

利用固定化Novozym 435脂肪 β 生產海藻糖脂肪酸酯類

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摘要

醣類脂肪酸酯是一種新的非離子界面活性劑，係由醣類與不同鏈長的脂肪酸或甘油經過酯化反應後所得之酯類化合物。由於他們具有兩性(親水性與疏水性)、生物可降解性、無毒及可被生產為再生的資源。因此可用於食品、化粧品、醫療等領域。產業界大都是以化學法來進行醣類脂肪酸之合成。目前亦開始發展以酵素合成方式來生產安全性高之酯類，碳水化合物脂肪酸酯類的合成，是在微水環境下藉由酵素走水解之逆反應，催化醣類與脂肪酸或甘油結合所產出。目前之文獻資料顯示，利用脂肪 β 合成醣酯類所使用的糖類大都以蔗糖、果糖及麥芽糖等為主。而以海藻糖進行醣酯類合成的相關文獻鮮少。本研究之主要目的在於建立海藻糖脂肪酸酯類之合成條件。試驗中以100mM海藻糖及300mM硫辛酸，以2-甲基-2-丙醇(tert-butanol)為溶劑，以0.5g固定化脂肪 β (Novozym 435)於50 $^{\circ}$ C催化醣類反應合成海藻糖脂肪酸酯類。目前已建立海藻糖脂肪酸之合成條件。反應終產物經高效液相層析質譜儀分析後，證實含有單酯衍生物。未來將進一步確認此醣酯類之化學結構。

關鍵詞：生物催化、碳水化合物、直接酯化、醣類、脂肪 β 、硫辛酸、海藻糖

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參考文獻

- 1.王浩昱。2002。在超臨界二氧化碳流體中以之解酵素催化合成乙酸乙酯之研究。大葉大學食品工程研究所碩士論文，彰化。
- 2.田蔚城。1997。生物技術的發展與應用。九州圖書文物有限公司，台北。
- 3.朱炫威。脂解酵素在有機溶劑中水解棕櫚核仁軟脂之研究。大葉大學食品工程研究所碩士論文，彰化。
- 4.何國慶和丁立孝。2007。食品酵素學。五南圖書出版股份有限公司，台中。
- 5.李素菁和華傑。2004。食品產業導入生物科技之效益評估。食品工業發展研究所。
- 6.高宇。2005。生物化學。鼎茂圖書出版股份有限公司，台北。
- 7.張淑微。2002。以反應曲面法研究酵素合成以己醇酯類之最優化。大葉大學食品工程研究所碩士論文，彰化。
- 8.許德發、鄭智交、楊濟華、李仰穿、溫慧萍及許照紅。2002。化粧品概論。第107-120頁。華格那企業有限公司，台中。
- 9.陳怡臻及蕭斯欣。1998。台灣生物科技產業1997/1998。財團法人生物科技開發中心，台北。
- 10.彭志英。2004。食品酵素學。九州圖書文物有限公司，台北。
- 11.蕭介夫。1996。酵素工技與蛋白質工程研究之新發展。生物產業，第29-35頁。
- 12.謝雯玲。2001。台灣生物科技產業的發展。淡江大學國際貿易研究所碩士論文，台北。
- 13.鐵翠娟、寇秀芬、徐家立。2002。固定化脂肪 β 催化合成6,6'-海藻糖月桂酸酯。催化學報，第一期

14. Addo, K., Slepak, M. and Akoh, C.C. 1995. Effects of sucrose fatty acid ester and blends on alveograph characteristics of wheat flour doughs. *Journal of Cereal Science*. 22: 123-127.
15. Aloksey, Z., Alexander, M. and Klibanov. 1984. Enzymatic catalysis in organic media at 100 °C. *Science*, 24: 1249-1251.
16. Brich, G.G., Wolfrom, M.L. and Tyson, R.S. 1963. *Advances in carbohydrate chemistry*. Academic Press New York., 18: 201-225.
17. Chakraborty, S., Sahoo, B., Teraoka, I., Miller, L. M. and Gross, R. A. 2005. Enzyme-catalyzed regioselective modification of starch nanoparticles. *Macromolecules*, 38: 61-68.
18. Chen, J., Kimura, Y., and Adachi, S. 2005. Synthesis of linoleoyl disaccharides through lipase-catalyzed condensation and their surface activities. *Journal of Bioscience and Bioengineering*. 100: 274-279.
19. Degn, B. and Zimmermann, W. 2001. Optimization of carbohydrate fatty acid ester synthesis in organic media by a lipase from *Candida antarctica*. *Biotechnology and Bioengineering*. 74: 483-491.
20. Devulapalle, K.S., Segura, A.G., Ferrer, M., Alcalde, Miguel., Mooser, G. and Plouim, F.J. 2004. Effect of carbohydrate fatty acid esters on *Streptococcus sobrinus* and glucosyltransferase activity. *Carbohydrate Research*, 339: 1029-1034.
21. Elbein, A.D., Tipson, R.S. and Horton, D. 1974. *Advances in Carbohydrate Chemistry and Biochemistry*. Academic Press, New York. 30: 227-256.
22. Ferrer, M., Cruces, M. A., Plou, F. J., Bernabe, M. and Ballesteros, A. 2000. A simple procedure for the regioselective synthesis of fatty acid esters of maltose, leucrose, maltotriose and n-dodecyl maltosides. *Tetrahedron*, 56: 4053-4061.
23. Ferrer, M., Soliveri, J., Plou, F.J., Lopez-Cortes, N., Reyes-Duarte, D., Christensen, M., Copa-Patino, J.L. and Ballesteros, A. 2005. Synthesis of sugar ester in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme and Microbial Technology*. 36: 391-398.
24. Garti, N., Aserin, A. and Fanun, M. 2000. Non-ionic sucrose esters microemulsions for food applications. *Colloids and Surfaces A : Physicochemical and Engineering Aspects*. 164: 27-38.
25. Harding, T.S. 1997. History of trehalose: its discovery and methods of preparation. *Sugar*, 25: 476-478.
26. Ishimoto, R., Sugimoto, M. and Kawai, F. 2001. Screening and characterization of trehalose-oleate hydrolyzing lipase. *FEMS Microbiology*. 195: 231-235.
27. Khaled, N., Montet, D., Pina, M. and Graille, J. 1991. Fructose oleate synthesis in a fixed catalyst bed reactor. *Biotechnology Letters*. 13: 167-172.
28. Kou, X. and Xu, J. 2000. Enzymatic synthesis of saccharide and saccharide alcohol fatty acid esters. *Wei Sheng Wu Xue Bao*. 40:193-197.
29. Ku, M.A. and Hang, Y.D. 1995. Enzymatic synthesis of esters in organic medium with lipase from *Byssoschlamys fulva*. *Biotechnology Letters*. 17: 1081-1084.
30. Marangon, K., Devaraj, S., Tirosh, O., Packer, L. and Jialal, I. 1999. Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress. *Free Radical Biology & Medicine*. 27: 1114-1121.
31. Muller, A.S., Gagnaire, J., Queneau, Y., Karaoglanian, M., Maitre, J.P. and Bouchu, A. 2002. Winsor behaviour of sucrose fatty acid esters : choice of the cosurfactant and effect of the surfactant composition. *Colloids and Surfaces A : Physicochemical and Engineering Aspects*. 203: 55-66.
32. Oosterom, M.W., Rantwijk, F. and Sheldon, R.A. 1996. Regioselective acylation of disaccharides in tert-butyl alcohol catalyzed by *Candida antarctica* lipase. *Biotechnology and Bioengineering*. 49: 328-333.
33. Osipow, L., Snell, F. D., York, W.C. and Finchler, A. 1956. Methods of preparation fatty acid esters of sucrose. *Industrial and Engineering Chemistry*. 48: 1459-1462.
34. Packer, L., Witt, E.H. and Tritschler, H.J. 1995. Alpha-Lipoic acid as a biological antioxidant. *Free Radical Biology & Medicine*, 19: 2278-50.
35. Pavia, D.L., Lampman, G.M. and Kriz, G.S. 2000. *Introduction to spectroscopy: A guide for students of organic chemistry*. (third ed) p168. Thomson Learning. USA.
36. Pedersen, N.R., Wimmer, R., Emmersen, J., Degn, P. and Pedersen, L.H. 2002. Effect of fatty acid chain length on initial reaction rates and regioselectivity of lipase-catalysed esterification of disaccharides. *Carbohydrate Research*. 337: 1179-1184.
37. Podda, M., Zollner, T.M., Grundmann-Kollmann, M., Thiele, J.J., Packer, L. and Kaufmann, R. 2001. Activity of alpha-lipoic acid in the protection against oxidative stress in skin. *Current Problems in Dermatology*, 204: 98-104.
38. Pouchert, C. J. and Behnke, J. 1992. *The Aldrich library of 1H and 13C FT NMR spectra*, Aldrich Chemical, Milwaukee, MI, USA, p819.
39. Quan, J., Xu, J.M., Liu, B.K., Zheng, C.Z. and Lin, X.F. 2007. Synthesis and characterization of drug-saccharide conjugates by enzymatic strategy in organic media. *Enzyme and Microbial Technology*. 41:756-763.
40. Raku, T., Kitagawa, M., Shimakawa, and Tokiwa, Y. 2003. Enzymatic synthesis of hydrophilic undecylenic acid sugar esters and their biodegradability. *Biotechnology Letters*, 25:161-166.
41. Raku, T., Kitagawa, M., Shimalawa, H. and Tokiwa, Y. 2003. Enzymatic synthesis of trehalose esters having lipophilicity. *Journal of Biotechnology*. 100: 230-208.
42. Richards, A.B., Krakowka, S. Dexter, L.B., Schmid, H. Wolterbeek, A.P.M., Waalkens-Berendsen, D.H. Shigoyuki, A. and Kurimoto, M. 2002. Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. *Food and Chemical Toxicology*, 40: 871-898.
43. Sabeder, S., Habulin, M. and Knez, Z. 2006. Lipase-catalyzed synthesis of fatty acid fructose esters. *Journal of Food Engineering*. 77: 880-886.
44. Sadtler, V.M., Guey, M. Marchal, P. and Choplin, L. 2004. Shear-induced phase transitions in sucrose ester surfactant. *Journal of Colloid and Interface Science*. 270: 270-275.
45. Scheckermann, C., Schlotterbeck, A. Schmidt, M., Wray, V. and Lang, Siegmund. 1995. Enzymatic monoacylation of fructose by two procedures. *Enzyme and Microbial Technology*. 17: 157-162.
46. Schiraldi, C., Di Lemia, I. and De Rose, M. 2002. Trehalose production :exploiting novel approaches. *Trends in Biotechnology*. 20: 420-425.
47. Schmid, R.D. and Verger, R. 1998. Lipases: Interfacial enzymes with attractive applications. *Angewandte Chemie International edition*. 37: 1608-1633.
48. Shieh, C.J. and Lou, Y.H. 2000. Five-factor response surface optimization of enzymatic synthesis of citronellyl butyrate by lipase IM77 from *Mucor miehei*. *Journal of the American Oil Chemists' Society*. 77: 521-525.
49. Tchinda, A.T., Tane, P., Ayafor, J.F. and Connolly, J.D. 2003. Stigmastane derivatives and isovaleryl sucrose esters from *Vernonia guineensis* (Asteraceae). *Phytochemistry*. 63: 841-864.
50. Thormar, H. and Hilmarsson, H. 2007. The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chemistry and Physics of Lipids*, 150: 1-11.
51. Tsuzuki, W., Kitamura, Y., Suzuki, T. and Kobayashi, S. 1999. Synthesis of Sugar fatty acid esters by modified lipase. *Biotechnology and Bioengineering*. 64: 267-271.
52. Tsuchido, T., Yokosuka, M. and Takano, M. 1993. Isolation and characteristics of a *Bacillus subtilis* mutant tolerant to the lytic action of sucrose esters of long-chain fatty acids. *Journal of Fermentation and Bioengineering*. 75: 191-195.
53. Tsuzuki, W., Kitamura, Y., Suzuki, T. and Kobayashi, S. 2008. Synthesis of sugar fatty acid esters by modified lipase. *Biotechnology*

and Bioengineering. 64: 267-271. 54. Vijayakumar, G. R., Lohith, K., Somashekar, B.R. and Divakar, S. 2004. Lipase catalyzed synthesis of l-alanyl, l-leucyl and l-phenylalanyl esters of d-glucose using unprotected amino acids. *Biotechnology Letters*. 26: 1323-1328. 55. Ward, O.P., Fang, J. and Li, Zuyi. 1997. Lipase-catalyzed synthesis of a sugar ester containing arachidonic acid. *Enzyme and Microbial Technology*. 20: 52-56. 56. Watanabe, Y., Miyawaki, Y., Adachi, S., Nakanishi, K. and Matsunno, R. 2000. Synthesis of lauroyl saccharides through lipase-catalyzed condensation in microaqueous water-miscible solvents. *Journal of Molecular Catalysis*. 10: 241-247. 57. Xie, J. and Hsieh, Y. L. 2001. Enzyme-catalyzed transesterification of vinyl esters on cellulose solids. *Biocmica Biophysica et Acta.*, 39:1931-1923. 58. Zhao, H., Baker, G.A., Song, Z., Olubajo, O., Grittle, T., and Peters, D. 2008. Designing enzyme-compatible ionic liquids that can dissolve carbohydrate. *Green Chemistry*. 10: 696-705.