Study on Enzymatic Activity of Pectinase for Degrading Methoxyl-Pectin

蔡佩貞、張耀南

E-mail: 9509698@mail.dyu.edu.tw

ABSTRACT

In this study, pectinase (pectolytic enzymes; PLEs including pectinlyase, polygalacturonase, pectinesterase) was used to study its enzymatic activity for degrading methoxyl-pectin. For both high and low methoxyl-group pectins (HMGP and LMGP, respectively), the optimal reaction temperature and time for PLEs was 60°C and first 30 min, respectively. For HMGP and LMGP, the optimal reaction pH of PLEs was 5.0 and 4.0, respectively. The enzymatic activity of PLEs decreased significantly when the reaction pH was above pH 6.0 and the temperature was above 70°C. This result may be due to its acidity and denaturation. For the stability test, the optimal temperature of PLEs thermostability was at 30~40°C, while the PLEs was stable at the pH range of the buffer solution from 3.0 to 5.0 at room temperature and its activity retained about 80~90% after 120 min incubation at this temperature. The storage temperature (4°C and 30°C) of PLEs was also investigated. PLEs still conserved about 80% activity during 7 days at 4°C, but the activity of PLEs after 4-day storage at 30°C decreased significantly from about 95% to below 40%. For HMGP and LMGP gels, the Km and Vm constants of PLEs were 0.16mg/mL HMGP gel and 2.31mg/mL reducing sugar per min and 1.53mg/mL LMGP gel and 7.71mg/mL reducing sugar per min, respectively.

Keywords : pectinase, pectin, enzymatic activity, reducing sugar

Table of Contents

REFERENCES

中文部分 1. 王姿惠。2001。益生菌於含果寡糖之培養環境中生長情形之探討:23-26。中興大學碩士論文。台中。 2. 李穎儒。1994。機能性寡糖應用於畜牧生產上。飼料營養雜誌12:89-92。 3. 周君蘭。1997。健康新主張—富力得健康活寡醣。食品資訊141:57-58。 4. 林瑩禎。1996。保健食品之簡介。食品工業28(11):31-36。 5. 林正彥。1976。果膠。食品工業8(4):18-22。 6. 林欣榜。1995。植物多醣體之應用—果膠。食品工業27(11):46-55。 7. 吳祝如。1994。果膠質及其在食品之應用。食品資訊1:32-34。 8. 香紅星、董仲華、劉亞力。2001。

功能性寡糖的研究應用進展(上)。飼料研究7:9-11。 9. 翁志華。1996。果膠的基本特性與應用。食品資訊122:24-30。 10. 許清森。1991。利用酵素合成法由蔗糖生產低聚果糖之研究:20-25。台灣大學博士論文。台北。 11. 張珍田。2001。木寡糖之特性與生理功能。食品工業 33(6):10-18。 12. 張振忠。1993。高壓下高甲氧基果膠之凝膠形成與品質特性:4-5。中興大學碩士論文。台中。 13. 郭文怡。1995。漫談果膠類產品—果醬、果凍及果糕。烘焙科學60:13-16。 14. 陳怡芳。2005。篩選分解蒟蒻菌株與其發酵液之酵素活性探討:52。大葉大學碩士論文。彰化。 15. 黃怡雯。2001。以纖維素水解?幾丁聚醣所得之水溶性產物對腸內細菌組成之影響:20。海洋大學碩士論文。基隆。 16. 裘仲元。1996。高壓下高甲氧基果膠凝膠作用中機能性代糖使用的研究:24-25。中興大學碩士論文。台中。 17. 羅淑卿、林木連。2001。認識果膠。農業試驗所技術服務45:30-33。 18. 譚靜芬。1999。機能性寡糖之介紹。食品工業31(7):1-8。 19. 譚靜芬。1992。利用酵素合成法由蔗糖生產巴拉金糖之研究:23。台灣大學碩士論文。台北。 20. 川端晶子。1990。植物多醣體　構造　機能。食品科學。pp.111-122。朝倉書局。東京。日本。 21. 草地道一。1984。食品工業　應用。食品工業11(2):44-55。 22. 黃海三雄。1988。食品　應用　技術的諸問題。食品工業3(2):59-65。 23. 渡邊浩、岩瀨弘士郎。1994。利用。食品　開發29(2):17-19。 24. 機能性甘味料　市場動向。1997。食品　開發32(11):39-44。 英文部分 1. Acuna-Arguelles, M. E., Gutierrez-Rajas, M., Viniegra-Gonzalez, G. and Favela-Toress, E. 1995. Production and properties of three pectinolytic activities produced by A. niger in submerged and solid state fermentaion. Appl. Microbiol. Biotechnol. 43:808-814. 2. Alana, A., Alkorta, I., Dominguez, J. B., Llama, M. J. and Serra, J. L. 1990. Pectin lyase activity in a Penicillium italicum strain. Appl. Environ. Microbiol. 56(12): 3755-3759. 3. Al-Obaidi, Z. S., Aziz, G. M. and Al-Bakir, A. Y. 1987. Screening of fungal strains for polygalacturonase production. J. Agric. Water Resour. Res. 6:125-182. 4. Arcamone, F., Cassinelli, G., Ferni, G., Penco, S., Pennella, P. and Pol, C. 1970. Ergotamine production and metabolism of Claviceps purpurea strain 275 FI in stirred fermenters. Can. J. Microbiol. 16:923-931. 5. Barfod, N. M. and Pedersen, K. S. 1990. Determining the setting temperature of high-methoxyl pectin gels. Food Technol. 44:139. 6. Bazzarre, T. L., Wu, S. L. and Yubes, J. A. 1983. Total and HDL- cholesterol concentration following yogurt and calcium supplementation. Nutr. Rep. Int. 28:1225-1232. 7. Bealing, F. J. and Bacon, J. S. D. 1953. The action of mold enzymes on sucrose. Biochem. J. 53:277-285. 8. Be Miller, J. N. 1986. An introduction to pectins: Structure and properties. In: Fishman, M. L., Jem, J. J. (Eds.), Chemistry and Functions of Pectin, ACS Symposium Series 310. American Chemical Society, Washington, DC. 9. Bernal, V. M., Smajda, J. L., Smith, J. L. and Stanley, D.W. 1987. Interactions in protein/polysaccharide/calcium gels. J. Food Sci. 52:112. 10. Bohdziewiez, J. and Bodzek, M. 1994. Ultrafiltration preparation of pectinolytic enzymes from citric acid fermentation broth. Proc. Biochem. 29:99-107. 11. Borin, M. D. F., Said, S. and Fonseca, M. J. V. 1996. Purification and biochemical characterization of an extracellular endo- polygalacturonase from Penicillium frequentans. J. Agric. Food Chem. 44:1616-1620. Brawman, J. W. 1981. Application of enzymes in fruit juice tech- nology. In: Brich, G. G., Blakcorough, N. and Barker, J. K. (Eds.), Enzymes and food processing. p. 247-261. Applied Science, London. 12. Bruhlmann, F., Kim, K. S., Zimmerman, W. and Fiechter, A. 1994. Pectinolytic enzymes from actinomycetes for the degumming of ramie bast fibers. Appl. Environ. Microbiol. 60(6):2107-2112. 13. Cao, J., Zheng, L. and Chen, S. 1992. Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of rammie. Enz. Microbiol. Technol. 14:1013-1016. 14. Carbonell, E., Costell, E. and Duran, L. 1991. Fruit content influence on gel strength of strawberry and peach jams. J. Food Sci. 56:1384. 15. Channe, P. S. and Shewal, J.G. 1995. Pectinase production by Sclerotium rolfsii: Effect of culture conditions. Folia Microbiol. 40:111-117. 16. Charley, V. L. S. 1969. Advances in food processing using pectic and other enzyme. Chem. Ind. 20:635-641. 17. Chesson, A. and Codner, R. C. 1978. Maceration of vegetable by a strain of Bacillus subtilis. J. Appl. Bacteriol. 44:347-364. 18. Christensen, S. H. 1986. Pectins. In Glicksman, M. (ed.), food Hydrocolloids, Vol. III. pp.205-212. CRC Press, Inc., Florida. 19. Darvill, A., Augur, C., Bergmann, C., Carlson, R. W., Cheong, J. J., Eberhard, S., Hahn, M. G., L and oacute, V. M., Marf and agrave, V., Meyer, B., Mohnen, D., O ’ Neill, M. A., Spiro, M. D., van Halbeek, H., York, W. S. and Albersheim, P. 1992. Oligosaccharins- oligo- saccharides that regulate growth, development and defence responses in plants. Glycobiology 2:181-198. 20. Dave, B. A. and Vaughn, R. H. 1971. Purification and properties of a polygalacturonic acid trans-eliminase produced by Bacillus pumilus. J. Bacteriol. 108:166-174. 21. Deuel, H. and Stutz, E. 1958. Pectic substances and pectic enzyme. Advances in Enzymology 20:341. 22. Dickerson, A. G. 1972. β-D-fructofuranosidase from Claviceps purpurea. Biochem. J. 129:263-272. 23. Duan, K. J., Chen, J. S. and Sheu, D. C. 1994. Kinetic studies and mathematical model for enzymatic production of fructooligo-saccharides from sucrose. Enzyme Microb. Technol. 16:334-339. 25. Dumville, J. C. and Fry, S. C. 2000. Uronic acid-containing oligo- saccharins: Their biosynthesis,degradation and signaling roles in non-diseased plant tissues. Plant Physiology and Biochemistry. 38(12):125-140. 26. Fogarty, M. V. and Kelly, C. T. 1983. Pectic enzymes. In: Fogarty, M. W. (Ed.), Microbial Enzymes and Biotechnology. p.131-182.Applied Science Publishers, London. 27. Fogarty, W. M. 1973. Bacteria, enzymes and wood permeability. Proc. Biochem. 8:30-34. 28. Fujita, K., Hara, K., Hashimoto, H. and Kitahara, S. 1990. Purification and some properties of β-fructofuranosidase from Arthrobacter sp. K-1. Agric. Biol. Chem. 54:913-919. 29. Glicksman, M. 1969. Pectins. In Glicksman, M. (ed.), Gum Technology in the Food Industry. p. 159-190. Academic Press, New York. 30. Gummadi, S. N. 2003. Purification and biochemical properties of microbial pectinases – a review. Process biochemistry. 38:987-996. 31. Gupta, A. K. and Bhatia, I. S. 1982. Glucofructosan biosynthesis in Fusarium oxysporum regulation and substrate specificity of fructosyl transferase and invertase. Phytochemistry 21:1249-1253. 32. Hankin, L. and Meintype, J. L. 1977. Production of kestose(fru- ctosyl sucroses) by phytophthora parasitica var. nicotianae. Appl. Environ. Microbial. 33:522-524. 33. Hata, Y., Hara, T., Oikawa, T., Yamamoto, M., Hirose, N., Nagashima, T., Torihama, N., Nakajima, K., Watabe, A., and Yamashita, M. 1983. The effects of fructooligosaccharides against hyperlipidemia. Geriatr. Med. 21:156-167. 34. Hayashi, S., Imada, K., Kushima, Y. and Ueno, H. 1989. Observation of the chemical structure of fructooligosaccharide produced by an enzyme from Aureobasidium sp. ATCC 20524. Curr. Microbiol. 19:175-177. 35. Hayashi, S., Nonokuchi, M., Imada, K. and Ueno, H.

1990. Production of fructosyl-tranferring enzyme by Aureobasidium sp. ATCC 20524. J. Ind. Microbiol. 5:395-400. 36. Hayashi, S., Ito, K., Nonoguchi, M., Takasaki, Y. and Imada, K. 1991. Immobilization of a fructosyl-transferring enzyme from Aureobasidium pullulans sp. on Shirasu porous glass. J. Ferment. Bioeng. 72:68-70. 37. Hershonhorn, J., Manulis, S. and Barash, I. 1990. Poly- galacturonase associated with infection of Valencia orange by Penicillium italicum . Phytopathology 80:1374-1376. 38. Hidaka, H. 1985. The role of intestinal flora in nutrition. Biseibutsu(Microbe)1:32-40. 39. Hidaka, H., Hirayama, M. and Sumi, N. 1988. A fructooligo- saccharide producing enzyme from Aspergillus niger ATCC 20611. Agric. Biol. Chem. 52:1181-1187. 40. Hidaka, H., Eida, T. and Saitoh, Y. 1989. Industrial production of fructooligosaccharides and its application for human and animals. Nippon Nogeikagaku Kaishi. 61:915-923. 41. Hirota, T. 1990. The health maintenance effects of lactic acid bacteria being used in milk products. New Food Ind. 32(10):9-12. 42. Horikoshi, K. 1990. Enzymes of alkalophiles. In: Fogarty, W. M., Kelly, C. T. (Eds.), Microbial Enzymes and Biotechnology, second ed. p. 275-294. Elsevier Applied Science, London. 43. Hughes, J. B. and Hoover, D. G. 1991. Bifidobacteria: Their potential for use in American dairy products. Food Technol. 45:74-83. 44. Ikeda, T., Kurita, T., Hidaka, H., Michalek, S. M. and Hirasawa, M. 1990. Low-cariogenicity of the tetrasaccharide nystose. Gen. Pharmacol. 21:9-175. 45. Ishii, S. and Yokotshuka, T. 1971. Maceration of plant tissues by pectin transeliminase. Agricultural and Biological Chemistry 35:1157-1162. 46. Joseph, G. H. and Baier, W. E. 1949. Methods of determining the firmness and setting time of pectin test jellies, Food Technology 3:18-21. 47. Joslyn, N. A., Mist, S. and Lambart, E. 1952. Clarification of apple juice by fungal PG preparations. Food Technol. 6:133-139. 48. Jung, K. H., Lim, J. Y., Yoo, S. J., Lee, J. H. and Yoo, M. Y. 1987. Production of fructosyltransferase from Aureobasidium pullulans. Biotechnol. Lett. 9:703-708. 49. Jung, K. H., Yun, J. W., Kang, K. R., Lim, J. Y. and Lee, J. H. 1989. Mathematical model for enzymatic production of fructooligo- saccharides from sucrose. Enzyme Microb. Technol. 11:491-494. 50. Kanbe, M. 1992. Chp.17: Cancer control and fermented milk. p. 377. Nakazawa and Hosono. 51. Karbassi, A. and Vaughn, R. H. 1980. Purification and properties of polygalacturonic acid trans-eliminase from Bacillus stearothermophilus. Can. J. Microbiol. 26:377-384. 52. Kashyap, D. R., Chandra, S., Kaul, A. and Tewari, R. 2000. Production purification and characterization of pectinase from a Bacillus sp. DT7. World J. Microbiol. Biotechnol. 16:277-282. 53. Kashyap, D. R., Vohra, P. K., Chopra, S. and Tewari, R. 2001. Applications of pectinases in the commercial sector: a review. Bioresour Technol. 77:216-285. 54. Kawabata, A., Sawayama, S., Nakahara, H. and Kamata, T. 1981. Mechanism of association of various demethylated pectins by calcium ions. Agricultural and Biological Chemistry 45(4):965-974. 55. Keijbets, M. J. H. and Pilnik, W. 1974. β-elimination of pectin in the presence of anions and cations. Carbohydrate Research 33:359-365. 56. Kida, M., Yoshikawa, T., Senda, T. and Yoshihiro, Y. 1988. Formation of fructooligosaccharides from sucrose catalyzed by immobilized β-fructofuranosidase originated from Aspergillus oryzae. Nippon Kagaku Kashi. 11:1830-1835. 57. Kilara, A. 1982. Enzymes and their uses in the processed apple industry: A Review. Proc. Biochem. 23:35-41. 58. Kramer, K. J. and Muthukrishnan, S. 1997. Insect chitinases: Molecular biology and potential use as biopesticides. Insect Biochemistry and Molecular Biology. 27(11):887-900. 59. Lang, C. and Dornenburg, H. 2000. Perspectives in the biological function and the technological application of polygalacturonases. Appl. Microbiol. Biotechnol. 53:366-375. 60. Lopez, A. and Li, L. H. 1968. Low-methoxyl pectin apple gels. J. Food Technol. 22:91. 61. Magro, P., Varvaro, L., Chilosi, G., Avanzo, C. and Balestra, G. M. 1994. Pectinolytic enzymes produced by Pseudomonas syringae pv. Glycinea. FEMS Microbiol. Lett. 117:1-6. 62. Marcus, L., Barash, I., Sneh, B., Koltin, Y. and Finker, A. 1986. Purification and characterization of pectolytic enzymes produced by virulent and hypovirulent isolates of Rhizoctonia solani KUHN. Physiol. Mol. Plant Pathol. 29:325-336. 63. Maruyama, Y. and Onodera, K. 1979. Production and some properties of invertase isozymes of Fusarium oxysporum. J. Gen. Appl. Microbiol. 25:361-366. 64. Masai, T., Wada, K., Hayakawa, K., Yoshihara, L. and Mitsuoka, T. 1987. Effects of soybean oligosaccharides on human intestinal flora and metabolic activities. Japan. J. Bacteriol. 42(1):313. 65. McCready, R. M., Owen, H. S. and Maclay, W. D. 1944. Alkali hydrolyzed pectins are potential industrial products. Food Ind. 16:69. 66. Mckellar, R. C. and Modler, H. W. 1996. Metabolism of fructo- oligosaccharides by bifidobacterium spp. Appl. Microbiol. Biotechnol. 31:537-541. 67. Muhammad, E. and Hiroshi, T. 1996. Lineweaver-burk analysis for the blocking effects of mammalian dopamine receptor antagonists on dopamine-induced currents in achatina giant neurones. Gen. Pharmac. 27(7):1209-1213. 68. Muramatsu, M., Kainuma, S., Miwa, T. and Nakakuki, T. 1988. Structures of some fructooligosaccharides produced from sucrose by mycelia of Aspergillus sydowi IAM 2544. Agric. Biol. Chem. 52:1303-1304. 69. Mutai, M. 1978. Characteristics of “ Mil-Mil ”, a Bifidobacterium fermented milk. New Food Ind. 20(8):17. 70. Muting, D., Escherich, D. and Mayer, J. P. 1968. The effect of Bifidobacterium bifidum on intestinal bacterial flora and toxic protein metabolites in chronic liver diseases. Am. J. Protol. 19:336-342. 71. Nagel, C. W. and Vaughn, R. H. 1961. The characterisitc of a polygalacturonase produced by Bacillus polymyxa. Arc. Biochem. Biophys. 93:344-352. 72. Naidu, G. S. N. and Panda, T. 1998. Production of pectolytic enzymes: a review. Bioproc. Eng. 19:355-361. 73. Nasumo, S. and Starr, M. P. 1967. Polygalacturonic acid trans- eliminase of Xanthomonas compestris. Biochem. J. 104:178-184. 74. Ohtsuka, K., Tsuji, K. Y., Nakagawa, H., Ueda, O., Ozawa, T. and Ichikawa, T. 1990. Availablity of 4 ’ -galactosyllatose (O-beta-D-galactopyranosyl-(1-4)-D-glucopyranose) in rats. Journal of nutritional science and vitaminology. 36:265-276. 75. Oku, T. and Nakamura, S. 2002. Digestion, absorption, fermentation, and metabolism of functional sugar substitutes and their available energy. Pure Appl. Chem. 74:1253-1261. 76. Olsen, A. G. 1993. Pectin studies III: General theory of pectin jelly formation. J. Physical Chem. 38:919. 77. Padival, R. A., Ranganna, S. and Manjrekar, S. P. 1979. Mechanism of gel formation by low methoxyl pectins. J. Food Technol. 14:277. 78. Patel, V., Saunders, G. and Bucke, C. 1994. Production of fructooligosaccharides by Fusarium oxysporum. Biotechnol. Lett. 11:1139-1144. 79. Pazur, J. H. 1952. Transfructosidation reactions of an enzyme of Aspergillus oryzae. J. Biol. Chem. 199:217-225. 80. Perez, S. M., Rodriguez-Carvajal, A. and Doco, T. 2003. A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. Biochimie. 85:109-121. 81. Rambouts, F. M. and Pilnik, W. 1980. Pectic enzyme. In: Rose, A. H. (ed), Economic microbial enzymes and bioconversions. p. 227-282.

Academic Press, New York. 82. Reid, I. and Ricard, M. 2000. Pectinase in papermaking solving retention problems in mechanical pulps bleached with hydrogen peroxide. Enzyme Microbiol. Technol. 26:115-123. 83. Rijssel, M. W., Gerwig, J. G. J. and Hausen, T. A. 1993. Isolation and characterization of an extracellular glycosylated protein complex from Clostridium thermosaccharolyticum with pectin methylesterase and polygalacturonate hydrolase activity. Appl. Environ. Microbiol. 59(3):828-836. 84. Rolin, C. and Vries, J. D. 1990. Pectin. In Harris, P. (Ed), Food Gels. p. 422-423. Elsevier Science Publishing Co, Inc., New York. 85. Sakai, T., Sakamoto, T., Hallaert, J. and vandamme, EI. 1993. Pectin, pectinase and protopectinase: production, properties and application. Adv. Appl. Microbiol. 39:213-294. 86. Smith, J. M., Grove, D., Luenser, S. J. and Park, L. 1980. Process for the production of fructose transferase enzyme. U.S. patent 4:309-505. 87. Straathof, A. J. J., Kieboom, A. P. G. and van Bekkum, H. 1986. Invertase-catalysed fructosyl transfer in concentrated solutions of sucrose. Carbohydr. Res. 146:154-159. 88. Takabe, H., Otsuki, Y. and Aoki, S. 1968. Isolation of tobacco mesophyll cells in intact and active state. Plant Cell Physiol. 9:115. 89. Thakur, B. R., Singh, R. K. and Handa, A. K. 1997. Chemistry and uses of pectins-a review. Critical Reviews in Food Science and Nutrition 37(1):47. 90. Takasoye, M., Inoue, N. and Knuma, C. 1990. Clinical investi- gation of feces improvements by soybean oligosaccharides. Rinsho tohkenykyu(Clinics and Research). 67:304-310. 91. Towel, G. A. and Christensen, O. 1973. Pectin. In Whistler, R. L. and Be Miller, J. N. (eds), Industrial Gums. p. 429-461. Academic Press, New York. 92. Usami, S., Ishii, T., Kirimura, K., Uehara, K. and Chen, J. 1991. Production of β-fructofuranosidase showing fructose-transferring activity by penicillium frequentans(P. glabrum). J. Ferment. Bioeng. 72:303-305. 93. Vaccari, G., Lodi, G., Tamburini, E., Dosi, E. and Mantovani, G. 2000. Planar chromatography applied to the analysis of oligo- saccharides in sugar product. Association Andrew Van Hook 7th Symposium: p. 28-38. 94. Van Buren, J. P. 1979. The chemistry of texture in fruits and vegetables. Journal of Texture Studies 10:1. 95. Wada, K., Watabe, J., Mizutani, J., Suzuki, H., Kiriu, N., Hayakama, K. and Yamaguchi, C. 1991. Effect of soybean oligosaccharides in- take on fecal microflora, enzyme activity, ammonia and frequency of evacuation in elderly person. Bifidus. 4:135-140. 96. Ward, O. P. and Young, M. M. 1989. Enzymatic degradation of cell wall and related plant polysaccharides. Crit. Rev. Biotechnol. 8:237-274. 97. Watanuki, M., Wada,Y. and K. Matsumoto. 1996. Digestibility and physiological heat of combustions of β1-4 and β1-6 galacto- oligosaccharides on vitro. Annual Reports of the Yakult Central Institute for Microbiological Research. 16:1-12. 98. Yamashita, K., Kawai, K. and Itakura, M. 1984. Effects of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutrition Research 4:961-965. 99. Yun, J. W. 1996. Fructooligosaccharide-Occurrence,preparation, and application. Enzyme and Microbial. Technol. 19:107-117. 100. Yun, J. W., Jung, K. H., Oh, J. W. and Lee, J. H. 1990. Semibatch production of fructo-oligosaccharides from sucrose by immobilized cells of Aureobasidium pullulans. Appl. Biochem. Biotechnol. 24(25):299-308. 101. Yun, J. W., Jung, K. H., Jeon, Y. J. and Lee, J. H. 1992. Continuous production of fructo-oligosaccharides from sucrose by immobilized cells of Aureobasidium pullulans. J. Microbiol. Biotechnol. 2:98-101. 102. Yun, J. W., Jeon, Y. J., Lee, M. G. and Song, S. K. 1993. Production of fructo-oligosaccharides by the fructosyltransferase immobilized onto a ion-exchange resin. Korean J. Biotechnol. Bioeng. 8:307-312.