Relationships between Antioxidant Properties and Protein Compositions of Bovine Colostrums and Their Enzymatic Hydrolysa

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ABSTRACT

The bovine colostrums collected within 7 days postpartum were used in this study to prepare skimmed milk, caseins and whey. The antioxidant properties of these samples and the relationships between the antioxidant activities and the protein compositions of these samples were analyzed. Then, the effect of enzymatic hydrolysis conditions with flavourzyme (from Aspergillus oryzae) on the antioxidant properties of bovine colostrum protein hydrolysates and the relationships between antioxidant properties and the obtained protein hydrolysates were investigated. We also used bovine colostrum protein hydrolysates and amino acids (His, Pro and His+Pro) as raw materials and flavourzyme as catalyst to undergo plastein reaction at different pH values (5.5, 7.5 and 9.5), and evaluated the antioxidant properties of the plastein products. Lastly, the changes in the antioxidant properties of the plastein products, together with bovine colostrums proteins and their hydrolysates, before and after digesting using gastrointestinal proteases (pepsin and pancreatin) were studied. The results showed: 1.The reducing powers of colostrum skimmed milk (SM), caseins (C), and whey (W) were in an order of SM＞W＞C; ferrous ion chelating abilities were SM＞W＞C; DPPH radical scavenging activities were SM＞W　C; superoxide anion scavenging activities were SM＞C＞W. The whey from the colostrums collected on the 1st and the 2nd days postpartum exhibited relatively high ferrous ion chelating ability. The whey from the colostrums collected between the 2nd and the 4th days and the caseins from the colostrums collected on the 2nd day had relatively high radical scavenging activities, more than 60 % at a concentration of 20 mg/mL. The inhibitory effect on lipid peroxidation of caseins was higher than those of whey, and was comparable to those of α-tocopherol and BHA within 8-hour reaction. 2.As for the relationships between antioxidant activities and protein compositions of bovine colostrums, we grouped the peaks on the gel filtration chromatograms of caseins and whey into four groups respectively. The results showed that Casein I (lactoferrin) was positively correlated to reducing power and DPPH radical scavenging activity. Whey I, II, III and IV were all positively correlated to ferrous ion chelating power, and Whey IV (β-lactoglobulin and α-lactalbumin) was positively correlated to DPPH radical scavenging activity. 3.The casein and whey hydrolysates exhibited higher ferrous ion chelating abilities, more than 60 % at a concentration of 1 mg/mL, than those unhydrolyzed, but had lower reducing power and DPPH radical scavenging activity than those unhydrolyzed. Based on the ridge max analysis, the hydrolysis conditions for the maximum DHs of caseins and wheys, 24.87 and 23.45 % respectively, were the hydrolysis time at 23.28 and 21.51 h, the temperature 36.77 and 41.65 ℃, and the E/S 2.26 and 1.35%, respectively. Both casein and whey hydrolysates, obtained through the hydrolysis using the conditions for maximum DH, had higher DPPH radical scavenging activities but lower reducing powers and ferrous ion chelating abilities than those obtained through ridge max analysis. This result revealed that the hydrolysate with higher degree of hydrolysis would have higher DPPH radical scavenging ability. 4.As for the amino acid compositions of bovine colostrum protein hydrolysates, it showed that the content of neutral amino acids in the hydrolysate was the highest, basic amino acids and aromatic amino acids were the next, and acidic amino acids and cyclic imino acid, proline, were the lowest. Both casein hydrolysates and whey hydrolysates were rich in leucine and lysine. The results of the analysis of relationships between the antioxidant properties and the amino acid compositions of bovine colostrum protein hydrolysates showed the more the neutral amino acids, the lower the reducing power and the superoxide anion scavenging activity. 5.The antioxidant activities of the products obtained through the plastein reaction using casein hydrolysates or whey hydrolysates as raw materials and flavourzyme as catalyst were higher than those of original hydrolysates. As compared to the casein and whey and its hydrolysates, it was found that the plastein products apparently had higher reducing power and ferrous ion chelating power at a concentration 1 mg/mL. 6.The reducing powers of skimmed milk, casein, whey, casein hydrolysate and whey hydrolysate after hydrolysis using gastrointestinal proteases were higher than those without hydrolysis by gastrointestinal proteases, however, the ferrous ion chelating abilities and the DPPH radical scavenging activities were lower than those without hydrolysis by gastrointestinal proteases. The antioxidant activities of the plastein products were decreased as hydrolyzed by gastrointestinal proteases.

Keywords : bovine colostrums ; casein ; whey ; antioxidant activity ; enzymatic hydrolysis ; plastein reaction

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REFERENCES

1. 王哲齡。1977。酪蛋白普拉斯丁性質之研究。國立台灣大學畜牧學系碩士論文，台北。 2. 行政院農業委員會。2000。八十九年農業統計年報。p. 136-137。 3. 佑生開發股份有限公司。1995。乳鐵蛋白--天然的保護性蛋白。畜牧半月刊，54 (7): 89-92。 4. 李怡萱。2002。衍自牛乳中血管收縮素轉化?抑制物活性之探討。國立中興大學畜產學系碩士論文，台中。 5. 吳則雄。1980。認識牛的初乳。乳業，98/99: 9-14。 6. 吳柏宏。2004。科學與技術自由基、老化與抗氧化配方。食品工業，36: 45-51。 7. 林玫欣。1999。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碩士論文，基隆。 8. 林淑媛。1986。利用普拉斯丁反應製備治療苯酮尿症的配方奶粉。國立中興大學食品科技系碩士論文，台中。 9. 皇輝貿易有限公司。2003。初乳之研究報告。畜牧半月刊，69 (8): 29-34。 10. 張為憲。1980。應用普拉斯丁反應於植物及微生物蛋白質之高度利用。食品工業，8: 10-12。 11. 陳昭誠、杜艷櫻、張鴻民。1999。牛乳IgG之酵素水解物性質。食品科學，26: 496-506。 12. 陳建中、劉春妙、蔣丙煌。1994。以共沉澱及超過濾膜濾洗方法分離全牛乳蛋白質及功能性測定。食品科學，21: 459-469。 13. 楊正護。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。 14. 楊詠翔。1999。食品中抗高血壓胜 ?的發展現況。食品工業，31: 9-18。 15. 鄭名凡。1999。蛋白質水解物的功能與應用。食品資訊，160: 49-54。 16. 劉新裕、王昭月、張同吳、林義恭、陳淑芬、朱良戩、王順成。1999。山藥之品種特性、生產潛力、物化性質與抗氧化研究。中華農業研究，48: 1-22。 17. 劉毓蕙。2004。水解蛋白的特性及應用。食品工業，36: 19-24。 18. 賴滋漢、金安兒。1991。食品加工學製品篇。p. 181-196。 19. 賴祥玲。2004。乳蛋白中的機能胜?。食品工業，36: 37-44。 20. 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。 21. 蘇家愷。1994。自牛初乳中分離免疫球蛋白與乳鐵蛋白。國立台灣大學食品科技研究所博士論文，台北。 22. 饒家麟、柯文慶。2001。鮪魚蒸煮液蛋白質水解物之抗氧化特性。台灣農業化學與食品科學，39: 363-369。 23. Adler-Nissen, J. 1986. Some fundamental aspects of food protein hydrolysis. In ”Enzymatic Hydrolysis of Food Protein”. pp. 20-21. Elesevier Science Publishing Co., Inc. New York, USA. 24. Al-Mashikhi, S. A. and Nakai, S. 1987. Isolation of bovine immunoglobulins and lactoferrin from whey protein by gel filtration techniques. J. Dairy Sci. 70: 2486-2492. 25. Arai, S., Yamashita, M. and Fujimaki, M. 1986. Plastein reaction and its application. Cereal Foods World 20: 107-112. 26. Aso, K., Yamashita, M. and Arai, S. 1974. Hydrophobic force as a main factor contributing to plastein chain assembly. J. Biochem. 76: 341-347. 27. Blosi, M. S. 1958. Antioxidant determination by the use of a stable free radical. Nature 26: 1199-1200. 28. Bouhallab, S., Molle, D. and Leonil, J. 1992. Tryptic hydrolysis of caseinomacropeptide in membrane reactor: preparation of bioactive peptides. Biotechnol. Lett. 14: 805-810. 29. Bowman, L. and Geiger, E. 1984. Optimization of fermentation conditions of alcohol production. Biotechnol. Bioeng. 26: 1492-1497. 30. Box, G. E. P. and Wilsnon, K. B. 1951. On the experimental attainment optimum conditions. J. Roy. Statist. Soc. B13: 1-45. 31. 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. Int. Dairy J. 9: 323-327. 32. Brunner, J. R. 1981. Cow milk proteins: Twenty-five years of progress. J. Dairy Sci. 64: 1038-1054. 33. Brownsell, V. L., Williams, R. J. H. and Andrews, A. T. 2001. Application of the plastein reaction to mycoprotein: Ⅱ. Plastein properties. Food Chem. 72: 337-346. 34. Cervato, G., Cazzola, R. and Cestaro, B. 1999. Studies on the antioxidant activity of milk caseins. Int. J. Food Sci. Nutr. 50: 291-296. 35. Cheynier, V., Feinberg, M., Chararas, C. and Ducauze, C. 1983. Application of response surface methodology to evaluation of bioconversion experimental conditions. Appl. Environ. Microbiol. 45: 634-639. 36. Chiang, W. D., Shih, C. J. and Chu, Y. H. 1999. Functional properties of soy protein hydrolysate produced from a continuous membrane reactor system. Food Chem. 65: 189-194. 37. Clemente, A. 2000. Enzymatic protein hydrolysates in human nutrition. Trends Food Sci. Technol. 11: 254-262. 38. Clemente, A. and Chambers, S. J. 2000. Development and production of hypoallergenic protein hydrolysates for use in infant formulas. Food Allergy Intoler. 1: 175-190. 39. Darkwa, J., Mundoma, C. and Simoyl, R. H. 1988. Antioxidant chemistry: reactivity and oxidation of dl-cysteine by some common oxidants. J. Chem. Soc. Faraday Trans. 94: 1971-1978. 40. Donnelly, J. L., Decker, E. A. and McClements, D. J. 1998. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. J. Food Sci. 63: 997-1000. 41. Fagbenro, O. and Jauncery, K. 1993. Chemical and nutritional quality of raw, cooked and salted fish silage. Food Chem. 48: 331-335. 42. Fang, Y. Z., Yang, S. and Wu, G. 2002. Free radicals, antioxidants, and nutrition. Nutri. 18: 872-879. 43. Fox, P. F. and Mulvihill, D. M. 1993. Developments in milk protein processing. Food Sci. Tech. Today. 7: 152-161. 44. Galas, E., Bieleeki, S., Antezak, T., Weiczorek, A. and Blaszezyk. 1981. Optimization of cultivation medium composition for lytic enzyme biosynthesis. In Moo-Young, M., Vezina, C. and Singh, K. (Eds) Advances in Biotechnology-Proceedings 6th International Fermentation Symposium, Vol.3, Pergamon Press, Canada. p. 301-306. 45. Gildberg, A. 1993. Enzymatic processing of marine raw materials. Process Biochem. 28: 1-15. 46. 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. J. Sci. Food Agric. 35: 1363-1369. 47. Gill, H. S. and Cross, M. L. 2000. Anticancer properties of bovine milk. Br. J. Nutri. 84: S161-S166. 48. Gill, H. S., Doull, F., Rutherfurd, K. J. and Cross, M. L. 2000. Immunoregulatory peptides in bovine milk. Br. J. Nutri. 84: S111-S117. 49. Green, M., Scott, K. J., Anderson, M., Griffin, M. C. and Glover, F. A. 1984. Chemical characterization of milk concentrated by ultrafiltratio. J. Dairy Res. 51: 267-278. 50. Gurr, M. I. 1981. Review of the progress of dairy science: Human and artificial milks for infant feeding. J. Dairy Res. 48: 519-554. 51. Halliwell, B. and Gutteridge, J. 1998. Free radicals in biology and medicine. pp. 316-319. Oxford university press, London, UK. 52. Hekmat, S. and McMahon, D. J. 1998. Distribution of iron between caseins and whey proteins in acidified milk. Lebensm- wiss. u. – Technol. 31: 632-638. 53. Himmelblau, D. M. 1970. Process analysis by statistical methods. John Wiley and Sons, New York. 230-292. 54. Hooijdonk, A. C. M. van, Kussendrager, K. D. and Steijns, J. M. 2000. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. Br. J. Nutri. 84: S127-S134. 55. Ishigawa, Y. 1992 Development of new types of antioxidants from microbial origin. J. Jpn. Oil Chem. Soc. 41: 762-771. 56. Jeon, S. M., Bok, S. H., Jang, M. K., Lee, M. K., Nam, K. T., Park, Y. B., Rhee, S. J. and Choi, M. S. 2001. Antioxidative activity of naringin and lovastatin in high cholesterol-fed rabbits. Life Sci. 69: 2855-2866. 57. Kayser, H. and Meisel, H. 1996. Stimulation of human peripheral blood lymphocytes by bioactive peptide derived from bovine milk proteins. FEBS Lett. 383: 18-20. 58.

Kosikowski, F. V. 1983. Low sodium cheddar cheese through whole milk retentate supplementation. J. Dairy Sci. 66: 2494-2500. 59. Kuo, K. P. and Cheryan, M. 1983. Ultrafiltration of acid whey in a spiral wound unit: effect of operating parameters on membrane fouling. J. Food Sci. 48: 1113-1118. 60. Lahl, W. J. and Braun, S. T. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48: 68-71. 61. Lee, Y. S., Noguchi, T. and Naito, H. 1980. Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. Br. J. Nutr. 43: 457-467. 62. Lindsay, D. G. and Astley, S. B. 2002. European research on the functional effects of dietary antioxidants-eurofeda. Mol. aspects med. 23: 1-38. 63. Lindmark-Mansson, H. and Akesson, B. 2000. Antioxidative factors in milk. Br. J. Nutri. 84: S103-S110. 64. Loukas, S., Varoucha, D., Zioudrou, C., Streaty, R. A. and Klee, W. A. 1983. Opioid activities and structures of β-casein-dervied exorphins. Biochem. 22: 4567-4573. 65. Lu, L. C., Chen, Y. W. and Chou, C. C. 2003. Antibacterial and DPPH free radical-scavenging activities of the ethanol extract of propolis collected in Taiwan. J. Food Drug Anal. 11: 277-282. 66. Mackie, I. M. 1982. Fish protein hydrolysates. Process Biochem. 31: 26-31. 67. Maddox, I. S. and Richert, S. H. 1977. Use of response surface methodology for the rapid optimization of microbiogical media. J. Appl. Bacterial 43: 17-204. 68. Manley, C. H. and Ahmedi, S. 1995. The development of process flavors. Trends Food Sci. Tech. 6: 46-51. 69. Maruyama, S. and Suzuki, H. 1982. A peptides inhibitor of angiotensin I-converting enzyme in the tryptic hydrolysate of casein. Agric. Biol. Chem. 46: 1393-1394. 70. Maruyama, S., Nakagomi, K. Tomozuka, N. and Suzuki, H. 1985. Isolation and characterization of angiotension I-converting enzyme inhibitor derived from an enzymatic hydrolysate of casein. II. Isolation and bradykinin-potentiating activity on the uterus and the ileum of rats. Agric. Biol. Chem. 49: 1405-1409. 71. McIntosh, H., Royle, J., Leu, K. Le, Regester, O., Johnson, A., Grinsted, L., Kenward, S and Smithers, W. 1998. Whey protein as functional food ingredients. Int. Dairy J. 8: 425-434. 72. Meisel, H. and Schlimme, E. 1996. Bioactivity peptides derived from milk proteins: Ingredients for functional foods? Kieler Milchw. Forsch. 48: 343-357. 73. Meisel, H. 1997. Biochemical properties of regulatory peptides derived from milk proteins. Biopoly. 43: 119-128. 74. Meisel, H. 1998. Overview on milk protein-derived peptides. Int. Dairy J. 8: 363-373. 75. Migliore-Samour, D., Floch, F. and Jolles, P. 1989. Biologically active casein peptide implicated in immunomodulation. J. Dairy Res. 56: 357-362. 76. Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H. and Maruyama, S. 1991. Structures and activity of Angiotensin I – converting enzyme inhibitors in an α-Zein hydrolysate. Agric. Biol. Chem. 55: 1313-1318. 77. Moresi, M., Colicchio, A. and Sansovini, F. 1980. Optimization of whey fermentation in a jar fermenter. Eur. J. Appl. Microbiol. Biotechnol. 9: 173-183. 78. Mullally, M. M., Meisel, H. and Fitzgerald, R. J. 1996. Synthetic peptides corresponding to α-lactalbumin and β-lactoglobulin sequences with angiotensin-I-converting enzyme inhibitory activity. Bio. Chem. Hoppe Seyler. 377: 259-260. 79. Orejana, F. M. and Liston, J., 1982. Agents of proteolysis and its inhibition in Patis (fish sauce) fermentation. J. Food Sci. 47: 198-203. 80. Oshima, G., Shimabukuro, H. and Nagasawa, K. 1979. Biochim. Biophys. Acta. 566: 128-137. 81. Paulson, D. J., Wilson, R. L. and Spatz, D. 1984. Cross-flow membrane technology and its application. Food Tech. 38: 77-91. 82. Pena-Ramos, E. A. and Xiong, Y. L. 2001. Antioxidative activity of whey protein hydrolysates in a liposomal system. J. Dairy Sci. 84: 2577-2583. 83. Quagila, G. B. and Orban, E. 1987. Enzymic solubilisation of sardin (Sardina pilchardus) by commercial protease. J. Sci. Food Agric. 38: 263-269. 84. Raynaud, M. 1959. Bifidus growth promoting of human casein. Sem. Hop. Paris. 35: 240-245. 85. Rebeca, B. D., Pena-Vera, M. T. and Diaz-castaneda, M. 1991. Production of fish protein hydrolysates with bacterial proteases, yield and nutritional value. J. Food Sci. 56: 309-314. 86. Rival, S. G., Boeriu, C. G. and Wicher, H. J. 2001. Casein and casein hydrolysates. II. antioxidative properties and relevance to lipoxygenase inhibition. J. Agric. Food Chem. 49: 295-302. 87. Sasaki, A., McClements, D. J. and Decker, E. A. 2000. Antioxidant activity of whey in a salmon oil emulsion. J. Food Sci. 65: 1325-1329. 88. Sakanaka, S., Tachibana, Y., Ishihara, N. and Juneja, L. R. 2004. Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. Food Chem. 86: 99-103. 89. Sasaki, A., Mcclements, D. J. and Decker, E. A. 2000. Antioxidant activity of whey in a salmon oil emulsion. J. Food Sci. 65: 1325-1329. 90. Satue-Gracia, M. T., Frankel, E. N., Nagendra-Rangavajhyala and German, J. B. 2000. Lactoferrin in infant formulas: effect on oxidation. J. Agric. Food Chem. 48: 4984-4990. 91. Shih, F. F. 1992. Modification of food protein by non-enzymatic methods. In: Biochemistry of Food Protein (B. J. F., Hudson ed.), Ch. 7., Elsevier Applied Science Publishers, London, England. 92. Suetsuna, K., Ukeda, H. and Ochi, H. 2000. Isolation and characterization of free radical scavenging activities peptides derived from casein. J. Nutr. Biochem. 11: 128-131. 93. Stadtman, E. R. 2002. Importance of individuality in oxidative stress and aging. Free Radical Bio. Med. 33: 597-604. 94. Tanimoto, S., Yamashita, M., Arai, S. and Fujimaki, M. 1975. Salt effect on plastein formation by α-chymotrypsin. Agr. Biol. Chem. 39: 1207-1210. 95. Tong, L. M., Sasaki, S., McClements, D. J. and Decker, E. A. 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. J. Agric. Food Chem. 48: 1473-1478. 96. Tsai, S. J., Yamashita, M., Arai, S. and Fujimaki, M. 1974. Polyacrylamide gel electrophoresis of plasteins. Agr. Biol. Chem. 38: 641-644. 97. Van Beresteijn, E. C., Peeters, R. A., Kaper, J., Meijer, R., Robben, A. and Schmidt, D. 1994. Molecular mass distribution, immunological properties and nutritive value of whey protein hydrolysates. J. Food Prot. 57: 619-625. 98. Varnam, H. and Sutherland, P. 1994. Milk and milk products. Chapman & Hall, London. 99. Walsh, G. 2002. Proteins biochemistry and biotechnology. John Wiley & Sons Ltd, England. 100. 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 peroxyl radical trapping antioxidant activity of human blood plasma. Biochem. Biophys. Acta. 924: 408-419. 101. Williams, R. J. H., Brownsell, V. L. A. and Andrews, T. 2001. Application of the plastein reaction to mycoprotein: I. Plastein synthesis. Food Chem. 72: 329-335. 102. Woodward, D. R. 1976. The chemistry of mammalian caseins: A review. Dairy Sci. Abstr. 38: 137-150. 103. Wu, H. C., Chen, H. M. and Shiau, C. Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Res. Int. 36: 949-957. 104. Yamamoto, S., Toida, I. and Iwai. K. 1980. Re-examination of the spectrophotometric assay for serum angiotensin-converting enzyme, Nippon Kyobu Shippei Kaishi 18: 297-303. 105. Yamashita, M., Arai, S., Matsuyama, J., Gonda, M., Kato, H. and Fujimaki, M. 1970.

Enzymatic modification of proteins in foodstuffs. III. Phenomenal survey on α-chymotryptic plastein synthesis from peptic hydrolyzate of soy protein. Agr. Biol. Chem. 34: 1484-1491. 106. Yamashita, M., Arai, A., Tsai, S. J., Kato, H. and Fujimaki, M. 1971. Enzymatic modification of proteins in foodstuffs. V. Plastein yields and their pH dependence. Agr. Biol. Chem. 35: 86-91. 107. Yamashita, M., Arai, S., Aso, K. and Fujimaki, M. 1972. Location and state of methionine residues in papain-synthesized plastein from a mixture of soybean protein hydrolyzate and L-methionive ethyl ester. Agr. Biol. Chem. 36: 1353-1360. 108. Zertuche, L. and Zall, R. R. 1985. Optimizing alcohol production from whey using computer technology. Biotechnol. Bioeng. 27: 547-554.