

同時利用納豆菌及寡孢根黴菌發酵黃豆

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摘要

日本的納豆（natto）是以枯草芽孢桿菌納豆變種（*Bacillus subtilis* var. *natto*，簡稱*Bacillus natto*或納豆菌）接種於無脫皮的熟黃豆發酵製成的黏稠物。印尼的天貝（tempeh）是以小孢根黴菌寡孢變種（*Rhizopus microsporus* var. *oligosporus*，簡稱*Rhizopus oligosporus*或天貝菌）接種於脫皮的熟黃豆發酵所製成的糕餅狀食物。兩者均具有相同但又獨特的保健效果。雖然兩菌種的生長速率不同，但若能使兩菌株平衛生長（balanced growth）於無脫皮的熟黃豆中，當能成功製成兼具兩種特色的保健發酵食品。因此，本研究以熟黃豆為原料，以不同方式製備的納豆菌和天貝菌同時接種於黃豆基質上進行固態發酵(solid-state fermentation)，探討兩種菌可以同時平衛生長的條件，並以納豆菌數、天貝菌絲量(以葡萄糖胺glucosamine含量為基準)、氨氮含量及黃豆上方之頂空（headspace）氧氣含量的變化做為評估產品品質是否有改善及兩菌株是否能同時平衛生長的標準。在單獨納豆菌的發酵下，不管緊密覆蓋在發酵盤上之錫箔紙有無戳洞及接種液如何被製備，納豆菌數皆可達109 CFU/g，而且生長愈好，生成的氨氮量愈多；有戳洞組別的氨氮可達0.46%，是無戳洞組別的三倍。基於頂空氧氣含量、黃豆中菌數及葡萄糖胺含量的測量值，可以知道何時菌種開始積極生長。例如，不管有無戳洞，在單菌發酵中，納豆菌均在第4小時才開始積極生長，天貝菌則在第6至8小時才開始。而且，納豆菌比天貝菌生長快速。在單獨天貝菌的發酵下，以不同方式製備的天貝菌在黃豆發酵上會有不同的生長速率及遲滯時間，且最終的葡萄糖胺含量介於9.53至10.63 mg/g之間，幾乎無氨氮產生。無接種的熟黃豆在此時段中也不產生氨氮，因此，混合菌發酵黃豆中氨氮的產生主要來自納豆菌。在黃豆蒸煮30分鐘及發酵溫度37 °C的操作下，混合菌發酵可達最佳的效果，且產生平衡的生長；納豆菌數超過109 CFU/g、葡萄糖胺含量達12 mg/g以上、氨氮含量為0.38%。在以不同方式單獨製備納豆菌和天貝菌然後同時接種之發酵中，以馬鈴薯葡萄糖培養液(potato dextrose broth)培養之天貝菌液及以市售納豆菌粉同時接種於黃豆中的發酵為最佳，兩菌可平衛生長；經24小時戳洞發酵後，納豆菌數可達109 CFU/g、葡萄糖胺含量12 mg/g以上、氨氮含量較低（0.29%），並在不戳洞下於9小時內耗盡頂空氧氣。若用同樣培養基同時培養混合菌，再予接種於黃豆上，則以酵母精麥芽液(yeast malt broth)同時培養納豆菌及天貝菌的發酵最好，兩菌也可平衛生長；經24小時戳洞發酵後，最後納豆菌數可達109 CFU/g、葡萄糖胺含量12 mg/g以上、氨氮含量最低（0.13%），但在不戳洞下則於12小時內耗盡頂空氧氣。因此，混合菌發酵可以降低氨氮及促進天貝菌的生長。另外，錫箔紙有無戳洞顯著影響混合菌的生長及氨氮含量；有戳洞的發酵可以促進納豆菌及天貝菌的生長，但會有較多的氨氮產生。

關鍵詞：混合菌發酵；納豆菌；天貝菌；納豆；天貝；平衛生長

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參考文獻

1. 李麒。2002。納豆的營養與保健價值中國食物與營養。 (1) :48-49。 2. 徐速、梁金鐘。2004。益康納豆的研製.大豆通報。 (3) :18-20。 3. 徐德平、高霞、江漢湖、霍成凱。2002。天貝異黃酮對腫瘤的抑制效應。南京農業大學學報。25 (1) :97-101。 4. 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。 5. 張南玲。1993。胃腸系統內 -氨基丁酸研究的進展。國外醫學:生理病理科學與臨床分冊。013 (003) :139-142。 6. 黃卓治，辛志勳，張文重。1977。納豆菌之研究: 納豆菌培養條件之檢討。屏東農專學報18:69-75。 7. 黃志立。羅立新。楊汝德。納豆激?。2000。生命的化學。20 (2) :82-83。 8. 董大成。1994。黃豆可比高麗參。元氣齋出版社。 9. 錢玉春,董明盛,江漢湖。1998。丹貝異黃酮的抗氧化作用。南京農業大學學報。21 (2) :104-108。 10. 鄭心怡。1993。磷脂質與健康。名望出版社。 11. 劉瓊淑。1994。幾丁質機丁聚醣極其相關酵素之特性應用。食品工業。26 (1) :26-36。 12. 謝秋玲、郭勇。1999。納豆一種多功能食品。食品工業科技 20 (1) :71-72。 13. Abouzed, M. M. and Reddy, C. A. 1986. Direct fermentation of potato starch to ethanol by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 52 (5) :1955-1959. 14. Anthony, M. S., Clarkson, T. B. and Williams, J. K. 1998. Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am. J. Clin. Nutr.* 68 (6 Suppl) :1390S-1393S. 15. Aoki, H., Uda, I., Tagami, K., Furuya, Y., Endo, Y. and Fujimoto, K. 2003. The production of a new tempeh-like fermented soybean containing a high level of gamma-aminobutyric acid by anaerobic incubation with *Rhizopus*. *Biosci. Biotechnol. Biochem.* 67 (5) :1018-1023. 16. Aoki, H., Furuya, Y., Endo, Y. and Fujimoto, K. 2003. Effect of gamma-aminobutyric acid-enriched tempeh-like fermented soybean (GABA-Tempeh) on the blood pressure of spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem.* 67 (8) :1806-1808. 17. Bhushan, B. 2000. Production and characterization of a thermostable chitinase from a new alkalophilic *Bacillus* sp. BG-11. *J. Appl. Microbiol.* 88 (5) :800-808. 18. Birrer, G. Crowmick, A. and Gross, R. A. 1994. -Poly (glutamic acid) formation by *Bacillus licheniformis* 9945A: physiological and biochemical studies. *Int. J. Biol. Macromol.* (16) :265 – 275. 19. Brezezinski, A., Adlerceutz, R., Shaoul, A., Rosler, A., Shmueli, V., Tanos, V. and Schenker, J. G. 1997. Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause*, (4) :89 – 94. 20. Chibnall, A. C., Ress, M. W. and Richards, F. M. 1958. Structure of the polyglutamic acid from *Bacillus subtilis*. *Biochem. J.* 68 (1) :129-135. 21. Cody, R. M. 1989. Disdtribution of chitinase and chitobiase in *Bacillus*. *Current Microbiol.* 19:201-205. 22. Crouse, J. R., Morgan, T., Terry, J. G., Ellis, J., Vitolins, M. and Burke, G. L. 1999. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch. Intern. Med.* 159 (17) :2070-2076. 23. Desgranges, C., Vergoignan, C., Georges, M. and Durand, A. 1991. Biomass estimation in solid state fermentation. I. Manual biochemical methods. *Appl. Microbiol. Biotechnol.* 35:200-205. 24. Durand, A., Vergoignan, C. and Desgrangefes, C. 1996. Biomass estimation in solid state fermentation. In: Roussos, S., Lonsane, B.K., Raimbault, M., Viniegra-Gonzales, G. (Eds.), *Advances in Solid State Fermentation*, Kluwer Academic Publisher, Montpellier. 23 – 37. 25. Esaki, H., Onozaki, H., Kawakishi, S. and Osawa, T. 1996. New antioxidant isolated from tempeh. *J. Agric. Food Chem.* (44) :696-700. 26. Glancer, M., and Ban, S. N. 1989. Biodegradation of lignin from the Acid Hydrolysate of Cornstover by Selected Mixed Culture of Yeasts. *Process Biochem.* 24 (3) :109-113. 27. GYORGY, P., Murata, K. and Ikehata, H. 1964. Antioxidants isolated from Fermented Soybeans (Tempeh). *Nature*. 203:870-872. 28. 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. 29. Hattori, T., Ohishi, H., Yokota, T., Ohoami, H. and Watanabe, K. 1995a. Antioxidative effect of crude antioxidant preparation from soybean fermented by *Bacillus natto*. *Food Sci. Technol. Res.* 28 (1) :135-138. 30. Hattori, T., Ohishi, H., Yokota, T., Ohoami, H. and Watanabe, K. 1995b. Beneficial effect of crude antioxidant preparation from fermented soybean food on xanthine oxidase-hypoxanthine-induced foot-edema in rats. *Food Sci. Technol. Res.* 28 (2) :169-173. 31. Hesseltine, C. W., Smith, M., Bradle, R. and Djien, K. S. 1963. Investigations of tempeh an Indonesian food. *Dev. Ind. Microbiol.* (4) :275-287. 32. Hendrich, A. B., Zugaj, J. and Michalak, K. 2002. Biochanin A similarly influences the fluidity of liposomes formed from charged and zwitterionic lipids. *Cell Mol. Biol. Lett.* 7 (2) :284. 33. 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. 34. Ikehata, H., Wakaizumi, M. and Murata, K. 1968. Antioxidant and antihemolytic activity of a new isoflavone "Factor 2" isolated from tempeh. *J. Agric. Biol. Chem.* 32 (6) :740-746. 35. Iwai, K., Nakaya, N., Kawasaki, Y. and Matsue, H. 2002. Antioxidative functions of natto, a kind of fermented soybeans: effect on LDL oxidation and lipid metabolism in cholesterol-fed rats. *J. Agric. Food Chem.* 50 (12) :3597-601. 36. Iwai, K., Nakaya, N., Kawasaki, Y. and Matsue, H. 2002. Inhibitory effect of natto, a kind of fermented soybeans, on LDL oxidation in vitro. *J. Agric. Food Chem.* 50 (12) :3592-3596. 37. 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. 38. Jurus,

- A. M. and Sundberg, W. J. 1976. Penetration of Rhizopus oligosporus into Soybeans in Tempeh. *Appl. Environ. Microbiol.* 32 (2) :284-287. 39.
- Kaneki, M., Hodges, S. J., Hosoi, T., Fujiwara, S., Lyons, A., Crean, S. J., Ishida, N., Nakagawa, M., Takechi, M., Sano, Y., Mizuno, Y., Hoshino, S., Miyao, M., Inoue, S., Horiki, K., Shiraki, M., Ouchi, Y. and Orimo, H. 2001. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition.* 17 (4) :315-321. 40.
- Krajcovicova-Kudlackova, M., Blazicek, P., Kopcová, J., Bederová, A. and Babinska, K. 2000. Homocysteine levels in vegetarians versus omnivores. *Ann. Nutr. Metab.* 44 (3) :135-138. 41. Liem, I. T., Steinkraus, K. H. and Cronk, T. C. 1977. Production of vitamin B-12 in tempeh, a fermented soybean food. *Appl. Environ. Microbiol.* 34 (6) :773-776. 42. Liu, K. 1997. Fermented oriental soyfoods. In: Liu, K. (Ed.), *Soybeans: Chemistry, Technology and Utilization*, Chapman and Hall, New York, 218 – 296. 43. Matsuura, M. and Obata, A. 1993. s-Glucosidases from Soybeans Hydrolyze Daidzin and Genistin. *J. Food Sci.* 58 (1) :144-147. 44. Matsuo, M., Nakamura, N., Shidoji, Y., Muto, Y., Esaki, H. and Osawa, T. 1997. Antioxidative mechanism and apoptosis induction by 3-hydroxyanthranilic acid, an antioxidant in Indonesian food Tempeh, in the human hepatoma-derived cell line, HuH-7. *J. Nutr. Sci. Vitaminol. (Tokyo).* 43 (2) :249-259. 45. McGahren, W. J., Perkinson, G. A. and Growich, J. A. 1984. Chitosan by fermentation. *J. Process Biochem.* 19:88-90. 46. Medwid, R. D. and Grant, W. 1984. Germination of Rhizopus oligosporus sporangiospores. *Appl. Environ. Microbial.* 48 (6) :1067-1071. 47. Messina, M. J. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* 70 (3 Suppl) :439S-450S. 48. Mital, B. K. and Garg, S. K. 1990. Tempeh-technology and food value. *Food Rev. Int.* 6 (2) :213 – 224. 49. Molteni, A., Brizio-Molteni, L. and Persky, V. 1995. In vitro hormonal effects of soybean isoflavones. *J. Nutr.* 125 (3 Suppl) :751S-756S. 50. Mori, Y. 1990. Characterization of a Symbiotic Coculture of Clostridium thermohydrosulfuricum YM3 and Clostridium thermocellum YM4. *Appl Environ. Microbiol.* 56 (1) :37-42. 51. Murata, K., Ikehata, H. and Miyamoto, T. 1967. Studies on the nutritional value of tempeh. *J. Food Sci.* 32:580 – 585. 52. Naim, M., Gestetner, B., Zilkah, S., Birk, Y. and Bondi, A. 1974. Soybean isoflavones. Characterization, determination, and antifungal activity. *J. Agric. Food Chem.* 22 (5) :806-810. 53. Nout, M. J. R. and Rombouts, F. M. 1990. Recent developments in Tempe research. *J. Appl. Bacteriol.* 69:609-633. 54. Odunfa, S.A. 1986. Dawadawa in Legume-based Fermented Foods. CRC Press, Boca Raton, FL, USA. 173 – 189. 55. Okamoto, A., Hanagata, H., Kawamura, Y. and Yanagida, F. 1995. Anti-hypertensive substances in fermented soybean, natto. *Plant Foods Hum. Nutr.* 47 (1) :39-47. 56. Okamoto, A., Hanagata, H., Matsumoto, E., Kawamura, T., Koizumi Y. and Yanagida, F. 1995. Angiotensin I converting enzyme inhibitory activities of various fermented foods. *Biosci. Biotechnol. Biochem.* 59 (6) :1147 – 1149. 57. Onozawa, M., Fukuda, K., Ohtani, M., Akaza, H., Sugimura, T. and Wakabayashi, K. 1998. Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Jpn. J. Clin. Oncol.* 28 (6) :360-363. 58. Oppermann-Sanio, F. B. and Steinbuchel, A. 2002. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. *Naturwissenschaften.* 89 (1) :11-22. 59. Osawa, R. and Matsumoto, K. 1997. Digestion of staphylococcal enterotoxin by Bacillus natto. *Antonie Van Leeuwenhoek.* 71 (4) :307-311. 60. Paredes-Lopez, O. and Harry, G. I. 1989. Changes in selected chemical and antinutritional components during tempeh preparation using fresh and hardened common beans. *J. Food. Sci.* 54: 968-970. 61. Pratt, D. E. and Birac, P. M. 1979. Source of antioxidant activity of soybeans and soy products. *J. Food Sci.* (44) :1720-1722. 62. 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. 63. Roche, N., Venague, A., Desgranges, C., and Durand, A. 1993. Use of chitin measurement to estimate fungal biomass in solid state fermentation. *Biotechnol Adv.* 11:677-683. 64. Ruiz-Tera 'n, F. and Owens, J. D. 1996. Chemical and enzymic changes during the fermentation of bacteria-free soya bean tempe. *J. Sci. Food Agric.* 71: 523 – 530. 65. Sarkar, P. K., Tamang, J. P., Cook, P. E. and Owens, J. D. 1994. Kinema-a traditional soybean fermented food: proximate composition and microflora. *Food Microbiol.* 11:47 – 55. 66. Sarkar, P. K. and Tamang, J. P. 1995. Changes in the microbial profile and proximate composition during natural and controlled polymers into water-soluble low molecular weight fermentations of soybeans to produce kinema. *Food Microbiol.* 12:317 – 325. 67. Sato, T., Yamada, Y., Ohtani, Y., Mitsui, N., Murasawa, H. and Araki, S. 2001. Production of menaquinone (vitamin K2) -7 by *Bacillus subtilis*. *J. Ind. Microbiol. Biotechnol.* 62 (7) :2482-8. 68. Shelp, B. J., Bown, A. W. and McLean, M. D. 1999. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* 4 (11) :446-452. 69. Shin, I. L. and Van, Y. T. 2001. The production of poly- (-glutamic acid) from microorganisms and its various application. *Bioresour. Technol.* 79:207-225. 70. Shurtleff, W. and Aoyagi, A. 1979. The book of tempeh. Harper and Row, New York. 71. Song, T., Barua, K., Buseman, G. and Murphy, P. A. 1998. Soy isoflavone analysis: quality control and a new internal standard. *Am. J. Clin. Nutr.* 68 (6) :1474s-1479s. 72. Stanley, W. L., Watters, G. G., Chan, B. G. and Mercer, J. M. 1975. Lactase and other Enzymes Bound to Chitin with Glutaldehyde. *Biotech. Bioeng.* 17:315- 326. 73. Stein, K. 2000. FDA approves health claim labeling for foods containing soy protein. *J. Am. Diet. Assoc.* 100 (3) :292. 74. 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. 75. 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 Tech.* 19:63 – 68. 76. Sumi, H., Hamada, H., Tsushima, H., Mihara, H. and Muraki, H. 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto, a typical and popular soybean food in the Japanese diet. *J. Experientia.* 43 (20) :1110-1111. 77. Sumi, H., Hamada, H., Nakanishi, K. and Hiratani, H. 1990. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. *Acta. Haematol.* 84:139-43. 78. Sumi, H., Yatagai, C. and Wada, H. 1995. Effect of *Bacillus natto*-fermented product (BIOZYME) on blood alcohol, aldehyde concentrations after whisky drinking human volunteers, and acute toxicity of acetaldehyde in mice. *Arakorū Kenkyūto Yakubutsu Ison.* 30 (2) :69-79. 79. Sumi, H. 1997. Antibacterial Activity of *Bacillus natto*-Growth inhibition against *Escherichia coli* O157. *Bioindustry.* 14:47. 80. Suzuki, Y., Kondo, K., Ichise, H.,

Tsukamoto, Y., Urano, T. and Umemura, K. 2003. Dietary supplementation with fermented soybeans suppresses intimal thickening. *Life Sci.* 73 (10) :1289-98. 81. Tamura, M. 1989. Development of low-smelling natto. *Lifesci. In Japanese. Biotechnol.* (5) :104 – 108. 82. Tanegashimia, chixuko, and Isehizaki. 1979. Studies on volatile flavor components of commercial natto. *Mukogawa Joshi Daigak Kiyo Shokumotsuhen.* 27:37-38. 83. Thamthiankul, S., Suan-Ngay, S., Tantimavanich, S. and Panbangred, W. 2001. Chitinase from *Bacillus thuringiensis* subsp. *pakistani*. *Appl. Microbiol. Biotechnol.* 56 (3-4) :395-401. 84. Thorne, C. B., Gomez, C. G., Noyes, H. E. and Housewright, R. D. 1954 Production of glutamyl polypeptide by *Bacillus subtilis*. *J. Bacteriol.* 68:307-315. 85. Tonouti, A., Oka, H., Kurotaki, K. and Takeda, K. 2000. Isolation of *Bacillus subtilis* (natto) useful for the manufacture of natto. *Bull. Facult. In Japanese. Agric. Hirosaki Univ.* (3) :14 – 18. 86. Tsuchida, K., Mizushima, S., Toba, M. and Soda, K. 1999. Dietary soybeans. intake and bone mineral density among 995 middle-aged women in Yokohama. *J. Epidemiol.* (9) :14 – 19. 87. Vattem, D. A. and Shetty, K. 2002. Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. *Food Biotechnol.* 16 (3) :189 – 210. 88. Vattem, D. A., Lin, Y. T., Labbe, R. G. and Shetty, K. 2004. Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bioprocessing using the food grade fungus *Rhizopus oligosporus*. *Process Biochem.* 39:1939-1946. 89. Xu, X., Harris, K. S., Wang, H. J., Murphy, P. A. and Hendrich, S. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. *J. Nutr.* 125 (9) :2307-2315. 90. Wang, H. J., and Murohy, P. A. 1996. Mass balance study of isoflavones during soybean processing. *J. Agric. Food Chem.* 44 (8) :2377-2383. 91. Wuryani, W. 1995. Isoflavones in tempe. *Asean. Food J.* 10: 99 – 102. 92. Yamaguchi, M., Kakuda, H., Gao, Y. H. and Tsukamoto, Y. 2000. Prolonged intake of fermented soybean (natto) diets containing vitamin K2 (menaquinone-7) prevents bone loss in ovariectomized rats. *J. Bone Miner. Metab.* 18 (2) :71-6. 93. Yokota, T., Hattori, T., Ohishi, H., Hasegawa, K. and Watanabe, K. 1996. The effect of antioxidant-containing fraction from fermented soybean food on atherosclerosis development in cholesterol-fed rabbits. *Lebensm.-Wiss. U.-Technol.* 29:751-755. 94. Yuki, Y., Nakagawat, T., Fujitam, M., Asadaa, A., Nakanishik, K. and Katok, K. 1994. A sandwich enzyme-linked immunosorbent assay for Nattokinase. *Biosci. Biotech. Biochem.* 58 (2) :366-370. 95. Zheng, Z. and Shetty, K. 2000. Solid state production of polygalacturonase by *Lentinus edodes* using fruit processing wastes. *Process Biochem.* 35:825 – 830.