

# Chemical Proximate Analysis Antioxidant and Immunomodulation Activity of Crude Polysaccharides from blanching water of *F. velutipes*

施雅芳、柳源德、林芳儀

E-mail: 345485@mail.dyu.edu.tw

## ABSTRACT

*Flammulina velutipes* is one of the common edible mushrooms on the market. The mainly edible mushrooms not only have many nutrient but also have high functional and medicinal values. Studies showed that the protein binding polysaccharide of *F. velutipes* has antioxidant, anti-tumor and immune regulation activities. In this study, investigation of the concentration of polysaccharide in the blanching water proceeded, nevertheless, antioxidant activity, anti-tumor activity, cytotoxicity and immune activity of *F. velutipes* polysaccharide were also analyzed. In this study, crude polysaccharide of *F. velutipes* was extracted from blanching water by ethanolic extraction. It contains many nutrients including carbohydrate (45.95%), crude protein (17.52%), crude fat (1.47%), crude fiber (0.99%) and ash (21.36%). The antioxidant activity on DPPH scavenging ability of blanching water is 33%; crude polysaccharide showed a 46%; As for the ferrous ion chelating ability, blanching water showed a 44% and crude polysaccharide has a ability of 98%. On reducing ability, blanching water has a 0.30, where crude polysaccharide showed a 1.73 at 1mg/mL. FTIR analysis of crude polysaccharide showed it contains  $\alpha$ -1,3-glycosidic groups. In cellular toxicity test showed that crude polysaccharide can promote the HPBMC proliferation for the first 24 hours, however, the HPBMC of proliferation was inhibited after 72 hours incubation. The anti-tumor activity with human cutaneous malignant melanoma showed no inhibition of cellular proliferation after 24 hours incubation; crude polysaccharide from multiple numbers of blanching can increase the ability of suppression of tumor cell proliferation after 72 hours incubation, however, the crude polysaccharide showed no inhibition on human oral cancer cell proliferation. Investigation of crude polysaccharide with HPBMC on the cytokine profile showed that after 24 hours incubation with 0.01 ~ 10  $\mu$ g/mL concentration of crude polysaccharide, highest TNF- $\alpha$  secretion was found (1144 pg/mL), as for 72 hours incubation, TNF- $\alpha$  secretion was dropped to 312 ~ 645 pg/mL. Mononuclear cells can be effectively stimulated by crude polysaccharide, the effect is not lower else fungi. The results can improve value of *Flammulina velutipes*, and the crude polysaccharide can replace synthetic antioxidants. Delop value-added waste water recycling plants and environmental.

Keywords : *Flammulina velutipes*、blanching water、polysaccharide、antioxidant、immunomodulation、chemical proximate

## Table of Contents

封面內頁 簽名頁 中文摘要iii 英文摘要v 誌謝vii 目錄viii 圖目錄xii 表目錄xvii 1.前言 1 2.文獻回顧4 2.1 金針菇簡介4 2.1.1 金針菇之分類地位4 2.1.2 金針菇之形態特徵4 2.1.3 分佈及生長條件5 2.2 金針菇之成分組成6 2.2.1 一般成分組成6 2.2.2 化學成分組成7 2.2.3 金針菇之活性成分8 2.2.3.1 蛋白質8 2.2.3.2 金針菇多醣12 2.3 金針菇之生理活性13 2.3.1 抗腫瘤、抗癌作用14 2.3.2 免疫調節作用14 2.3.3 抗氧化作用14 2.3.4 保肝作用15 2.3.5 幫助智力發育16 2.3.6 抗病毒作用16 2.3.7 降膽固醇16 2.3.8 抗疲勞作用17 2.4 研究動機17 3.材料與方法18 3.1 名詞對照表18 3.2 實驗流程圖19 3.3 實驗材料21 3.3.1 試驗樣品及菌種21 3.3.2 金針菇殺菁水前處理21 3.3.3 金針菇殺菁水澱析粗多醣體之製備21 3.4 金針菇殺菁水粗多醣體一般成分分析22 3.4.1 溶解度22 3.4.2 粗蛋白含量測定22 3.4.3 粗脂肪測定23 3.4.4 灰分含量測定24 3.4.5 粗纖維含量測定24 3.4.6 碳水化合物含量測定25 3.5 抗氧化活性之分析25 3.5.1 還原力之測定25 3.5.2 亞鐵離子螯合能力26 3.5.3 DPPH 自由基清除率26 3.6 細胞實驗27 3.6.1 細胞活化27 3.6.2 細胞繼代方法28 3.6.3 人類周邊血液單核細胞 ( Human Peripheral Blood Mononuclear Cells ; HPBMC ) 之製備28 3.6.4 人類周邊血液單核細胞之培養29 3.6.5 MTT 測試法29 3.6.6 錐藍排除法(cell viability assay ; Trypan blue exclusion)30 3.7 細胞激素之測定30 3.8 傅立葉紅外線光譜儀(FTIR)圖譜分析 31 3.9 統計分析32 4. 結果與討論33 4.1 金針菇殺菁水及粗多醣體乾燥處理33 4.2 溶劑量及溫度對金針菇粗多醣體溶解度之影響36 4.3 金針菇殺菁水粗多醣體一般成分分析41 4.4 金針菇殺菁水及其粗多醣體之抗氧化試驗44 4.5 FTIR 圖譜分析58 4.6 細胞毒性試驗61 4.7 腫瘤細胞存活率試驗70 4.8 金針菇不同殺菁次數澱析粗多醣體對HPBMC產生 TNF- $\alpha$  之產量88 5.結論98 參考文獻100 圖目錄 圖1.1 *Flammulina velutipes*子實體3 圖2.1 FIP-fve晶體衍射結構圖9 圖4.1 以0.01g粗多醣體溶於1mL水溶液在不同溫度下溶解度之影響38 圖4.2 以0.01g粗多醣體3mL水溶液在不同溫度下溶解度之影響39 圖4.3 以0.01g粗多醣體5mL水溶液在不同溫度下溶解度之影響40 圖4.4 金針菇不同殺菁次數處理殺菁水對亞鐵離子螯合能力46 圖4.5 不同殺菁次數處理金針菇殺菁水澱析粗多醣體對亞鐵離子螯合能力 47 圖4.6 金針菇不同殺菁次數處理殺菁水之還原能力48 圖4.7 不同殺菁次數處理金針菇殺菁水澱析粗多醣體之還原能力49 圖4.8 金針菇不同殺菁次數處理殺菁水之DPPH 自由基清除能力 50 圖4.9 不同殺菁次數處理金針菇殺菁水澱析粗多醣體之DPPH 自由基清除能力51 圖4.10  $\alpha$ -1,3-glucan、FVP-1、FVP-2、FVP-3和FVP-4之FTIR圖譜分析60 圖4.11 不同殺菁

次數處理金針菇殺菁水澱析粗多醣體於濃度0.01  $\mu\text{g/mL}$ 與HPBMC共培養24、72小時後對HPBMC增生之影響63 圖4.12不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度0.1  $\mu\text{g/mL}$ 與HPBMC共培養24、72小時後對HPBMC增生之影響64 圖4.13不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度1  $\mu\text{g/mL}$ 與HPBMC共培養24、72小時後對HPBMC增生之影響65 圖4.14不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度10  $\mu\text{g/mL}$ 與HPBMC共培養24、72小時後對HPBMC增生之影響66 圖4.15不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度100  $\mu\text{g/mL}$ 與HPBMC共培養24、72小時後對HPBMC增生之影響67 圖4.16不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與HPBMC共培養24小時後對HPBMC增生之影響68 圖4.17不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與HPBMC共培養72小時後對HPBMC之增生之影響 69 圖4.18 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度10  $\mu\text{g/mL}$ 與KOSC-3細胞株共培養24、72小時後對KOSC-3細胞株之影響74 圖4.19 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度100  $\mu\text{g/mL}$ 與KOSC-3細胞株共培養24、72小時後對KOSC-3細胞株之影響 75 圖4.20 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度250  $\mu\text{g/mL}$ 與KOSC-3細胞株共培養24、72小時後對KOSC-3細胞株之影響 76 圖4.21 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度500  $\mu\text{g/mL}$ 與KOSC-3細胞株共培養24、72小時後對KOSC-3細胞株之影響 77 圖4.22 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度1000  $\mu\text{g/mL}$ 與KOSC-3細胞株共培養24、72小時後對KOSC-3細胞株之影響 78 圖4.23 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與KOSC-3細胞株共培養24小時對KOSC-3細胞株之影響79 圖4.24不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與KOSC-3細胞株共培養72小時對KOSC-3細胞株之影響80 圖4.25 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度10  $\mu\text{g/mL}$ 與A-375細胞株共培養24、72小時後對A-375細胞株之影響81 圖4.26 不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度100  $\mu\text{g/mL}$ 與A-375細胞株共培養24、72小時後對A-375細胞株之影響82 圖4.27不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度250  $\mu\text{g/mL}$ 與A-375細胞株共培養24、72小時後對A-375細胞株之影響83 圖4.28不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度500  $\mu\text{g/mL}$ 與A-375細胞株共培養24、72小時後對A-375細胞株之影響84 圖4.29不同殺菁次數處理金針菇殺菁水澱析粗多醣體於濃度1000  $\mu\text{g/mL}$ 與A-375細胞株共培養24、72小時後對A-375細胞株之影響85 圖4.30不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與A-375細胞株共培養24小時對A-375細胞株之影響86 圖4.31不同殺菁次數處理金針菇殺菁水澱析粗多醣體於不同濃度下與A-375細胞株共培養72小時對A-375細胞株之影響87 圖4.32 金針菇不同殺菁次數澱析粗多醣體在濃度0.01  $\mu\text{g/mL}$ 與HPBMC培養24、72小時對TNF- $\alpha$ 產量之影響91 圖4.33 金針菇不同殺菁次數澱析粗多醣體在濃度0.1  $\mu\text{g/mL}$ 與HPBMC培養24、72小時對TNF- $\alpha$ 產量之影響92 圖4.34 金針菇不同殺菁次數澱析粗多醣體在濃度1  $\mu\text{g/mL}$ 與HPBMC培養24、72小時對TNF- $\alpha$ 產量之影響93 圖4.35 金針菇不同殺菁次數澱析粗多醣體在濃度10  $\mu\text{g/mL}$ 與HPBMC培養24、72小時對TNF- $\alpha$ 產量之影響94 圖4.36 金針菇不同殺菁次數澱析粗多醣體在濃度100  $\mu\text{g/mL}$ 與HPBMC培養24、72小時對TNF- $\alpha$ 產量之影響95 圖4.37 金針菇不同殺菁次數澱析粗多醣體在不同濃度下與HPBMC培養24小時對TNF- $\alpha$ 產量之影響96 圖4.38 金針菇不同殺菁次數澱析粗多醣體在不同濃度下與HPBMC培養72小時對TNF- $\alpha$ 產量之影響97 表目錄 表4.1殺菁水次數處理對金針菇殺菁水固形物乾重(mg/mL)之影響34 表4.2金針菇殺菁水不同殺菁次數處理之酒精澱析粗多醣體乾重(mg/mL) 35 表4.3金針菇殺菁萃取粗多醣體一般成分分析42 表4.4金針菇子實體一般成分分析43 表4.5 金針菇不同殺菁次數殺菁水之亞鐵離子螯合能力52 表4.6 金針菇不同殺菁次數澱析粗多醣體之亞鐵離子螯合能力 53 表4.7 金針菇不同殺菁次數殺菁水之清除DPPH自由基能力54 表4.8 金針菇不同殺菁次數澱析粗多醣體之清除DPPH自由基能力55 表4.9 金針菇不同殺菁次數殺菁水之還原能力56 表4.10 金針菇不同殺菁次數澱析粗多醣體之還原能力57

## REFERENCES

- 1.于榮利、秦旭昇、宋鳳菊。2004。金針菇研究概況。食用菌學報 11(4):63-68。
- 2.孔祥輝、孫宇峰、任永春、奚新偉、楊志興。2006。金針菇免疫調節蛋白的研發與應用。生物技術 16(4):84-88。
- 3.文鏡、陳文。1993。金針菇抗疲勞的實驗研究。營養學報 15(1):79-81。
- 4.王玉峰、王昱、尹鴻萍。2008。金針菇菌絲體中多醣的分離、結構鑒定及免疫學活性。中國天然藥物 16(4):312-315。
- 5.王伯徹。2009。菇類之食藥用價值及其多樣化市場產品開發。農業生技產業季刊 18: 34-40。
- 6.王亞榮。2008。金針菇的特徵特性及高產栽培技術。現代農業科技 (9):34。
- 7.付鳴佳、吳祖建、林奇英、謝聯輝。2003。金針菇中一種抗病毒蛋白的純化及其抗煙草花葉病毒特性。福建農林大學學報(自然科學版) 32(1):84-88。
- 8.朱曙東、嚴茂祥、陳芝芸、曹俊敏。2001。金針菇多醣免疫活性的研究。浙江中醫學院學報 25(4)。
- 9.何軒輝、廖森泰、劉吉平。2008。金針菇的食用和藥用價值研究開發進展。廣東農業科學 (3):70-94。
- 10.吳希哲、高向東。2002。金針菇提取物的保肝及抗腫瘤作用。中國生化藥物雜誌 23(4):176-178。
- 11.吳政蔚。2007。金針菇熱水萃取物中beta-D-葡萄糖聚糖與多酚氧化酶抑制劑之分離:8-9。
- 12.汪金玉。2008。金針菇揮發性化學成分的研究。時珍國醫國藥 19(5):1145-1146。
- 13.林景衛、孫非、張韜、張春玉、劉立俠。2005。真菌免疫調節蛋白的研究進展。中國免疫學雜誌 21(6):477-480。
- 14.邵穎。2007。食用菌多醣含量和清除自由基活性的研究。徐州工程學院學報 22(10):36-39。
- 15.金湘、姿愷、毛培宏。2007。金針菇生物活性物質結構與功能的研究進展。中草藥38(10):1596-1598。
- 16.苗立成、王立強、吳迪。2003。金針菇多醣對小鼠抗腫瘤及抗白血病效應的實驗研究。解放軍藥學學報 19(3):171-173。
- 17.夏俊、陳治文、胡守芬、馬佳、陳素蓮。2004。蘆筍提取液抑制黑色素細胞瘤A375細胞增殖的研究。蚌埠醫學院學報 29(2):95-97。
- 18.孫宇峰。2006。金針菇功能性蛋白的研究進展。微生物學雜誌 26(4)。
- 19.晏文潔、李家璞、杜平。2000。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。
- 20.秦小明、余娟、寧恩創、林華娟。2005。金針菇子實體多醣成分的初步研究。食用菌學報 12(2):27-31。
- 21.袁仲。2005。金針菇的保健功能與加工利用。農產品加工

學刊 (6):125-128. 22.許清祥。2004。免疫學。合記圖書出版社。 23.楊帆。2010。金針菇栽培技術。中國林副特產108(5):70-72。 24.董玉宏。2010。談金針菇栽培技術。現代農業(1)。 25.趙建英。2001。金針菇營養成份分析。呂梁高等專科學校學報 16(1):43。 26.劉淑雲、張春淑。2008。金針菇高產栽培技術。中國農村小康科技 (12):39-41。 27.蔡和暉、廖森泰、葉運壽、劉學銘。2008。金針菇的化學成分、生物活性及加工研究進展。食品研究與開發 29(11):171-174。 28.韓愛華、張燁燁。2010。金針菇栽培技術要點。北京農業 (3):19-22。 29.魏文樹、譚建權。1997。雲芝多醣對活性氧清除機制的增強作用。中國藥學雜誌 32(4):199-201。 30.魏鵬。2010。金針菇栽培技術(一)。農村科技 (1):50-51。 31.饒玉鵬、張李陽、謝玉芹、張曼。2007。金針菇液體發酵條件的研究。南京曉莊學院學報 23(3):117-119。 32.AOAC, 1995. Official methods of analysis of AOAC International 16th ed. Association of Official Analytical Chemists, Washington, D. C., USA. 33.Bao, H. N. D., Ochiai, Y. and Ohshima, T. 2010. Antioxidative activities of hydrophilic extracts prepared from the fruiting body and spent culture medium of *Flammulina velutipes*. *Bioresource Technology* 101(15): 6248-6255. 34.Bohn, J. A. and BeMiller, J. N. 1995. (1-3)-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers* 28(1): 3-14. 35.Chang, H. H., Hsieh, K. Y., Yeh, C. H., Tu, Y. P. and Sheu, F. 2010. Oral administration of an Enoki mushroom protein FVE activates innate and adaptive immunity and induces anti-tumor activity against murine hepatocellular carcinoma. *International Immunopharmacology* 10(2): 239-246. 36.Cutler, R. G. 1991. Recent progress in testing the longevity determinant and dysdifferentiation hypotheses of aging. *Gerontology and Geriatrics* 12(2-3): 75-98. 37.Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *Food Chemistry* 38(3): 674 – 677. 38.Di Luzio, N. R., Williams, D. L., McNamee, R. B., Edwards, B. F. and Kitahama, A. 1979. Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. *International Journal of Cancer* 24(6): 773-779. 39.Dubosta, N. J. O. B. and Beelman, R. B. 2007. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry* 105(2): 727-735. 40.Dziezak, J. D. 1986. Preservatives:Antioxidants. *Food Technology* 40:94-102. 41.Fu, H. Y., Shieh, D. E. and Ho, C. T. 2002. Antioxidant and free radical scavenging activities of edible mushrooms. *Food Lipids* 9(9): 35-43. 42.Fukushima, M., Ohashi, T., Fujiwara, Y., Sonoyama, K. and Nakano, M. 2001. Cholesterol-lowering effects of maitake (*Grifola frondosa*) fiber, shiitake (*Lentinus edodes*) fiber, and enokitake (*Flammulina velutipes*) fiber in rats. *Biology and Medicine* 226(8): 758-765. 43.Gloeckler, R., Ohsawa, I., Speck, D., Ledoux, C., Bernard, S., Zinsius, M., Villeval, D., Kisou, T., Kamogawa, K. and Lemoine, Y. 1990. Cloning and characterization of the *Bacillus sphaericus* genes controlling the bioconversion of pimelate into dethiobiotin. *Gene* 87(1): 63-70. 44.Kochnar, S. P. and Rossel, J. B. 1990. Detection, estimation and evaluation of autoxidants in food systems. Ch.2, in food antioxidant, B. J. F. Huson (Ed.), Elsevier Applied Science, London and New York pp: 67-70. 45.Goldsby, R. A., Kindt, T. J., Osborne, B. A. and Kuby, J. 2003. *Immunology* 5th ed. Freeman and Company New York. 46.Halliwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I. 1995. Free radicals and antioxidants in food and in vivo : what they do and how they work. *Critical Reviews Food Science and Nutrition* 35(1-2): 7-20. 47.Hartley, M. R. and Lord, J. M. 2004. Genetic of ribosome-inactivating proteins. *Medicinal Chemistry* 4(5): 487-92. 48.Hsieh, K. Y., Hsu, C. I., Lin, J. Y., Tsai, C. C. and Lin, R. H. 2003. Oral administration of an edible-mushroom-derived protein inhibits the development of food-allergic reactions in mice. *Clinical and Experimental Allergy:Journal of the British Society for Allergy and Clinical Immunology* 33(11): 1595-1602. 49.Hsu, H. C. , Hsu, C. I. , Lin, R. H. , Kao, C. L. and Lin, J. Y. 1997. Fip-vvo, a new fungal immunomodulatory protein isolated from *Volvariella volvacea*. *Biochemistry*: 557-565. 50.Ikekawa, T., Ikeda, Y., Yoshioka, Y., Nakanishi, K., Yokoyama, E. and Yamazaki, E. 1982. Studies on antitumor polysaccharides of *Flammulina velutipes* (Curt. ex Fr.) Sing.II. The structure of EA3 and further purification of EA5. *Pharmacobiodynamics* 5(8): 576-81. 51.Ishikawa, N. K., Yamaji, K., Tahara, S., Fukushi, Y. and Takahashi, K. 2000. Highly oxidized cuparene—type sesquiterpenes from a mycelial culture of *Flammulina velutipes*. *Phytochemistry* 54(5): 777-782. 52.Jeurink, P., Noguera, C., Savelkoul, H. and Wichers, H. 2008. Immunomodulatory capacity of fungal proteins on the cytokine production of human peripheral blood mononuclear cells. *International Immunopharmacology* 8(8): 1124-1133. 53.Ko, J. L., Hsu, C. I., Lin, R. H., Kao, C. L. and Lin, J. Y. 1995. A new fungal immunomodulatory protein, FIP-fve isolated from the edible mushroom, *Flammulina velutipes* and its complete amino acid sequence.*European Biochemical Societies Journal* 228(2): 244-249. 54.Ko, J. L., Hsu, C. I., Kao, C. L. and Lin, J. 1997. Molecular cloning and expression of a fungal immunomodulatory protein, FIP-fve, from *Flammulina velutipes*. *Formosan Medical Association* 96(7): 517-24. 55.Kraus, J., Blaschek, W., Schutz, M. and Franz, G.. 1992. Antitumor activity of cell wall beta-1,3/1,6-glucans from *Phytophthora* species. *Planta Medica* 58(1): 39-42. 56.Lee, C. L., Yang, X. and Wan, J. M. F. 2006. The culture duration affects the immunomodulatory and anticancer effect of polysaccharopeptide derived from *Coriolus versicolor*. *Enzyme and Microbial Technology* 38: 14-21. 57.Legdeur, M. C., Bontje, P. M., Ossenkoppele, G. J., Beelen, R. H., Van de, L. A. A., Broekhoven, M. G., Langenhuijsen, M. M., Thijsen, S. F., Hofstee, H. and Schuurhuis, G. J. 1996. The role of BCL-2 and bax protein in monocyte-mediated apoptosis in human leukemic cell lines. *Experimental Hematology* 24(13): 1530-1539. 58.Leung, M. Y. K., Fung, K. P. and Choy, Y. M. 1997. The isolation and characterization of an immunomodulatory and anti-tumor polysaccharide preparation from *Flammulina velutipes*. *Immunopharmacology* 35(3): 255-263. 59.Lin, J. Y., Wu, H. L. and Shi, G. Y. 1975. Toxicity of the cardiotoxic protein, flammutoxin, isolated from the edible mushroom *Flammulina velutipes*. *Toxicon* 13(5): 323-326. 60.Liu, Z., Wang, P. and Liu, Y. 1992. Studies on Bio-antioxidants. *Science in China, series B* 35(11): 1307-1314. 61.Liu, Z., Wang, L. and Liu, Y. 1991. Studies on Bio-antioxidants. *Science in China, series B* 34(7): 787-795. 62.Liu, Z., Han, Z. and Cheng, P. 1991. Studies on Bio-antioxidants. *Chin J Chemistry* 9(2): 144-151. 63.Mackerras, D. 1995. Antioxidants and health : fruits and vegetables or supplements. *Food Australia* 47(11): 1-24. 64.Matsuzaki, K., Sato, T., Enomoto, K., Yamamoto, I., Oshima, R., Hatanaka, K. I., Uryu, T., Kaku, H. and Sone, Y. Synthesis of water-soluble, branched polysaccharides having d-mannopyranose, d-arabinofuranose, or oligo-d-arabinofuranose side-chains and their antitumor activity. *Carbohydrate Research* 157(1): 171-182. 65.Ng, T. B., Chan, W. Y. and Yeung, H. W. 1992. Proteins with

abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants. *General pharmacology* 23(4): 579-90. 66.Ng, T. B. and Wang, H. 2004. Flammulin and velin: new ribosome inactivating polypeptides from the mushroom *Flammulina velutipes*. *Peptides* 25(6): 929-33. 67.Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition* 44: 307. 68.Paaventhana, P., Joseph, J. S., Seow, S. V., Vaday, S., Robinson, H., Chua, K. Y. and Kolatkar, P. R. 2003. A 1.7A Structure of Fve, a Member of the New Fungal Immunomodulatory Protein Family. *Journal of Molecular Biology* 332(2): 461-470. 69.Pang, X., Yao, W., Yang, X., Xie, C., Liu, D., Zhang, J. and Gao, X. 2007. Purification, characterization and biological activity on hepatocytes of a polysaccharide from *Flammulina velutipes* mycelium. *Carbohydrate Polymers* 70(3): 291-297. 70.Quang, D. N., Hashimoto, T. and Asakawa, Y. 2006. Inedible mushrooms: a good source of biologically active substances. *Chemical Record* 6(2): 79-99. 71.Rincon, M., Enslin, H., Raingeaud, J., Recht, M., Zapton, T., Su, M. S. S., Penix, L. A., Davis, J. R. and Flavell, A. 1998. Interferon-gamma expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway. *EMBO Journal* 17(10): 2817 – 2829. 72.Sakurai, N., Imaia, Y. and Komatsubara, S. 1995. Instability of the mutated biotin operon plasmid in a biotin-producing mutant of *Serratia Marcescens* *Biotechnology* 43(1): 11-19. 73.Seow, S. V., Kuo, I. C., Paaventhana, P., Kolatkar, P. R. and Chua, K. Y. 2003. Crystallization and preliminary X-ray crystallographic studies on the fungal immunomodulatory protein Fve from the golden needle mushroom (*Flammulina velutipes*). *Biological Crystallography* 59(8): 1487-1489. 74.Shamtsyan, M., Konusova, V., Maksimova, Y., Goloshchev, A., Panchenko, A., Simbirtsev, A., Petrishchev, N. and Denisova, N. 2004. Immunomodulating and anti-tumor action of extracts of several mushrooms. *Journal of Biotechnology* 113(1-3): 77-83. 75.Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Food Chemistry* 40(6): 945-948. 76.Slater, T. F. 1987. Free radicals and tissue injury: fact and fiction. *BrJ Cancer* 55(8): 5-10. 77.Smith, R. A. and Baglioni, C. 1987. The active form of tumor necrosis factor is a trimer. *Biological Chemistry* 262(15): 6951-6954. 78.Tomita, T., Mizumachi, Y., Chong, K., Ogawa, K., Konishi, N., Sugawara, T., Tomita, N., Dohmae, N., Hashimoto, Y. and Takio, K. 2004. Protein sequence analysis, cloning, and expression of flammutoxin, a pore-forming cytolysin from *Flammulina velutipes*. Maturation of dimeric precursor to monomeric active form by carboxyl-terminal truncation. *Biological chemistry* 279(52): 54161-54172. 79.Tsuda, M. 1979. Purification and Characterization of a Lectin from the Mushroom, *Flammulina Veltipes*. *Biochem* 86(5): 1463-1468. 80.Wang, H. and Ng, T. B. 2001. Isolation and characterization of velutin, a novel low-molecular-weight ribosome-inactivating protein from winter mushroom (*Flammulina velutipes*) fruiting bodies. *Life Sciences* 68(18): 2151-2158. 81.Wang, H. X. and Ng, T. B. 2000. Flammulin: A novel ribosome-inactivating protein from fruiting bodies of the winter mushroom *Flammulina velutipes*. *Cell Biology* 78(6): 699-702. 82.Wang, P. H., Hsu, C. I., Tang, S. C., Huang, Y. L., Lin, J. Y. and Ko, J. L. 2004. Fungal immunomodulatory protein from *Flammulina velutipes* induces interferon-gamma production through p38 mitogen-activated protein kinase signaling pathway. *Food Chemistry* 52(9): 2721-2725. 83.Wang, Y. J., Yao, S. J., Guan, Y. X., Wu, T. X. and Kennedy, J. F. 2005. A novel process for preparation of (1 → 3)-β-D-glucan sulphate by a heterogeneous reaction and its structural elucidation. *Carbohydrate Polymers* 59(1): 93-99. 84.Wasser, S. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology* 60(3): 258-274. 85.Wiley, J. 1999. *Current protocols in toxicology*. New York. 86.Williams, W. B., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* 28(1): 25-30. 87.Yang, J. H., Lin, H. C. and Mau, J. L. 2001. Non-volatile taste components of several commercial mushrooms. *Food Chemistry* 72(4): 465-471. 88.Yang, J. H., Lin, H. C. and Mau, J. L. 2002. Antioxidant properties of several commercial mushrooms. *Food Chemistry* 77(2): 229-235. 89.Yang, Q. Y. and Zhou, Y. F. 1993. A protein bound polysaccharide-PSP. In *Proceedings of PSP International Symposium* (Edited by Yang Q. Y. and Kwok C. Y.). Fundan University press. Shanghai, China 22-34. 90.Zeng, Q. 1990. The antitumor activity of *Flammulina velutipes* polysaccharide (FVP), edible fungi of China. *Szechuan Institute of Materia Medica* 10:2-19. 91.Zhang, M., Cui, S. W., Cheung, P. C. K. and Wang, Q. 2007. Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology* 18(1): 4-19.