# 海藻糖-硫辛酸酯之合成及其自由基清除能力之研究 = Studies on synthesis of trehalose-Lipoic acid ester and its free-radical


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**摘 要**

糖酯衍生物是一種非離子界面活性劑，可被生物降解，無毒和再生的功能且也可經由酵素催化來合成。這種“ 天然 ”的產品，因此在食品，化妝品和藥品的應用有極大的作用。為了提高其應用價值和範圍，糖（即海藻糖）和脂肪酸（即硫辛酸）因為它們有特殊的生活活性作用，因此選定此原料再經由脂肪?催化合成多功能產品（即海藻糖單雙糖脂肪酸酯）。在這項研究中核磁共振（NMR）的判別，已經成功確定海藻糖酯衍生的實際結構，且將進一步探討產率的優化反應條件如下；包括反應時間（1-6天），反應溫度（30 - 50℃），莫耳濃度比（海藻糖：硫辛酸=1:1-1:5），和脂肪?量（0.1-0.5克），水分添加(0-25%)，基質階梯式添加1(莫耳/天)，共溶液比1:1-8:1(DMSO:tert-Butyl alcohol、2M-2B( 2-methyl-2-butanol)、Acetonitrile、Hexane)。 從結果可得知，最適化條件為：反應天數4天、基質莫耳比1:4(海藻糖:硫辛酸)、酵素添加量0.3g、反應溫度40℃、水份添加量0%、基質階梯式添加1(莫耳/天)、共溶液8:1(DMSO:2M-2B)，以此條件的實際實驗值為66.82±0.01%。進一步檢測產物的生物功能和海藻糖脂肪酸酯產率最優化的研究和探討，且將應用在市場或工業上。



關鍵詞 : 生物表面活性劑、海藻糖、脂肪酸、直接酯化、脂肪?、核磁共振（NMR）、最優化、生理活性


**目錄**

**參考文獻**

1.謝岱紘。2004。界面活性劑類型與含量對四氯乙烯在氣液間質傳現象影響之探討。國立高雄第一科技大學環境與安全衛生工程學系碩士論文。 2.陳秀芬。2005。含界面活性劑水溶液中有機汙染物揮發特性之探討。環境工程所碩士論文。 3.卓昔弘。2003。醚基連接型之Gemini界面活性劑之合成及性質研究。國立台灣科技大學高分子工程研究所碩士論文。 4.刈米孝夫原著、王鳳英編譯。2008。界面活性劑的原理與應用。高立圖書有限公司。 5.蔡維娟。2003。藉由混合反應曲面法探討溶劑極性對酵素催化甘油解產物選擇性之影響。大葉大學食品工程學系碩士班碩士論文。 6.陳威丞。2004。兼具酪胺酸?抑制力及抗氧化力，中藥材之篩選。大同大學生物工程研究所碩士論文。 7.段世鋒，王萬興。1990。非離子表面活性劑。北京:中國鐵道出版社，1—5。 8.劉程。1995。表面活性劑應用手?。北京:化學工業出版社，23-25。 9.嚴群芳。2005。非離子表面活性劑的性質及應用。?州化工，30(5):4-7 10.馮雷剛。2004。非水相脂肪?催化合成糖酯的研究。天津科技大學碩士論文。 11.劉巧瑜、張曉鳴。2010。糖酯的表面活性及其在食品加工中的應用。6月第31卷第6期。 12.王銀珍。2009。脂肪?合成糖酯的催化作用研究．廣西大學碩士學位論文。 13.孫月娥、夏文水、陸洁。2009。非水相?法合成亞麻油酸海藻糖單醣工藝的研究。第25卷第3期。 14.孫月娥。2009。不飽和脂肪酸海藻糖單酯?法合成及水相氧化研究，江南大學，博士學位論文。 15.鐵翠娟，寇秀芬，徐家立。2002。固定化脂肪?催化合成6,6,-海藻糖月桂酸酯。第23卷第1期。 16.張淑微。2001。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論文。 17.賴麗羽。2010。利用固定化Novozym435脂肪?生產海藻糖脂肪酸酯類。大葉大學生物產業科技研究所論文。 18.劉欣怡。2002。透過抗發炎、抗氧化及抗腫瘤促進作用評估硫辛酸在化學預防上可能扮演的角色。國立成功大學環境醫學研究所碩士論。 19.李俊穎。2008。海藻糖合成?的基因表現及其應用於合成海藻糖。國立中正大學化學工程所碩士論文。 20.郭孟欣。2003。海藻糖生成?的生產與特性探討。國立臺灣海洋大學食品科學系碩士學位論文。 21.A.Fiechter. 1992. Biosurfactants moving to wards industrial application, Trends Biotechnol,10208,217. 22.A.Kato, K. Arima. 1971. Inhibitory effect of sucrose ester of lauric acid on the growth of Escherichia coli, Biochemical and Biophysical Research Communications, 42:596 – 601. 23.A.K. Hathcox, L. R. Beuchat. 1996. Inhibitory effects of sucrose fatty acid esters , alone and in combination wiethylenediaminetetraacetic acid and other organic acids, on viability of Escherichia coli.International Journal of Food Microbiology, 13:213 – 225. 24.A, M.S.M. Annuar, T. Heidelberg, Y. Chisti . 2011. Lipasemediated synthesis of sugar fatty acid esters. Process Biochemistry, 462079 – 2090. 25.A.M. Gumel. 2011. Artificial neural network analysis of lipase-catalyzed synthesis of sugar alcohol ester. Industrial Crops and Products, 33:42 – 48. 26.Biewenga GP. Haenen GR. Bast A. 1997 .The pharmacology of the antioxidant lipoic acid. General Pharmacology, 29:315 – 31. 27.Coulon D, Ghoul M. 1998. The enzymatic synthesis of non-ionic surfactants: the sugar esters – an overview. Agro Food Industry Hi-Tech, 9 : 22 – 6. 28.Dastoli,F.R.,Musto,N.A.,and Price,S. 1966.Reactivity of active sites of chymotrypsin suspended in an organic medium. Archives of Biochemistry and Biophysics, 115:44 – 47. 29.Dordick, J. D. 1991.Designing enzymes for use in organic solvents, Biotechnology Progress, 8:259 – 267. 30.Degn P, Pedersen LH, Duus J, Zimmermann W. 1999. Lipase-catalysed synthesis of glucose fatty acid esters in tertbutanol. Journal of Biotechnology Letters, 21:275 – 80. 31.Elbein, A. D., Pan, Y. T., Pastuszak, I., Carroll, D. 2003 .New insights on trehalose a multifunctional molecule. Journal of Glycobiology, 13:17R – 27R. 32.Fersht,A. 1985.Stereochemistry of enzymatic reactions, Yale Journal of Biology & Medicine, 221-247. 33.Gurer H. Ozgunes H. Oztezcan S. Ercal N. 1999. Antioxidant role of alpha-lipoic acid in lead toxicity. Journal of the Society for Free Radical Biology and Medicine, 27(1-2):75 – 81. 34.Hounsa, C. G., Brandt, E. V., Trevelyan, J., Hohmann, S., Prior, B. A. 1998.Role of trehalose in survival of Saccharomyces cerevesiae under osmotic stress. The American Society For Microbiology, 144: 671 – 680. 35.Klibanov,A.M.,1989.Enzymatic catalysis in anhydrous organic solvent.Trends in Biochemical Sciences, 14:141-144. 36.Klibanov, A.M. 1983. Basic biology of new developments in biotechnology. Plenum, New York, 497 – 517. 37.Klibanov, A.M. 1989.Enzymatic catalysis in anhydrous organic solvent.Trends in Biochemical Sciences, 14:141 – 144. 38.Klibanov, A.M.,Samokhin,G.P.,Martinek,K.,and Berezin, I.V. 1977.A new approach to preparative enzymatic synthesis. Biotechnology Bioengineering, 19:1351 – 1361. 39.Kirk,O.and Christensen,M.W. 2002.Lipase from Candida antarctica:Unique biocatalysts from a unique origin.Organic. Process Research & Development, 6:446-451. 40.Laane, C., Boneren, S., Vos, K., and Veeger, C. 1987.

Rulesfor optimizations of biocatalysis in organic solvents. Biotechnol Bioeng, 30 : 81 – 87. 41.M.A.Godshall.2001.Future directions for the sugar industry, international sugar journal, 103 ,378 – 384. 42.Muller, J., Wiemken, A., Aeschbacher, R. 1999.Trehalose metabolism in sugar sensing and plant development. An international journal of experimental plant biology, 147:37 – 47 . 43.Marangon K. Devaraj S. Tirosh O. Packer L. Jialal I. 1999. Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress. Journal of the Society for Free Radical Biology and Medicine, 27:1114 – 21. 44.Maja Habulin, Sasa Sabeder, Zeljko Knez. 2008. Enzymatic synthesis of sugar fatty acid esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. The Journal of Supercritical Fluids, 45:338 – 345. 45.Manuel Ferrer, Juan Soliveri, Francisco J. Plou, Nieves L ' opez-Cort ' es, Dolores Reyes-Duarte, Morten Christensen , Jos ' e L. Copa-Pati?no , AntonioBallesteros . 2005. Synthesis of sugar esters in solvent mixtures by lipases from Thermomyces lanuginosus and Candida antarctica B, and their antimicrobial properties. Enzyme and Microbial Technology, 36:391 – 398. 46.No, J. K., D. Y. Soung, Y. J. Kim, K. H. Shim, Y. S. Jun, S. H.Rhee, T.Yokozawa, and H. Y. Chung. 1999. Inhibition of tyrosinase by green teacomponents. LSc, 65:241 – 246. 47.Oosterom WM, van Rantwijk F, Sheldon RA. 1996.Regioselective acylation of disaccharides in t-butyl alcohol catalysed by Candidaantartica lipase. Biotechnology and Bioengineering, 49:328 – 33. 48.Oguntimein GB, Erdmann H, Schmid RD. 1993. Lipase catalyzedsynthesis of sugar ester in organic solvent. Journal of Biotechnology Letters, 15:175 – 80. 49.Ly Villo , Malle Kreen, Marina Kudryashova, Andrus Metsala, Sven Tamp, Tonis Pehk, Omar Parve. 2011. A chemoenzymatic synthesis of deoxy sugar esters involving stereoselective acetylation of hemiacetals catalyzed by CALB. Journal of Molecular Catalysis B: Enzymatic, 68:44 – 51. 50.Podda M. Zollner TM. Grundmann-Kollmann M. Thiele JJ. Packer L. Kaufmann R. 2001. Activity of alpha-lipoic acid in the protection against oxidative stress in skin. Current Problems in Dermatology, 29:43 – 5 51.Packer L. Witt EH. 1995.Tritschler HJ. Alpha-Lipoic acid as a biological ntioxidant. Free Radical Biology & Medicine, 19:227 – 50. 52.Rupley, Y. A., and Careri, G. 1991 . Protein hydration and function.Advances in Protein Chemistry, 41,:37-172. 53.Pan, Y. T., Edavana, V. K., Jourdian, W. J., Edmondson, R., Carroll, J. D., Pastuszak, I., Elbein, A. D. 2004.Trehalose synthase of Mycobacterium smegmatis, purification, cloning, expression,and properties of the enzyme .European Journal of Biochemistry, 271:4259 – 4269. 54.Rosa Margesin, F.S. 1999.Biotechnological applications of cold-adapted organisms.Springer, 338pp. 55.Richards, A. B., Krakowka, S., Dexter, L. B., Schmid, H., Wolterbeek, A.P. M., Waalkens-Berendsen, D. H., Shigoyuki, A.,Kurimoto, M. 2002.Trehalose: a review of properties, history of useand human tolerance,and results of multiple safety studies. Food and Chemical Toxicology, 40:871-898. 56.Saha,K., N. H. Lajis, A. S. Hamzah, S. Khozirah, S. Khamis, and A.Syahida. 2004. Evaluation of antioxidant and nitric inhibitory activities of selected Malaysian medicinalplants. Journal of Ethnopharmacology, 92:263 – 267. 57.Schiraldi, C., Lernia, I. D., Rosa, M. D. 2002.Trehalose production :exploiting novel approaches. Trends in Biotechnology, 20:420-425. 58.Soichi Furukawa , Yuko Akiyoshi , George A. O'Toole , Hirokazu Ogihara , Yasushi Morinaga. 2010. Sugar fatty acid esters inhibit biofilm formation by food-borne pathogenic bacteria. International Journal of Food Microbiology, 138:176 – 180. 59.Sabeder S, Habulin M, Knez Z. 2006.Lipase-catalyzed synthesis of fatty acid fructose esters. Journal of Food Engineering, 77:880 – 6. 60.Soultani S, Engasser J-M, Ghoul M. 2001. Effect of acyl donor chain length and sugar/acyl donor molar ratio on enzymatic synthesis of fatty acid fructose esters. Journal of Molecular Catalysis B:Enzymatic, 11:725 – 31. 61.Sharma A, Chattopadhyay S. 1993.Lipase catalysed acylation of carbohydrates. Journal of Biotechnology Letters, 15:1145 – 93. 62.Tarahomjoo S, Alemzadeh I. 2003 . Surfactant production by an enzymatic method. Journal of Enzyme and Microbial Technology, 33:33 – 7. 63.T. Polat, R.J. Linhardt. 2001.Syntheses and applications of sucrose-based esters, Journal of Surfactants Deterg, 4:415 – 421. 64.Volkin, D. B., Staubli, A., Langer, R., and Klibanov, A. M. 1991. Enzyme thermoinactivation in anhydrons organic solvent. Journal of Biotechnol. Bioeng.37 , 843 – 853. 65.Villeneuve P.and Foglia T.A. 1997.Lipase specificities:potential application in lipid bioconversions. Journal of Financial Statement Analysis, 8:640-651. 66.Ward OP, Fang J, Li Z. 1997. Lipase-catalyzed synthesis of a sugar ester containing arachidonic acid. Enzyme and Microbial Technology, 20:52 – 6. 67.Y. Yan, U.T. Bornscheuer, L. Cao, R.D. Schmid. 1999 .Lipase-catalyzed solidphase synthesis of sugar fatty acid esters,.Enzyme Microb.Technol, 25:725 – 728. 68.Y. Nishikawa, M. Okabe, K. Yoshimoto, G. Kurono, F. 1976. Fukuoka,Antitumor activitiy of saturated fatty acids and their ester derivates against Ehrlich ascites carcinoma,chemical & pharmaceutical bulletin, 24:387 – 393. 69.Yu J, Zhang J, Zhao A, Ma X. 2008. Study of glucose ester synthesis by immobilized lipase from Candida sp. Catalysis Communications, 9:1369 – 74.