

# The Antioxidant Stability of Microencapsulated Bovine Colostrum Protein Hydrolysates

陳志璋、張基郁

E-mail: 9606891@mail.dyu.edu.tw

## ABSTRACT

The bovine colostrums collected on the second day postpartum were used to prepare skimmed milk, caseins and whey in this study, and the antioxidant properties of these samples were analyzed. In addition, an enzyme preparation from porcine small intestine was used to hydrolyze these samples under different enzyme/substrate (E/S) ratios (10-60 %), and the antioxidant properties of the colostrum protein samples after hydrolysis by the enzyme preparation were also investigated. Moreover, five different encapsulating materials, including Acryl-EZE, Surelease, chitosan, gum arabic and - cyclodextrin, were used in this study to encapsulate the colostrum protein samples and their hydrolysates with different encapsulating material/protein (or hydrolysate) ratios (9:1, 8:2, and 7:3) and the microencapsulated samples were digested by the enzymes simulated in gastrointestinal tracts in order to investigate the effects of the encapsulating materials and the protein (or hydrolysate)/encapsulating material ratios on the antioxidant properties of the colostrum protein and their hydrolysates. The results were as follows: 1. (a) The ferrous ion chelating abilities of colostrum skimmed milk, caseins and whey (10 mg/mL) were in order of caseins > whey > skimmed milk. The ferrous ion chelating abilities of these samples were all higher than 60 %. (b) The Trolox equivalent antioxidant capacities (TEAC) of these samples were in order of whey > casein > skimmed milk. The TEAC values of these samples were all higher than 10 m mol Trolox. (c) The superoxide anion scavenging activities of these samples at a concentration of 1 mg/mL were in order of skimmed > casein > whey. 2. In the enzyme/substrate (E/S) ratios (10-60 %) test for the hydrolysis of protein samples, the degree of hydrolysis of caseins and whey proteins were 29.16 and 23.55 %, respectively, when the proteins were hydrolyzed under a 60 % E/S ratio for 12 and 18 hours. 3. As for the results of antioxidant properties of the protein samples (at a concentration of 10 mg/mL) hydrolyzed under a 30 % E/S ratio, (a) the ferrous ion chelating abilities were in order of caseins hydrolysate > whey hydrolysate > skimmed milk hydrolysate. The ferrous ion chelating ability of the hydrolysates of caseins and whey proteins were 85.17 and 71.99 %, respectively when the proteins were hydrolyzed for 24 hours. The ferrous ion chelating abilities of caseins and whey were significantly increased when they were hydrolyzed, and the ferrous ion chelating abilities of the enzymatic hydrolysates were increased with the increasing concentration. (b) The TEAC values of the hydrolysates were in order of whey hydrolysate > casein hydrolysates > skimmed milk hydrolysates. The TEAC values of the hydrolysates of caseins and whey were 13.39 and 13.46 m mol Trolox/g, respectively, when the proteins were hydrolyzed under a 60 % E/S ratio for 24 hours. The TEAC values of the hydrolysates were also increased with increasing concentration. (c) The superoxide anion scavenging activities of the skimmed milk, caseins and whey were all decreased significantly after hydrolysis. 4. As for the antioxidant properties of the microencapsulated colostrum proteins and their hydrolysates after digestion by the enzymes simulated in gastrointestinal tracts, the result showed: (a) The best encapsulating material for keeping antioxidant activity was gum Arabic, and the worst was - cyclodextrin. (b) The best encapsulating material/protein (or hydrolysate) ratio was 7:3.

Keywords : bovine colostrums ; casein ; whey ; enzymatic hydrolysis ; antioxidant properties ; microencapsulated

## Table of Contents

封面內頁 簽名頁 授權書.....	iii 中文摘要.....	iv 英文摘要.....
要 要.....	vi 謹謝.....	viii 目 錄.....
錄.....	x 圖目錄.....	xiv 表目 錄.....
的.....	xvii 1. 緒論.....	1 2. 研究目 的.....
乳.....	3 3. 文獻回顧.....	4 3.1 牛初 成.....
成.....	4 3.1.1 牛乳的成分.....	5 3.1.2 牛乳的蛋白質組 用.....
用.....	5 3.1.3 牛乳蛋白的機能性.....	6 3.1.4 牛初乳蛋白的研究與應 解.....
解.....	8 3.1.5 乳鐵蛋白(lactoferrin).....	9 3.2 酵素水 置.....
置.....	10 3.2.1 水解方式及條件.....	10 3.2.2 酵素種類及其水解位 值.....
值.....	11 3.2.3 酵素與基質比例.....	11 3.2.4 溫度與pH
用.....	11 3.2.5 食鹽濃度與抑制劑.....	12 3.3 蛋白質水解物之應 12 3.4 乳蛋白及其水解物之機能性.....
		13 3.5 蛋白質水解物之抗氧化

性.....	17 3.6自由基、老化與抗氧化物質.....	18 3.6.1自由基與活性
氧.....	18 3.6.2老化機制.....	19 3.6.3抗氧化物
質.....	19 3.7小腸及腸液酵素.....	20 3.7.1小腸之功能與作
用.....	20 3.7.2小腸酵素與其功用.....	22 3.8微膠
囊(microcapsule).....	23 3.8.1微膠囊之簡介.....	23 3.8.2微膠囊之應
用.....	24 3.8.3微膠囊之包覆材質.....	27 4. 材料與方
法.....	29 4.1材料藥品及儀器設備.....	29 4.1.1材
料.....	29 4.2 藥品.....	29 4.2.1 儀器設
備.....	31 4.3方法.....	32 4.3.1本試驗之流
程.....	32 4.3.2基本組成分析.....	32 4.3.3脫脂全乳、乳清及酪蛋白之抗氧
化性.....	34 4.3.4豬腸液酵素之粗萃取與活性測定.....	36 4.3.5 SDS-聚丙醯胺膠體電泳
法.....	37 4.3.6牛初乳蛋白之水解.....	39 4.3.7乳清蛋白、酪蛋白水解物之抗氧化力
測定.....	41 4.3.8微膠囊化.....	41 4.3.9模擬腸胃道試驗及抗氧化
性.....	43 4.3.10統計分析.....	43 5. 結果與討論.....
44 5.1 牛初乳組成分分析及乳清與酪蛋白之蛋白質定分析.....	44 5.2 豬小腸之粗酵素液活性與安定性試	
驗.....	46 5.3 豬小腸粗酵素液之電泳分析.....	46 5.4 牛初乳蛋白質之水
解.....	50 5.5 脫脂全乳、酪蛋白與乳清及其水解物之電泳分析.....	53 5.6 牛初乳脫脂全乳、
酪蛋白與乳清及其水解物之抗氧化性.....	55 5.6.1脫脂全乳、酪蛋白與乳清之亞鐵離螯核能力.....	55 5.6.2牛
初乳脫脂全乳、酪蛋白與乳清水解物之亞鐵離子敖核能力.....	57 5.6.3脫脂全乳、酪蛋白與乳清之Trolox當量抗氧化能	力.....
力.....	62 5.6.5脫脂全乳、酪蛋白、乳清	
及其水解物之超氧陰離子清除能力.....	63 5.7牛初乳蛋白之微膠囊與模擬胃腸道消化試驗之抗氧化安定性.....	67 5.7.1
模擬胃腸道消化.....	67 5.7.2微膠囊化最適包覆比例.....	70 5.7.3微膠囊化最佳
包覆材質.....	78 5.7.4利用阿拉伯膠微膠囊化處理所得牛初乳脫脂全乳、酪蛋白、乳清及其水解物經胃	
腸道酵素消化前後之抗氧化安定性.....	86 6. 結論.....	93 參考文
獻.....	95	

## REFERENCES

- 1.大澤。1995。微膠囊化技術。食品開發。30 (8): 41-44。2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。私立大葉大學生物產業科技學系博士論文，彰化。
- 3.沈立言、林淑媛、蔡順仁。1991。不同精油含量之羅勒、蒜、薑精油之微膠囊之製備及其精油成分在噴霧乾燥過程中之變化。食品科學。18 (4):344-355。
- 4.沈立言、蔡順仁。1991。薑、羅勒、蒜噴霧乾燥精油微膠囊之製備及其性質之研究。中農業化學會誌。29 (2): 226-237。
- 5.林淑媛、沈立言、蔡順仁。1992。蒜、薑精油微膠囊在儲藏後之香味變化。中國農業化學會誌。30 (4): 544-552。
- 6.林慶文、蘇和平、張兆德。1995。微膠囊化硫酸亞鐵之製備對全脂乳粉脂肪安定性之影響。食品科學。22 (2): 141-148。
- 7.林致欣。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.吳則雄。1980。認識牛的初乳。乳業。98/99: 9-14。
- 9.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。
- 10.吳蕙君。1998。魚貝類抽出物抗氧化性之探討。國立海洋大學水產食品科學系碩士論文，基隆。
- 11.原田淳、西村信明。1995。酵素分解調味料的特殊用途，食品加工包裝技術。34 (11): 35-38。
- 12.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。
- 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.楊珮琪、陳炯堂。1995。相分離膠體包覆油溶性香味物質及其安定性之研究。食品科學。22 (2): 172-184。
- 15.楊詠翔。1999。食品中抗高血壓勝?的發展現況。食品工業。31: 9-18。
- 16.鄭名凡。1999。蛋白質水解物的功能與應用。食品資訊。160: 49-54。
- 17.賴茲漢、金安兒。1991。食品加工學製品篇。p.181-196。
- 18.盧健宇、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。
- 19.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。
- 20.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。
21. AOAC.1995. Official method of Analysis, 14th ed.
- Association of Official American Chemists, Washington, D.C., U.S.A. 22. Al-Mashikhi, S. A. and Nakai, S. 1987. Isolation of bovine immuneoglobulins and lactoferrin from whey protein by gel filtration techniques. J. Dairy Sci. 70: 2486-2492.
23. Anonymous. 1998. Alcalase Food Grade. B 318b-GB 2000. bagsvaerd. Denmark: Novo Ind.A/S.
24. Anonymous. 1991. Enzymatic Modification of Proteins using Novo Nordisk Proteases. B 163g-GB 2500. Bagsvaerd. Denmark: Novo Ind.A/S.
25. Arnao B.M., Cano A, Hernandez-Ruiz J, Garcia-Canovas. F. and Acosta, M. 1996: Inhibition by L - ascorbic acid and other antioxidants of the 2, 2 ' -azino-bis ( 3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of food. Ana. Biol. Chem. 236: 255-261.
26. Bangs, W. E. and Reineccius, G.A. 1990. Isolation of bovine immuneoglobulins and lactoferrin from whey protein by gel filtration techniques. J. Dairy Sci. 70: 2486-2492.
27. Bhandari, B., D'Arcy, B. R. and Padukka, I. 1999. Encapsulation of lemonoil by paste method using a-cyclodextrin: encapsulation efficiency and profile of oil volatiles. J. Agric Food Chem. 47:5194-5197.
28. Bhandari, B. R., Dumoulin, E. D., Richard, H., Noleau, I. and Lebert, A. M. 1992. Flavor encapsulation by spray drying:application to citral and linalyl acetate. J. Food Sci. 57(1): 217-221.
29. Bouhallab, S., Molle, D. and Leonil, J.

1992. Tryptic hydrolysis of caseinomacropeptid in membrane reactor: preparation of bioactive peptides. *Biotechnol. Bioeng.* 26: 1492-1497. 30.
- Brantl, V. and Teschemacher, H. 1979. A material with opioid activity in bovine milk and milk products. *Naunyn-Schmeidebergs Arch Pharmacol.* 306: 301-304. 31. Brun, J. M. and Dalglish, D. G. 1999. Some effects of heart on the competitive adsorption of casein and whey proteins in oil-in water emulsions. *Int. Daily J.9:* 323-327 32. Chen, H. M., Muramoto, K. and Yamauchi, F. 1995. Structural analysis of antioxidative peptides from soybean -conglycinin. *J. Agric. Food Chem.* 43(3): 574-578. 33. Chen, H. M., Muramoto, K., Yamauchi, F. and Nokihara, K. 1996. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *J. Agric. Food Chem.* 44(9): 2619-2622.
34. Chen, H. M., Muramoto, K., Yamauchi, F., Fujimoto, K. and Nokihara, K. 1998. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *J. Agric. Food Chem.* 46(1): 49-53. 35. Cheung, H. S. and Chushman, D. W. 1971. spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem. Pharmacol.* 20: 1637-1648. 36.
- Chiang, S. H. and Chang, C. Y. 2005. Antioxidant properties of casein and whey proteins from colostrums. *J. Food Drug Anal.* 13(1):57-63. 37.
- Clement, A. 2000. Enzymatic protein hydrolysates in human nutrition. *Trends Food Sci. Technol.* 11: 254-262. 38. Clement, A. and Chambers, S. J. 2000. Development and production of hypoallergenic protein hydrolysates for use in infant formulas. *Food Allergy Int.* 1: 175-190. 39. Colbert, L. B. and Decker, E. A. 1991. Antioxidant activity of ultrafiltration permeate from acid whey. *J. Food Sci.* 56: 1248-1250. 40. Debeaufort, F. and Voilley, A. 1995. Effect of surfactants and drying rate on barrier properties of emulsified edible films. *Int. J. Food Sci & Tech.* 30: 183-190. 41.
- Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidants. *J. Chem. Soc. Faraday Trans.* 94: 1971-1978. 42. Dezarn, T. J. 1995. Food ingredient encapsulation: an overview. Risch S. J., Reineccius G. A, editors. *Encapsulation and controlled release of food ingredients.* p74-86.
43. Donhowe,I.G.and Fennema,O.1996.Edible films andcoatings:character formation,definitions, and testing methods.Krochta JM,Baldwin EA, Nisperos-Carriedo MO,editors.*Edible coatings and films to improve ood quality.* Capter 1. Techhomic Publishing CO INC, USA. p1-24.
- 44.Dziezak,J.D.1988.Microencapsulation and encapsulated ingredients. *Food Tech.* April: 136-148. 45. Francisco, J. C., Aguilarb, R., Mar? ' a, F. L.and Toroc. A. 2005. Isolation and characterization of trypsin from pyloric caeca of Montere sardine sardinops sagax caerulea. Comparative Biochemistry and Physiology, Part B 140: 91 – 98. 46. Fagbenro, O. and Tauncery, K. 1993. Chemical and nutritional quality of raw,cooked and salted fish silage. *Food Chem.* 48: 331-335. 47. Fiat, A-M, Migliore-Samour. D. and Tolles, P. 1993. biologically active peptides from milk proteins with emphasis on two samples concerning antitrombotic and immuneomodulating activeities. *J. Daily Sci.* 76: 301-310. 48. Frokjaer, S. 1994. Use of hydrolysates for protei supplementation. *Food ehnol.*10: 86-88. 49. Gildberg, A. 1993. Enzymatic processing of marine raw materials. *Process Biochem.* 28: 1-15. 50. Gildberg, A., Hermes, J. E. and Orejana, F. M. 1984. Acceleration of autolysis during fish sauce fermentatation by adding acid and reducing the salt content. *J. Sci. Food Agric.* 35: 1363 1369. 51. Gill, H. S. and Cross, M. L. 2000. Anticancer propertyes of bovine milk. *Br. J. Nutri.* 84: S161-S166. 52. Grant, L. A. and Burns, J. 1994. Application of coating. Krochta JM, Baldwin EA, Nisperos-Carriedo MO, editors .*Edible coatings and films to improve food quality.*Capter 8. Techhomic Publishing CO INC, USA. p189-200. 53. Gunning, Y. M., Gunning, P. P., A., Kemsley, E. K., Parker, R., Ring, S.G., Wilson, R. H. and Blake, A. 2000. Factors Affecting the Release of Flavor Encapsulated in Carbohydrate Matrixes. *J. Agric Food Chem.* 47:519-5205. 54. Halliwel, B. and Gutteridge, J. 1998. Free radicals in biology and medicine. Pp. 316-319. Oxford university press, London, UK. 55. Hekmat, S. and MaMahon, D. J. 1998. Distribution of iron between caseins and whey proteins in acidified milk. *Lebensm. -Wissu. Technol.* 31: 632-638. 56. Hooijdonk, A. C. M. van, 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. *Br. J. Nutri.* 84: S127-S134. 57. Hummel, B. 1959. A modified spectrophotometeric determineation of chymotrypsin, trypsin, and thrombin. *Can. J. Biochem Physiol.* 371-393. 58. I. Kurtovic, S. N., Marshall, B. K. and Simpson. 2006 Isolation and characterization of a trypsin fraction from the pyloric ceca of chinok salmon (*Oncorhynchus tshawytscha*).Comparative Biochemistry and Physiology. 143: 432-440. 59.Jolles,P.and Migliore-Samour,D.1986.Preparation of immunological agents by treating lipid-free bovine casein with proteolytic enzyme and fractionating the product Patent Assignee: Rhone-Poulenc Sante. United States Patent 4:851- 509, European Patent: 170- 550. 60.Jolles,P.,Migore-Samour,D.and Parker F.1988.Immuno stimulant substances derived from bovine casein and compositions containing the same. Patent Assignee: Rhone-Poulenc Sante. United States Patent 4:777-243. 61. 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. 62. Keryn, D. J., Clark, A. and Marshall, S. 2002. A functional comparison of ovein and porcine trypsins. Comparative Biochemistry and Physiology Part B 131: 423-431. 63. King, A. H. 1995. Encapsulation of food ingredients: A review ofavailable technology, focusing on hydrocolloids. Risch SJ, Reineccius GA, editors. *Encapsulation and controlled release of food ingredients.* p26-39. 64.
- Konstance, R. P., Onwulata, C. I. and Holsinger, V. H. 1995. Flow properties of spray-dried encapsulated butteroil. *J Food Sci.*60(4): 841-844. 65.
- Laemml, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.*227, 680-685 66. Lahl, W. J. and Brum, S. T. 1994. Enzymatic production of protein hydrolysates for food use. *Food Technol.* 48(10): 68-71. 67. Lin, C. C., Lin, S. Y.and Hwang, L. S. 1995. Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. *J. Food Sci.* 60(1):36-39. 68. Lindmark-Mansson, H. and Akesson, B. 2000. Antioxidative factors in milk. *Br. J. Nutri.* 84: S103-S110. 69. Mackie, I. M. 1982. Fish protein hydrolysates. *Process Biochem.* 31: 26-31. 69. Mackie, I. M. 1982. Fish protein hydrolysates. *Process Biochem.* 17: 26-32. 70.
- Mahmoud, M. I. 1994. Physicochemical and functional propertyes of protein hydrolysates in nutriational products. *Food Technology* 48 (10): 89-95. 71. Manlry, C. H. and Ahmed, S. 1995. The development of processs flavors. *Trends in Food Sci. & Technol.* 6: 46-51.
72. McNamee,F.,O'Riordan,E.D.and O'Sullivan,M.1998.Emulsification and microenclsulation properties of gum arabic. *J.Agric.FoodChem.*46:4551 -4555. 73. Meisel, H. 1998. Overview on milk protein-derived peptides. *Int. Dairy J.* 8: 363-373.

74. Meisel, H. and Frister, H. 1988. Chemical characterization of a casein phosphopeptides isolated from in vivo digests of a casein diet. *Biol. Chem. Hoppe-Seyler*. 369:1275-1279.
75. Meisel, H. and Schlimme, E. 1990. Milk proteins: precursors of bioactive peptides. *Trends Food Sci. Technol.* 8: 41-43.
76. Minemoto, Y., Adachi, S. and Matsuno, R. 1997. Comparison of oxidation of methyl linoleate encapsulated with gum arabic by hot-air-drying and freeze-drying. *J. Agri. Food Chem.* 45 (12):4530-4534.
77. Migliore-Samour, D., Floch, F. and Jolles, P. 1989. Biologically active casein peptide implicated in immune modulation. *J. Dairy Res.* 56: 357-363.
78. Morato, A. F., Carreira, R. L., Junqueira, R. G. and Silvestre, M. P. C. 2000. Optimization of casein hydrolysis for obtaining high contents of small peptides: use of subtilisin and trypsin. *J. Food Compos. Anal.* 13: 843-875.
79. Mykanen, H. M. and Wassermann, R. H. 1980. Enhanced absorption of calcium by casein phosphopeptides in Rachitic and normal chicks. *J. Nutr.* 110: 2141-2148.
80. Nakadai, T., Nansuno, S. and Iguchi. 1972. The action of peptidase from *Aspergillus oryzae* in digestion of soybean protein. *Agric. Biol. Chem.* 36:261.
81. Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S., and Takano, T. 1995. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *J. Dairy Sci.* 78: 1253-1257.
82. Orejana, F. M. and Liston, J. 1982. Agents of proteolysis and its inhibition in patis (fish sauce) fermentation. *J. Food Sci.* 47: 198-203.
83. Petersen, R.B. 1981. The impact of the enzymic hydrolysis process or recovery and use of proteins. In *Enzymes and Food Processing*. G.G. Birch, N. Blakebrough, and K. J. Parker. (Ed.): 149. Applied Science Publishers Ltd., London, U.K.
84. Pintado, M. E., Pintado, A. E. and Malcata, F. X. 1999. Controlled whey protein hydrolysis using two alternative proteases. *J. Food Eng.* 42: 1-13.
85. Quaglia, G. B. and Orban, E. 1987. Enzymic solubilisation of sardine (*Sardina pilchardus*) by commercial protease. *J. Sci. Food Agric.* 38: 263-269.
86. Rebeca, B. D., Pena-Vera, M. T. and Diaz-castañeda, M. 1991. Production of fish protein hydrolysates with bacterial protease, yield and nutritional value. *J. Food Sci.* 56(2): 309-314.
87. Regenstein, M. and Regenstein, E. 1984. In "Food Protein Chemistry Int." Food Risch SJ, Reineccius GA, editors. Encapsulation and controlled release of food ingredients. Sci. Pp. 56-66. Academic press, New York.
88. Reineccius, G.A., Ward, F.M., Whorton, C. and Andon, S.A. 1995a. Development in gum acacias for the encapsulation of flavors. Risch S.J., Reineccius G.A., eds. Encapsulation and controlled release of food ingredients. p33-46.
89. Risch, S.J. 1995. Encapsulation: overview of use and techniques. Risch S.J., Reineccius G.A., editors. Encapsulation and controlled release of food ingredients. p2-7.
90. Rival, S. G., Fornaroli, S., Boeriu, C. G. and Wickers, H. J. 2001a. Caseins and casein hydrolysates. I. Lipoxygenase inhibitory properties. *J. Agric. Food Chem.* 49: 287-294.
91. Satue-Gracia, M.T., Frankel, E.N., Rangavajhyala, N. and German, J.B. 2000. Lactoferrin in infant formulas: effect on oxidation. *J. Agric. Food Chem.* 48:4984 -4990.
92. Saviha, K., Kshirsagara, A. C. and Singhal, R. S. 2005. The use of gum Arabic and modified starch in the microencapsulation of a food flavoring agent. *Carbohydrate Polymers* 62 (2005) 309-315.
93. Sen, D. P., Sripathy, N. V., Lahiry, N. L., Sreenivasan, A. and Subrahmanyam, V. 1962. Fish hydrolysates. I. Rate of hydrolysis of fish flesh with papain. *Food Technol.* 5: 138-141.
94. Sheu, T. Y. and Rosenberg, M. 1998. Microstructure of microcapsules consisting of whey proteins and carbohydrates. *J. Food Sci.* 63(3):491-494.
95. 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.
96. Shinmoto, H., Dosako, S. and Nakajima, I. 1992. Antioxidant activity of bovine lactoferrine on iron/ascorbate induced lipid peroxidation. *Biosic Biotech. Biochem.* 56: 2079-2080.
97. Slocum, S. A., Jasinski, E. M., Anantheswaran, R. C. and Kilare, A. 1988. Effects of sucrose on proteolysis in yogurt during incubation and storage. *J. Dairy Sci.* 71:589-595.
98. Spellman, D., McEvoy, E., Cuinn, G. O. and Fitzgerald, R.J. 2003. Proteinase and exopeptidase hydrolysis of whey protein: Comparison of the TNBS, OPA and pH stat methods for quantification of degree of hydrolysis. *Int. Dairy J.* 13:447-453.
99. Sutas, Y., Soppi, E. and Korhonen, H. 1996. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes. *J. Allergy Clin. Immunol.* 98(1): 216-224.
100. Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int. J. Food Microbiol.* 62:47 – 55.
101. Tamime, A. Y. and Robinson, K. 1988. Fermented milks and their future trends. Part II. Technological aspects. *J. Dairy Res.* 55:281-307.
102. Thevenet, F. 1995. Acacia gums: natural encapsulation agent for food ingredients. Risch S.J., Reineccius G.A. Encapsulation and controlled release of food ingredients. p51-59.
103. Tsuda, T., Fujii, M., Watanabe, M., Nakakuki, H., Ohshima, K., Osawa, T. and Kawakishi, S. 1994. Antioxidative activity of red bean extract and its application to food. *Nippon Shokuhin Kogyo Gakkaishi* 41(7): 475-480.
104. Tsuge, N., Eikawa, Y., Nomura, Y., Yamamoto, M. and Sugisawa, K. 1991. Antioxidative activity of peptides prepared by enzymatic hydrolysis of egg-white albumin. *Nippon Nogeikagaku Kaishi* 65(11): 1635-1641.
105. Varnam, H. and Sutherland, P. 1994. Milk and milk products. Chapman & Hall, London.
106. Wu, J. P. and Ding, X. L. 2002. Characterization of inhibition, immunological properties and nutritive value of whey protein hydrolysates. *J. Food Prot.* 57: 619-625.
107. Yamauchi, K., Tomita, M., Giehl, T. J. and Ellison, R.T. 1993. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infect. Immun.* 61: 719-728.
108. Yamaguchi, N., Yokoo, Y. and Fujimaki, M. 1975. Studies on antioxidative activities of amino compounds of fats and oils. Part III. Antioxidative activities of soybean protein hydrolysates and synergistic effect of hydrolyzate on tocopherol. *Nippon Shokuhin Kogyo Gakkaishi* 22(9): 431-435.
109. Yamaguchi, N., Yokoo, Y. and Fujimaki, M. 1979. Antioxidative activities of protein hydrolysates. *Nippon Shokuhin Kogyo Gakkaishi* 26(2): 65-70.
110. Young, S. L., Sarda, X. and Rosenberg, M. 1993a. Microencapsulating properties of whey proteins. I. microencapsulation of anhydrous milk fat. *J. Dairy Sci.* 76:2868-2877.
111. Young, S. L., Sarda, X. and Rosenberg, M. 1993b. Microencapsulating properties of whey proteins. 2 combination of whey protein with carbohydrate. *J. Dairy Sci.* 76:2878-2885.
112. Zinn, S. 1997. Bioactive components in milk: introduction. *Livest. Prod. Sci.* 50:101-103.
113. Zioudrou, C., Streaty, R. A. and Klee, W. A. 1979. Opioid peptides derived from food proteins. *J. Biol. Chem.* 254: 2446-2449.