

Comparison on the Functional Component Contents in Fermented Dehulled Black Soybeans Inoculated with *Bacillus subtilis*

劉馨璘、陳明造 柯文慶

E-mail: 9417942@mail.dyu.edu.tw

ABSTRACT

Natto is a traditional fermented food in Japan which is made by fermenting steamed soybeans with *Bacillus subtilis*. It is characterized by unique viscous material and flavor. Tempe is a protein source of Indonesian diet, which is made by fermenting steamed dehulled soybeans inoculated with *Rhizopus oligosporus* and packed with banana leaf. The cotton-like hypha grow and make the fermented soybeans become pressed cake-form product with desirable flavor. In this study, the steamed dehulled black soybeans are used as raw material which is inoculated with *Bacillus subtilis* var. natto(Bsn, a commercial strain obtained from Japan), *Rhizopus microsporus* var. *oligosporus*(Rmo, a commercial strain obtained from Indonesia) individually or combination. Minitab 10 software is utilized to analyze the data to investigate the effects of organisms, inoculating order and temperature on the production of functional compounds. The incubation temperature : 40 °C and 42 °C for Bsn ; 37 °C and 40 °C for Rmo ; 40 °C for the combination of both. The chemical composition including moisture, crude protein, amino nitrogen, degree of hydrolysis ; functional compounds content including flavonoids, -PGA and Q10 ; antioxidant enzymes including GSH-Px and SOD activities, DPPH free radical scavenging ability ; protease and lipase activities and ACE inhibitory ability are determined and compared the different data among the treatments. The results are used to study the optimal condition-time, temperature and inoculating sequence for functional compound production. The results are as follows: The chemical composition of the products obtained from different strains did not change as temperature increased obviously. However, amino nitrogen increased to some extent with fermenting time increased. The production of functional components from individual strain fermented products are influenced by fermenting temperature and time. And the production of these components from both strains combination are influenced by inoculating sequence and fermenting time. GSH-Px and SOD activities of the products obtained from both the individuals and combination increased with fermenting time increased, and the removing rate of free radical also increased with fermenting time. The activities of protease and lipase are influenced by fermenting temperature, however, as compared to the combination fermentation protease and lipase activities and ACE inhibitory ability for the individual strain fermentation increased as time increased.

Keywords : fermented food, functional component, antioxidant activity , enzyme activity, angiotensin-converting enzyme

Table of Contents

第一章 緒言.....	1 第二章 文獻回顧.....	2 2.1 黑豆.....
豆.....	2 2.1.1 黑豆簡介.....	2 2.1.2 黑豆的醫療保健效果.....
果.....	2 2.2 納豆.....	4 2.2.1 納豆簡介.....
豆的醫療保健效果.....	4 2.3 天貝.....	7 2.3.1 天貝簡介.....
介.....	7 2.3.2 天貝的醫療保健效果.....	7 2.4 蛋白質水解.....
解.....	8 2.4.1 蛋白質水解物.....	8 2.4.2 蛋白質水解程
度.....	9 2.5 機能性成分.....	10 2.5.1 黃酮類化合物.....
10 2.5.2 超氧化物歧化.....	12 2.5.3 血管收縮素轉化.....	13 2.5.4 聚穀胺酸.....
酸.....	13 2.5.5 DPPH自由基清除能力測定.....	14 2.5.6
泛.....	15 第三章 實驗方法.....	17 3.1 材料.....
料.....	17 3.2 儀器設備.....	17 3.3 發酵菌種菌數計
算.....	17 3.4 發酵方法.....	18 3.5 萃取液製
備.....	19 3.6 化學組成分析.....	20 3.6.1 粗蛋白.....
白.....	20 3.6.2 水分.....	21 3.6.3 肽基態氮.....
3.6.4 水解率.....	22 3.7 機能性成分.....	23 3.7.1 類黃酮化合物定量分析.....
析.....	23 3.7.2 泛定量分析.....	24 3.7.3 -聚穀胺酸含量測定.....
25 3.8 超氧歧化活性測定.....	26 3.9 DPPH自由基清除能力測定.....	27 3.10 脲胱甘過氧化活性測定.....
甘過氧化活性測定.....	27 3.11 蛋白活性測定.....	28 3.12 解脂活性測定.....
定.....	29 3.13 血管收縮轉化抑制活性測定.....	30 3.14 統計分析與繪

圖.....	31 第四章 結果與討論.....	32 4.1 產品性
狀.....	32 4.2 一般化學組成.....	33 4.3 機能性成
分.....	46 4.4 麥胱甘.過氧化.、超氧歧化.活性與自由基清除率比較.....	60 4.5 蛋白.、解脂.活
性與血管收縮轉化.抑制活性比較.....	74 第五章 結論.....	88 參考文
獻.....	90	

REFERENCES

- 周祺鈞。2004。高生理活性黑豆及豆渣納豆最適加工條件之探討。私立中國文化大學生活應用科學研究所碩士論文。台北。姜淑繡。
- 2001。省產蘿蔔之抗氧化性研究。私立大葉大學食品工程研究所碩士論文。彰化。翁千惠。2004。利用酵素水解明膠及卵白蛋白製備抗高血壓勝。私立東海大學食品科學研究所碩士論文。台中。
- 郭士榮。1998。黑豆豆腐及豆漿加工中抗氧化力與異黃酮之改變。私立中國文化大學生活應用科學研究所碩士論文。台北。
- 郭淑姿。2003。黑豆納豆最適加工條件之探討。私立中國文化大學生活應用科學研究所碩士論文。台北。
- 彭德蘭。1999。烘烤黑豆對血脂膽固醇正常者低密度脂蛋白氧化敏感性之影響。私立中國文化大學生活應用科學研究所碩士論文。台北。
- 曾慶瀛、余哲仁、余哲禮。1993。烏豆脂氧化酵素之純化與特性之探討。中華生質能源學會會誌 12:171-178.
- 黃宗慶。1999。攝食草桿菌發酵大豆、黑豆及藝仁對老鼠血液 繖維蛋白水解活性、凝血作用之影響。私立靜宜大學食品營養研究所碩士論文。台中。
- 劉昌峰。2001。探討不同培養基組成對光合菌 *Rhodobacter sphaeroides* 產生 Coenzyme Q10 之研究。中央大學化工所碩士論文。桃園。
- 劉毓蕙。2004。水解蛋白的特性及應用。食品工業 30(3):19-24 - 91 - 11.
- 戴文禎、胡雪萍、趙璧玉。1996。黑豆的抗氧化物質含量。中華民國營養學會雜誌 21:210-216.
- 戴文禎。1997。黑豆萃取物之抗氧化效用。私立中國文化大學生活應用科學研究所碩士論文。台北。
- Ariyoshi, Y. 1993. Angiotensin-converting enzyme inhibitor derived from food protein. Trends Food Sci. Technol 4: 139- 144.
- Bernard, F. G. Alexandre, Z. Robert, M. and Catherine, M. 2004. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. Food Research Int. 37: 123-131.
- Cha, M. J. and Kim, I. H. 1996. Biochem. Biophys. Res. Commun 222: 619-625.
- Chan, K. M. Decker, E. A. and Means, W. J. 1993. Extraction and activity of carnosine , a naturally occurring antioxidant in beef muscle. J. Food Sci. 58: 1-4.
- Chen, H. M. Muramoto, K. Yamauchi, F. Fujimoto, K. and Nokihara, K. 1998. Antioxidative properties of histidinecontaining peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 46: 49-53.
- Chin-Wen Lin, Jeng-Huh Yang, and Lieh-Chi Su. 1997. The extraction and properties of superoxide dismutase from porcine blood. Meat Science 46: 303-312.
- Christel, Q. D. Bernard, G. Jacques, V. Thierry, D. Claude, B. Michel, L. Micheline, C. Jean-Claude, C. Francois, B. Francis, T. - 92 - 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacology. 72: 35- 42.
- Fasial, M. Schafhauser, D. Y. Garreis, K. A. Elsaye, E. L. and Peyre, J. F. 1999. Isolation and characterization of *Perkinsus marinus* proteases using bactracin-sepharose affinity chromatography. CBP Part B 123:417-426.
- Fischer, A. Bommarius, A. S. Drauz, K. and Wandrey, C. 1994. A novel approach to enzymatic peptide synthesis using highly solubilizing N -protecting groups of amino acid. Biocatalysis 8: 289-307.
- Francisco, J. Ustariz, A. L. Luis, A. and Mario, D. 2004. Fermentation of proteins for protease production by *Serratia marcescens*. Biochemical Engineering Journal 19:147-153.
- Frokjaer, S. 1994. Use of hydrolysates for protein supplementation. Food Technol. 48(10): 86- 88.
- Fujita, M. Nomura, K. Hong, K. Ito, T. Asada, A. and Nishiumura, S. 1993. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheeses Natto, a popular soybean fermented food in Japan. Biochemical and Biophysical Research 197(3): 1340-1347.
- Gill, I. L. Jorba, R.X. and Vulfson, E. N. 1996. Biologically active peptides and enzymatic approaches to their production. Enzyme Microbial Technol. 18: 162-183. - 93 - 26.
- Gulcin, I. Sat, I. G. Beydemir, S. Elmastas, M. and Kufrevioglu, O. I. 2004. Comparison of antioxidant activity of clove (*Eugenia caryophyllea* Thunb) buds and lavender (*Lavandula Stoechas* L.) . Food Chemistry 87: 393-400.
- Hachmeister, K. A. and Fung, D. Y. C. 1993. Tempeh: a moldmodified indigenous fermented food made from soybeans and / or cereal grains. Critical Reviews in Microbiology 19: 137-188.
- Hertog, M. G. L. Hollman, P. C. H. Katan, M. B. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J. Agric. Food Chem. 40:2379.
- Hideo, E. Hiromichi, O. Shunro, K. and Toshihiko, O. 1999. New antioxidant isolated from Tempeh. J. Agric. Food Chem 44: 696- 700.
- Hosoi, T. Ametani, A. Kiuchi, K. and Kaminogawa, S. 1996. Changes on fecal microflora induced by incubation of mice with *bacillus subtilis* (Natto) spores are dependent upon dietary components. Can. J. Microbiology. 45: 59-66.
- Iyengar, R. and McEvily, A. J. 1992. Anti-browning agents: Alternatives to the use of sulfites in foods. Trends Food Sci. Technol. 3: 60- 64.
- Jens, A. N. 1986. Enzymic hydrolysis of food proteins. Novo Industri A/S, Bagsvaerd, Denmark.
- Kanno, A. and Takamatsu, H. 1995. Determination of - 94 - Polyglutamic acid in Natto using cetyltrimethylammonium bromide. Nippon Shokuhin Kagaku Kaishi 42(11): 878-887.
- Kequan, Z. and Liangli, Yu. 2004. Effect of extraction solvent on wheat bran antioxidant activity estimation. Lebensm-Wiss . U-Technol. 37:717-721.
- Kitts, D. D. and Yuan, Y. V. 1992. Caseinophospho-peptides and calcium bioavailability. Trends Food Sci. Technol. 3: 31- 35.
- Lahl, W. J. and Braun, S. D. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48(10): 68- 71.
- Margaret, M. M. Hans, M. and Richard, J. F. 1997. Angiotensin-I-converting enzyme inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. Int. Dairy Journal 7: 299-303.
- Maruo, B. and Yoshikawa, H. 1989. Industrial application of *B subtilis*. In Topics in secondary metabolism Bacillus subtilis: Molecular biology and industrial application. Maruo, B. and Yoshikawa, H.(Eds.). Elsevier Science Publishing Co. Inc, New York, USA, 143-161.
- Matsuura, M. Sasaki, J. and Sawao, M. 1995. Studies on -glucosidases from soybeans that hydrolyze daidzin and genistin: isolation and characterization of an isozyme. Biosci. Biotech. Biochem. 59: 1623-1627.
- Meisel, H.

and Schlimme, E. 1990. Milk proteins: precursors of bioactive peptides. *Trends Food Sci. Technol.* 2: 41-43. - 95 - 41. Merkler, D. J. 1994. C-terminal amidated peptides: Production by in vitro enzymatic amidation of glycine-extended peptides and the importance of the amide to bioactivity. *Enz. Microbiol. Technol.* 16: 450- 456. 42. Messina, M. and Messina, V. 1991. Increasing use of soyfoods and their potential role in cancer prevention. *J. Am. Diet. Assoc.* 91:836-840. 43. Meyers, S. A. Cuppett, S. L. and Hutkins, R. W. 1996. Lipase production by lactic acid bacteria and activity on butter oil. *Food Microbiology* 13: 383-389. 44. Mill, E. N. C. Alcocer, M. J. C. and Morgan, M. R. A. 1992. Biochemical interactions of food derived peptides. *Trends Food Sci. Technol.* 3:64-68. 45. Muramatsu, K. Nagai, T. Sato, S. Ochiai, Y. Ishimura, N. Ito, Y. and Kiuchi, K. 1997. Stimulative effect of phytone on the production of sticky materials in *Bacillus subtilis*(natto). *Nippon Shokuhin Kagaku Kaishi* 44: 812-815. 46. Nagai, T. Nishimura, K. Suzuki, H. Banba, Y. Sasaki, H. and Kiuchi, K. 1994. Isolation and characterization of *Bacillus subtilis* strain producing natto with strong umami-taste and high viscosity. *Nippon Shoukuhin Kogyo Gakkaishi*. 42: 338- 343. 47. Nelson, D. L. and Cox, M. M. 2000. *Lehninger Principles of Biochemistry*. Worth Publ. 3: 659-672. - 96 - 48. Ogawa, Y. Yamaguchi, F. Yuasa, K. and Thara, Y. 1998. Efficient production of γ -polyglutamic acid by *Bacillus subtilis* (natto) in jar fermenters. *Biosci. Biotechnol. Biochem.* 61:1684-1687. 49. Ohta, T. 1986. Natto. In: Reddy, N. R., Pierson, M. D. and Salunkhe, D. K. (Eds.). *Legume-based fermented foods*. CRC Press, Boca Roton, FL, USA, 85-93. 50. Okamoto, A. Hanagata, H. Kawamura, Y. and Yanagide, F. 1995. Angiotensin converting enzyme inhibitory activities of various fermented foods. *Biosci. Biotechnol. Biochem.* 59: 1147-1149. 51. Penas, E. Prestamo, G. and Gomez, R. 2004. High pressure and the enzymatic hydrolysis of soybean whey proteins. *Food Chemistry* 85: 641-648. 52. Peter, P. 1995. Production , regulation and some properties of lipase activity from *Fusarium oxysporum* F. sp. *vasinfectum*. *Enzyme and Microbial Technology* 17: 832-838. 53. Reena, R. Dhiraj, V. and Kalidas, S. 2004. Solid-state bioconversion of fava bean by *Rhizopus oligosporus* for enrichment of phenolic antioxidants and L-DOPA. *Innovative Food Science & Emerging Technologies* 5:235-244. 54. Roberto, M. L. Gabriella, C. Simon, M. C. and Martin, J. W. 2001. *Bacillus subtilis* spores competitively exclude *Escherichia coli* O78:K80 in poultry. *Veterinary Microbiology* 79: 133-142. - 97 - 55. Sasikala, C. and Ramana, C. V. 1995. Biotechnological potentials of anoxygenic phototrophic bacteria.I. Production of single-cell protein, vitamins, ubiquinones, hormones and enzymes and use in waste treatment. *Adv. Appl. Microbiol.* 41:173-226. 56. Saydam, N. Kirb, A. Demir, O. Hazan, E. Oto, O. Saydam, O. and Guner, G. 1997. Determination of glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase levels in human lung cancer tissues. *Cancer Letters* 119: 13-19. 57. Schagger, H. and Jagow, G. 1987. Tricine-sodium dodecyl- sulfatepolyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* 166: 378- 379. 58. Schauer, K. H. Nielsen, A. H. Dantzer, V. and Poulsen, K. 2001. Angiotensin-converting enzyme activity in the bovine uteroplacental unit changes in relation to the cycle and pregnancy. *Placenta* 22: 852-862. 59. Sumi H., Yatagai C. and Sumi A. 1999. Superoxide radical scavenging enzymes detected in the fermented soybean natto. *J. Brew. Sci. Japan* 94: 1016-1018. 60. Synder, H. E. and Kwon, T. W. 1987. *Soybean Utilization*. AVI Book, New York. 61. Spellman, D. McEvoy, E. Cuinn, O. G. and FitzGerald, R. J. 2003. Proteinase and exopeptidase hydrolysis of whey protein : Comparison of the TNBS, OPA and pH stat methods of quantification of degree of hydrolysis. *International Dairy Journal* - 98 - 13: 447-453. 62. Tamura, Y. and Takenawa, T. 1999. Antioxidative activity of water soluble extracts from okara fermented with *Bacillus natto* and *Rizopus oligosporus*. *Nippon Shokuhin Kogyo Gakkaishi* 46: 561- 569. 63. Ursini, F. Maiorino, R. Brigelius-Flohe, Aumann, K. D. Roveri, A. Schomburg, D., and Flohe, L. 1995. In *Enzyme* 252: 38-53. 64. Wang, H. and Murphy, P. A. 1994. Isoflavone content in commercial soybean food. *J. Agric. Food Chem.* 42:1666-1673. 65. Wang, H. J. and Murphy, P. A. 1996. Mass balance study of isoflavones during soybean processing . *J. Agric. Food Chem.* 44: 2377-2383. 66. Wei, Q. Wolf-Hall, C. and Chang, K. C. 2001. Natto characteristics as affected by streaming time, *Bacillus* Strain , and fermentation time. *J. food Sci.* 66: 167- 173. 67. Wei, H. Bonald, B. Cai, Q. Barnes, S. and Wang, Y. 1995. Antioxidant and antipromotional effect of soybean isoflavone genistein. *PSEBM* 208: 124-130.62. 68. Williams, W. B., Cuvelier, M. E., and Berest, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm-Wiss. Technol.* 28(1): 25- 30. 69. Yamaguchi, M. Taguchi, H. Gao, Y. Igarashi, A. and Yoshinori, T. 2000. Prolonged intake of fermented soybean(Natto) diet containing vitaminK2(menaquinone-7) prevents bone loss in - 99 - ovariectomized rats. *Journal of Bone and Mineral Metabolism* 18 : 71-76. 70. Yokota, T. Hattori, T. Ohishi, H. Hasegawa, K. and Watanabe, K. 2002. Antioxidative functions of natto, a kind of fermented soybean: Effect on LDL oxidation and lipid metabolism in cholesterol-fed rats . *J. Agric. Food Chem* 50: 3597-3601.