

Inhibition on tyrosinase activity and melanoma cell growth by bovine colostrum hydrolysates / 吳秋慧 撰 .- 彰化縣大村鄉

吳秋慧、張基郁

E-mail: 350795@mail.dyu.edu.tw

ABSTRACT

In recent years, there is an increasing interest in finding natural tyrosinase inhibitors from animals, plants and microorganisms. The tyrosinase inhibitors should have broad applications, especially, in food, medicinal and cosmetics products in relation to hyperpigmentation. This research used the bovine colostrums collected on the first to fifth days postpartum and normal milk to prepare skimmed colostrums and skimmed normal milk, and used two different enzymes, Alcalase and α -Chymotrypsin, to hydrolyze the samples, followed by investigating the inhibition of the hydrolysates on tyrosinase activity, and finally selected day 2 colostrums and normal milk hydrolysates for cell experiments, including melanoma cell viability, tyrosinase activity and melanin content in melanoma cells. The results are as follows: 1. The inhibitory effect of the Alcalase hydrolysates on tyrosinase activity was more profound than that of the α -Chymotrypsin hydrolysates. At a concentration of 5 mg/mL day 2 colostrum hydrolysates had the highest inhibition rate, 23.99 %; at a concentration of 10 mg/mL day 2 colostrum hydrolysates also had the highest inhibition the rate, 32.96 %; at a concentration of 15 mg/mL both day 2 colostrum and normal milk hydrolysates had the inhibition rate of 37.48 and 35.59 %, respectively. Overall the day 2 colostrum hydrolysate obtained by Alcalase hydrolysis for 4 hrs had the highest inhibitory activity on tyrosinase. The inhibitory activity of normal milk hydrolysates on tyrosinase increased with increasing hydrolysis time. 2. In the results of melanoma cell viability the hydrolysates of day 2 colostrums and normal milk could decrease the cell viability, and the cell viability decreased with increasing the hydrolysate concentration. 3. In the results of tyrosinase activity in melanoma cells the inhibitory effect of the Alcalase hydrolysates on tyrosinase activity was more profound than that of the α -Chymotrypsin hydrolysates. The inhibition rate of the Alcalase hydrolysate of day 2 colostrums at a concentration of 7.5 mg/mL was 54.01%. 4. In the results of melanin content in cells, both Alcalase and α -Chymotrypsin hydrolysates had comparable ability to reduce the melanin content in cells, and both colostrum and normal milk hydrolysates also had a ability to reduce the cellular melanin content.

Keywords : bovine colostrums、hydrolysis、tyrosinase、melanoma cell (B16-F10)

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 ix 圖目錄 xiii 表目錄 xiv 1. 緒論 1 2. 文獻回顧 3 2.1 牛初乳 3 2.1.1 酪蛋白 3 2.1.2 乳清蛋白 4 2.1.3 常乳 4 2.1.4 牛初乳蛋白的研究與應用 4 2.2 酵素水解 6 2.2.1 水解方式及條件 6 2.2.2 水解種類及位置 7 2.2.3 水解與基質比例 7 2.2.4 水解蛋白的應用 8 2.3 皮膚簡介 9 2.3.1 皮膚的結構及功能 9 2.3.2 皮膚老化因素 11 2.4 黑色素細胞(Melanocytes) 11 2.4.1 黑色素細胞簡介 11 2.4.2 黑色素細胞生成 14 2.4.3 黑色素細胞刺激素 15 2.4.4 黑色素瘤 17 2.5 酪胺酸(Tyrosinase) 18 2.5.1 酪胺酸?簡介 18 2.5.2 酪胺酸?作用機制 20 2.5.3 胺酸?活性抑制 21 2.6 天然美白劑 23 2.6.1 植物天然美白劑 23 3. 材料與方法 24 3.1 實驗材料 24 3.1.1 原料 24 3.1.2 藥品 24 3.1.3 儀器設備 25 3.1.4 蛋白質分解酵素 26 3.1.5 細胞株 26 3.2 實驗架構 28 3.3 牛初乳及常乳脫脂製備 29 3.4 基本組成成分分析 29 3.5 酵素水解 31 3.5.1 Alcalase 酵素水解 31 3.5.2 α -Chymotrypsin 酵素水解 31 3.5.3 氨基態氮測定 32 3.6 聚丙烯醯胺凝膠電泳 33 3.6.1 膠體配置 33 3.6.2 實驗方法 34 3.7 酪胺酸?抑制法 37 3.7.1 酪胺酸?抑制測定 37 3.8 細胞培養 38 3.8.1 細胞培養液 38 3.8.2 細胞冷凍保存 38 3.8.3 細胞活化 38 3.8.4 細胞繼代 39 3.8.5 細胞增生試驗 39 3.8.6 酪胺酸?活性分析 40 3.8.7 黑色素含量測定 41 3.9 統計分析 42 4. 結果與討論 43 4.1 牛初乳及常乳之基本組成成分分析 43 4.2 牛初乳及常乳蛋白質之電泳分析 43 4.3 牛初乳及常乳蛋白質的水解 46 4.3.1 水解物之收率 49 4.4 牛初乳及常乳水解物對酪胺酸?活性之抑制 51 4.5 牛初乳及常乳蛋白水解物對黑色素細胞存活率之影響 54 4.6 牛初乳及常乳蛋白水解物對黑色素細胞內酪胺酸?活性分析 57 4.7 牛初乳及常乳蛋白水解物對黑色素細胞內黑色素含量分析 61 5. 結論 66 參考文獻 68 圖目錄 圖2.1 皮膚的構造 10 圖2.2 黑色素合成路徑 16 圖2.3 黑色素瘤形成 19 圖2.4 熊果素結構圖 22 圖3.1 實驗設計流程圖 28 圖4.1 不同天數牛初乳及常乳之電泳 45 圖4.2 以Alcalase 水解牛初乳及常乳之水解率 47 圖4.3 以 α -Chymotrypsin 牛初乳及常乳之水解率 48 圖4.4 不同濃度之熊果素對酪胺酸?抑制率 52 圖4.5 以Alcalase 水解第二天牛初乳和以 α -Chymotrypsin 水解常乳之所得水解物對酪胺酸?活性抑制率 53 圖4.6 第二天牛初乳及常乳之Alcalase 水解物對細胞內酪胺酸?活性之抑制 58 圖4.7 第二天牛初乳及常乳之 α -Chymotrypsin 水解物對細胞內酪胺酸?活性之抑制 60 圖4.8 第二天牛初乳及常乳之Alcalase 水解物對細胞內黑色素含量之影響 62 圖4.9 第二天牛初乳及常乳之 α -Chymotrypsin 水解物對細胞內黑色素含量之影響 64 表目錄 表2.1 牛乳中的成分 5 表2.2 自然老化與光老化區分 12 表2.3 紫外線對人體的影響

REFERENCES

- 1.石原和之。2001。紫外線與皮膚癌。林鬱文化事業有限公司。臺北。
- 2.江淑華，謝淳仁，張基郁。2005。酵素水解條件對牛初乳蛋白水解物抗氧化性之影響。中華生質能源學會誌，24(1,2): 9-18。
- 3.服部淳彥。1997。褪黑激素的功效。青春出版社。臺北。
- 4.林慶文。1993。乳品加工學。華香園出版社。臺北。
- 5.許惠悆。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。
- 6.郭智宏。2001。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研究所碩士論文。基隆。
- 7.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學系研究所碩士論文。彰化。
- 8.楊正獲。1983。剩餘初乳利用性之研究。國立中興大學畜牧研究所碩士論文。台中。
- 9.楊佳璋，陳宜嫻，黃宜純，黃淑桂，傅如嶽，溫慧萍，鄭智交。2004。皮膚生理學(二版)。華格那企業有限公司。台中。
- 10.鄭名凡。1999。蛋白水解物的功能與應用。食品資訊。160:49-54。
- 11.鄭靜桂。1997。蛋白質水解與水解液之利用。食品工業，29(5): 10-17。
- 12.Al-Mashikhi S. A. and Nakai S. 1987. Isolation of bovine immunoglobulins and lactoferrin from whey protein by gel filtration techniques. *Journal of Dairy Science* 70: 2486-2492.
- 13.Amiota J., Germain L., Turgeon S., Lemaya M. and Francois C. O. S. 2004. Peptides from milk protein hydrolysates to improve the growth of human keratinocytes in culture. *International Dairy Journal* 14: 619-626.
- 14.AOAC. 1984. Official Methods of Analysis of the AOAC, 16th ed. Association of Official Analytical Chemists. Washington, DC.
- 15.Baurin N., Arnoult E., Scior T., Do Q. T. and Bernard P. 2002. Preliminary screening of some tropical plants for anti-tyrosinase activity. *Journal of Ethnopharmacology* 82: 155-158.
- 16.Brun J. M. and Dalgleish D. G. 1999. Some effects of heat on the competitive adsorption of caseins and whey proteins in oil-in-water emulsions. *International Dairy Journal* 9: 323-327.
- 17.Bryant C. M. and McClements D. J. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trend in Food Science and Technology* 9: 143-151.
- 18.Chang C. Y., Wu K. C., Chiang S. H. 2007. Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. *Food Chemistry* 100: 1537-1543.
- 19.Chen C. Y., Kuo P. L., Chen Y. H., Huang J. C., Ho M. L., Lin R. J., Chan J. S. and Wang H. M. 2010. Tyrosinase inhibition, free radical scavenging, antimicroorganism and anticancer proliferation activities of *Sapindus mukorossi* extracts. *Journal of the Taiwan Institute of Chemical Engineers* 41: 129-135.
- 20.Chiari M. E., Joray M. B., Ruiz G., Palacios S. M., Carpinella M. C. 2010. Tyrosinase inhibitory activity of native plants from central Argentina: Isolation of an active principle from *Lithrea molleoides*. *Food Chemistry* 120: 10-14.
- 21.Chicon R., Belloque J., Alonso E. and Lopez-Fandino R. 2009. Antibody binding and functional properties of whey protein hydrolysates obtained under high pressure. *Food Hydrocolloids* 23: 593-599.
- 22.Cumby N., Zhong Y., Naczek M. and Shahidi F. 2008. Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food Chemistry* 109: 144-148.
- 23.Fujimoto A., Shingai Y., Nakamura M., Maekawa T., Sone Y. and Masud T. 2010. A novel ring-expanded product with enhanced tyrosinase inhibitory activity from classical Fe-catalyzed oxidation of rosmarinic acid, a potent antioxidative Lamiaceae polyphenol. *Bioorganic & Medicinal Chemistry Letters* 20: 7393-7396.
- 24.Gallaher J. J., Hollender R., Peterson D. G., Roberts R. F. and Coupland J. N. 2005. Effect of composition and antioxidants on the oxidative stability of fluid milk supplemented with an algae oil emulsion. *International Dairy Journal* 15: 333-341.
- 25.Gildberg A. 1993. Enzymatic processing of marine raw materials. *Process Biochemistry* 28: 1-15.
- 26.Gill H. S. and Cross M. L. 2000. Anticancer properties of bovine milk. *British Journal of Nutrition* 84: S161-S166.
- 27.Hekmat S. and McMahon D. J. 1998. Distribution of iron between caseins and whey proteins in acidified milk. *Lebensmittel-Wissenschaft und-Technologie* 31: 632-638.
- 28.Ha Y. M., Kim J. A., Park Y. J., Park D., Kim J. M., Chung K. W., Lee E. K., Park J. Y., Lee J. Y., Lee H. J., Yoon J. H., Moon H. R., Chung H. Y. 2011. Analogs of 5-(substituted benzylidene)hydantoin as inhibitors of tyrosinase and melanin formation. *Biochimica et Biophysica Acta* 1810: 612-619.
- 29.Hekmat S. and McMahon D. J. 1998. Distribution of iron between caseins and whey proteins in acidified milk. *Lebensmittel-Wissenschaft und-Technologie* 31: 632-638.
- 30.Hooijdonk A. C. M., Kussendrager K. D. and Strijns J. M. 2000. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrums involved in non-specific defence. *British Journal of Nutrition* 84: S127-S134.
- 31.Im S. J., Kim K. N., Yun Y. G., Lee J. C., Mun Y. J., Kim J. H. and Woo W. H. 2003. Effect of radix ginseng and radix trichosanthis on the melanogenesis. *Biological and Pharmaceutical Bulletin* 26: 849-853.
- 32.Kentaro T. N., Tomoko H., Takeshi O. and Takao T. 2007. Modulating effects of a novel skin-lightening agent, a-lipoic acid derivative, on melanin production by the formation of DOPA conjugate products. *Bioorganic & Medicinal Chemistry* 15: 1967-1975.
- 33.Kong X. Z., Guo M. M., Hua Y. F., Cao D. and Zhang C. M. 2008. Enzymatic preparation of immunomodulating hydrolysates from soy proteins. *Bioresource Technology* 99: 8873-8879.
- 34.Lahl W. J. and Brum S. T. 1994. Enzymatic production of protein hydrolysates for food use. *Food Technology* 48(10): 68-71.
- 35.Lee C. W., Son E. M., Kim H. S., Xu P., Batmunkh T., Lee B. J. and Koo K. A. 2007. Synthetic tyrosyl gallate derivatives as potent melanin formation inhibitors. *Bioorganic & Medicinal Chemistry Letters* 17: 5462-5464.
- 36.Lee J., Kim Y. S. and Park D. 2007. Rosmarinic acid induces melanogenesis through protein kinase A activation signaling. *Biochemical pharmacology* 74: 960-968.
- 37.Lin Y. P., Hsu F. L., Chen C. S., Chern J. W. and Lee M. H. 2007. Constituents from the Formosan apple reduce tyrosinase activity in human epidermal melanocytes. *Phytochemistry* 68: 1189-1199.
- 38.Lindmark-Mansson H. and Akesson B. 2000. Antioxidative factors in milk. *British Journal of Nutrition* 84: S103-S110.
- 39.Loa Y. H., Linb R. D., Lind Y. P., Liue Y. L. and Lee M. H. 2009. Active constituents from *Sophora japonica* exhibiting cellular tyrosinase inhibition in human epidermal melanocytes. *Journal of Ethnopharmacology* 124: 625-629.
- 40.Mackie I. M. 1982. Fish protein hydrolysates. *Process Biochemistry*. 31: 26-31.
- 41.Mahendra Kumar C., Sathisha U. V., Shylaja D., Rao A. A.

G. and Sridevi S. A. 2011. Interaction of sesamol (3,4-methylenedioxyphenol) with tyrosinase and its effect on melanin synthesis. *Biochimie* 93: 562-569.

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

42. McCord C. P. and Allen F. P. 1997. Evidences associating pineal gland function with alternation in pigmentation. *Journal of Experimental Zoology* 23: 207-227.

43. Momtaz S., Mapunya B. M., Houghton P. J., Edgerly C., Hussein A., Naidoo S. and Lall N. 2008. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. *Journal of Ethnopharmacology* 119: 507-512.

44. Mun Y. J., Lee S. W., Jeong H. W., Lee K. G., Kim J. H. and Woo W. H. 2004. Inhibitory effect of miconazole in melanogenesis. *Biological and Pharmaceutical Bulletin* 27: 806-809.

45. Nerya O., Musa R., Khatib S., Tamir S. and Vaya J. 2004. Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. *Phytochemistry* 65: 1389-1395.

46. No J. K., Soung D. Y., Kim Y. J., Shim K. H., Jun Y. S., Rhee S. H., Yokozawa T. and Chung H. Y. 1999. Inhibition of tyrosinase by green tea components. *Pharmacology letters* 65(21): 241-246.

47. Noh J. M., Kwak S. Y., Seo H. S., Seo J. H., Kim B. G. and Lee Y. S. Kojic acid – amino acid conjugates as tyrosinase inhibitors. 2009. *Bioorganic and Medicinal Chemistry Letters* 19: 5586-5589.

48. Ozeki H., Ito S., Wakamatsu K. and Ishiguro I. 1997. Chemical characterization of pheomelanogenesis starting from dihydroxyphenylalanine or tyrosine and cysteine. Effects of tyrosinase and cysteine concentrations and reaction time. *Biochimica et Biophysica Acta* 1336: 539-548.

49. Pena-Ramosa E. A. and Xiong Y. L. 2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science* 64: 259-263.

50. Peng X., Kong B., Xia X., Liu Q. 2010. Reducing and radical-scavenging activities of whey protein hydrolysates prepared with Alcalase. *International Dairy Journal* 20: 360-365.

51. Rout S. and Banerjee R. 2007. Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*. *Bioresource Technology* 98: 3159-3163.

52. Satue-Gracia M. T., Frankel E. N., Rangavajhala N. and German J. B. 2000. Lactoferrin in infant formulas: effect on oxidation. *Journal Agriculture and Food Chemistry* 48: 4984-4990.

53. Thapa B. R. 2005. Health factors in colostrums. *Indian Journal of Pediatrics* 72 (7): 579-581.

54. Too J. R. and Tsai M. F. 1999. Functional properties of hydrolysates from porcine blood cells. *Food Science* 26: 36-46.

55. Varnam H. and Sutherland P. 1994. Milk and milk products. Chapman and Hall, London.

56. Wang K. H., Lin R. D., Hsu F. L., Huange Y. H., Chang H. C., Huang C. Y. and Lee M. H. 2006. Cosmetic applications of selected traditional Chinese herbal medicines. *Journal of Ethnopharmacology* 106: 353-359.

57. Wei Y., Rihui C., Huan W., Qin Y., Binhua Z., Yiqian W., Lin A. and Huacan S. 2008. Synthesis and biological evaluation of heligid analogues as mushroom tyrosinase inhibitors. *Bioorganic & Medicinal Chemistry Letters* 18: 6490-6493.

58. Wu J. J., Lin J. C., Wang C. H., Jong T. T., Yang H. L., Hsu S. L. and Chang C. J. 2009. Extraction of antioxidative compounds from wine lees using supercritical fluids and associated anti-tyrosinase activity. *The Journal of Supercritical Fluids* 50: 33-41.

59. Ye Y., Chou G. X., Mu D. D., Wang H., Chu J. H., Leung A. K. M., Fong W. f., Yu Z. L. 2010. Screening of Chinese herbal medicines for antityrosinase activity in a cell free system and B16 cells. *Journal of Ethnopharmacology* 129: 387-390.

60. Yi W., Wu X., Cao R., Song H. and Ma L. 2009. Biological evaluations of novel vitamin C esters as mushroom tyrosinase inhibitors and antioxidants. *Food Chemistry* 117: 381-386.

61. Zhu K., Zhou H. and Qian H. 2006. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. *Process Biochemistry* 41: 1296-1302.