

Studies on the optimization of ultrasound-assisted enzymatic synthesis of caffeic acid phenethyl ester

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ABSTRACT

Phenolic acids are good radical scavengers for anti-inflammatory and anti-oxidant performances. Caffeic acid, one kind of phenolic acid, increase the solubility in oil-based formulas and emulsions is to esterify the compounds with alcohols and enhance anti-oxidant ability in the food and cosmetics applications. However, the reagents used in chemical synthesis of caffeic acid phenethyl ester (CAPE) are harmful to natural environmental. In contrast, enzymatic synthesis offers the advantages of specificity, milder reaction conditions, and minimization of side reactions and byproduct formation. Therefore, the value of using continuous ultrasound-assisted packed-bed bioreactor for the lipase-catalyzed processing should also permit an easier approach to producing commercial amount of CAPE. In this study, optimum conditions for the enzymatic synthesis of CAPE, catalyzed by immobilized lipase (NovozymR 435) were investigated. NovozymR 435 was used to catalyze caffeic acid and 2-phenyl ethanol in an isoctane system. 5-level-4-factor central-composite rotatable design (CCRD), Box-Behnken experiment design and response surface methodology (RSM) were employed to evaluate the effects of synthesis parameters on percentage conversion of CAPE by esterification for three part experiments. In the first part, immobilized enzymes were used to catalyze the esterification of caffeic acid with phenyl ethanol. The esterification improved the stability and hydrophobicity of phenolic acid. On the basis of ridge max analysis, the optimum conditions for synthesis were: reaction time 59 h, reaction temperature 69 oC, substrate molar ratio 1:72, and enzyme amount 351 PLU. The molar conversion of predicted value was 91.86% and actual experimental value was 91.65 ± 0.66%, respectively. In the second part, ultrasonication causes cavitations in the liquid medium. Subsequent collapses of the cavitation bubbles appear to cause a thorough mixing and stirring of the liquid solution, and the energy thus released should accelerate the enzymatic reactions. Ultrasound provides a very effective mixing and stirring in the reaction solution and increases the contacts between substrates and enzyme. The optimum condition for CAPE synthesis were reaction time 9.6 h, substrate molar ratio 1:71, enzyme amount 2938 PLU, and ultrasonic power 2 W/cm². The molar conversion of predicted values and actual experimental values were 96.03% and 93.08 ± 0.42%, respectively. In the third part, the ultrasound-acceleration synthesis of CAPE in a continuous packed-bed bioreactor was investigated. A three-level-three-factor Box-Behnken and RSM were employed on percent molar conversion of CAPE. The optimum conditions for synthesis CAPE were: reaction temperature of 72.66 °C, flow rate of 0.046 mL/min, and ultrasonic power of 1.64 W/cm². The molar conversion of predicted values and actual experimental values were 97.84% and 92.11 ± 0.75%, respectively. This work demonstrates of lipase in a continuous ultrasound-acceleration packed-bed bioreactor for industrial production of CAPE. The use of continuous ultrasound-acceleration packed-bed bioreactor in NovozymR 435-catalyzed synthesis of CAPE from caffeic acid and 2-phenyl ethanol in isoctane was investigated. Compared with chemical synthesis was more natural and milder synthesis process reduced the environmental damage, while the synthesized product of CAPE was also relatively safe for food or cosmetic applications. According to our results, used the natural enzyme catalysis and ultrasound to accelerate improve time-consuming for synthesis CAPE. The value of using packed-bed bioreactors for the lipase-catalyzed processing should also permit an easier approach to producing commercial amount of the product.

Keywords : biocatalysis、bioreactor、caffeic acid phenethyl ester、lipase、optimization、phenolic acid、ultrasonication

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REFERENCES

- 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。
- 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。
- Adamczak, M. and Krishna, S. H. 2004. Strategies for improving enzymes for efficient biocatalysis. *Food Technol. Biotechnol.* 42: 251-264.
- 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.
- 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.
- Bai, S., Guo, Z., Liu, W. and Sun, Y. 2006. Resolution of (\pm)-menthol by immobilized *Candida rugosa* lipase on superparamagnetic nanoparticles. *Food Chem.* 96: 1-7.
- Balachandran, S., Kentish, S. E., Mawson, R. and Ashokkumar, M. 2006. Ultrasonic enhancement of the supercritical extraction from ginger. *Ultrason. Sonochem.* 13: 471-479.
- 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.
- Balcao, V. M. and Malcate, F. X. 1998. Lipase catalyzed modification of milk fat. *Biotechnol. Adv.* 16: 309-341.
- Bankova, V., Popov, S. and Marekov, N. 1983. A study of flavonoids of propolis. *J. Nat. Prod.* 46: 471-474.
- 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. Inhibitory 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 transesterification. *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. Soc.* 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 pharmaceutical 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 (\pm)-2-methylbutanoate ester from racemic (\pm)-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-caffeooyl 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-deficient 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-inflammatory activity of caffeic acid phenethyl ester (CAPE) extracted from *Rhodiola sacra* against lipopolysaccharide-induced inflammatory 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-Louis, 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 effect 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. *Phytochemistry* 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 flavanoide caffeic acid phenethyl ester blocks 6-hydroxdopamine-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 emulsification. *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. Stepanović, S., Antić, N., Dakić, I. and Vabrić-Vlahović, 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. Sudina, 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 cafeic 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., Stanculescu, 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.