培養基組成與液態培養條件對Rhizopus oligosporus發酵產物之理化性質的影響

張啟霆、陳明造

E-mail: 9608239@mail.dyu.edu.tw

摘 要

天貝（tempeh）為印尼的一種傳統醱酵食品，一般以蒸煮黃豆接種天貝菌Rhizopus oligosporus，以固態培養而成。本研究擬以3%、5%及10%單離大豆蛋白（含90%粗蛋白），分別添加20%的馬鈴薯萃取液（馬鈴薯：無菌水＝1：5）、0.5%可溶性澱粉、0.5% KH2PO4及0.25% MgSO4．7H2O作為基質培養Rhizopus oligosporus，並以125 rpm、150 rpm及175 rpm之不同轉速震盪液態培養，經醱酵24、36、48及60小時，比較各醱酵時間與培養基之代謝物成分，包括多醣減少程度、天貝菌數、澱粉?活性蛋白?活性、蛋白質水解率及γ-胺基丁酸（GABA）含量之差異性。由結果得知：添加5%單離大豆蛋白，於125 rpm轉速醱酵60小時有最高多醣減少程度85.12%；添加10%單離大豆蛋白，於125 rpm轉速醱酵48小時有最高的天貝菌數1.84×109CFU/ml，於150 rpm轉速醱酵60小時有最高的澱粉?、蛋白?及蛋白質水解率，分別為39.48U/ml、420.00U/ml及91.88%，於175 rpm轉速醱酵48小時有最高的GABA含量4.51 mg/ml。 綜合以上結果，建議添加10%大豆蛋白，以150 rpm轉速醱酵60小時為較好的發酵條件。

關鍵詞 : 天貝菌、單離大豆蛋白、液態培養、 γ-胺基丁酸（GABA）

目錄

目錄 封面內頁 簽名頁 授權書..... iii 中文摘要..... iv 英文摘要..... v 誌謝..... vi 目錄..... vii 圖目錄..... xiii 表目錄..... xv 1. 前言..... 1 2. 文獻回顧..... 3 2.1大豆蛋白..... 3 2.1.1大豆蛋白簡介..... 3 2.1.2大豆蛋白之組成..... 4 2.1.3大豆蛋白之胺基酸組成..... 6 2.1.4大豆蛋白之抗營養因子..... 9 2.2天貝..... 10 2.2.1天貝菌..... 10 2.2.2天貝之簡介..... 11 2.2.3天貝之液態培養優點..... 12 2.3天貝之機能性成分..... 12 2.3.1異黃酮..... 12 2.3.2 γ-胺基丁酸..... 13 2.3.3牛乳凝乳活性..... 16 2.4胺基酸..... 17 3.材料與方法..... 19 3.1實驗藥品與儀器..... 19 3.1.1材料..... 19 3.1.2藥品..... 19 3.1.3儀器..... 20 3.2實驗方法..... 21 3.2.1Rhizopus oligosporus培養..... 21 3.2.1.1菌種活化..... 21 3.2.1.2試驗處理..... 21 3.3化學成分分析..... 22 3.3.1水分測定..... 22 3.3.2pH值..... 23 3.3.3多醣..... 23 3.3.3.1多醣萃取液之製備..... 23 3.3.3.2多醣之測定..... 23 3.3.4總生菌數..... 24 3.3.5澱粉?活性..... 25 3.3.5.1澱粉?酵素液之製備..... 25 3.3.5.2澱粉?活性測定..... 25 3.3.6蛋白?活性..... 27 3.3.6.1蛋白?酵素液之製備..... 27 3.3.6.2蛋白?活性測定..... 27 3.3.7水解率..... 28 3.3.7.1水解率萃取液之製備..... 28 3.3.7.2水解率之測定..... 28 3.3.8GABA分析..... 29 3.3.8.1GABA之萃取及衍生..... 30 3.3.8.2GABA濃度之測定..... 30 3.3.8.3GABA之HPLC條件..... 31 3.4統計分析..... 32 4.結果與討論..... 33 4.1添加不同比例之單離大豆蛋白於不同震盪轉速對培養Rhizopus oligosporus之水份比較..... 33 4.1.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之水分比較.... 33 4.1.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之水分比較.... 35 4.1.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之水分比較.... 37 4.2添加不同比例之單離大豆蛋白於不同震盪轉速對 培養Rhizopus oligosporus之pH值比較..... 39 4.2.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之pH值比較.... 39 4.2.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之pH值比較.... 41 4.2.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之pH值比較.... 43 4.3添加不同比例之單離大豆蛋白於不同震盪轉速對培 養Rhizopus oligosporus之多醣減少程度比較... 45 4.3.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之多醣減少程度比較 45 4.3.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之多醣減少程度比較 47 4.3.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之多醣減少程度比較 49 4.4添加不同比例之單離大豆蛋白於不同震盪轉速對培 養Rhizopus oligosporus之天貝菌數比較..... 51 4.4.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之天貝菌數比較... 51 4.4.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之天貝菌數比較 53 4.4.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之天貝菌數比較 55 4.5添加不同比例之單離大豆蛋白於不同震盪

轉速對培 養Rhizopus oligosporus之澱粉?活性比較..... 57 4.5.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之澱粉?活性比較.. 57 4.5.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之澱粉?活性比較.. 59 4.5.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之澱粉?活性比較.. 61 4.6添加不同比例之單離大豆蛋白於不同震盪轉速對培 養Rhizopus oligosporus之蛋白?活性比較..... 64 4.6.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之蛋白?活性比較 64 4.6.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之蛋白?活性比較 66 4.6.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之蛋白?活性比較 68 4.7添加不同比例之單離大豆蛋白於不同震盪轉速對培 養Rhizopus oligosporus之蛋白質水解率比較 71 4.7.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之蛋白質水解率比較 71 4.7.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之蛋白質水解率比較 73 4.7.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之蛋白質水解率比較 75 4.8添加不同比例之單離大豆蛋白於不同震盪轉速對 培養Rhizopus oligosporus之GABA含量比較 78 4.8.1 添加不同比例之單離大豆蛋白在轉速125rpm震 盪培養Rhizopus oligosporus之GABA含量比較 78 4.8.2 添加不同比例之單離大豆蛋白在轉速150rpm震 盪培養Rhizopus oligosporus之GABA含量比較 80 4.8.3 添加不同比例之單離大豆蛋白在轉速175rpm震 盪培養Rhizopus oligosporus之GABA含量比較 82 5. 結論..... 85 參考文獻 87

參考文獻

1.王三郎。1994。應用微生物學。高立出版社。台北。 2.王三郎。2005。應用微生物學四版。高立出版社。台北。 3.田村哲彥。1993。健康醋大豆。正義出版社。台北。 4.朱燕華。1994。淺談大豆蛋白質及其應用。食品工業26（1）:15-18。 5.吳獻花、王樹坤。1999。植物性微生物發酵食品的研究進展。中國微生態學雜誌。11（2）:87-91。 6.徐德平、江漢湖。2005。天貝異黃酮生物活性增強的機理研究。食品與生物技術學報。24（3）:1673-1689。 7.財團法人生物技術發展中心。1989。胺基酸產業市場調查。台北。 8.凌美月、周正俊。1995。Aspergillus oryzae在不同擠壓醬油發酵基質上之生長及發酵生產。中國農業會誌。33:521-532。 9.陳仲仁。黃豆蛋白的組織、 功能特性及在食品上的應用。食品工業29（3）:19-28。 10.張南玲。1993。胃腸系統內γ-胺基丁酸研究的進展。國外醫學: 生理病理科學與臨床分冊。013（003）:139-142。 11.張為憲、李敏雄、呂政義、張永和、陳昭雄、孫璐西、陳怡宏、張基郁、顏國欽、林志城、林慶文。1995。食品化學。台北。 12.游芸悌。2003。以納豆菌生產生物性高分子之研究。私立大葉大學環境工程所碩士論文。彰化。 13.董大成。黃豆可比高麗參。1993。元氣齋出版社。台北。 14.蔡孟貞。2001。大豆蛋白之凝膠機制。食品工業月刊。33:32-38。 15.劉馨璘。2005。Bacillus subtilis var. natto及Rhizopus microsporus var. oligosporus混合發酵對去種皮黑豆機能性成分生成之影響。私立大葉大學生物產業科技所碩士論文。彰化。 16.鄭心怡。1993。磷脂質與健康。名望出版社。台北。 17.鄭斯元。2003。噴霧乾燥條件對鯖柴魚水解物胜?活性之影響。國立臺灣海洋大學食品科學所碩士論文。基隆。 18.Abe, Y., Umemura, S., Sugimotto, K., Hirawa, N., Kato, Y., Yokoyama, T., Iwai, J. and Ishii, M., 1995. Effect of green tea rich in γ-aminobutyric acid on blood pressure on dahl salt-sensitive rats. Am. J. Hypertens. 8: 74-79. 19.Aoki, H., Furuya, Y., Endo, Y. and Fujimoto, K., 2003. Effect of γ-aminobutyric acid-enriched tempeh-like fermented soybean (GABA-tempeh) on the blood pressure of spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. 67: 1806-1808. 20.AOAC, 1990. In: Helrich, E. (istyed.), Official Methods of Analysis of the Association of Official Chemists, (Fifteenth edition) Association of Official Chemists, Virginia. 21.Bailey, C. R., Arst, H. N. and Penfold, H. A. 1980. A third gene affecting GABA transaminase levels in Aspergillus nidulans. Gen. Res. 36: 167-180. 22.Bayley, H. S. and W. E. Carlson. 1970. Comparisons of simple and complex diets for baby pigs: Effects of form of feed and glucose addition. J. Anim. Sci. 30: 394-401. 23.Beizhong, H., Jeroen, L. K. and Rombouts, M. J. and Robert Nout. 1999. Solid-substrate fermentation of soybean with Rhizopus spp.: Comparison of dicontinuous rotation with stationary bed fermentation. J. Biosci. Bioeng. 88(2): 205-209. 24.Beizhong, H., Yong, M., Frans, M., Rombouts, M. J. and Robert, Nout. 2003. Effects of temperature and relative humidity on growth and enzyme production by Actinomucor elegans and Rhizopus oligosporus during sufu pehtze preparation. Food Chem. 81: 27-34. 25.Bernard Gibbs, B.F., Zougman A., Masse R., Mulligan C. 2004. Production and characterization of bioactive from soy hydrolysate and soy-fermented food. Food Res. Int. 37: 123-131. 26.Bertola, N. C., Califano, A. N., Bevilacqua, A. E. and Zaritzky, N. E. 2000. Effect of ripening conditions on the texture of Gounda cheese. Int. J. Food Sci. Tech. 35: 207-214. 27.Buckel, W. 1986. Substrate stereochemistry of the biotindependent sodium pump glutaconyl-CoA decarboxylase from Acidaminococcus fermentans. Eur. J. Biochem. 156: 259-263. 28.Cato, E. P., Johnson, J. L., Hash, D. E. and Holdeman, L. V. 1983. Synonymy of Peptococcus glycinophilus with Peptostreptococcus micros and electrophoretic differentiateon of Peptostreptococcus micros from Peptococcus magnus. Inst. J. Syst. Bacteriol. 33: 207-210. 29.Catsimpoolas, N. 1969. A note on proposal of an immunochemical system of reference and nomenclature for the major soybean globulin. Cereal Chem. 46: 369-372. 30.Church, F. C., Swaisgood, H. E., Porter, D. H. and Catignani, G. L. 1983. Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. J. Dairy Sci. 66: 1219-1227. 31.Dibois, M., Gilles, K. A., Hamilton, J. K., Reber, P. A. and Smith F. 1956. Colorimetric method for determineation of sugars and related substances. Analytical Chem. 28(3): 350-356. 32.Dreau, D., J. P. Lalles, V. Philouze, R. Toullec and H. Salmon. 1994. Local and systemic immune responses to soybean protein ingestion in early weaned pigs. J. Anim. Sci. 72: 2090-2098. 33.Durre, P., Spahr, R. and Andreesen, J. R. 1983. Glycine fermentation via glycine reductase in Peptococcus glycinophilus and Peptococcus magnus. Arch. Microbiol. 134: 127-135. 34.Egounlety, M. and Awoth, O. C. 2003. Effect of soaking, dehulling, cookingand fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna

unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). J. Food Eng. 56: 249-254. 35.Erdman, J. W. 2000. AHA Science Advisory: Soy protein and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee of the AHA. Circul. 102: 2555-2559. 36.Escobar, J. and Barnett, S. M. 1993. Effect of agitateon speed on the synthesis of Mucor miehei acid protease. Enzyme Microb. Tech. 15: 1009-1013. 37.FDA. 1992. Bacteriological Analytical Manual. Association of official chemists. Washington, D. C. 38.Feng, J. and Y. L. Xiong. 2002. Interaction of myofibrillar and preheated soy protein. J. Food Sci. 67: 2851-2856. 39.Feng, X. M., Anders R.B. Eriksson, Johan Schnurer. 2005. Growth of lactic acid bacteria and Rhizopus oligosporus during barley tempeh fermentation, International J. Food Micro. 104: 249-256. 40.Food and Drug Administration. 1999. Food labeling: health claims; soy protein and coronary heart disease. Fed Reg. 64: 57699-57733. 41.Fox, PF, Law, J., McSweeney, P. L. H. and Wallace, J. 1993. Biochemistry of cheese ripening. In: Fox PF, editor. Cheese Chemistry Physics and Microbiology, vol. II. London: Chapman & Hall. 389-438. 42.Friesen, K. G., J. L. Nelssen, R. D. Goodband, K. C. Behnke, and L. J. Kats. 1993. The effect of moist extrusion of soy products on growth performance and nutrient utilization in the early-weaned pig. J. Anim. Sci. 71: 2099-2109. 43.Fukushima, D. 1968. Internal structure of 7S and 11S globulin molecules in soybean proteins. Cereal Chem. 45: 203-207. 44.Garcia, M. C., M. Torre, M. L. Marina and F. Laborda. 1997. Composition and characterization of soybean and related products. CRC. Crit. Rev. Food Sci. Nutr. 37: 361-391. 45.Gyorgy, P., Murata, K. and Ikehata, H. 1964. Antioxidants isolated from Fermented Soybeans (Tempeh). Nature 203: 870-872. 46.Hachmeister, K. A. and Fung, D. Y. 1993. Tempeh: A mold-modified indigenous fermented food made from soybeans and/or cereal grains. Crit. Rev. Microbiol. 19 (3): 137-188. 47.Hayakawa, K., Kimura, M., Kasaha, K., Matsumoto, K., Sansawa, H. and Yamori, Y., 2004. Effect of a gamma-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. Br. J. Nutr. 92: 411-417. 48.Hermsnsson, A. M. 1986. Soy proteins gelation. J. Am. Oil. Chem. 63: 658-672. 49.Hesseltine, C. W., Smith, M., Bradle, R. and Djien, K. S. 1963. Investigations of tempeh an Indonesian food. Dev. Ind. Microbiol. (4): 275-287. 50.Hesseltine, C. W. 1965. A millennium of fungi food and fermentation. Mycol. 57: 149-197. 51.Hesseltine, C. W., Smith, M., Wang, H. L., 1967. New fermented cereal products. Devel. Ind. Micro. 8: 179-186. 52.Ibrahim, S. S., Habiba, R. A., Shatta, A. A. and Embaby, H. E. 2002. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. Nahrung. 46(2): 92-95. 53.Ikehata, H., Wakaizumi, M. and Murata, K. 1968. Antioxdant and antihemolytic activity of a new isoflavone "Factor 2" isolated from tempeh. J. Agric. Biol. Chem. 32(6): 740-746. 54.Ingram, D., Sanders, K., Kolybaba, M and Lopez, D. 1997. Case-control study of phytoestrogens and breast cancer. Lancet 350: 990-994. 55.Jennessen, J., Nielsen, K. F., Houbraken, J., Lyhne, E. K., Schnurer, J., Frisvad, J. C. and Samson, R. A. 2005. Secondary metabolite and mycotoxin production by the Rhizopus microsporus group. J. Agric. Food Chem. 53: 1833-1840. 56.Jones, D. L., Darrah, P. R., 1994. Amino-acid influx at the soil-root interface of Zea mays L. and its implications in the rhizosphere. Pla. Soi. 163: 1-12. 57.Kandeler, E., Luxhoi, J., Tscherko, D., Magid, J., 1999. Xylanase, invertase and protease at the soil-litter interface of a loamy sand. Soi. Bio. Biochem. 31: 1171-1179. 58.Kerley, S. J., Read, D. J., 1998. The biology of mycorrhiza in the Ericaceae XX. Plant and mycorrhizal necromass as nitrogenous substrates for the ericoid mycorrhizal fungus Hymenscyphus Ericae and its host. New Phytol. 139: 353-360. 59.Kielland, K., 1994. Amino acid absorption by arctic plants: Implications for plant nutrition and nitrogen cycling. Ecol. 75: 2372-2383. 60.Kilara, A. and Sharkasi. T. W 1986. Effect of temperature on food protein and its implications on functional properties. CRC. Crit. Rev. Food Sci. Nutri. 23: 323-395. 61.King, A. and Young, G. 1999. Characteristics and occurrence of phenolic phytochemicals. J. Am. Diet Assoc. 99: 213-218. 62.Ko, S. D. and Hesseltine, C. W. 1979. Tempe and related foods. In: Economic Microbiology, Vol. 4: Microbial Biomass (Ed. A H. Rose), London, Academic Press. pp. 115-140. 63.Kronenberg, H. J. 1984. Reduction of incubation time for tempeh fermentation by use of pregerminated inoculum. Econ. Bot. 38: 433-438. 64.Kruger, J. E. 1973. Change in the levels of proteolytic enzymes from hard red spring wheat during growth and maturation. Cereal Chem. 5: 122-131. 65.Kubicek, C. P., Hampel, W. and Rohr, M. 1979. Manganese deficiency leads to elevated amino acid pools in citric acid accumulating Aspergillus niger. Arch. Microbiol. 123: 73-79. 66.Liener, I. E. 1987. Detoxifying enzyme. In: R. D. King and P. S. J. Cheetham (Eds). Food Biotechnology. Elseyier Applied Science. London. pp.249-271. 67.Litchfield, J. H. 1967. Submerged culture of mushroom mycelium. In: Microbial. Technology (Peppler, H. J., ed), Reinhold, New York, pp. 107-144. 68.Liu, K. 1997. Fermented oriental soyfoods. In: Liu, K. (Ed.), Soybeans: Chemistry, Technology and Utilization, Chapman and Hall, New York, pp. 218-296. 69.Mahmoud, M. I. 1994. Physicochemical and functional properties of protein hydrolysates in nutritional product. Food Techno. 48: 89-95. 70.Medwid, R. D. and Grant, W. 1984. Gremination of Rhizopus oligosporus sporangiospores. Appl. Environ. Microbial. 48(6): 1067-1071. 71.Mital, B. K. and Garg, S. K. 1990. Tempeh-technology and food value. Food Rev. Int. (62): 213-224. 72.Messina, M. J., Persky, V., Setchell, K. D. R. and Barnes, S. 1994. Soybean and cancer risk: A review of the in vitro and in vivo data. Nutr. Cancer. 21: 113-131. 73.Messina, M. 1995. Modern uses for an ancient bean: Soy-food and disease. Chem. Ind. 11, 412-415. 74.Messina, M, J. 1999. Legumes and soybeans: Overview of their nutritional profiles and health effects. Am. J. Clin. Nutr. 70(3 Suppl): 439S-450S. 75.Nakamura, T., Matsubaysahi, T., Kamachi, K., Hasegawa, T., Ando, Y., Omori, M., 2000. γ -Aminobutyric acid (GABA)-rich chlorella depresses the elevation of blood pressure in spontaneously hypertensive rats (SHR). Nippon Nogeikagaku Kaishi 74: 907-909 (in Japanese). 76.Nanninga, H. J., Drent, W. J. and Gottschal, J. C. 1986. Major differences between glutamate-fermenting species isolated from chemostat enrichments at different diluteion rates. FEMS Microbiol Ecol. 38: 321-329. 77.Nasholm, T., Ekblad, A., Nordin, A., Giesler, R., Hogberg, M., Hogberg, P. 1998. Boreal forest plants take up organic nitrogen. Nature 392: 914-916. 78.Newby, T. J., B. Miller, C. R. Stokes and F. J. Bourne. 1985. Hypersensitivity to dietary antigen as the predisposeing factor in post-weaning diarrhoea. Pig Vet. Sci. Proc. 10: 50-58. 79.Newton, R. J., 1974. Dual uptake pattern of DL-leucine absorption by duckweed root tips. Pla. Cell Physio. 15: 249-254. 80.Nout, M. J. R. and Rombouts, F. M. 1990. Recent developments in Tempe research. J. Appl. Bacteriol. 69: 609-633. 81.Pedersen, H. E. 1995. Application of soya protein concentrated

in processed meat products. Fleishwirtsch. 75: 798-802. 82.Peng, I. C., Quass, D. W., Dayton, W. R., and Allen, C. E. 1984. The physicochemical and functional properties of soybean 11S Globulin-a review. Cereal Chem. 61(6): 480-490. 83.Ramarathnam, N., Ochi, H., and Takeuchi, M. 1997. Antioxidative Defense system in vegetable extracts. In: Natural Antioxidants. Editor: Shahidi, F. AOCS Press. 84.Rehms, H. and Barz, W. 1995. Degradation of stachyose, raffinose, melibiose and sucrose by different tempe-producing Rhizopus fungi. Appl. Microbiol. Biotechnol. 44: 47-52. 85.Rogers, A. H., Gully, N. J., Pfennig, A. L. and Zlim, P. S. 1992. The breakdown and utilization of peptides by strains of Fusobacterium nucleatem. Oral Microbiol. Immunol. 7: 299-303. 86.Santosh, K. and Narayan, S. P. 1997. The metabolism of γ -aminobutyrate(GABA) in fungi. Mycol. Res. 101: 403-409. 87.Sarker, P. K., Jones, L. J., Craven, G. S., Somerset, S. M. and Palmer, C. 1997. Amino acid profiles of kinema, a soybean-fermented food. Food Chem. 59: 69-75. 88.Seker, S., Beyenal, H. and Tanyolac, A. 1999. Modeling milk clotting activeity in the continuous production of microbial rennet from Mucor miehei. J. Food Sci. 64: 525-529. 89.Shizuka, F., Kido, Y., Nakazawa, T., Kitajima, H., Aizawa, C., Kayamura, H. and Ichijo, N. 2004. Antihypertensive effect of γ -aminobutyric acid enriched soy products in spontaneously hypertensive rats. Biofactors 22: 165-167. 90.Stams, A. J. M. and Hansen, T. A. 1984. Fermentation of glutamate and other compounds by Acidaminobacter hydrogenoformans gen. nov. sp. Nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate reduceing and methanogenic bacteria. Arch. Microbiol. 137: 329-337. 91.Staswick, P. E., M. A. Hermodson and N. C. Nielsn. 1984. Identification of the cysteine which link the acidic and basic components of the glycinin subunits. J. Biol. Chem. 259: 13431-13436. 92.Steinkraus, K. H., Yap, B. H., Van Buren, J. P., Provvidenti, M. J. and Hand, D. B. 1960. Studies on tempeh-an Indonesian fermented soybean food. Food Res. 25: 777-788. 93.Steinkraus, K. H., van Buren, J. P., Hackler, L. R. and Hand, D. B. 1965. A pilot-plant process for the production of dehydrated tempeh. Food Technol. 19: 63-68. 94.Sternberg, M. J. 1970. Crystalline milk clotting protease from Mucor miehei and some of its propertyes. J. Dairy Sci. 54: 159-167. 95.Tamura, Y. and Takenawa, T. 1999. Antioxidative activeity of water soluble extracts from okara fermented with Bacillus natto and Rhizopus oligosporus. Nippon Shokuhin Kogyo Gakaishi. 46: 561-569. 96.Tsuji, K., Ichikawa, T., Tanabe, N., Abe, S., Tarui, S., Nakagawa, Y., 1992. Antihypertensive activities of beni-koji extracts and γ -aminobutyric acid in spontaneously hypertensive rats. Eiyogaku Zasshi 50: 285-291 (in Japanese). 97.Turhan, M. and Mutlu, M. 1998. Kinetics of κ -casein/immobileized chymosin hydrolysis. Enzyme Microb. Tech. 22: 342-347. 98.Usmani, N. F. and Noorani, R. 1986. Studies on soybean tempeh. II. Propagation and preservation of Rhizopus oligosporus spores for commercial production of tempeh from soybean. J. Sci. Ind. Res. 29: 148-150. 99.Valeria Rossetti and Anna Lombard. 1996. Determination of glutamate decarboxylase by high-performance liquid chromatography. Chromato. B. 681: 63-67. 100.Vanharanta, M., Voutilainen, S., Lakka, T. A., van der Lee, M., Adlercreutz, H. and Salonen, J. T. 1999. Reduced risk of acute coronary events at high levels of mammalian lignan, enterolactone: A prospective population-based cohort study. Lancet 354: 2112-2115. 101.Vioque, M., Gomez, R., Sanchez, E., Mata, C. Tejada, L. and FernandezSalguero, J. 2000. Chemical and microbiological characteristics of ewe ' s milk cheese manufactured with extracts from flowers of Cynara cardunculus and Cynara humilis as coagulants. J. Agric. Food Chem. 48: 451-456. 102.Wadud, S., Kosar, S., Ara, H. and Durrani, H. 1988. A process for the pilot plant production of tempeh. Pakstan J. Sci. Res. 31: 435-438. 103.Wang, H. and Murphy, P. A. 1994a. Isoflavone compositeon of American and Japanese soybeans in lowa: effects of variety, crop year and locateon. J. Agr. Food Chem. 42: 1674-1677. 104.Wang, H. and Murphy, P. A. 1994b. Isoflavone content in commercial soybean foods. J. Agr. Food Chem. 42: 1666-1673. 105.Wang, H. L., Ruttle, D. I., and Hesseltine, C. W. 1969. Milk-clotting activeity of proteinases produced by Rhizopus. Can. J. Microbiol. 15: 99-104. 106.Watson, R., Fowden, L., 1975. The uptake of phenylalanine and tyrosine by seeding root tips. Phytochem. 15: 249-254. 107.Whiteley, H. R. 1957. Fermentation of amino acid by Micrococcus aerogenes. J. Bacteriol. 74: 324-330. 108.Wolf, W. J. 1970. Soy protein: Their functional, chemical, and physical properties. J. Agric. Food Chem. 18: 969-976. 109.Wuryani, W. 1995. Isoflavones in tempe. Asean. Food J. 10: 99-102. 110.Yagasaki, K., T. Toshio, S. Miyo and K. Keisuke. 1997. Biochemical characterization of soybean protein consisting of different subunits of glycinin. J. Agric. Food Chem. 45: 656-660. 111.Ziegler, G. R. and E. A. Foegeding. 1990. The gelation of proteins. Adv. Food Nutr. Res. 34: 203-298. 112.Zindel, U., Freudenberg, W., Rieth, M. Andreeseen, J. R., Schnell, J. and Widdel, F. 1988. Eubacterium acidaminophilum sp. Nov., a versatile amino acid-degrading anaerobe producing or utilizing H2 or formate. Description and enzymeatic studies. Arch. Microbiol. 150: 254-266.