo generation of oxygen free radicals on immune responsiveness in rabbits. Immunol. Lett. 59: 127-137. 52.Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 277: 680-685. 53.Lahl, W. J. and Braun, S. T. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48(10): 68-71. 54.Liu, C. C., Huang, L. C., Chang, C. T., Sung, H. Y. 2006. Purification and characterization of soluble invertases from suspension-cultured bamboo (Bambusa edulis) cells. Food Chem. 96: 621-631. 55.Mackie, I. M. 1982. Fish protein hydrolysates. Process Biochem. 17: 26-32. 56.Mackie, I. M. 1982. Fish protein hydrolysates. Process Biochem. 31: 26-31. 57.Meister, A. 1984. New aspects of glutathione biochemistry and transport: selective alteration of glutathione metabolism. Fed. Proc. 43: 3031-3042. 58.Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307. 59.Pihlanto, A., Akkanen, S., Korhonen, H. J. 2008. ACE-inhibitory and antioxidant properties of potato (Solanum tuberosum). Food Chem. 109: 2744- 2748. 60.Quagila, G. B. and Orban, E. 1987. Enzymic solubilisation of sardine (Sardina pilchardus) by comm.ercial protease. J. Sci. Food Argic. 38: 263-269. 61.Ray, G. and Husain, S. A. 2002. Oxidants, antioxidants and carcinogenesis. Indian J. Exp. Biol. 40: 1213-1232. 62.Rebeca, B. D., Pena-Vera, M. T. and Diaz-castaneda, M. 1991. Production of fish protein hydrolysates with bacterial protease, yield and nutritional value. J. Food Sci. 56(2): 309-314. 63.Salah, N., Miller, N. J., Paganga, G.., Tijburg, L., Bolwell, G. P., and Rice- Evans, C. 1995. Polyphenolic flavanols as scavengers of aqueous phase radicals and as

chain-breaking antioxidants. Arch. Biochem. Biophys. 322:339-346. 64.Shih, F. F. 1992. Modification of food protein by non-enzymatic methods. In: Biochemistry of Food Protein (B. J. F., Hudson ed.), Ch. 7., Elsevier Applied Science Publishers, London, England. 65.Smith, M. A., Perry, G., Richey, P. L., Sayrec, L. M., Anderson, V. E., Beal, M. F. and Kowall, N. 1996. Oxidative damage in Alzheimer ' s. Nature 382: 120-121. 67.Xie, Z., Huang, J., Xu, X., Jin, Z. 2008. Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chem. 111: 370-376. 68.Yamaguchi, T., Takamura, H., Matoba, T., and Terao, J. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1- diphenyl-2-picrylhydrazyl. Biosci. Biotech. Biochem. 62(6): 1201 – 1204. 69.Yamamoto, N. 1997. Antihypertensive peptides derived from food proteins. Biopolymer 43: 129-134. 70.Yokoyama, k., Chiba, H. and Yoshikawa, M. 1992. Peptide inhibitors for ACE from thermolysin digest of dried bonito. Biosci. Biotech. Biochem. 56: 1541-154.