

# 以反應曲面法探討羥苯丙酸辛酯之最優化酵素合成

凌逸凡、謝淳仁

E-mail: 9318514@mail.dyu.edu.tw

## 摘要

酚酸廣泛地存在於天然植物界中，其不只具有抗氧化活性，還具有抗癌、抗突變、抗過敏及抗衰老等的生物活性。許多天然的酚酸表現出低溶解度以及在各種有機溶劑系統中的低穩定性，故在商業應用上有某部分的限制，如酚酸的親水特性，在油相系統中會減弱其抗氧化活性，因此透過與脂肪醇的酯化作用，改變其溶解度，使其可被應用於以油為基礎的配方和乳化劑中，提升他們在油相系統中的有效性。酚酸酯類可由化學方法合成，但此等化學方法所合成的酯類並不適合添加於食品中；近年來，經由酵素催化合成酚酸酯類的方法雖已有文獻發表，但受到酚酸立體結構的影響，其酵素反應速率低，致使產率無法提升。故本研究選用立體阻礙較小之對羥苯丙酸 (p-Hydroxyphenylpropionic acid, HPPA)，於無有機溶劑系統中與辛醇 (Octanol) 合成酚酸酯類，再利用反應曲面法 (Response surface methodology, RSM) 及五階層四變數之中心混層實驗設計，探討酚酸與脂肪醇合成酚酸酯類之酵素合成條件，包括反應時間 (24?72 h)、反應溫度 (25?65 ?C)、酵素用量 (10?50%, w/w) 和酵素pH記憶值 (5?9) 對酚酸酯類莫耳轉換率的影響。研究結果發現，反應時間、反應溫度和酵素用量對酚酸酯類之莫耳轉換率有顯著影響。利用脊形分析可得到酚酸酯類之最優化莫耳轉換率為96%，其反應條件為：反應時間58 h、反應溫度53 ?C、酵素用量38%和酵素pH記憶值7。

關鍵詞：酚酸；直接酯化；脂解酵素；最優化；反應曲面法

## 目錄

目錄封面內頁 簽名頁 授權書 III 中文摘要 V 英文摘要 VI 誌謝 VIII 目錄 IX 圖目錄 XII 表目錄 XIII
第一章 緒論 1
第二章 文獻回顧 5
2.1 商業界天然抗氧化劑之使用近況 5
2.1.1 抗氧化劑之定義 6
2.1.2 抗氧化劑的種類 7
2.1.3 抗氧化劑的作用機制 9
2.1.3.1 第一類抗氧化劑 9
2.1.3.2 第二類抗氧化劑 9
2.1.3.3 第三類抗氧化劑 12
2.1.3.4 第四類抗氧化劑 13
2.1.3.5 第五類抗氧化劑 13
2.2 酚酸之抗氧化活性 14
2.2.1 來源與抗氧化作用機制 14
2.2.2 結構與抗氧化活性 18
2.2.3 酚酸酯化對低密度脂蛋白氧化之影響 21
2.3 酚酸酯類合成之國內外相關研究 22
2.3.1 化學合成 23
2.3.2 酵素合成 24
2.4 脂解酵素於油脂工業上之優勢 26
2.4.1 Novozyme?435之簡介 27
2.4.2 脂解酵素之專一性 28
2.4.3 影響酵素活性之因子 31
2.4.3.1 溫度 31
2.4.3.2 pH值 32
2.4.3.3 水活性 32
2.4.3.4 離子強度 32
2.4.3.5 界面不活化 33
2.5 反應曲面法之應用 34
2.5.1 反應曲面法之原理 34
2.5.2 二水準因子設計 35
2.5.3 中心混層設計 38
2.5.4 回應曲面模式適切性之統計檢驗 41
2.5.5 正則分析 41
第三章 材料與方法 43
3.1 實驗材料 43
3.1.1 儀器設備 43
3.1.2 藥品 43
3.2 實驗設計與方法 44
3.2.1 實驗設計 44
3.2.2 酵素pH記憶值之調整 46
3.2.3 酵素含水量之校正 46
3.2.4 羥苯丙酸辛酯之合成方法 46
3.2.5 萃取與分析 48
3.2.6 統計分析 48
3.3 結果與討論 50
3.3.1 時間對莫耳轉換率的影響 51
3.3.2 溫度對重量轉換率的影響 52
3.3.3 酵素用量對重量轉換率的影響 55
3.3.4 酵素pH記憶值對莫耳轉換率的影響 55
3.3.5 最優化合成之研究 59
3.3.6 綜合討論 65
第四章 結論 68
參考文獻 70
附錄一 酚酸酯類酵素合成之文獻整理 80
圖目錄 圖1-1 以脂解酵素催化對羥苯丙酸與辛醇之直接酯化反應 4
圖2-2 常見之人工合成抗氧化劑 11
圖2-3 植物組織中之酚酸衍生物結構 16
圖2-4 植物中苯丙胺酸之合成路徑 17
圖2-5 抗氧化能力測試之酚酸結構 19
圖2-6 三甘油酯以sn-命名的代表分子 30
圖2-7 中心混層設計法之星點及中心點補充實驗圖 40
圖3-1 氣相層析儀分析羥苯丙酸辛酯之標準圖譜 49
圖3-2 反應時間對羥苯丙酸辛酯合成產率之影響，反應條件分別為：反應溫度45 ?C、酵素用量30 % 及酵素pH記憶值7 56
圖3-3 酵素用量與反應時間對羥苯丙酸辛酯莫耳轉換率影響 57
圖3-4 酵素用量與反應溫度對羥苯丙酸辛酯莫耳轉換率影響 58
圖3-5 酵素用量與酵素pH記憶值對羥苯丙酸辛酯莫耳轉換率影響之反應曲面圖 60
圖3-6 羥苯丙酸辛酯百分比莫耳轉換率之等高線圖。在等高線圖內之數字是用來表示不同的反應條件下之莫耳轉換率 61
圖3-7 正則分析建議之最優化酵素合成 64
表目錄 表2-1 天然抗氧化劑之來源 8
表2-2 農業廢棄物中之多酚萃取物 15
表2-3 酚酸在不同測試方法中之抗氧化活性 20
表2-4 脂解酵素的分類與來源 29
表2-5 23因子設計表 37
表2-6 中心混層設計之補充實驗 39
表3-1 五階層四變數中心混層實驗設計反應參數實驗值之範圍 45
表3-2 羥苯丙酸辛酯之五階層四變數中心混層實驗設計與數據 47
表3-3 羥苯丙酸辛酯莫耳轉換率對合成變數之變異數分析 53
表3-4 羥苯丙酸辛酯合成變數之聯合檢測分析 54
表3-5 利用脊形分析評估羥苯丙酸辛酯莫耳轉換率之最大值 63

## 參考文獻

參考文獻 1. 朱燕華。2000。植物類機能性成分介紹。食品工業月刊。32: 48—52。 2. 江文德。1999。脂肪酶在油脂加工上的應用。食品工業月刊。31: 10—19。 3. 李昌憲、洪哲穎及熊光濱。1992。利用反應曲面法進行以 *Streptococcus faecalis* 生產酪胺酸脫羧酶之培養基最

適化研究。中國農業化學會誌。30: 264—272。4. 李根永和李孟修。1998。Corynebacterium glutamicum 在高濃度鹽份培養基脯胺酸發酵之研究。中國農業化學會誌。36: 57—64。5. 林志城和孫璐西。1995。食品添加物。食品化學/張為憲等編著。華香園出版社。台北市。6. 拱玉郎。1997。天然抗氧化劑發展近況。食品工業月刊。29: 29—35。7. 郁凱衡。1999。酚類抗氧化劑(上) - 合成類。食品資訊。157: 34—38。8. 高馥君。1992。反應曲面法在食品開發上的應用。食品工業月刊。24: 32—41。9. 張基郁和張為憲。1995。酵素。食品化學/張為憲等編著。華香園出版社。台北市。10. 張淑微。2002。以反應曲面法研究酵素合成己醇酯類之最優化。大葉大學食品工程研究所碩士論文。11. 張曉莉和黃世佑。1997。生物轉換法 - 有機溶劑中維持酵素活之研究。化工。44: 71—84。12. 陳如茵。2002。抗氧化成分對動脈粥樣硬化影響的機制。食品工業月刊。34: 34—42。13. 陳昭雄和孫璐西。1995。脂質。食品化學/張為憲等編著。華香園出版社。台北市。14. 楊海明。1986。脂解?在油脂交酯化上的應用。食品工業月刊。18: 34—43。15. 劉冠汝。1996。應用脂肪?在有機溶劑與超臨界流體中合成高價產品。國立海洋大學水產食品科學研究所博士論文。16. 賴永沛。1999。小籽立大功 - 葡萄籽抽出物。食品資訊。162: 20—23。17. Arouma, O. I. 1994. Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxic. 32: 671—683。18. Ballesteros, A., Bornscheuer, U., Capewell, A., Combes, D., Condoret, J. S., Koenig, K., and Kolisis, F. N. 1995. Enzymes in non-conventional phases. Biocatal. Biotransfor. 13: 1—42。19. Bowman, L. and Geiger, E. 1984. Optimization of fermentation conditions of alcohol production. Biotechnol. Bioeng. 26: 1492—1497。20. Box, G. E. P. and Wilson, K. B. 1951. On the experimental attainment optimum conditions. J. Roy. Statist. Soc. B13: 1—45。21. Box, G. E. P., Hunter, W., and Hunter, J. S. 1978. Statistics for experimenters. John Wiley and Sons, New York。22. Branen, A. L. 1975. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chem. Soc. 52: 59—65。23. Buisman, G. J. H., Helteren, C. v., Kramer, G. F. H., Veldsink, J. W., Derksen, J. T. P., and Cuperus, F. P. 1998. Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol. Lett. 20: 131—136。24. Chalas, J., Claise, C., Edeas, M., Messaoudi, C., Vergnes, L., Abella, A., and Lindenbaum, A. 2001. Effect of ethyl esterification of phenolic acids on low-density lipoprotein oxidation. Biomed. Pharmacother. 55: 54—60。25. Chen, Q. Y., Shi, H., and Ho, C. T. 1992. Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. J. Am. Oil Chem. Soc. 69: 999—1002。26. Chen, W. K., Tsai, C. F., Liao, P. H., Kuo, S. C., and Lee, Y. J. 1999. Synthesis of caffeic acid esters as antioxidants by esterification via acyl chlorides. Chin. Pharm. J. 51: 271—278。27. Cheynier, V., Feinberg, M., Chararas, C., and Ducauze, C. 1983. Application of response surface methodology to evaluation of bioconversion. Appl. Environ. Microbiol. 45: 634—639。28. Chimi, H., Cillard, J., Cillard, P., and Rahmani, M. 1991. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. J. Am. Oil Chem. Soc. 68: 307—312。29. Pirozzi, D. and Greco Jr., G. 2004. Activity and stability of lipases in the synthesis of butyl lactate. Enzyme Microb. Tech. 34: 94—100。30. Dziejak, J. D. 1986. Antioxidants: the ultimate answer to oxidation. Food Technologist 40: 94—102。31. Frankel, E. N., Kanner, J., German, J. B., Parks, E., and Kinsella, J. E. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 341: 454—457。32. Graf, E. 1992. Antioxidant potential of ferulic acid. Free Radic. Biol. Med. 13: 435—448。33. Gutman, A. L., Shkolnik, E., and Shapira, M. 1992. A convenient method for enzymatic benzyl-alkyl transesterification under mild neutral conditions. Tetrahedron 48: 8775—8780。34. Guyot, B., Bosquette, B., Pina, M., and Graille, J. 1997. Esterification of phenolic acids from green coffee with an immobilized lipase from Candida antarctica in solvent-free medium. Biotechnol. Lett. 19: 529—532。35. Himmelblau, D. M. 1970. Process analysis by statistical methods. John Wiley and Sons, New York。36. Ito, N., Fukushima, S., and Tsuda, H. 1985. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. Crit. Rev. Toxicol. 15: 109—150。37. Jaeger, K. E., Ransac, S., Dijkstra, B. W., Colson, C., and Misset, O. 1994. Bacterial lipases. FEMS Microbiol. Rev. 15: 29—63。38. Jogiekar, A. M. and May, A. T. 1987. Product excellence through design of experiments. Cereal Food World 32: 857—868。39. John, V. T. and Abraham, G. 1991. Lipase catalysis and its applications. In Biocatalysis for Industry. By Dodrick, J. S. Plenum Press, New York。40. Kajiyama, T. and Ohkatsu, Y. 2001. Effect of para-substituents of phenolic antioxidants. Polym. Degrad. Stabil. 71: 445—452。41. Karra-Chaabouni, M., Pulvin, S., Touraud, D., and Thomas, D. 1998. Parameters affecting the synthesis of granyl butyrate by esterase 30,000 from Mucor miehei. J. Am. Oil Chem. Soc. 75: 1201—1206。42. Kitts, D. 1997. An evaluation of the multiple effects of the antioxidant vitamins. Trends Food Sci. Tech. 8: 198—203。43. Kochnar, S. P. and Rossel, J. B. 1990. Detection, estimation and evaluation of antioxidants in food systems. In Food antioxidant. By Huson, B. J. F. Elsevier Science Publishers, New York。44. Kris-Etherton, P. M. and Nicolosi, R. J. 1995. Trans fatty acid and coronary heart disease risk. ILSI press, Washington DC。45. Lee, Y. J., Liao, P. H., Chen, W. K., and Yang, C. C. 2000. Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells. Cancer Lett. 153: 51—56。46. Liu, X. Y., Guo, F. L., Wu, L. M., and Liu, Z. L. 1996. Remarkable enhancement of antioxidant activity of Vitamin C in an artificial bilayer by making it lipo-soluble. Chem. Phys. Lipids 62: 345—350。47. Marangoni, A. G. and Rousseau, D. 1998. The influence of chemical interesterification on physicochemical properties of complex fat systems 1. Melting and crystallization. J. Am. Oil Chem. Soc. 75: 1265—1271。48. Marinova, E. M. and Yanishlieva, N. V. 1994. Effect of lipid unsaturation on the antioxidative activity of some phenolic acid. J. Am. Oil Chem. Soc. 71: 427—434。49. Massaeli, H., Sobrattee, S., and Pierce, G. N. 1999. The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein peroxidation. Free Radic. Biol. Med. 26: 1524—1530。50. Maugard, T., Boulonne, M., Rejasse, B., and Legoy, M. D. 2001. Enzymatic synthesis of water-soluble derivatives of salicylic acid in organic media. Biotechnol. Lett. 23: 989—993。51. Montgomery, D. C. 1984. Design and analysis of experiments. John Wiley and Sons, New York。52. Moresi, M., Colicchio, A., and Sansovini, F. 1980. Optimization of whey fermentation in a jar fermenter. Eur. J. Appl. Microbiol. Biotechnol. 9: 173—183。53. Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., and Nunez, M. J. 2001. Natural antioxidants from residual sources. Food Chem. 72: 145—171。54. Nardini, M., D'Alessandro, N., Tomassi, G., Gentili, V., Di Felice, M., and Scaccini, C. 1995. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radic.

Biol. Med. 19: 541—552. 55. Natella, F., Nardini, M., Felice, M. D., and Scaccini, C. 1999. Benzoic and cinnamic acid derivatives as antioxidants: structure-activity relation. *J. Agr. Food Chem.* 47: 1453-1459. 56. Novozymes Co. 2001. Product Sheet. <https://www.novozymes.com> 57. Ohta, T., Nakano, T., Egashira, Y., and Sanada, H. 1997. Antioxidant activity of ferulic acid beta-glucuronide in the LDL oxidation system. *Biosci. Biothchnol. Biochem.* 61: 1942—1943. 58. Pokorny, J. 1987. Major factors affecting the antioxidant of lipids. In *Autoxidation of unsaturated lipids*. By Chan, H. Academic Press, London. 59. Pratte, D. E. and Hudson, B. J. F. 1990. Natural antioxidants not exploited commercially. In *Food Antioxidants*. By Hudson, B. J. F. Elsevier Science Publishers, New York. 60. Retsky, K. L., Freeman, M. W., and Frei, B. 1993. Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification: anti-rather than prooxidant activity of vitamin C in the presence of transition metal ions. *J. Biol. Chem.* 268: 1304—1309. 61. Rice-Evans, C. A., Miller, N. J., and Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 2: 152—159. 62. Schuler, P. 1990. Natural antioxidants exploited commercially. In *Food Antioxidants*. By Hudson, B. J. F. Elsevier Science Publishers, New York. 63. Seriburi, V and Akoh, C. C. 1998. Enzymatic transesterification of triolein and stearic acid and solid fat content of their products. *J. Am. Oil Chem. Soc.* 75: 511—516. 64. Shahidi, F., Amarowicz, R., Yuehua, H., and Mahinda, W. 1997. Antioxidant activity of phenolic extracts of evening primrose (*Oenothera biennis*): a preliminary study. *J. Food Lipids* 4: 75—86. 65. Sherwin, E. R. 1990. Antioxidants. In *Food antioxidants*. By Branen, A. L., Davidson, P. M., and Salminen, S. Marcel Dekker Inc., New York. 66. Stamatis, H., Sereti, V., and Kolisis, F. N. 1999. Studies on the enzymatic synthesis of lipophilic derivatives of natural antioxidants. *J. Am. Oil Chem. Soc.* 76: 1505—1510. 67. Stamatis, H., Sereti, V., and Kolisis, F. N. 2001. Enzymatic synthesis of hydrophilic and hydrophobic derivatives of natural phenolic acids in organic media. *J. Mol. Catal. B-Enzym.* 11: 323—328. 68. Thomson, D. 1982. Response surface experimentation. *J. Food Process. Pres.* 6: 155—188. 69. Villeneuve, P. and Foglia, T. A. 1997. Lipase specificities: potential application in lipid bioconversions. *INFORM* 8: 640—651. 70. Wada, S. and Fang, X. 1992. The synergistic antioxidant effect of rosemary extract and  $\alpha$ -tocopherol in sardine oil model system and frozen-crushed fish meat. *J. Food Process Preserv.* 16: 263-274. 71. Wanasundara, U. N. and Shahidi, F. 1998. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chem.* 63: 335—342. 72. Yagi, K. and Ohishi, N. 1979. Action of ferulic acid and its derivatives as antioxidants. *J. Nutr. Sci. Vitaminol.* 25: 127—130. 73. Zertuche, L. and Zall, R. R. 1985. Optimizing alcohol production from whey using computer technology. *Biotechnol. Bioeng.* 27: 547—554.