

牛初乳乳清水解物對LDL及細胞DNA氧化性傷害之抑制研究

王鏗葦、張基郁、江淑華

E-mail: 9707228@mail.dyu.edu.tw

摘要

本研究以母牛分娩後第二天之牛初乳為原料，將其分離出乳清後，利用alcalase和flavourzyme酵素進行兩階段水解，產生乳清水解物，再以10 kDa超過濾膜過濾乳清水解物，取得乳清水解物之劃分物。本實驗以乳清水解物、10 kDa以上劃分物及10 kDa以下劃分物為分析樣品，分別對三者進行DNA (deoxynucleic acid)氧化傷害之抗氧化性及抑制LDL氧化進行探討。研究結果如下：1.乳清水解物及其劃分物對Fenton reaction 誘導生物分子氧化傷害之影響：(1)Fe³⁺-EDTA/H₂O₂/Asc誘導去氧核糖之氧化傷害：乳清水解物及其劃分物均具有抑制去氧核糖氧化傷害之效果。乳清水解物、10 kDa以上劃分物及10 kDa以下劃分物於1 mg/mL濃度時，各別可抑制41.27、43.17及43.09 %之氧化傷害。(2)Fenton reaction 誘導DNA 單股斷裂：乳清水解物及10 kDa以上劃分物於各實驗濃度下並不會促進DNA 單股斷裂，但是10 kDa以下劃分物於10 mg/mL濃度時卻會促進DNA 單股斷裂。(3)Fenton reaction 誘導2'-dG氧化生成8-OH-2'-dG：乳清水解物及其劃分物均具有降低8-OH-2'-dG生成量之功效。三者之抑制能力依序為10 kDa以上劃分物>10 kDa以下劃分物>乳清水解物。2.乳清水解物及其劃分物對bleomycin-Fe³⁺誘導DNA傷害之影響：乳清水解物及其劃分物並沒有明顯的促氧化效果。此結果顯示乳清水解物及其劃分物並不會提高bleomycin-Fe³⁺所誘導之DNA傷害。3.乳清水解物及其劃分物對銅離子誘導低密度脂蛋白氧化之影響：(1)銅離子誘導低密度脂蛋白氧化形成TBARS：乳清水解物及其劃分物均具有減少TBARS 形成之作用。在濃度0.001及0.01 mg/mL時，三者之抑制能力依序為10 kDa以上劃分物>10 kDa以下劃分物>乳清水解物。在濃度0.1 mg/mL時，三者之抑制能力依序為10 kDa以上劃分物>乳清水解物>10 kDa以下劃分物，10 kDa以下劃分物於此濃度會造成促氧化及低密度脂蛋白氧化之效果。(2)銅離子誘導低密度脂蛋白氧化形成共軛雙烯：在只添加銅離子及低密度脂蛋白之控制組方面，其延滯時間為90分鐘。而添加乳清水解物劃分物則具有較佳抑制能力，在1.0 及10 mg/mL濃度下，其延滯時間為270分鐘。關鍵字：牛初乳、乳清蛋白、乳清水解產物、抗氧化性、氧化傷害。

關鍵詞：牛初乳;乳清蛋白;乳清水解產物;抗氧化性;氧化傷害

目錄

封面內頁 簽名頁 授權書 中文摘要 英文摘要 誌謝 目錄 圖目錄 表目錄 1. 前言 2. 文獻回顧 2.1 初乳之簡介 2.1.1 牛乳的蛋白質組成 2.2 乳清蛋白及其組成成分之生物特性 2.2.1 -乳球蛋白(-lactoglobulin) 2.2.2 -乳白蛋白(-lactalbumin) 2.2.3 乳鐵蛋白(lactoferrin) 2.2.4 免疫球蛋白(immunoglobulins) 2.2.5 牛血清白蛋白(bovine serum albumin) 2.2.6 乳過氧化酶(lactoperoxidase) 2.3 蛋白質水解的特性與運用 2.3.1 水解方式及條件 2.3.2 蛋白質水解酵素 2.3.3 酵素水解之影響因子 2.3.4 乳清水解物之機能性 2.4 氧化作用 2.4.1 自由基 2.4.2 氧化壓力 2.4.3 費頓反應(Fenton reaction) 2.5 DNA氧化傷害 2.5.1 8-氫氧-2'-去氧鳥糞嘌呤核糖 2.5.2 Bleomycin 2.6 人類低密度脂蛋白(Low density lipoprotein, LDL) 2.6.1 氧化修飾低密度脂蛋白(OxLDL) 2.6.2 丙二醛(Malondialdehyde)及TBA反應物 2.7 抗氧化物質與其作用機制 3. 材料與方法 3.1 實驗材料 3.1.1 原料 3.1.2 藥品 3.1.3 儀器設備 3.1.4 蛋白質分解酵素 3.2 實驗方法與分析項目 3.2.1 本實驗流程 3.2.2 乳清水解物之製備 3.2.3 以超過濾法進行水解物之劃分 3.2.4 乳清水解物對生物分子氧化傷害之抗氧化性 3.2.5 乳清水解物及其劃分物對生物分子保護及降低氧化傷害之研究 3.2.6 初乳乳清於抑制LDL氧化修飾之探討 3.2.5 統計分析 4. 結果與討論 4.1 兩階段酵素水解乳清蛋白之水解率 4.2 對生物分子氧化傷害之抗氧化性 4.2.1 乳清水解物及其不同分子量超過濾劃分物對Fenton reaction誘導的deoxyribose氧化傷害之影響 4.2.2 乳清水解物及其不同分子量超過濾劃分物對Fenton reaction誘導的DNA單股斷裂之影響 4.2.3 乳清水解物及其不同分子量超過濾劃分物對Fenton reaction誘導2'-deoxyguanosine (2'-dG)氧化形成8-hydroxy-2'-deoxy-guanosine (8-OH-2'-dG)之影響 4.2.4 乳清水解物及其不同分子量超過濾劃分物對bleomycin-Fe³⁺誘導DNA氧化傷害之影響 4.2.5 乳清水解物及其劃分物對生物分子保護及降低氧化傷害之研究 4.3 乳清水解物及其不同分子量超過濾劃分物對銅離子誘導LDL氧化修飾之影響 4.3.1 乳清水解物及其不同分子量超過濾劃分物對Cu²⁺誘導LDL氧化生成TBARS之影響 4.3.2 乳清水解物及其不同分子量超過濾劃分物對Cu²⁺誘導LDL氧化生成共軛雙烯(conjugated diene, CD)之影響 5. 結論 參考文獻

參考文獻

1.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。 2.吳則雄。1980。認識牛的初乳。乳業。98/99: 9-14。 3.江淑華。2005。牛初乳及其酵素水解物之抗氧化性與其蛋白質組成之相關性研究。大葉大學生物產業科技學系研究所

博士論文。彰化。4.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。5.陳志璋。2007。微膠囊化牛初乳蛋白質水解物之抗氧化安定性。大葉大學生物產業科技學系碩士論文。彰化。6.陳瑩龍。2002。啤酒廢棄酵母有用成份回收方法之探討。大同大學生物工程系碩士論文。7.楊詠翔。1999。食品中抗高血壓胜?的發展現況。食品工業。31: 9-18。8.劉毓蕙。2004。水解蛋白的特性及運用。食品工業。36: 19-24。9.鄭名凡。1999。蛋白質水解物的功能與應用。食品資訊。160: 49-54。10.盧建宇、陳全木、林慶文。2003。乳鐵蛋白素之抗菌表現及其應用。食品工業。35: 61-65。11.Almeida, I. F., Fernandes, E., Lima, J. L. F. C., Costa P. C. and Bahia, M. F. 2008. Protective effect of *Castanea sativa* and *Quercus robur* leaf extracts against oxygen and nitrogen reactive species. *Journal of Photochemistry and Photobiology B: Biology* 91: 87 – 95。12.Amatore, C., Arbault, S., Ferreira, D. C. M., Tapsoba, I. and Verchier, Y. 2008. Vitamin C stimulates or attenuates reactive oxygen and nitrogen species (ROS, RNS) production depending on cell state: Quantitative amperometric measurements of oxidative bursts at PLB-985 and RAW 264.7 cells at the single cell level. *Journal of Electroanalytical Chemistry* 615: 34-44。13.Anandharamakrishnan, C., Rielly, C. D. and Stapley, A. G. F. 2008. Loss of solubility of α -lactalbumin and β -lactoglobulin during the spray drying of whey proteins. *LWT - Food Science and Technology* 41: 270-277。14.Andersen, J. H., Osbakk, S. A., Vorland, L. H., Traavik, T., & Gutteberg, T. J. 2001. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. *Antiviral Research* 51: 141 – 149。15.Andersen, J. H., Jenssen, H. and Gutteberg, T. J. 2003. Lactoferrin and lactoferricin inhibit Herpes simplex 1 and 2 infection and exhibit synergy when combined with acyclovir. *Antiviral Research* 58: 209 – 215。16.Andersen, J. H., Jenssen, H., Sandvik, K. and Gutteberg, T. J. 2004. Anti-HSV activity of lactoferrin and lactoferricin is dependent on the presence of heparan sulphate at the cell surface. *Journal of Medical Virology* 74: 262 – 271。17.Anonymous. 1998. *Alcalase? Food Grade. B 318b-GB 2000*. Bagsvaerd. Denmark: Novo Industry A/S。18.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。19.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。20.Aslan, M., Horoz, M., Kocycigit, A., Ozgonu", 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。21.Berkhout, B., Van Wamel, J. L., Beljaars, L., Meijer, D. K., Visser, S. and Floris, R. 2002. Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. *Antiviral Research* 55: 341 – 355。22.Borek, C. 2004. Dietary antioxidants and human cancer. *Integrative Cancer Therapies* 3: 333 – 341。23.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。24.Bramanti, E., Sortino, C. and Raspi, G. 2002. New chromatographic method for separation and determination of denatured α 1-, α 2-, β - and κ -caseins by hydrophobic interaction chromatography. *Journal of Chromatography A* 958: 157 – 166。25.Brock, J. 1995. Lactoferrin: a multifunctional immunoregulatory protein? *Immunology Today* 16: 417-419。26.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. *Trends in Food Science & Technology* 9: 143 – 151。27.Chatterton, D. E.W., Smithers, G., Roupas, P. and Brodtkorb, A. 2006. Bioactivity of α -lactoglobulin and β -lactalbumin- technological implications for processing. *International Dairy Journal* 16: 1229 – 1240。28.Cheung, H. S. and Chushman, D. W. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology* 20: 1637-1648。29.Clement, A. 2000. Enzymatic protein hydrolysates in human nutrition. *Trends in Food Science and Technology* 11: 254-262。30.Costa, E. L. D., Gontijo, J. A. D. R. and Netto, F. M. 2007. Effect of heat and enzymatic treatment on the antihypertensive activity of whey protein hydrolysates. *International Dairy Journal* 17: 632 – 640。31.Di Biase, A. M., Pietrantonio, A., Tinari, A., Siciliano, R., Valenti, P. and Antonini, G. 2003. Heparin-interacting sites of bovine lactoferrin are involved in anti-adenovirus activity. *Journal of Medical Virology* 69: 495 – 502。32.Donnely, J. L., Decker, E. A. and McClements, D. J. 1998. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. *Journal of Food Science* 63: 997-1000。33.Erdem, G., O"ner, C., O"nal, A. M., Kisaku"rek, D. and O"gu"s, A. 1994. Free radical mediated interaction of ascorbic acid and ascorbate/Cu (II) with viral and plasmid DNAs. *Journal of Biosciences* 19: 9-17。34.Esterbauer, H., Gebicki, J., Puhl, H. and Ju"rgens, G. 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine* 13: 341-390。35.Filippou, D., Papadopoulou, 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。36.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 & Medicine* 7: 597 – 601。37.Foley, J. A. and Otterby, D. E. 1978. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A Review. *Journal of Dairy Science* 61:1033-1061。38.Fujita, H., Yamagami, T. and Ohshima, K. 2001. Effects of an ACE inhibitory agent, katsuobushi oligopeptide, in the spontaneously hypertensive rat and in borderline and mildly hypertensive subjects. *Nutrition Research* 21: 1149 – 1158。39.Gallagher, 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。40.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。41.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。42.Gildberg, A. 1993. Enzymatic processing of marine raw materials. *Process Biochemistry* 28: 1-15。43.Gliszczyńska-Swig"o, A. 2006. Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing

antioxidant power) assays. *Food Chemistry* 96: 131 – 136. 44.Groff, J. L. and Gropper, S. S. 1999. *Advanced nutrition and human metabolism* (3rd ed). Belmont, Wadsworth/Thomson Learning. 45.Harauma, A., Murayama, T., Ikeyama, K., Sano, H., Arai, H., Takano, R., Kita, T., Hara, S., Kamei, K. and Yokode, M. 2007. Mulberry leaf powder prevents atherosclerosis in apolipoprotein E-deficient mice. *Biochemical and Biophysical Research Communications* 358: 751 – 756. 46.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. 47.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. 48.Ikeda, M., Nozaki, A., Sugiyama, K., Tanaka, T., Naganuma, A. and Tanaka, K. 2000. Characterization of antiviral activity of lactoferrin against hepatitis C virus infection in human cultured cells. *Virus Research* 66: 51 – 63. 49.Iyer, S. and Lonnerdal, B. 1993. Lactoferrin, lactoferrin receptors and iron metabolism. *European Journal of Clinical Nutrition* 47: 232-241. 50.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. 51.Kleinvel, H. A., Hak-Lemmers, H. L., Stalenhoef, 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. 52.Kobayashi, S., Uead, 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. 53.Lahl, W. J. and Braun, S. D. 1994. Enzymatic production of protein hydrolysates for food use. *Food Technology* 48: 68-71. 54.Levay, P. F. and Viljoen, M. 1995. Lactoferrin: A general review. *Haematologica* 80: 252-267. 55.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. 56.Lindmark-Mansson, H. and Akesson, B. 2000. Antioxidative factors in milk. *British Journal of Nutrition* 84: S103-S110. 57.Madureira, A. R., Pereira, C. I., Gomes, A. M. P., Pintado, M. E. and Malcata, F. X. 2007. Bovine whey proteins – Overview on their main biological properties. *Food Research International* 40: 1197 – 1211. 58.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. 59.Mahmud, R., Matin, M. A. and Otani, H. 2004. Mitogenic effect of bovine α -lactoglobulin and its proteolytic digests on mouse spleen resting cells. *Pakistan Journal of Biological Sciences* 7: 2045-2050. 60.Mantry, C. H. and Ahmed, S. 1995. The development of process flavors. *Trends in Food Science and Technology* 6: 46-51. 61.Mercier, A., Gauthier, S. F. and Fliss, I. 2004. Immunomodulating effects of whey proteins and their enzymatic digests. *International Dairy Journal* 14: 175 – 183. 62.Moore, S. A., Anderson, B. F., Groom, C. R., Haridas, M. and Baker, E. N., 1997. Three-dimensional structure of diferric bovine lactoferrin at 2.8 Å resolution. *Journal of Molecular Biology* 274: 222-236. 63.Moskovitz, J., Yim, M. B. and Chock P. B. 2002. Free radicals and disease. *Archives of Biochemistry and Biophysics* 397: 354-359. 64.Nagaoka, S., Futamura, Y., Miwa, K., Awano, T., Yamauchi, K., Kanamaru, Y., Tadashi, K. and Kuwata, T. 2001. Identification of novel hypocholesterolemic peptides derived from bovine milk α -lactoglobulin. *Biochemical and Biophysical Research Communications* 281: 11 – 17. 65.Nurminen, M. L., Sipola, M., Kaarto, H., Pihlanto-Leppä "la", A., Piilola, K., Korpela, R., Tossavainen, O., Korhonen, H. and Vapaatalo, H. 2000. α -Lactophin lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sciences* 66: 1535 – 1543. 66.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. 67.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. 68.Pen-a-Ramos, E. A. and Xiong, Y. L. 2001. Antioxidative activity of whey protein hydrolysates in a liposomal system. *Journal of Dairy Science* 84: 2577 – 2583. 69.Pen-a-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.SAS Institute, Inc.: SAS User 's guide: Statistics version 5th ed. SAS Inst. Cary, NC, U.S.A. 1985. 72.Sasaki, A., McClements, D. J. and Decker, E. A. 2000. Antioxidant activity of whey in a salmon oil emulsion. *Journal of Food Science* 65: 1325-1329. 73.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. 74.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. 75.Siciliano, R., Rega, B., Marchetti, M., Seganti, L., Antonini, G. and Valenti, P. 1999. Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. *Biochemical and Biophysical Research Communications* 264: 19 – 23. 76.Sipola, M., Finckenberg, P., Vapaatalo, H., Pihlanto-Leppä "la", A., Korhonen, H., Korpela, R. and Nurminen, M. L. 2002. α -Lactophin and β -lactophin improve arterial function in spontaneously hypertensive rats. *Life Sciences* 71: 1245 – 1253. 77.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. 78.Solomons, N. W. 2002. Modulation of the immune system and the response against pathogens with bovine colostrums concentrates. *European Journal of Clinical Nutrition* 56: 24-28. 79.Stejjns, J. M. and Hooijdonk, A. C. M. 2000. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. *British Journal of Nutrition* 1: 1-17. 80.Sternhagen, L. G. and Allen, J. C. 2001. Growth rates of a human colon adenocarcinoma cell line are regulated by the milk protein α -lactalbumin. *Advances in Experimental Medicine and Biology* 501: 115 – 120. 81.Suji, G. and Sivakami, S. 2008. Malondialdehyde, a

lipid-derived aldehyde alters the reactivity of Cys34 and the esterase activity of serum albumin. *Toxicology in Vitro* 22: 618-624. 82. Superti, F., Siciliano, R., Rega, B., Giansanti, F., Valenti, P. and Antonini, G. 2001. Involvement of bovine lactoferrin metal saturation, sialic acid and protein fragments in the inhibition of rotavirus infection. *Biochimica et Biophysica Acta* 1528: 107 – 115. 83. Tamime, A. Y. and Robinson, K. 1988. Fermented milks and their future trends. Part II. Technological aspects. *Journal of Dairy Research*. 55: 281-307. 84. Thapa, B. R. 2005. Health factors in colostrums. *Indian Journal of Pediatrics* 72: 579-581. 85. Tomita, M., Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H. and Kawase, K. 1991. Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. *Journal of Dairy Science* 74: 4137-4142. 86. Tong, L. M., Sasaki, S., McClements, D. J. and Decker, E. A. 2000. Antioxidant activity of whey in a salmon oil emulsion. *Journal of Food Science* 65:1325-1329. 87. Valenzuela, R., Contreras, D., Oviedo, C., Freer J. and Rodri'guez, J. 2008. Copper catechol-driven Fenton reactions and their potential role in wood degradation. *International Biodeterioration & Biodegradation*, In Press, Corrected Proof, Available online. 88. Van der strate, B. W., Beljaars, L., Molema, G., Harmsen, M. C. and Meijer, D. K. 2001. Antiviral activities of lactoferrin. *Antiviral Research* 52: 225-239. 89. Van der Ven, C., Grupen, H., Bont, D. B. A. and Voragen, A. G. J. 2002. Optimization of the angiotensin converting enzyme inhibition by whey protein hydrolysates using response surface methodology. *International Dairy Journal* 12: 813 – 820. 90. Varnam, H. and Sutherland, P. 1994. *Milk and milk products*. Chapman and Hall, London. 91. Wayner, D. D. M., Burton, G. W., Ingold, K. U., Barclay, L. R. C. and Locke, S. J. 1987. The relative contributions of vitamin E, urate, ascorbate, and proteins to the total peroxy radical trapping antioxidant activity of human blood plasma. *Biochimica et Biophysica Acta* 924: 408-419. 92. Weinberg, E. D. 2001. Human lactoferrin: a novel therapeutic with broad spectrum potential. *Journal of Pharmacy and Pharmacology* 53: 1303-1310. 93. Wiseman, H., Kaur, H. and Halliwell, B. 1995. DNA damage and cancer: measurement and mechanism. *Cancer Letters* 93: 113-120. 94. 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 ? p. 215. 95. Yamauchi, K., Tomita, M., Giehl, T. J. and Ellison, R. T. 1993. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infection and Immunity*. 96. 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. 97. 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. 98. Yoshino, M., Haneda, M., Naruse, M., Htay, H. H., Iwata, S., Tsubouchi, R. and Murakami, K. 2002. Prooxidant action of gallic acid compounds: copper-dependent strand breaks and the formation of 8-hydroxy-2'-deoxyguanosine in DNA. *Toxicology in Vitro* 16: 705 – 709. 99. Zhang, P. and Omaye, S. T. 2001. Beta-carotene: interactions with alpha-tocopherol and ascorbic acid in microsomal lipid peroxidation. *Journal of Nutritional Biochemistry* 12: 38 – 45.