

牛初乳水解物對酪胺酸?活性和黑色素細胞生長之抑制作用 = Inhibition on tyrosinase activity and melanoma cell growth by bovine colostrum hydrolysate

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摘要

近年來許多研究嘗試從動、植物與微生物中，尋求可以有效抑制酪胺酸?之活性物質，並應用於食品、醫藥以及化妝品等相關產業。本研究以第1至第5天牛初乳與常乳作為材料，製備成脫脂牛初乳及常乳後，利用兩種不同酵素進行水解(Alcalase 和 -Chymotrypsin)，接著進行酪胺酸?活性抑制，最後以第2天牛初乳及常乳水解物進行黑色素細胞試驗，包含細胞存活率、細胞內酪胺酸?活性與黑色素含量的探討。研究結果顯示：1. 水解物在酪胺酸?活性抑制結果方面，Alcalase 水解物抑制效果優於 -Chymotrypsin水解物，在濃度5 mg/mL時，以第2天牛初乳效果最好，其抑制率為23.99 %；在濃度10 mg/mL時，以第2天牛初乳效果最好，其抑制率為32.96 %；在濃度15 mg/mL時，以第2天牛初乳與常乳水解物其抑制率分別為37.48 與35.59 %。整體而言，以第2天牛初乳以Alcalase 水解4小時所得水解物對酪胺酸?活性抑制效果最佳，而常乳水解物之抑制酪胺酸?活性則隨著水解的時間增加而效果變大。2. 水解物在細胞存活率結果方面，第2天牛初乳及常乳水解物對黑色素細胞有降低其存活率之趨勢，且隨樣品濃度之增加而存活率有下降之趨勢。3. 水解物對黑色素細胞內酪胺酸?活性之抑制結果方面，Alcalase 水解物之抑制效果優於 -Chymotrypsin水解物。第2天牛初乳水解物在濃度為7.5 mg/mL時其抑制率最高，其值為54.01 %。4. 水解物對細胞內黑色素含量之影響結果，Alcalase 與 -Chymotrypsin 兩種酵素水解物對細胞內的黑色素含量之降低能力相當，且初乳與常乳水解物皆有降低細胞內黑色素含量的效果。

關鍵詞：牛初乳、酵素水解、酪胺酸?、黑色素細胞(B16-F10)

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參考文獻

- 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。退黑激素的功效。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。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.陳志璋。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 immuneoglobulins and lactoferrin from whey protein by gel filtrateion techniques. *Journal of Dairy Science* 70: 2486-2492. 13.Amiota J., Germainb L., Turgeona 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., Naczk 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.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. 28.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. 29.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. 30.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. 31.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. 32.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. 33.Lahl W. J. and Brum S. T. 1994. Enzymatic production of protein hydrolysates for food use. *Food Technology* 48(10): 68-71. 34.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. 35.Lee J., Kim Y. S. and Park D. 2007. Rosmarinic acid induces melanogenesis through protein kinase A activation signaling. *Biochemical pharmacology* 74: 960-968. 36.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. 37.Lindmark-Mansson H. and Akesson B. 2000. Antioxidative factors in milk. *British Journal of Nutrition* 84: S103-S110. 38.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. 39.Mackie I. M. 1982. Fish protein hydrolysates. *Process Biochemistry*. 31: 26-31. 40.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.Manlry C. H. and Ahmed 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., Rangavajhyala 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 helicid 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.