以超音波輔助酵素合成咖啡酸苯乙酯之最優化研究

陳曉菁、謝淳仁、?耀國

E-mail: 319700@mail.dyu.edu.tw

摘 要

酚酸化合物已被證實對人體具有抗發炎(anti-inflammatory)、抗氧化(anti-oxidant)等功效。自然界大量存在之咖啡酸(caffeic acid)為水溶性多酚類，其酯類衍生物的親油性高，可強化其抗氧化能力，並提高其應用價值。利用脂解酵素(lipase)合成酚酸酯類，藉此改變酚酸的親油特性，使抗氧化及自由基清除力提高的功能更穩定。脂解酵素可催化咖啡酸及苯乙醇(phenyl ethanol)合成咖啡酸苯乙酯(caffeic acid phenethyl ester, CAPE)，酵素合成比起化學合成方式不僅更天然，且反應條件溫和，製程對環境較友善，所合成出來的產物亦偏向天然，在食品或化妝品的應用上，對於消費者之接受度較高。為了縮短催化酚酸酯化物(phenolic acid ester)的合成時間，本研究藉由生物催化 (biocatalysis)技術並配合超音波(ultrasound)系統及連續式酵素填充床生物反應器(continuous packed-bed bioreactor)進行催化合成反應，以符合未來工業量產之應用。 將基質咖啡酸與苯乙醇(phenyl ethanol)於異辛烷(isooctane)溶劑中，利用固定化脂解酵素(NovozymR 435)，經由中心混層實驗設計(central composite rotatable design, CCRD)或Box-Behnken實驗設計(Box-Behnken design)，以直接酯化(direct esterification)方式合成咖啡酸苯乙酯，再以反應曲面法(response surface methodology, RSM)進行分析，分別探討合成反應參數對莫耳轉換率的影響，最後，利用脊型分析(analysis of ridge max)詳細探討酵素合成咖啡酸苯乙酯之最優化反應條件。 本研究分成三階段進行，第一階段為利用固定化酵素NovozymR 435合成咖啡酸苯乙酯，使提高咖啡酸在親油性環境下之穩定性，增加其有效利用率。將咖啡酸及苯乙醇以直接酯化方式，並利用五階層四變數之中心混層實驗設計，探討合成咖啡酸苯乙酯之最優化條件。實驗結果顯示，反應時間59小時、反應溫度69 °C、咖啡酸與苯乙醇莫耳比1:72及酵素用量351 PLU (propyl laurate unit)，咖啡酸苯乙酯莫耳轉換率之理論值與實驗值分別為91.36%及91.65±0.66%。 第二階段藉由超音波輔助系統合成咖啡酸苯乙酯。超音波於液相溶液中形成空穴現象(cavitation)，產生許多微小氣泡及超臨界區域，提供了化學介質力量，使增加了酵素與基質間作用的接觸機會，進而提高合成酯化作用，縮短咖啡酸苯乙酯合成時間。結果顯示，超音波輔助系統利用NovozymR 435於反應溫度70 °C，合成咖啡酸苯乙酯之最優化條件為：反應時間9.6 小時、咖啡酸與苯乙醇莫耳比1:71、酵素用量2938 PLU及超音波功率為2 W/cm2，咖啡酸苯乙酯莫耳轉換率之理論值與實驗值分別為96.03%及93.08±0.42%。 第三階段為超音波輔助系統結合連續式酵素填充床生物反應器合成咖啡酸苯乙酯，利用超音波加速酵素作用並在連續式生物反應器下，模擬工業連續化生產流程，提供業界大量製備之依據。結果顯示，當咖啡酸與苯乙醇莫耳比1:100，填充15,000 PLU固定化酵素NovozymR 435於連續式酵素填充床生物反應器，合成咖啡酸苯乙酯之最優化條件為：反應溫度72.66 °C、體積流量0.046 mL/min及超音波功率為1.64 W/cm2，莫耳轉換率之理論值與實驗值分別為97.84%及92.11±0.75%。 綜合以上實驗結果，利用固定化酵素NovozymR 435合成咖啡酸苯乙酯，並在超音波輔助系統下，克服了酚酸酯類合成耗時的障礙。藉由連續式生物反應器，達到連續生產咖啡酸苯乙酯之目的，探討合成咖啡酸苯乙酯之最優化條件，找出最佳莫耳轉換率，節省操作時間及能源，進一步提供予業界量化的參考。

關鍵詞 : 生物反應器、生物催化、咖啡酸苯乙酯、脂解酵素、酚酸、超音波、最優化

目錄

參考文獻

1. 陳國誠。2000。生物固定化技術與產業應用。第121-155頁。茂昌圖書有限公司。台北，台灣。 2. 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(3): 32-41。 3. Adamczak, M. and Krishna, S. H. 2004. Strategies for improving enzymes for efficient biocatalysis. Food Technol. Biotechnol. 42: 251-264. 4. Aga, H., Shibuya, T., Sugimoto, T., Kurimoto, M. and Nakajima, S. 1994. Isolation and identification of antimicrobial compounds in Brazilian propolis. Biosci. Biotechnol. Biochem. 58: 945-946. 5. Babicz, I., Leite, S. G. F., Souza, R. O. M. A. D. and Antunes, O. A. C. 2010. Lipase-catalyzed diacylglycerol under sonochemical irradiation. Ultrason. Sonochem. 17: 4-6. 6. Bai, S., Guo, Z., Liu, W. and Sun, Y. 2006. Resolution of (±)-menthol by immobilized Candida rugosa lipase on superparamagnetic nanoparticles. Food Chem. 96: 1-7. 7. Balachandran, S., Kentish, S. E., Mawson, R. and Ashokkumar, M. 2006. Ultrasonic enhancement of the supercritical extraction from ginger. Ultrason. Sonochem. 13: 471-479. 8. Balca?o, V. M., Paiva, A. L. and Malcata, F. X. 1996. Bioreactors with immobilized lipases: state of the art. Enzyme Microb. Technol. 18: 392-416. 9. Balcao, V. M. and Malcate, F. X. 1998. Lipase catalyzed modification of milk fat. Biotechnol. Adv. 16: 309-341. 10. Bankova, V., Popov, S. and Marekov, N. 1983. A study of flavonoids of propolis. J. Nat. Prod. 46: 471-474. 11. Banskota, A. H., Nagaoka, T., Sumioka, Y. L., Tezuka, Y., Awale, S., Midorikawa, K., Matsushige, K. and Kadota, S. 2002. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. J. Ethnopharmacol. 80: 67-73. 12. Banskota, A. H., Tezuka, Y. and Kadota, S. 2001. Recent progress in pharmacological research of propolis. Phytother. Res. 15: 561-571. 13. Borrelli, F., Izzo, A. A., Di Carlo, G., Maffia, P., Russo, A., Maiello, F. M., Capasso, F. and Mascolo, N. 2002. Effect of a propolis extract and caffeic acid phenethyl ester on formation of aberrant crypt foci and tumors in the rat colon. Fitoterapia. 73: 38-43. 14. Borrelli, F., Maffia P., Pinto, L., Ianaro, A., Russo, A., Capasso, F. and Ialenti, A.

2002. Phytochemical compounds involved in the anti-inflammatory effect of propolis exract. Fitoterapia. 73: 53-63. 15. 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. 16. Burdock, G. A. 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem. Toxicol. 36: 347-363. 17. Castaldo, S. and Capasso, F. 2002. Propolis, an old remedy used in modern medicine. Fitoterapia. 73: 1-6. 18. Chang, A. C. and Chen, F. C. 2002. The application of 20 kHz ultrasonic waves to accelerate the aging of different wines. Food Chem. 79: 501-506. 19. Chang, C., Chen, J. H., Chang, C. M. J., Wu, T. T. and Shieh, C. J. 2009. Optimization of lipase-catalyzed biodiesel by isopropanolysis in a continuous packed-bed reactor using response surface methodology. New Biotech. 26: 187-192. 20. Chang, S. W., Shaw, J. F., Yang, C. K. and Shieh, C. J. 2007. Optimal continuous biosynthesis of hexyl laurate by packed bed bioreactor. Process Biochem. 42: 1362-1366. 21. Chen, J. H., Shao, Y., Huang, M. T., Chin, C. K. and Ho, C. T. 1996. Inhibitiory effect of caffeic acid phenethyl ester on human leukemic HL-60 cells. Cancer Lett. 108: 211-214. 22. Chen, J. W. and Wu, W. T. 2003. Regeneration of immobilized Candida antarctica lipase for transesterfication. J. Biosci. Bioeng. 95: 466-469. 23. Chen, Q. H., Fu, M. L., Jin, L., Zhang, H. F., He, G. Q. and Ruan, H. 2009. Optimization of ultrasonic-assisted extraction (UAE) of betulin from white birch bark using response surface methodology. Ultrason. Sonochem. 16: 599-604. 24.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. 69: 999-1002. 25. Chen ,W. K., Tsai, C. F., Liao, P. H., Kuo, S. C. and Lee, Y. J. 1999. Synthesis of caffeic acid esters as antioxidant by esterification via acyl chlorides. Chin. Pharm. J. 51: 271-278. 26. Chisti, Y. 2003. Sonobioreactors: using ultrasound for enhanced microbial productivity. Trends Biotechnol. 21: 89-93. 27. Cirasino, L., Pisati, A. and Fasani, F. 1987. Contact dermatitis from propolis. Contact Dermatitis. 16:110-111. 28. Compton, D. L., Laszlo, J. A. and Berhow, M. A. 2000. Lipase-catalyzed synthesis of ferulate esters. J. Am. Oil Chem. Soc. 77: 513-519. 29. Cuvelier, M. E., Richard, H. and Berset, C. 1992. Comparison of the antioxidant activity of some acid-phenols: structure-activity relationship. Biosci. Biotechnol. Biochem. 56: 324-327. 30. De Castro, S. L. 2001. Propolis: biological and pharmacological activities. Therapeutic uses of this bee-product. Annu. Rev. Boimed. Sci. 3: 49-83. 31. Demirel, D. and Mutlu, M. 2005. Performance of immobilized pectinex ultra SP-L on magnetic duolite-polystyrene composite particles. Part II: A magnetic fluidized bed reactor. J. Food Eng. 70: 1-6. 32. Figueroa-Espinoza, M. C. and Villeneuve, P. 2005. Phenolic acid enzymatic lipophilization. J. Agric. Food Chem. 53: 2779-2787. 33. Freitas, S., Hielscher, G., Merkle, H. P. and Gander, B. 2006. Continuous contact and contamination free ultrasonic emulsification- A useful tool for pharmacuetical development and production. Ultrason. Sonochem. 13: 76-85. 34. Gandhi, N. N. 1997. Applications of lipase. J. Am. Oil Chem. Soc. 74: 621-634. 35. Garcia, R., Renedo, A., Martinez, M. and Aracil, J. 2002. Enzymatic synthesis of n-octyl (±)-2-methylbutanoate ester from racemic (±)-2-methylbutanoic acid by immobilized lipase: optimization by statistical analysis. Enzyme Microb. Technol. 30: 110-115. 36. Gomez-Romero, M., Arraez-Roman, D., Moreno-Torres, R., Garcia-Salas, P., Segura-Carretero, A. and Fernandez-Gutierrez., A. 2007. Antioxidant compounds of propolis determined by capillary electrophoresis – mass spectrometry. J. Sep. Sci. 30: 595-603. 37. Graf, E. 1992. Antioxidant potential of ferulic acid. Free Radic. Biol. Med. 13: 435-448. 38. Grunberger, D. Banerjee, R., Eisinger, K., Oltz, E. M., Efros, L., Caldwell, M., Estevez, V. and Nakanishi, V. 1988. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Cell. Mol. Life Sci. 44: 230-232. 39. Gupta, M. N. and Roy, I. 2004. Enzyme in organic media: forms, function and applications. Eur. J. Biochem. 271: 2575-2583. 40. Guyou, B., Bosquette, B., Pina, M. and Graille, J. 1997. Esterification of phenolic acids from green coffee with an immobilized lipase from Candida antarcitca in solvent-free medium. Biotechnol. Lett. 19: 529-532. 41. Guyou, B., Gueule, D., Pina, M., Graille, J., Farines, V. and Farines, M. 2000. Enzymatic synthesis of fatty ester in 5-caffeoyl quinic acid. Eur. J. Lipid Sci. Technol. 102: 93-96. 42. Halim, S. F. A., Kamaruddin, A. H. and Fernando, W. J. N. 2009. Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer studies. Bioresour. Technol. 100: 710-716. 43. Halldorsson, A., Magnusson, C. D. and Haraldsson, G. G. 2003. Chemoenzymatic synthesis of structured triacylglycerols by highly regioselective acylation. Tetrahedron 59: 9101-9109. 44. Hasan, F., Shah, A. A. and Hameed, A. 2006. Industrial application of microbial lipase. Enzyme Microb. Technol. 39: 235-251. 45. Heo, M. Y., Sohn, S. J. and Au, W. W. 2001. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. Mutat. Res. 488: 135-150. 46. Hernandez, C. E., Chen, H. H., Chang, C. I. and Huang, T. C. 2009. Direct lipase-catalyzed lipophilization of chlorogenic acid from coffee pulp in supercritical carbon dioxide. Ind. Crop. Prod. 30: 359-365. 47. Hishikawa, K., Nakaki, T. and Fujita, T. 2005. Oral ?avonoid supplementation attenuates atherosclerosis development in apolipoprotein E-de?cient mice. Arterioscler. Thromb. Vasc. Biol. 25: 442-446. 48. H-Kittikun, A., Kaewthong, W. and Cheirsilp, B. 2008. Continuous production of monoacylglycerols from palm olein in packed-bed reactor with immobilized lipase PS. Biochem. Eng. J. 40: 116-120. 49. Hollman, P. C. H. 2001. Evidence for health benefits of plant phenols: local or systemic effects? J. Sci. Food Agr. 81: 842-852. 50. Horchani, H., Salem, N. B., Zarai, Z., Sayari., A, Gargouri, Y. and Chaabouni, M. 2010. Enzymatic synthesis of eugenol benzoate by immobilized Staphylococcus aureus lipase: optimization using response surface methodology and determination of antioxidant activity. Bioresour. Technol. 101: 2809-2817. 51. Hsu, A. F., Jones, K. C., Foglia, T. A. and Marmer, W. N. 2004. Continuous production of ethyl esters of grease using an immobilized lipase. J. Am. Oil Chem. Soc. 81: 749-752. 52. Ilhan, A., Iraz, M., Gurel, A., Armutcu, F. and Kyol, O. 2004. Caffeic acid phenethyl ester exerts a neuroprotective effect on CNS against pentylenetetrazol-induced seizures in mice. Neurochem. Res. 29: 2287-2292. 53. Jayaprakasam, B., Vanisree, M., Zhang, Y., Dewitt, D. L. and Nair, M. G. 2006. Impact of alkyl esters of caffeic and ferulic acids on tumor cell proliferation, cyclooxygenase enzyme, and lipid peroxidation. J. Agric. Food Chem. 54: 5376-5381. 54. Jennings, B. H. and Akoh, C. C. 2001. Lipase catalyzed modification of fish oil to incorporate capric acid. Food Chem. 72: 273-278. 55. Ji, J., Wang, J., Li, Y., Yu, Y. and Xu, Z. 2006. Preparation of biodiesel with the help of ultrasonic and hydrodynamic cavitation. Ultrasonics 44: 411-414. 56. Ju, H. Y., Yang, C. K., Yen, Y. H. and Shieh, C. J. 2009. Continuous lipase-catalyzed synthesis of hexyl laurate in a packed-bed reactor: optimization of the reaction conditions in a solvent-free system. J. Chem. Biotechnol. 84: 29-33. 57. Jung, W. K., Lee, D. Y., Kim, J. H., Choi, I., Park, S. G., Seo, S. K., Lee, S. W., Lee, C. M., Park, Y. M., Jeon, Y. J., Lee, C. H., Jeon, B. T., Qian, Z. J., Kim, S. K. and Choi, I. W. 2008. Anti-in?ammatory activity of caffeic acid phenethyl ester (CAPE) extracted from Rhodiola sacra against lipopolysaccharide-induced in?ammatory responses in mice. Process Biochem. 43: 783-787. 58. Kajiyama, T. and Ohkatsu, Y. 2001. Effect of para-substituents of phenolic antioxidants. Polym. Degrad. Stabil. 71: 445-452. 59. Karam, R., Karboune, S., St-Louis, R. and Kermasha, S. 2009. Lipase-catalyzed acidolysis of fish liver oil with dihydroxyphenylacetic acid in organic solvent media. Process Biochem. 44: 1193-1199. 60. Karboune, S., Safari, M. Lue, B. M., Yeboah, F. K. and Kermasha, S. 2005. Lipase-catalyzed biosynthesis of cinnamoylated lipids in a selected organic solvent medium. J. Biotechnol. 119: 281-290. 61. Karboune, S., St-Louie, R. and Kermasha, S. 2008. Enzymatic synthesis of structured phenolic lipids by acidolysis of flaxseed oil with selected phenolic acids. J. Mol. Catal. B-Enzym. 52-53: 96-105. 62. Kimbaris, A. C., Siatis, N. G., Daferera, D. J., Tarantilis, P. A., Pappas, C. S. and Polissiou, M. G. 2006. Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (Allium sativum). Ultrason. Sonochem. 13: 54-60. 63. Kirk,O., Borchert, T. V. and Fuglsang, C. C. 2002. Industrial enzyme applications. Curr. Opin. Biotechnol. 13: 345-351. 64. Kristensen, J. B., Xu, X. and Mu, H. 2005. Diacylglycerol synthesis by enzymatic glycerolysis: screening of commercially available lipases. J. Am. Oil Chem. Soc. 82: 329-334. 65. Laszlo, J.A., Compton, D. L., Eller, F. J., Taylor, S. L. and Isbell, T. A. 2003. Packed-bed bioreactor synthesis of feruloylated monoacyl- and diacyl-glycerols: clean production of a “ green ” sunscreen. Green Chem. 5: 382-386. 66. Lee, G. S., Widjaja, A. and Ju, Y. H. 2006. Enzymatic synthesis of cinnamic acid derivatives. Biotechnol. Lett. 28: 581-585. 67. Lee, S. H., Nguyen, H. M., Koo, Y. M. and Ha, S. H. 2008. Ultrasound-enhanced lipase activity in the synthesis of sugar ester using ionic liquids. Process Biochem. 43: 1009-1012. 68. Lee, K. T., Akoh, C. C. and Dawe, D. L. 1999. Effects of structured lipid containing omega-3 and medium chain fatty acids on serum lipids and immunological variables in mice. J. Food Biochem. 23: 197-208. 69. Lee, Y. J., Liao, P. H., Chen, W. K. and Yang, C. Y. 2000. Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells. Cancer Lett. 153: 51-56. 70. Liu, L., Robert Hudgins, W., Shack, S., Yin, M. Q. and Samid, D. 1995. Cinnamic acid: A natural product with potential use in cancer intervention. Int. J. Cancer. 62: 345-350. 71. 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 marking it lipo-soluble. Chem. Phys. Lipids 62: 345-350. 72. Liu, Y., Jin, Q., Shan, L., Liu, Y., Shen, W. and Wang, X. 2008. The e?ect of ultrasound on lipase-catalyzed hydrolysis of soy oil in solvent-free system. Ultrason. Sonochem. 15: 402-407. 73. Long, W. S., Kamaruddin, A. and Bhatia, S. 2005. Chiral resolution of racemic ibuprofen ester in an enzymatic membrane reactor. J. Membr. Sci. 247: 185-200. 74. LOpez Giraldo, L. J., Laguerre, M., Lecomte, J., Figueroa-Espinoza, M. C., Barouh, N., Barea, B. and Villeneuve, P. 2007. Lipase-catalyzed synthesis of chlorogenate fatty esters in solvent-free medium. Enzyme Microb. Technol. 41: 721-726. 75. Malcata, F. X., Reyes, H. R., Garcia, H. S., Hill Jr., C. G. and Amundson, C. H. 1990. Immobilized lipase reactors for modification of fats and oils. A review. J. Am. Oil Chem. Soc. 67: 890-910. 76. Margulis, M. A. 1992. Fundamental aspects of sonochemistry. Ultrasonics 30: 152-154. 77. Marinova, E. M. and Yanishlieva, N. V. 1994. Effect of lipid unsaturation in the antioxidative activity of some phenolic acid. J. Am. Oil Chem. Soc. 71: 427-434. 78. Markham, K. R., Mitchell, K. A., Wilkins, A. L., Daldy, J. A. and Lu, Y. 1996. HPLC and GC-MS identification of the major organic constituents in NEW Zealand propolis. Phytochemisty 42: 205-211. 79. Masudo, T. and Okada, T. 2001. Ultrasonic Irradiation-Novel principle for microparticle separation. Anal. Sci. 17: 341-344. 80. Michaluart, P., Masferrer, J. L., Carothers, A. M., Subbaramaiah, K., Zweifel, B. S., Koboldt, C., Mestre, J. R., Grunberger, D., Sacks, P. G., Tanabe, T. and Dannenberg, A. J. 1999. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in rat model of inflammatory. Cancer Res. 59: 2347-2352. 81. Miethchen, R. 1992. Selected applications of sonochemistry in organic chemistry. Ultrasonics 30: 173-179. 82. Mirzoeva, O. K. and Calder, P. C. 1996. The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins Leukot. Essent. Fatty Acids 55: 441-449. 83. Nagaoka, T., Banskota, A. H., Tezuka, Y., Saiki, I. and Kadota, S. 2002. Selective antiproliferative activity of caffeic acid phenethyl ester analogues on highly liver-metastatic murine colon 26-L5 carcinoma cell line. Bioor. Med. Chem. 10: 3351-3359. 84. Natella, F., Nardini, M., Felice, M. D. and Scaccini, C. 1999. Benzoic and cinnamic acid derivatives as antioxidant: structure-activity relation. J. Agric. Food Chem. 47: 1453-1459. 85. Nielsen, N. S., Yang, T., Xu, X. and Jacobsen, C. 2006. Production and oxidative stability of a human milk fat substitute produced from lard by enzyme technology in a pilot packed-bed reactor. Food Chem. 94: 53-60. 86. Noelker, C., Bacher, M., Gocke, P., Weib, X., Klockgether, T., Du, Y. and Dodel, R. 2005. The ?avanoide caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. Neurosci. Lett. 383: 39-43. 87. Papay, V., Toth, L., Soltesz, M., Nagy, E. and Litkei, G. 1986. Isolated compounds from Hungarian propolis and populi gemma. Stud. Org. Chem. 23: 233-240. 88. Patist, A. and Bates, D. 2008. Ultrasonic innovations in the food industry: From the laboratory to commercial production. Innov. Food Sci. Emerg. Technol. 9: 147-154. 89. Pitt, W. G. and Rodd, A. 2003. Ultrasound increases the rate of bacterial growth. Biotechnol. Prog. 19: 1030-1044. 90. Posorske, L. H. 1984. Industrial-scale application of enzymes to the fats and oil industry. J. Am. Oil Chem. Soc. 61: 1758-1760. 91. Puri, S., Kaur, B., Parmar, A. and Kumar, H. 2009. Ultrasound-promoted greener synthesis of 2H-chromen-2-ones catalyzed by copper perchlorate in solventless media. Ultrason. Sonochem. 16: 705-707. 92. Piao, J., Kobayashi, T., Adachi, S., Nakanshi, K. and Matsuno, R. 2004. Continuous synthesis of lauroyl and oleoyl erythritol by a packed bed reactor with an immobilized lipase. Process Biochem. 39: 113-119. 93. Ribeiro, C. M. R., Passaroto, E. N. and Brenelli, E. C. S. 2001. Ultrasound in enzymatic resolution of ethyl 3-hydroxy-3-phenylpropanoate. Tetrahedron Lett. 42: 6477-6479. 94. Ramachandran, K. B., Al-Zuhair, S., Fong, C. S. and Gak, C. W. 2006. Kinetic study on hydrolysis of oils by lipase with ultrasonic emulsi?cation. Biochem. Eng. J. 32: 19-24. 95. Ratoarinoro, Contamine, F., Wilhelm, A. M., Berlan, J. and Delmas, H. 1995. Activation of a solid-liquid chemical reaction by ultrasound. Chem. Eng. Sci. 50: 554-558. 96. Royon, D., Daz, M., Ellenrieder, G. and Locatelli, S. 2007. Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. Bioresour. Technol. 98: 648-653. 97. Sabally, K., Karboune, S., St-Louis, R. and Kermasha, S. 2006. Lipase-catalyzed transesterification of trilinolein or trilinolenin with selected phenolic acids. J. Am. Oil Chem. Soc. 83: 101-107. 98. Shahidi, F. and Ho, C. T. 2005. Phenolic compounds in foods and natural health products. p. 2-5. American Chemical Society. Washington, DC, USA. 99. 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. 100. Stavarache, C., Vinatoru, M., Nishimura, R. and Maeda, Y. 2005. Fatty acids methyl esters from vegetable oil by means of ultrasonic energy. Ultrason. Sonochem. 12: 367-372. 101. Stepanovi?, S., Anti?, N., Daki?, I. and ?vabi?-Vlahovi?, M. 2003. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol. Res. 158: 353-357. 102. Stevenson, D. E., Parkar, S. G., Zhang, J., Stanley, R. A., Jensen, D. J. and Cooney, J. M. 2007. Combinatorial enzymic synthesis for functional testing of phenolic acid esters catalysed by Candida antarctica lipase B (NovozymR 435). Enzyme Microb. Technol. 40: 1078-1086. 103. Sud ’ ina, G. F., Mirzoeva, O. K., Pushkareva, M. A., Korshunova, G. A., Sumbatyan, N. V. and Varfolomeev, S. D. 1993. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett. 329: 21-24. 104. Talukder, M. M. R., Zaman, M. M., Hayashi, Y., Wui, J. C. and Kawanishi, T. 2006. Ultrasonication enhanced hydrolytic activity of lipase in water/isooctane two-phase systems. Biocatal. Biotransfor. 24: 189-194. 105. Twu, Y. K., Shih, I. L., Yen, Y. H., Ling, Y. F. and Shieh, C. J. 2005. Optimization of lipase-catalyzed synthesis of octyl hydroxyphenylpropionate by response surface methodology. J. Agric. Food Chem. 53: 1012-1016. 106. Vilkhu, K., Mawson, R., Simons, L. and Bates, D. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry-A review. Innov. Food Sci. Emerg. Technol. 9: 161-169. 107. Vosmann, K., Wiege, B., Weitkamp, P. and Weber, N. 2008. Preparation of lipophilic alkyl (hydroxy) benzoates by solvent-free lipase-catalyzed esterification and transesterification. Appl. Microbiol. Biotechnol. 80: 29-36. 108. Watanabe, Y., Shimada, Y., Sugihara, A. and Tominaga, Y. 2001. Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed-bed bioreactor. J. Am. Oil Chem. Soc. 78: 703-707. 109. Wei, X., Zhao, L., Ma, Z., Holtzman, D. M., Yan, C., Dodel, R. C., Hampel, H., Oertel, W., Farlow, M. R. and Du, Y. 2004. Caffeic acid phenethyl ester prevents neonatal hypoxicischaemic brain injury. Brain 127: 2629-2635. 110. Weitkamp, P., Vosmann, K. and Weber, N. 2006. Highly efficient preparation of lipophilic hydroxycinnamates by solvent-free lipase-catalyzed transesterification. J. Agric. Food Chem. 54: 7062-7068. 111. Wu, W. M., Lu, L., Long, Y., Wang, T., Liu, L., Chen, Q. and Wang, R. 2007. Free radical scavenging and antioxidative activities of ca?eic acid phenethyl ester (CAPE) and its related compounds in solution and membranes: A structure – activity insight. Food Chem. 105: 107-115. 112. Xiao, Y. M., Wu, Q., Cai, Y. and Lin, X. F. 2005. Ultrasound-accelerated enzymatic synthesis of sugar esters in nonaqueous solvents. Carbohyd. Res. 340: 2097-2103. 113. Xin, J. Y., Zhang, L., Chen, L. I., Zhang, Y., Wu, X. M. and Xia, C. G. 2009. Lipase-catalyzed synthesis of ferulyl oleins in solvent-free medium. Food Chem. 112: 640-645. 114. Xu, X. 2000. Production of specific-structured triacylglycerols by lipase- catalyzed reactions: a review. Eur. J. Lipid Sci. Tech. 102: 287-303. 115. Yadav, G. D. and Dhoot, S. B. 2009. Immobilized lipase-catalyzed synthesis of cinnamyl laurate in non-aqueous media. J. Mol. Catal. B-Enzym. 57: 34-39. 116. Yang, T., Rebsdore, M., Engelrud, U. and Xu, X. 2005. Enzymatic production of monoacylglycerols containing polyunsaturated fatty acid through an efficient glycerolysis system. J. Agric. Food Chem. 53: 1475-1481. 117. Yu, D., Tian, L., Wu, H., Wang, S., Wang, Y., Ma, D. and Fang, X. 2010. Ultrasonic irradiation with vibration for biodiesel production from soybean oil by NovozymR 435. Process Biochem. 45: 519-525. 118. Yu, X., Li, Y. and Wu. D. 2004. Enzymatic synthesis of gallic acid esters using microencapsulated tannase: effect of organic solvents and enzyme specificity. J. Mol. Catal. B-Enzym. 30: 69-73. 119. Yu, Z. R., Chang, S. W., Wang, H. Y. and Shieh, C. J. 2003. Study on synthesis parameters of lipase-catalyzed hexyl acetate in supercritical CO2 by response surface methodology. J. Am. Oil Chem. Soc. 80: 139-144. 120. Zhang, J., Chen, M., Ju, W., Liu, S., Xu, M., Chu, J. and Wu, T. 2010. Liquid chromatograph/tandem mass spectrometry assay for the simultaneous determination of chlorogenic acid and cinnamic acid in plasma and its application to a pharmacokinetic study. J. Pharm. Biomed. Anal. 51: 685-690. 121. Zhang, Y., Stanciulescu, M. and Ikura, M. 2009. Rapid transesterification of soybean oil with phase transfer catalysts. Appl. Catal. A-Gen. 366: 176-183 122. Zheng, L. and Sun, D. W. 2006. Innovative applications of power ultrasound during food freezing processes-A review. Trends Food Sci. Technol. 17: 16-23.