

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

廖恬瑤、張基郁

E-mail: 9806846@mail.dyu.edu.tw

摘要

本研究以蜂王蛹蛋白為材料，利用alcalase及flavourzyme進行一階段與兩階段水解，探討蜂王蛹蛋白經酵素水解前後對細胞DNA氧化性傷害及LDL氧化之抑制作用。

在蜂王蛹蛋白水解研究方面，蜂王蛹蛋白以1、1.5及2 % alcalase進行水解20小時，水解率分別為8.89、8.99及9.19 %；以1、1.5及2 % flavourzyme進行水解16小時，水解率分別為9.57、8.59及9.91 %；蜂王蛹蛋白以1.5% alcalase水解4小時後，再以1、1.5及2 % flavourzyme水解12小時，水解率分別為9.03、9.49及8.98 %。

在Fenton reaction誘導DNA氧化傷害之抑制研究方面，蜂王蛹蛋白、一階段（alcalase、flavourzyme）及兩階段水解物皆有抑制去氧核糖氧化傷害的效果，其中以蜂王蛹蛋白效果最大，於1 mg/mL濃度時，分別可抑制47.06、33.70、24.19及43.09 %之氧化傷害。蜂王蛹蛋白及其水解物皆有降低8-OH-2'-dG之生成，其抑制能力大小順序為蜂王蛹蛋白 < flavourzyme一階段水解物 < alcalase一階段水解物 兩階段水解物。在Bleomycin-Fe³⁺ 誘導DNA氧化傷害研究方面，蜂王蛹蛋白及水解物皆無明顯的促氧化效果。

在LDL的氧化修飾研究方面，對Cu²⁺誘導LDL氧化生成TBARS之影響，以1 mg/mL之兩階段水解物之效果最大，其抑制能力大小順序為兩階段水解物 > alcalase一階段水解物 > 蜂王蛹蛋白 > flavourzyme一階段水解物。在共軛雙烯的生成量方面，蜂王蛹蛋白及水解物皆可延滯共軛雙烯的生成。在濃度1 mg/mL時，蜂王蛹蛋白及水解物皆有明顯的抑制效果，其lag time約為240 min，為控制組的2倍。

關鍵詞：蜂王蛹、抗氧化性、氧化傷害、低密度脂蛋白

目錄

封面內頁

簽名頁

授權書iii

中文摘要iv

英文摘要vi

誌謝viii

目錄ix

圖目錄xiii

表目錄xv

1.前言1

2.文獻回顧3

2.1蜂蛹之簡介3

2.1.1蜂蛹之成份3

2.1.2蜂王蛹的研究及應用4

2.2蛋白質水解7

2.2.1蛋白質水解酵素7

2.2.2水解方式及條件7

2.2.3蛋白質水解特性及應用8

2.2.4 酵素水解之影響因子9

2.3蛋白質及水解物之機能性11

2.3.1血管緊縮素轉化?抑制胜?11

2.3.2抗氧化胜?12

2.3.3類鴉片胜? (opiod peptides) 12

2.3.4免疫活性胜?(immunopeptides)13

2.3.5礦物質結合胜? - 酪蛋白磷酸胜?13

2.3.6抗菌活性14

2.4氧化作用	14
2.4.1自由基	14
2.4.2自由基的來源與種類	15
2.4.3氧化壓力	18
2.4.4抗氧化防禦系統	19
2.4.5費頓反應(Fenton reaction)	21
2.4.6DNA氧化傷害	23
2.5人類低密度脂蛋白	24
2.5.1氧化修飾低密度脂蛋白(OxLDL)	26
2.5.2丙二醛(Malondialdehyde, MDA)	27
2.5.3硫代巴比妥酸反應物質(TBARS)	27
3.材料與方法	29
3.1實驗材料	29
3.1.1原料	29
3.1.2藥品	29
3.1.3儀器設備	30
3.1.4蛋白質分解酵素	32
3.2實驗方法與分析項目	32
3.2.1本實驗流程	32
3.2.2基本組成分析	32
3.2.3蜂王蛹蛋白試液製備	35
3.2.4水解物之製備	35
3.2.4.1一階段水解	35
3.2.4.2兩階段水解	36
3.2.5蜂王蛹蛋白及水解物對生物分子氧化傷害之抗氧化性	37
3.2.6蜂王蛹蛋白及水解物於抑制LDL氧化之探討	39
3.2.6.1LDL製備	39
3.2.6.2蜂王蛹蛋白及水解物對銅離子誘導LDL氧化之影響	40
3.2.7統計分析	41
4.結果與討論	42
4.1蜂王蛹之基本成分分析	42
4.2蜂王蛹蛋白之酵素水解	43
4.2.1以不同濃度alcalase水解蜂王蛹蛋白之水解率變化	43
4.2.2以不同濃度flavourzyme水解蜂王蛹蛋白之水解率變化	47
4.2.3兩階段酵素水解蜂王蛹蛋白之水解率變化	49
4.3對生物分子氧化傷害之抗氧化性	49
4.3.1蜂王蛹蛋白及水解物對Fenton reaction誘導的deoxyribose氧化傷害之影響	49
4.3.2蜂王蛹蛋白及水解物對Fenton reaction誘導2'-deoxyguanosine(2'-dG)氧化形成8-hydroxy-2'-deoxy-guanosine(8-OH-2'-dG)之影響	51
4.3.3蜂王蛹蛋白及水解物對bleomycin-Fe ³⁺ 誘導DNA氧化傷害之影響	56
4.4蜂王蛹蛋白及水解物對銅離子誘導LDL氧化修飾之影響	58
4.4.1蜂王蛹蛋白及水解物對Cu ²⁺ 誘導LDL氧化生成TBARS之影響	58
4.4.2蜂王蛹蛋白及水解物對Cu ²⁺ 誘導LDL氧化生成共軛雙烯(conjugated diene, CD)之影響	63
5.結論	69
參考文獻	71
圖3.1實驗流程圖	33
圖4.1以alcalase水解蜂王蛹蛋白之水解率	44
圖4.2以flavourzyme水解蜂王蛹蛋白之水解率	46
圖4.3使用酵素(alcalase和flavourzyme)水解蜂王蛹蛋白	48
圖4.4蜂王蛹蛋白及水解物對Fe ³⁺ -EDTA/H ₂ O ₂ /Asc誘導去氧核糖氧化傷害之影響	50
圖4.5蜂王蛹蛋白及水解物對bleomycin-Fe ³⁺ 誘導DNA傷害之影響	57
圖4.6蜂王蛹蛋白對Cu ²⁺ 誘導LDL氧化生成TBARS之影響	59
圖4.7蜂王蛹蛋白水解物(Alcalase)對Cu ²⁺ 誘導LDL氧化生成TBARS之影響	60

- 圖4.8蜂王蛹蛋白水解物(Flavourzyme)對Cu²⁺誘導LDL氧化生成TBARS之影響61
- 圖4.9蜂王蛹蛋白水解物(Alcalase 和Flavourzyme)對Cu²⁺誘導LDL氧化生成TBARS之影響62
- 圖4.10蜂王蛹蛋白對銅離子誘導LDL形成共軛雙烯之影響65
- 圖4.11蜂王蛹蛋白水解物(Alcalase)對銅離子誘導LDL形成共軛雙烯之影響66
- 圖4.12蜂王蛹蛋白水解物(Flavourzyme)對銅離子誘導LDL形成共軛雙烯之影響67
- 圖4.13蜂王蛹蛋白水解物(Alcalase 和Flavourzyme)對銅離子誘導LDL形成共軛雙烯之影響68
- 表2.1蜂王蛹所含的營養素5
- 表2.2蜂蛹及蜂王漿胺基酸含量6
- 表2.3內源性及外源性的抗氧化系統22
- 表2.4健康人類低密度脂蛋白的組成25
- 表4.1蜂王蛹之一般組成分42
- 表4.2蜂王蛹蛋白對Fenton reaction所誘導2'-dG形成8-OH-2'-dG之影響52
- 表4.3蜂王蛹蛋白水解物(Alcalase)對Fenton reaction所誘導2'-dG形成8-OH-2'-dG之影響53
- 表4.4蜂王蛹蛋白水解物(Flavourzyme)對Fenton reaction所誘導2'-dG形成8-OH-2'-dG之影響54
- 表4.5蜂王蛹蛋白水解物(Flavourzyme和Alcalase)對Fenton reaction所誘導2'-dG形成8-OH-2'-dG之影響55

參考文獻

- 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。
- 王鐸葦。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。
- 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。
- 林姿儀。2004。豬血漿蛋白質之酵素水解及抗氧化活性研究。大葉大學生物產業科技學系碩士論文。彰化。
- 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。
- 林天送。1995。羥基自由基:毒性極高的破壞分子。健康世界。112:6-10。
- 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技學系碩士論文。彰化。
- 周碧青、張金彪、孫向華。2009。氣相色譜法測定工蜂幼蟲和蛹中脂肪酸組成。福建分析測試。18:58-61。
- 後藤克夫。1994。神奇蜂王乳。第52-54頁。國際村文庫書店。台北。
- 洪銘育。2009。抗氧化物質與後期糖化終產物抑制劑對生理的正面效應。食品工業。41:35-49。
- 郭智宏。2001。腸道吸收-胺基酸與胜?。食品工業。33:15-25。
- 梁俊煌。2006。運動訓?對人體體內自由基產生及抗氧化酵素之影響。嘉南學報。32:433-442。
- 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。
- 張海生、陳錦屏。2008。蜂蛹酮的提取及体外抗氧化作用的研究。食品科學。29:159-162。
- 張海生、陳錦屏。2007。蜂蛹脂肪超音波提取工藝研究及脂肪酸成分分析。天然產物研究及開發。19:299-302。
- 張其康、吳珍紅、杜迎剛、徐昌利、繆曉青。2004。蜂王幼蟲抗疲勞作用研究。中國養蜂。55:6-7。
- 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。
- 程竹青。2003。綜論食品胜?之機能。食品工業。35:1-3。
- 塗佳琪。2006。蜂王蛹蛋白及其水解物對血管收縮素轉換酵素之抑制活性研究。大葉大學生物產業科技學系碩士論文。彰化。
- 楊詠翔。1999。食品中抗高血壓胜?的發展現況。食品工業。31: 9-18。
- 劉毓蕙。2004。水解蛋白的特性及運用。食品工業。36: 19-24。
- 劉海萍、邵有全、張云毅。2005。雄蜂幼蟲粉抗疲勞和增強記憶力試驗。中國養蜂。56:9。
- 鄭名凡。1999。蛋白質水解物的功能與應用。食品資訊。160: 49-54。
- 賴祥玲。2004。乳蛋白中的機能胜?。食品工業36(2):37-44。25。謝坤霖。2007。番木瓜果實水萃取物之抗氧化能力研究。靜宜大學食品營養學系碩士論文。台中。
- 黃志強。2008。牛初乳水解物中之抗氧化胜?研究。大葉大學生物產業科技學系碩士論文。彰化。
- Anderson, D. and Phillips, B. J. 1999. Comparative in vitro and in vivo effects of antioxidants. Food and Chemical Toxicology 37: 1015-1025.
- Alessio, H.M. 1993. Exercise-induced oxidative stress. Medicine and Science in Sport and Exercise 25(2): 218-224.
- Aruoma, O. I., Murcia, A., Butler, J. and Halliwell, B. 1993. Evaluation of antioxidant and prooxidant actions of gallic acid and its derivatives. Journal of Agricultural and Food Chemistry 41: 1880-1885.
- Asami, S., Manabe, H., Miyake, J., Tsurudome, Y., Hirano, T., Yamaguchi, R., Itoh, H. and Kasai, H. 1997. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 18: 1763 – 1766.
- Aslan, M., Horoz, M., Kocyigit, A., Ozgon?l, S., Celik, H., Celik, M. and Erel, O. 2006. Lymphocyte DNA damage and oxidative stress in patients with iron deficiency anemia. Mutation Research 601: 144 – 149.
- Bejma, J. & Ji, L.L. 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. Journal of Applied Physiology 87(1): 465-470.
- Bourne, L. C. and Rice-Evans, C. A. 1997. The effect of the phenolic antioxidant ferulic acid on the oxidation of low density lipoprotein depends on the pro-oxidant used. Free Radical Research 27: 337-344.
- Borek, C. 2004. Dietary antioxidants and human cancer. Integrative Cancer Therapies 3: 333 – 341.
- Channel, S. R., Latendresse, J. R., Grabau, J. H., Lane, J. W., Goodwin, L. S., and Gothaus, M. C. 1998. A subchronic exposure to trichloroethylene causes lipid peroxidation and hepatocellular proliferation in male B6C3F1 mouse liver. Toxicological sciences 43:145-154.
- Child, R., Brown, S., Day, S., Donnelly, A., Roper, H. & Saxton, J. 1999. Change in indices of antioxidant status, lipid peroxidation and inflammation in human skeletal muscle after eccentric muscle actions. Clinical Science 96: 105-115.
- Clement, A. 2000. Enzymatic protein hydrolysates in human nutrition. Trends in Food Science and Technology 11: 254-262.
- Cojocel, C., Beuter, W., Muller, W., and Mayer, D. 1989. Lipid peroxidation: a possible mechanism of trichloroethylene-induced nephrotoxicity. Toxicology 55: 131-141.
- Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A. 2006. Biomarkers of oxidative damage in human disease. Clinical Chemistry 52: 601-623.
- Esterbauer, H., Gebicki, J., Puhl, H. and J?rgens, G.

1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine* 13: 341-390.

41. Foksinski, M., Kotzbach, R., Szymanski, W. and Olinski, R. 2000. The level of typical biomarker of oxidative stress 8-Hydroxy-2-Deoxyguanosine is higher in uterine myomas than in control tissues and correlates with the size of the tumor. *Free Radical Biology and Medicine* 7: 597 – 601.

42. Filippou, D., Papadopoulos, V. P., Triga, A., Filippou, G., Rizos, S., Skandalakis, P. and Manolis, E. 2007. Nitric oxide, antioxidant capacity, nitric oxide synthase and xanthine oxidase plasma levels in a cohort of burn patients. *Burns* 33: 1001 – 1007.

43. Gildberg, A. 1993. Enzymatic processing of marine raw materials. *Process Biochemistry* 28: 1-15.

44. Gauthier, S. F., Pouliot, Y. and Saint-Sauveur, D. 2006. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. *International Dairy Journal* 16: 1315-1323.

45. Gerry, A. B., Satchell, L. and Leake, D. S. 2008. A novel method for production of lipid hydroperoxide- or oxysterol-rich low-density lipoprotein. *Atherosclerosis* 197: 579-587.

46. Gildberg, A. 1993. Enzymatic processing of marine raw materials. *Process Biochemistry* 28: 1-15.

47. Gildberg, A., Hermes, J. E. and Orejana, F. M. 1984. Acceleration of autolysis during fish sauce fermentation by adding acid and reducing the salt content. *Journal of the Science of Food and Agriculture* 35: 1363-1369.

48. Halliwell, B. 1995. Oxidation of low-density lipoproteins: questions of initiation, propagation, and the effect of antioxidants. *American Journal of Clinical Nutrition* 61: 670-677.

49. Huet, J. and Laval, F. 1985. Potentiation of cell killing by inhibitors of poly (adenosine diphosphate-ribose) synthesis in bleomycin-treated Chinese hamster ovary cells. *Cancer Research* 45: 987-991.

50. Horsley, E. T. M., Burkitt, M. J., Jones, C. M., Patterson, R. A., Harris, L. K., Moss, N. J., del Rio, J. D. and Leake, D. S. 2007. Mechanism of the antioxidant to pro-oxidant switch in the behavior of dehydroascorbate during LDL oxidation by copper (II) ions. *Archives of Biochemistry and Biophysics* 465: 303 – 314.

51. Hsieh, C. L. and Yen, G. C. 2000. Antioxidant actions of *Du-Zhong* (*Eucommia ulmoides* Oliv.) toward oxidative damage in biomolecules. *Life Science* 66: 1387-1400.

52. Jang, A. and Lee, M. 2004. Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Science* 69: 653-661.

53. Kang, J. H. 2003. Oxidative damage of DNA by the reaction of amino acid with methylglyoxal in the presence of Fe(III). *Biological Macromolecules* 33: 43-48.

54. Kasai, H. and Nishimura, S. 1984. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Research* 12: 2137-2145.

55. Kent, K. D., Harper, W. J. and Bomser, J. A. 2003. Effect of whey protein isolate on intracellular glutathione and oxidant-induced cell death in human prostate epithelial cells. *Toxicology in vitro* 17: 27 – 33.

56. Kleinveld, H. A., Hak-Lemmers, H. L., Stalenoef, A. F. H. and Demacker, P. N. M. 1992. Improved measurement of low-density-lipoprotein susceptibility to copper-induced oxidation: application of a short procedure for isolating low-density lipoprotein. *Clinical Chemistry* 38: 2066-2072.

57. Kobayashi, S., Ueda, K. and Komano, T. 1990. The effects of metal ions on the DNA damage induced by hydrogen peroxide. *Agricultural and Biological Chemistry* 54: 69-76.

58. Lahl, W. J. and Braun, S. D. 1994. Enzymatic production of protein hydrolysates for food use. *Food Technology* 48: 68-71.

59. Larson, J. L., and Bull, R. J. 1992. Metabolism and lipoperoxidation activity of trichloroacetate and dichloroacetate in rats and mice. *Toxicology and Applied Pharmacology* 115: 268-277.

60. Lee, K. J., and Jeong, H. G. 2007. Protective effects of kahweol and cafestol against hydrogen peroxide-induced oxidative stress and DNA damage. *Toxicology Letters* 173: 80-87.

61. Li, T. H., Jia, W. L., Wang, H. S. and Liu, R. M. 2007. Electrochemical performance of 8-hydroxy-2-deoxyguanosine and its detection at poly (3-methylthiophene) modified glassy carbon electrode. *Biosensors and Bioelectronics* 22: 1245 – 1250.

62. Mahadik, S. P., Evans, D. and Lal, H. 2001. Oxidative stress and role of antioxidant and ω -3 essential fatty acid supplementation in schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 25: 463-493.

63. Manly, C. H. and Ahmedi, S. 1995. The development of process flavors. *Trends in Food Science and Technology* 6: 46-51.

64. Mackie, I. M. 1982. Fish protein hydrolysates. *Process Biochemistry* 31: 26-31.

65. Migliore-Samour, D., Floch, F. and Jolles, P. 1989. Biologically active casein peptide implicated in immune modulation. *Journal Dairy Research* 56: 357-363.

66. 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. *Agricultural and biological chemistry* 55: 1313-1318.

67. Nurminen, M. L., Sipola, M., Kaarto, H., Pihlanto-Leppä, A., Piilola, K., Korpela, R., Tossavainen, O., Korhonen, H. and Vapaatalo, H. 2000. Lactorphin lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sciences* 66: 1535 – 1543.

68. Papadopoulos, A. E., Fatta, D. and Loizidou, M. 2007. Development and optimization of dark Fenton oxidation for the treatment of textile wastewaters with high organic load. *Journal of Hazardous Materials* 146: 558-563.

69. Peñalva-Ramos, E. A. and Xiong, Y. L. 2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science* 64: 259 – 263.

70. Pereira, V. G., Martins, A. M., Micheletti, C. and Almeida V. D. 2008. Mutational and oxidative stress analysis in patients with mucopolysaccharidosis type I undergoing enzyme replacement therapy. *Clinica Chimica Acta* 387: 75 – 79.

71. Pellegrini, A., Dettling, C., Thomas, U. and Hunziker, P. 2001. Isolation and characterization of four bactericidal domains in the bovine α -lactoglobulin. *Biochimica et Biophysica Acta* 1526: 131 – 140.

72. Puhl, H., Waeg, G. and Esterbauer, H. 1994. Methods to determine oxidation of low-density lipoproteins. *Methods in enzymology* 233: 425-441.

73. Raal, F. J., Areias, A. J., Waisberg, R. and Von, A. M. 1995. Susceptibility of low-density lipoprotein to oxidation in familial hypercholesterolemia. *Atherosclerosis* 115: 9-15.

74. Ray, G. and Husain, S. A. 2002. Oxidants, antioxidants and carcinogenesis. *Indian Journal of Experimental Biology* 40: 1213-1232.

75. 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. *Archives of Biochemistry and Biophysics* 322: 339-346.

76. SAS Institute, Inc.: SAS User's guide: Statistics version 5th ed. SAS Inst. Cary, NC, U.S.A. 1985.

77. Seljeskog, E., Hervig, T. and Mansoor, M. A. 2006. A novel HPLC method for the measurement of thiobarbituric acid reactive substances (TBARS). A comparison with a commercially available kit. *Clinical Biochemistry* 39: 947 – 954.

78. Shimoda, R., Nagashima, M., Sakamoto, M., Yamaguchi, N., Hirohashi, S., Yokota, J. and Kasai, H. 1994. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. *Cancer Research* 54: 3171 – 3172.

79. Sies, H. 1993. Strategies of antioxidant

defense. *European Journal of Biochemistry* 215: 213-219.80. Smith, C., Halliwell, B. and Aruoma, O. I. 1992. Protection by albumin against the pro-oxidant actions of phenolic dietary components. *Food and Chemical Toxicology* 30: 483-489.81. Taccone-Gallucci, M., Lubrano, R., Meloni, C., Morosetti, M., Adolfo, C. M. and Casciani, C. U. 1998. Malonyldialdehyde content of cell membranes is the most important marker of oxidative stress in haemodialysis patients. *Nephrology Dialysis Transplantation*. 13: 2711-2712.82. Takagi, A., Sai, K., Umemura, T., Hasegawa, R. and Kurokawa, Y. 1995. Inhibitory effects of vitamin E and ellagic acid on 8-hydroxydeoxyguanosine formation in liver nuclear DNA of rats treated with 2-nitropropane *Cancer Letters* 91: 139-144.83. Valenzuela, R., Contreras, D., Oviedo, C., Freer J. and Rodriguez, J. 2008. Copper catechol-driven Fenton reactions and their potential role in wood degradation. *International Biodeterioration & Biodegradation*, In Press, Corrected Proof, Available online.84. Wiseman, H., Kaur, H. and Halliwell, B. 1995. DNA damage and cancer: measurement and mechanism. *Cancer Letters* 93: 113-120.85. Yagi, K. 1989. A simple fluorometric assay for lipid peroxides in blood serum or plasma. *CRC hand book of free radicals and antioxidants in biomedicine Vol 7* p. 215.86. Yamamoto, N. 1997. Antihypertensive peptides derived from food proteins. *Biopolymer* 43: 129-134.87. Yen, G. C., Chen, H. Y. and Peng, H. H. 1997. Antioxidant and pro-oxidant effects of various tea extracts. *Journal of Agricultural Food Chemistry* 45: 30-34.88. Yen, G. C. and Hsieh, C. L. 2002. Inhibitory effects of Du-zhong (*Eucommia ulmoides* Oliv.) against low-density lipoprotein oxidative modification. *Food Chemistry* 77: 449 – 456.89. Yokoyama, H., Chiba, H. and Yoshikawa, M. 1992. Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Bioscience, biotechnology, and biochemistry* 56: 1541-1545.