A Study on Enzymatic Hydrolysis and Antioxidant Properties of Porcine Plasma Protein

林姿儀、張基郁

E-mail: 9318493@mail.dyu.edu.tw

ABSTRACT

ABSTRACT In this study, the antioxidant properties of porcine plasma proteins before and after enzymatic hydrolysis and their heat stability were investigated in order to be as a reference of the use of porcine blood. This study was divided into three parts. In the first part, porcine plasma protein was used as a sample to analyze its proximate composition and to evaluate its antioxidant properties. The results showed that the moisture of the plasma sample was 7.10 %, the crude protein was 73.82%, the crude fat was 0.74%, and the ash was 9.61%. The plasma protein sample is rich in the protein of molecular weight 50-75 kDa. The plasma protein sample had relatively high ferrous ion chelating ability; however, low reducing power and DPPH radical scavenging activity. When the concentration of plasma protein sample was at 20 mg/mL, the reducing power of the plasma protein sample was 0.32 times as high as those of BHA and alpha-tocopherol; the DPPH radical scavenging activity was only 0.25 times as high as those of BHA and alpha-tocopherol; however, the ferrous ion chelating ability of the plasma protein sample was high up to 90 %. In the second part, the conditions of single-stage hydrolysis using Alcalase or Flavourzyme and two-stage were investigated, and the antioxidant properties of the hydrolysates were also evaluated. The degree of hydrolysis (DH) using 2 % Alcalase for 14 hours was 6.29 %; the DH using 2 % of Flavourzyme for 14 hours was 12.68 %; the DH using 2 % Alcalase for 4 hours followed by 2 % Flavourzyme for 10 hours was the highest, valued at 14.86 %. As for the results of the antioxidant properties of hydrolysates, the reducing power of the hydrolysate obtained through hydrolysis using 2 % Alcalase for 4 hours followed using 2 % Flavourzyme for 10 hours was the highest at a concentration of 20mg/mL. For the ferrous ion chelating ability, the hydrolysate obtained through hydrolysis using 2 % Alcalase for 1 hour had the highest value at a concentration of 20mg/mL. For the DPPH radical scavenging activity, the hydrolysate obtained through hydrolysis using 2 % Alcalase for 10 hours had the highest value at a concentration of 20 mg/mL. In summary, the ferrous ion chelating ability of the hydrolysate obtained through using Alcalase was higher than that using Flavourzyme; the reducing power and DPPH radical scavenging activity of porcine plasma protein could be enhanced through enzymatic hydrolysis. In the third part, the thermal stability of the antioxidant properties of porcine plasma proteins before and after enzymatic hydrolysis using 2 % Alcalase for 4 hours followed using 1 % Flavourzyme for 10 hours was studied. For the reducing power, the heating treatments at 50°C and 80°C for 60 minutes did not damage the reducing power of plasma protein before and after enzymatic hydrolysis, inversely, a heating process at 80°Ccould enhance the reducing power of the samples. For the ferrous ion chelating ability, the plasma protein without enzymatic hydrolysis had higher thermal stability than that after enzymatic hydrolysis. The heating treatment at 80°Ccould decrease the ferrous ion chelating ability of the hydrolysate obtained through two-stage enzymatic hydrolysis. For the DPPH radical scavenging activity, both the plasma proteins before and after and enzymatic hydrolysis had high thermal stability during the heating treatment at 50°C. During the heating treatment at 80°C, the DPPH radical scavenging activity of plasma protein before and after enzymatic hydrolysis could be maintained or enhanced. Key words: porcine plasma protein, enzymatic hydrolysis, antioxidant properties, reducing power, ferrous ion chelating ability, DPPH radical scavenging activity, thermal stability.

Keywords : porcine plasma protein ; enzymatic hydrolysis ; antioxidant properties ; reducing power ; ferrous ion chelating ability ; DPPH radical scavenging activity ; thermal stability

Table of Contents

REFERENCES

參考文獻 1.行政院農委會 (2002) 毛豬交易行情統計。 2.行政院農委會畜產試驗所 (2002) 台灣畜產種原資訊網。 http://www. tlri. gov. tw. 3.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): 1-11。 4.王建龍 (2002) 阿根廷魷及赤魷眼窩組織酵素水解物之抗氧化活性。 海洋大學食品科學系碩士論文。 5.阮進惠、張為憲 (1984) 豬血血漿與血球蛋白質之分離回收及其功能性 質。食品科學，11(3,4): 178-188。 6.江美昭 (2003) 酵素水解豬血漿中白蛋白以製備高血壓抑制胜?。東海 大學食品科學系碩士論文。 7.林彥均 (2001) 以脫脂花生粉之酵素水解液製備花生香料。大葉大學食 品工程學系碩士論文。 8.林玫欣 (1999) 鯖魚肉與內臟水解物之抗氧化性研究。海洋大學食品科 學系碩士論文。 9.余碧、許振忠、邱文石 (1996) 蛋白質水解酵素力價估測之研究。中國 畜牧學會會誌，25(2): 149-159。 10.李宜娟 (2003) 洛神花多酚酸及花青素萃取物對含有突變粒體DNA之人 類腫瘤細胞。中山醫學大學生物化學系碩士

論文。 11.吳春惠 (2003) 可果美市販蕃茄製品抗氧化抑變異能力之評比。台北 醫學大學醫學系碩士論文。 12.林慶文 (1991) 豬血之利用。現代肉品，pp.14-16。 13.胡韻笙 (2002) 發酵乳製品生理活性之研究。中山醫學大學食品營養 學系碩士論文。 14.晏文潔 (2002) 類黃酮抗氧化力與其結構之關係。台灣農業化學與食 品科學，38 (1) : 80-88。 15.陳正宗 (1992) 胺基酸與蛋白質。生物化學指引，pp.86-87。 16.陳明造、劉登城 (1988) 血液的利用。屠宰場副產物之加工利用研討 會論文集。行政院農委會，pp.32-40。 17.陳明造 (1999) 素食食品-營養、特性與加工。藝軒圖書出版社， pp.67-68。 18.陳怡宏 (1997) 蛋白質酵素水解液之生產技術。食品工業月刊，29 (11): 34-40。 19.陳螢龍 (2002) 啤酒廢棄酵母有用成分回收方法之探討。大同大學生 物工程系碩士論文。 20.郭悅雄 (1995) 自由基、活性氧及抗氧化劑。台灣科學，48(2): 164- 177。 21.郭淑綾 (2004) 不同甘藷品種皮、葉及藤之抗氧化功能評估。實踐大 學食品營養系碩士論文。 22.許嘉慧 (2002) 應用酵素水解豬血漿蛋白以製備調味料之研究。東海 大學食品科學系碩士論文。 23.許雅芳 (2003) 鯖柴魚水解物對血管升壓素轉換?之抑制與其純化。 海洋大學食品科學系碩士論文。 24.張為憲 (1995) 食品化學。國立編譯館，pp.90-96。 25.張鈺驪 (1993) 基礎食品化學。藝軒圖書出版社，pp.104-105。 26.張嘉倫 (1994) 屠宰場廢棄豬血之利用。大葉大學食品工程學系碩士 論文。 27.張玉琴 (2000) 以豬肉酵素水解液製備豬肉香料。大葉大學食品工程 學系碩士論文。 28.葉震浩 (1998) 雞蛋白之水解與應用之研究。台灣大學農業化學系碩 士論文。 29.彭明月 (1992) 紡絲法之豬血組織化食品製造。食品科學，19(1): 46-56。 30.曾吉偉 (2001) 酵素水解魚肉生產胜?及其抗氧化特性之研究。海洋 大學食品科學系碩士論文。 31.楊正護、林慶文 (1994) 凍乾豬血粉之特性。中國農業化學會誌，32 (4): 355-360。 32.楊海明、段盛秀 (1995) 食品化學實驗。藝軒圖書出版社。 33.劉益忠 (1995) 不同集血方式豬血液於貯藏期間品質之變化。台灣大 學畜產學研究所碩士論文。 34.劉伯康、陳惠英、顏國欽 (1999) 數種傳統食用植物甲醇萃取物抗氧 化力之研究。中國農業化學會誌，37(1):105-116。 35.鄭靜桂 (1997) 蛋白質之水解與水解液之利用。食品工業月刊，29 (5): 10-17。 36.蔡明芳 (1997) 屠宰場豬血之回收與利用。大葉大學食品工程學系碩 士論文。 37.蔡采芳 (1997) 雞蛋蛋白水解物之製備及其性質之研究。台灣大學農 業化學系碩士論文。 38.魏耀輝 (1986) 生物化學原理。國立編譯館，pp.124~125。 39.魏韶宏 (2003) 利用酵素水解雞頭與黑豆生產含生物活性胜?之機能 性產品。台灣大學食品科技系碩士論文。 40.饒家麟 (2001) 鮪魚蒸煮液蛋白質水解物之抗氧化特性。台灣農業化 學與食品科學，39(5): 363-369。 41.Adler-Nissen, J. (1977) Enzymatic hydrolysis of food proteins. Process Biochemistry 12:18-23. 42.Astawan, M., Wahyuni, M.T., Yasuhara, K., Yamada, T. and Maekawa, A. (1995) Effects of angiotensin I-converting enzyme inhibitory substances derived from Ind-onesian dried- salted fish on blood pressure of rats. Bioscience, Biotechnology, and Biochemistry 59: 425-429. 43.AOAC. (1986) Official Methods of Analysis, 15thed. Association of Official Analytical Chemists, Washington, DC. 44.Bishov, S. J. and Henick, A. S. (1975) Antioxidants effect of protein hydrolysates in freeze-dried model system. Food Science 40: 345-348. 45.Blenford, D. E. (1994) Protein hydrolysates functionalityes and uses in nutritional products. Neurochemical Research. 3: 45-49. 46.Blosi, M. S. (1958) Antioxidants determination by the use of a stable free radical. Nature 26: 1199-1200. 47.Clemente, A., Vioque, J., Sanchez-Vioque, R., Pedroche, J. and Millan, F. (1999) Production of extensive chick-pea (Cicer arietinum L.) protein, hydrolysates with reduced antigenic activity. Journal of Agricultural and Food Chemistry 47: 3776-3781. 48.Chen, H. M., Murumoto, K. and Yamauchi, F.(1995)Structural analysis of antioxidative peptides from soy bean conglycinin. Journal of Agricultural and Food Chemistry 43: 74-578. 49.Chen, X. and Ahn, D. U. (1998) Antioxidant activites of six natural phenolics against lipid oxidation induced by Fe2+ or ultraviolet light. Journal of American and Oil Chemistry Society 75: 717-1712. 50.Decker, E. A. and Welch, B. (1990) Role of ferritin as a lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry 38: 674. 51.Denison, E. T. and Emanuel, N. M. (1956) Kinetic characteristics of cyclohexane oxidation in the presence of cobalt stearate. Zhurnal. Fiz. Khim. 30: 2327-2336. Chemistry Abstrate 51: 9274d. 52.Djenane, D., Martinez, L ., Sanchez-Escalante, A., Beltran, J. A., and Roncales, P. (2004) Antioxidant effect of carnosine and carnitine in fresh beef steaks stored under modified atmosphere. Food Chemistry 85 : 453-459. 53.Farmer, E. H., Koch, H. P. and Sutton, D. A. (1943) The course of autoxidation reaction in polyisoprenes and allied compounds. Part VII. Rearrangement of double bonds during autoxidation. Journal of American Chemistry pp.541-547. 54.Fereidoon, S., Jozef, S. and Jerzy, B. (1994) Proteolytic hydrolysis of muscle proteins of harp seal (Phocagroen landica).Journal of Agricultural and Food Chemistry 42: 2634- 2638. 55.Giese, B. (1996) Antioxidant: Tools for preventing lipid oxidation. Food Technology 50(11): 73-81. 56.Giovanni, M. (1983) Response surface methodology and product optimization. Food Technology 37(11): 41-45. 57.Godfrey, T. (1986) Comparison of key characteristics of industrial enzyme by type and source. Industrial Enzymology pp.466-557. 58.Halliwell, B., Gutteridge, J. M. C. and Cross, C. E. (1992) Free radical, antioxidants and human disease; Where are we now? Journal of Clinical Laboratory Medicine 119: 598-620. 59.Halliwell, B. and Gutteridge, K. (1989) Free radical in biology and medicine. Journal of Medicinal Chemistry 8: 484- 487. 60.In, M. J., Chae, H. J. and Oh, N. S. (2002) Process development for heme-enriched peptide by enzymatic hydrolysis of hemoglobin Bioresource Technology 84: 63-68. 61.Kanner, J., German, J. B. and Kinsella, J. E. (1987) Initiation of lipid peroxidation in biological systems. Critical Reviews in Food Science Nutrition 25(4): 317-363. 62.Labuza, T. P.(1984). Application of chemical kinetics to deterioration of foods. Journal of Chemical Editor 61:348. 63.Lahl, W. J. and Braun, S. D. (1994) Enzymatic production of protein hydrolysates for food use. Food Technology 48(10): 68-71. 64.Ledward, D. A. and Lawire, R. A. (1984) Recovery and utilization of by-product protein of the meat industry. Journal of Chemical and Biotechnology 34B: 223-228. 65.Lee, S. and Song K. B. (2003) Effect of gamma-irradiation on the physicochemical properties of porcine and bovine blood plasma proteins. Food Chemistry 82: 521-526. 66.Loukas, S., Varoucha, D. C., Zioudrou, R., Streaty, A. and Klee, W. A. (1983) Opioid activities and structures of α - casein-derivedexor-phins. Biochemistry 22 : 4567-4573. 67.Lu, L. C., Chen, Y. W. and Chou, C. C. (2003) Anti-bacterial and DPPH radical-scavenging activities of the ethanol extract of propolis collected in taiwan. Journal of Food and Drug Analysis 11(4): 277-282. 68.Ockerman, H. W. and Hansen, C. L. (1988) Animal by-Product Processing. VCH Publishers, chichester, VK pp.58-88. 69.Oyaizu, M. (1986) Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese. Journal of Nutrition 44: 307-315. 70.Pares, D., Saguer, E., Toldra, M. and Carretero, C. (2000) Effect of high pressure

processing at different temperatures on protein functionality of porcine blood plasma. Journal of Food Science 65 (3): 486-490. 71.Park, E., Lee, H. and Song, K. B. (1996) Characterization of plasma proteins from bloods of slaughtered cow and pig and utilization of the proteins as adhesives. Agricultural and Biological Chemistry 39(2): 123-126. 72.Park, K. J. and Hyun, C. K. (2002) Antigenotoxic effects of the peptides derived from bovine blood plasma proteins. Enzyme and Microbial Technology pp.633-638. 73.Pokorny, J. (1991) Natural antioxidants for food use. Trends in Food Science & Technology pp.223. 74.Raeker, M. O. and Johnson, L. A. (1995). Thermal and functional properties of bovine blood plasma and egg white proteins. Journal of Food Science 60: 685-690. 75.Ranken, M. D. (1980) Applications of blood proteins. In " Applied protein Chemistry ", Ed. Grant, R. A., pp.169-180. Applied Science. Publishers, LTD. London. 76.Sela, M., White, F. H., Jr. and Anfinsen, C.B. (1957) Reductive cleavage of disulfide bridges in ribonuclease. Science 125: 691-692. 77.Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992) Antioxidative properties of xanthane on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40:945. 78.Simic, M. G. (1998) Mechanisms of inhibition of free- radical processes in mutagenesis and carcinogenesis. Mutation Research 202: 377-386. 79.Tappel, A. L. (1962) Hematin compounds and lipo-xidase as biocatalysts. In Symposium on Foods: Lipids and their oxidation. H. W. Schultz, E. A. Day, and R. O. Sinnhuber (Editors). AVI Publishing Co., Inc. Westport, CT. 80.Tecator Co. (1983) Instruction manual: Soxtec System HT6. Sweden. 81.Williams, W. B., Cuvelier, M. E. and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss. Technology 28(1): 25-30. 82.Wu, H. C., Chen, H. M. and Shiau, C.Y. (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Research International 36: 949-957. 83.Yagi, K. (1987) Lipid peroxides and human disease. Chemistry of Physiology Lipids 45: 337-341. 84.Yamaguchi, N., Yokoo, Y. and Fujimaki, M. (1979) Antioxidative activities of protein hydrolysates. Nippon Shokuhin Kogyo Gakkaishi 26: 65-70. 85.Yashiro, A., Oda, S. and Sugano, M. (1985) Hypocholester- olemic effect of soybean protein in rats and mice after peptic digestion. Journal of Nutritional 115: 1325-1336. 86.Yee, J. J., Shipe, W. F. and Kinsella, J. E. (1980) Antioxidant effects of soy protein hydrolysates on copercatalyzed methyl linoleate oxidation. Journal of Food Science 45:1082-1083. 87.Zioudrou, C., Streaty, R. A. and Klee, W. A. (1979) Opioid peptides derived from food proteins. Journal of Biological Chemistry 254:2446-2449.