# 以脂解酵素合成丙二醇脂肪酸單酯之研究


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**摘 要**

丙二醇單酯 (PROPYLENE GLYCOL MONOESTERS, PGME) 是一種具有低 HLB (HYDROPHILIC-LIPOPHILIC BALANCE) 值的親脂型乳化劑，已被美國食品藥物管理局核准使用於食品和藥品等用途上。丙二醇單酯可利用化學法和酵素法進行合成，但化學法會因高溫高壓造成產率低及生成不必要的副產物等缺點；相較於化學法，酵素法具有反應條件溫和、降低成本及產物容易分離純化等優點。 本研究利用脂解酵素 IM77 催化丙二醇和脂肪酸 (月桂酸 C12:0、硬脂酸 C18:0) 之間的直接酯化反應，實驗中套用反應曲面法 (RESPONSE SURFACE METHODOLOGY, RSM) 和三階層四變數的部份因子實驗設計 (FRACTIONAL FACTORIAL EXPERIMENTAL DESIGN) 來探討反應變數，如反應時間 (3-9 H)、反應溫度 (25-65 oC)、脂肪酸與丙二醇之基質莫耳比 (1:1-3:1) 及酵素用量 (15-45%) 之間的關係及對丙二醇單酯之莫耳轉換率的影響，並求得丙二醇單酯最優化的合成條件。 研究結果發現不同碳鏈長度的脂肪酸對丙二醇單酯的莫耳轉換率會造成影響，以 C12:0 優於 C18:0 的丙二醇單酯產率。最後可由等高線圖預測丙二醇月桂酸單酯之最優化合成條件：反應時間 7.6 H、反應溫度 37.6 oC、基質莫耳比 2.6:1、酵素用量 37.1%，產率可高達 100%;而丙二醇硬脂酸單酯 (PROPYLENE GLYCOL MONOSTEARATE, PGMS) 最優化之合成條件為：反應時間 7.9 H、反應溫度 53.4 oC、基質莫耳比 2.6:1、酵素用量 35.2%，產率可高達 98.5%。



關鍵詞 : 丙二醇單酯、直接酯化、反應曲面法、部份因子實驗設計、最優化


**目錄**


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**參考文獻**


1.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 ?之培養基最適化研究。中國農業化學會誌。30:264-272。 2.李敏雄和鍾玉明。1993。以酵素法生產單甘油酯。食品工業。25:10-18。 3.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。 4.陳俊成。2000。乳化劑之合成與組成。食品資訊。172:29-35。 5.陳俊成。1985。酵素在油脂工業上之應用。食品工業。17:26-33。 6.陳國誠。1989。酵素工程學。藝軒圖書出版社。PP. 1-57。 7.陳樹人。1999。以 ACINETOBACTER RADIORESISTENS 生產耐鹼性脂肪酵素-醱酵生理之探討。化學工 程研究所博士論文。國立成功大學。PP. 1-129。 8.張曉莉和黃世佑。1997。生物轉換法-有機溶劑中維持酵素活性之研究。化工。44:71-84。 9.楊海明。1986。脂解?在油脂交酯化上的應用。食品工業。18:34-43。 10.AKOH, C. C. 1993. LIPASE-CATALYZED SYNTHESIS OF A PARTIAL GLYCERIDE. BIOTECHNOL. LETT. 15: 949-954. 11.AKOH, C. C., COOPER, C., AND NWOSU, C. V. 1992. LIPASE G- CATALYZED SYNTHESIS OF MONOGL YCERIDES IN ORGANIC SOLVENT AND ANALYSIS BY HPLC. J. AM. OIL CHEM. SOC. 69: 257-260. 12.ARTMAN, N. R. 1975. SAFETY OF EMULSIFIERS IN FATS AND OILS. J. AM. OIL CHEM. SOC. 52: 4 9-52. 13.ASLAN, A. AND GENC, M. 1996. CONDITIONS OF MONOGLYCERIDE PRODUCTION BY USING LIPASE IN A TRIGLYCERIDE GLYCEROL SYSTEM. TURKISH J. BIOL. 20: 37-47. 14.BARTON, M. J., HAMMAN, J. P., FICHTER, K. C., AND CALTON, G. J. 1990. ENZYMATIC RESOLUT -ION OF (R,S)-2-(4-HYDROXY- PHENOXY) PROPIONIC ACID. ENZYME MICROB. TECH. 12: 577-583. 15.BATTISTEL, E., BIANCHI, D., CESTI, P., AND PINA, C. 1991. ENZYMATIC RESOLUTION OF (S)-(+)-NAPROXEN IN A CONTINUOUS REACTOR. BIOTECHNOL. BIOENG. 38: 659-664. 16.BERGER, M., LAUMEN, K., AND SCHNEIDER, M. P. 1992. LIPASE-CATALYZED ESTERIFICATION OF H -YDROPHILIC DIOLS IN ORGANIC SOLVENTS. BIOTECHNOL. LETT. 14: 553-558. 17.BERGER, M. AND SCHNEIDER, M. P. 1993. REGIOISOMERICALLY PURE MONO- AND DIACYLGLYCEROLS AS SYNTHETIC BUILDING BLOCKS. FAT SCI. TECHNOL. 95: 169-175. 18.BERGER, M. AND SCHNEIDER, M. P. 1992. ENZYMATIC ESTERIFICATION OF GLYCEROL II. LIPASE-C ATALYZED SYNTHESIS OF REGIOISOMERICALLY

PURE 1(3)-RAC-MONOACYLGLYCEROLS. J. AM. OIL CHE M. SOC. 69: 961-965. 19.BLOOMER, S., ADLERCREUTZ, P., AND MATTIASSON, B. 1990. TRIGLYCERIDE INTERESTERIFICATION BY LIPASES. 1. COCOA BUTTER EQUIVALENTS FROM A FRACTION OF PALM OIL. J. AM. OIL CHEM. S OC. 67: 519-523. 20.BORNSCHEUER, U. T. AND YAMANE, T. 1995. FATTY ACID VINYL ESTERS AS ACYLATING AGENTS: A NEW METHOD FOR THE ENZYMATIC SYNTHESIS OF MONOACYLGLYCEROLS. J. AM. OIL CHEM. SOC. 72: 193-197. 21.BORNSCHEUER, U. T. 1995. LIPASE-CATALYZED SYNTHESIS OF MONOACYLGLYCEROLS. ENZYME MICROB. TECH. 17: 578-586. 22.BORNSCHEUER, U. T., STAMATIS, H., XENAKIS, A., YAMANE, T., AND KOLISIS, F. N. 1994. A C -OMPARISON OF DIFFERENT STRATEGIES FOR LIPASE-CATALYZED SYNTHESIS OF PARTIAL GLYCERIDES. BIOTECHNOL. LETT. 16: 697-702. 23.BORNSCHEUER, U. T. AND YAMANE, T. 1994. ACTIVITY AND STABILITY OF LIPASE IN THE SOLID-P -HASE GLYCEROLYSIS OF TRIOLEIN. ENZYME MICROB. TECH. 16: 864-869. 24.BROCKMAN, H. L. 1984. IN "LIPASE", ED. B. BORGSTROM AND H. L. BROCKMAN, ELSEVIER, AMSTE -RDAM. PP. 3-47. 25.CAO, S. G., YANG, H., MA, L., AND GUO, S. Q. 1996. ENHANCING ENZYMATIC PROPERTIES BY TH -E IMMOBILIZATION METHOD. APPL. BIOCHEM. BIOTECHNOL. 59: 7-14. 26.CHANG, M. K., ABRAHAM, G., AND JOHN, V. T. 1990. PRODUCTION OF COCOA BUTTERLIKE FAT FRO -M INTERESTERIFICATION OF VEGETABLE OILS. J. AM. OIL CHEM. SOC. 67: 832-834. 27.CHEN, C. S. AND SIH, C. J. 1989. GENERAL ASPECTS AND OPTIMIZATION OF ENANTIOSELECTIVE B -IOCATALYSIS IN ORGANIC SOLVENTS: THE USE OF LIPASES. ANGEW. CHEM. INT. ED. ENGL., 28: 695-707. 28.CHEN, S. L. 1981. OPTIMIZATION OF BATCH ALCOHOLIC FERMENTATION OF GLUCOSE SYRUP SUBSTRA -TE. BIOTECHNOL. BIOENG. 23: 1827-1836. 29.CHOPINEAU, J., MCCAFFERTY, F. D., THERISOD, M., AND KLIBANOV, A. M. 1988. PRODUCTION OF -BIOSURFACTANTS FROM SUGAR ALCOHOLS AND VEGETABLE OILS CATALYZED BY LIPASES IN A NONAQU -EOUS MEDIUM. BIOTECHNOL. BIOENG. 31: 208-214. 30.CHOPINEAU, J., MCCAFFERTY, F. D., THERISOD, M., AND KLIBANOV, A. M. 1987. PRODUCTION OF -BIOSURFACTANTS FROM SUGAR ALCOHOLS AND VEGETABLE OILS CATALYZED BY LIPASES IN A NONAQU -EOUS MEDIUM. BIOTECHNOL. BIOENG. 31: 208-214. 31.DAE, Y., HYO, N., AND SUK, H. 1997. ESTERIFICATION PATTERNS OF LIPASES FOR SYNTHESIZING -TRICAPROYLGLYCEROLS IN ORGANIC SOLVENT. J. AM. OIL CHEM. SOC. 74: 1287-1290. 32.DORDICK, J. S. 1992. DESIGNING ENZYMES FOR USE IN ORGANIC SOLVENTS. BIOTECHNOL. PROG. 8 : 259-267. 33.ENGEL, K. H. 1991. LIPASE-CATALYZED ENANTIOSELECTIVE ESTERIFICATION OF 2-METHYLALKANOIC -ACIDS. TETRAHEDRON: ASYMMETRY. 2: 165-168. 34.FABER, K. 1992. BIOTRANSFORMATIONS IN ORGANIC CHEMISTRY. SPRINGER-VERLAG, GERMANY. 2-4. 35.FERREIRA-DIAS, S. AND FONSECA, M. M. R. 1993. ENZYMATIC GLYCEROLYSIS OF OLIVE OIL: A RE -ACTIONAL SYSTEM WITH MAJOR ANALYTICAL PROBLEMS. BIOTECHNOL. TECHNOL. 7: 447-452. 36.FITZPATRICK, P. A. AND KLIBANOV, A. M. 1991. HOW CAN THE SOLVENT AFFECT ENZYME ENANTIOS -ELECTIVITY ? J. AM. OIL CHEM. SOC. 113: 3166-3171. 37.FLETCHER, P. D. I., FREEDMAN, R. B., ROBINSON, B. H., REES, G. D., AND SCHOMACKER, R. 1 987. BIOCHIM. BIOPHYS. ACTA. 912: 278-282. 38.FRANCISCO J. PLOU, MARIA BARANDIARAN, MARIA V. CALVO, ANTONIO BALLESTEROS, AND EITEL PA STOR. 1996. HIGH-YIELD PRODUCTION OF MONO- AND DI-OLEYLGLYCEROL BY LIPASE-CATALYZED HYD ROLYSIS OF TRIOLEIN. ENZYME MICROB. TECHNOL. 18: 66-71. 39.GANCET, C. 1990. CATALYSIS BY RHIZOPUS ARRHIZUS MYCELIAL LIPASE. ANN. N. Y. ACAD. SCI. 613: 600-604. 40.GAO, X. G., CAO, S. G., AND ZHANG, K. C. 2000. PRODUCTION, PROPERTIES AND APPLICATION T O NONAQUEOUS ENZYMATIC CATALYSIS OF LIPASE FROM A NEWLY ISOLATED PSEUDOMONAS STRAIN. EN ZYME MICROB. TECHNOL. 27: 74-82. 41.GIOVANNI, M. 1983. RESPONSE SURFACE METHODOLOGY AND PRODUCT OPTIMIZATION. FOOD TECHNOL. 11: 41-45, 83. 42.GOLDBERG, M., THOMAS, D., AND LEGOY, M. D. 1990. THE CONTROL OF LIPASE-CATALYSED TRANSE -STERIFICATION AND ESTERIFICATION REACTION RATES. EFFECTS OF SUBSTRATE POLARITY, WATER ACTIVITY AND WATER MOLECULES ON ENZYME ACTIVITY. EUROPEAN J. BIOCHEM. 190: 603-609. 43.GUPTA, M. N. 1992. ENZYME FUNCTION IN ORGANIC SOLVENTS. EUR. J. BIOCHEM. 203: 25-32. 44.HAYES, D. G. AND GULARI, E. 1992. FORMATION OF POLYOL-FATTY ACID ESTERS BY LIPASES IN R -EVERSE MICELLAR MEDIA. BIOTECHNOL. BIOENG. 40: 110-118. 45.HALLING, P. J. 1987. IN: BIOCATALYSIS IN ORGANIC MEDIA (LAANE, C., TRAMPER, J. AND LILL -Y, M. D., EDS.). ELSEVIER, AMSTERDAM. 125-132. 46.HAYES, D. G. AND GULARI, E. 1991. 1-MONOGLYCERIDE PRODUCTION FROM LIPASE-CATALYZED ESTE -RIFICATION OF GLYCEROL AND FATTY ACID IN REVERSE MICELLES. BIOTECHNOL. BIOENG. 38: 507 -517. 47.HOQ, M. M., YAMANE, T., SHIMIZU, S., FUNADA, T. AND ISHIDA, S. 1985. BIOREACTOR FOR EN -ZYMIC REACTION OF FAT AND FATTY ACID DERIVATIVES. III CONTINUOUS HYDROLYSIS OF OLIVE OI -L BY LIPASE IN A MICROPOROUS HYDROPHOBIC MEMBRANE REACTOR. J. AM. OIL CHEM. SOC. 62: 10 16-1021. 48.HUANG, K. H. AND AKOH, C. C. 1994. LIPASE-CATALYZED INCORPORATION OF N-3 POLYUNSATURATE -D FATTY ACID INTO VEGETABLE OILS. J. AM. OIL CHEM. SOC. 71: 1277-1280. 49.IBRAHIM, C. O., NISHIO, N., AND NAGAI, S. 1987. FAT HYDROLYSIS AND ESTERIFICATION BY A LIPASE FROM HUMICOLA LANUGINOSA. AGRIC. BIOL. CHEM. 51: 2153-2159. 50.JANSSEN, A. E. M., PADT, A., AND RIET, K. 1993. SOLVENT EFFECTS ON LIPASE-CATALYZED EST -ERIFICATION OF GLYCEROL AND FATTY ACIDS. BIOTECHNOL. BIOENG. 42: 953-962. 51.JANSSEN, A. E. M., PADT, A., SONSBEEK, H. M., AND RIET, K. 1992. THE EFFECT OF ORGANIC SOLVENTS ON THE EQUILIBRIUM POSITION OF ENZYMATIC ACYLGLYCEROL SYNTHESIS.

BIOTECHNOL. B -IOENG. 41: 95-103. 52.JOGIEKAR, A. M. AND MAY, A. T. 1987. PRODUCT EXCELLENCE THROUGH DESIGN OF EXPERIMENTS. CEREAL FOOD WORLD. 32: 857-868. 53.KARRA-CHAABOUNI, M., PULVIN, S., TOURAUD, D., AND THOMAS, D. 1998. PARAMETERS AFFECTING THE GERANYL BUTYRATE BY ESTERASE 30,000 FROM MUCOR MIEHEI. J. AM. OIL CHEM. SOC. 75: 12 01-1206. 54.KAZLAUSKAS, R. J. 1994. ELUCIDATING STRUCTURE-MECHANISM RELATIONSHIPS IN LIPASES: PROSP -ECTS FOR PREDICTING AND ENGINEERING CATALYTIC PROPERTIES. TRENDS BIOTECHNOL. 12: 464-4 72. 55.KHAN, S. A. AND HALLING, P. J. 1990. MEASUREMENT AND CONTROL OF WATER ACTIVITY WITH AN ALUMINUM OXIDE SENSOR IN ORGANIC TWO-PHASE REACTION MIXTURES FOR ENZYMATIC CATALYSIS. E -NZYME MICROB. TECHNOL. 12: 453-458. 56.KILARA, A. 1985. ENZYME-MODIFIED LIPID FOOD INGREDIENTS. PROC. BIOCHEM. 20: 35-45. 57.KITAGUCHI, H., FITZPATRICK, P. A., HUBER, J. E. AND KILBANOV, A. M. 1989. ENZYMATIC RES -OLUTION OF RACEMIC AMINES: CRUCIAL ROLE OF THE SOLVENT. J. AM. OIL CHEM. SOC. 111: 309 4-3095. 58.KLIBANOV, A. M. 1991. ENZYME THERMOINACTIVATION IN ANHYDROUS ORGANIC SOLVENTS. BIOTECHN -OL. BIOENG. 37: 843-853. 59.KLIBANOV, A. M. 1990. ASYMMETRIC TRANSFORMATIONS CATALYZED BY ENZYMES IN ORGANIC SOLVEN -TS. ACC. CHEM. RES. 23: 114-120. 60.KLIBANOV, A. M. 1986. ENZYMES THAT WORK IN ORGANIC SOLVENTS. CHEM. TECH. 354-359. 61.KLIBANOV, A. M. 1985. ENZYME-CATALYZED PROCESSES IN ORGANIC SOLVENTS. PROC. NATL. ACAD. 82: 3192-3196. 62.LAANE, C., BONEREN, S., VOS, K., AND VEEGER, C. 1987. RULES FOR OPTIMIZATIONS OF BIOCAT -ALYSIS IN ORGANIC SOLVENTS. BIOTECHNOL. BIOENG. 30: 81-87. 63.LI, Z. AND WARD, O. P. 1994. SYNTHESIS OF MONOGLYCERIDE CONTAINING OMEGA-3 FATTY ACIDS BY MICROBIAL LIPASE IN ORGANIC SOLVENT. J. IND. MICROBIOL. 13: 49-52. 64.LI, Z. AND WARD, O. P. 1993. ENZYME CATALYSED PRODUCTION OF VEGETABLE OILS CONTAINING O -MEGA-3 POLYUNSATURATED FATTY ACID. BIOTECHNOL. LETT. 15: 185-188. 65.LIU, K. J.,SHAW, J. F., AND CHEN, S. T. 1998. LIPASE-CATALYZED TRANSESTERIFICATION OF P -ROPYLENE GLYCOL WITH TRIGLYCERIDE IN ORGANIC SOLVENTS. J. AGRIC. FOOD CHEM. 46: 3835-3 838. 66.LO, S. AND SHAW, J. F. 1994. PRODUCTION OF PROPYLENE GLYCOL FATTY ACID MONOESTERS BY LI -PASE-CATALYZED REACTIONS IN ORGANIC SOLVENTS. J. AM. OIL CHEM. SOC. 71: 715-719. 67.MACRAE, A. R. 1983. LIPASE-CATALYZED INTERESTERIFICATION OF OILS AND FATS. J. AM. OIL C -HEM. SOC. 60: 291-294. 68.MARTINELLE, M. AND HULT, K. 1994. KINETICS OF TRIGLYCERIDE LIPASES. IN: LIPASE: THEIR S -TRUCTURE, BIOCHEMISTRY AND APPLICATION (WOODLEY, P. AND PETERSEN, S. B., EDS.). CAMBRI -DGE UNIVERSITY PRESS, CAMBRIDGE. 159-180. 69.MCNEILL, G. P. AND BERGER, R. G. 1993. ENZYMATIC GLYCEROLYSIS OF PALM OIL FRACTIONS AND A PALM OIL BASED MODEL MIXTURE: RELATIONSHIP BETWEEN FATTY ACID COMPOSITION AND MONOGLY -CERIDE YIELD. FOOD BIOTECHNOL. 7: 75-87. 70.MCNEILL, G. P., AND YAMANE, T. 1991. FURTHER IMPROVEMENTS IN THE YIELD OF MONOGLYCERIDE -S DURING ENZYMATIC GLYCEROLYSIS OF FATS AND OILS. J. AM. OIL CHEM. SOC. 68: 6-10. 71.MCNEILL, G. P., SHIMIZU, S., AND YAMANE, T. 1990. SOLID PHASE ENZYMATIC GLYCEROLYSIS OF BEEF TALLOW RESULTING IN A HIGH YIELD OF MONOGLYCERIDE. J. AM. OIL CHEM. SOC. 67: 779-7 83. 72.MILLER, C., AUSTIN, H., POSORSKE, L., AND JESUS, G. 1988. CHARACTERISTICS OF AN IMMOBIL IZED LIPASE FOR THE COMMERCIAL SYNTHESIS OF ESTERS. J. AM. OIL CHEM. SOC. 65: 927-931. 73.MORESI, M., COLICCHIO, A., AND SANSOVINI, F. 1980. OPTIMIZATION OF WHEY FERMENTATION IN A JAR FERMENTER. EUR. J. APPL. MICROBIOL. BIOTECHNOL. 9: 173-183. 74.MUKHERJEE, K. D. 1998. LIPID BIOTECHNOLOGY. IN "FOOD LIPIDS: CHEMISTRY, NUTRITION, AND BIOTECHNOLOGY." ED. C. C. AKOH AND D. B. MIN, MARCEL DEKKER, NEW YORK. 589-640. 75.NASH, N. H. AND BRICKMAN, L. M. 1972. FOOD EMULSIFIERS-SCIENCE AND ART. J. AM. OIL CHEM. SOC. 49: 457-461. 76.NOUREDDINI, H. AND HARMEIER, S. E. 1998. ENZYMATIC GLYCEROLYSIS OF SOYBEAN OIL. J. AM. OIL CHEM. SOC. 75: 1359-1365. 77.OHTA, Y., YAMANE, T., AND SHIMIZU, S. 1989. INHIBITION AND INACTIVATION OF LIPASE BY FA -T PEROXIDE IN THE COURSE OF BATCH AND CONTINUOUS GLYCEROLYSES OF FAT BY LIPASE. AGRIC. BIOL. CHEM. 53: 1885-1890. 78.OMAR, I. C., SAEKI, H., NISHIO, N., AND NAGAI, S. 1989. SYNTHESIS OF ACETONE GLYCEROL A -CYL ESTERS BY IMMOBILIZED LIPASE OF MUCOR MIEHEI. BIOTECHNOL. LETT. 11: 161-166. 79.OTERO, C., PASTOR, E., RUA, M. L., FERNANDEZ, V. M., AND BALLESTEROS, A. 1990. INFLUENC -E OF THE SUPPORT ON THE REACTION COURSE OF TRIBUTYRIN HYDROLYSIS CATALYZED BY SOLUBLE -AND IMMOBILIZED LIPASES. APPL. BIOCHEM. BIOTECHNOL. 23: 237-247. 80.PASTOR, E., OTERO, C., AND BALLESTEROS, A. 1995. SYNTHESIS OF MONO- AND DIOLEYLGLYCEROL -S USING AN IMMOBILIZED LIPASE. APPL. BIOCHEM. BIOTECHNOL. 50: 251-263. 81.PECNIK, S. AND KNEZ, Z. 1992. ENZYMATIC FATTY ESTER SYNTHESIS. J. AM. OIL CHEM. SOC. 69 : 261-265. 82.RUBIO, E., FERNANDEZ-MAYORALES, A., AND KLIBANOV, A. M. 1991. EFFECT OF THE SOLVENT ON ENZYME REGIOSELECTIVITY. J. AM. OIL CHEM. SOC. 113: 695-696. 83.SAKURAI, T., MARGOLIN, A. L., RUSSELL, A. J., AND KLIBANOV, A. M. 1988. CONTROL OF ENZY -ME ENANTIOSELECTIVITY BY THE REACTION MEDIUM. J. AM. OIL CHEM. SOC. 110: 7236-7237. 84.SCHMID, R. D. AND VERGER, R. 1998. LIPASES: INTERFACIAL ENZYMES WITH ATTRACTIVE APPLICA -TIONS. ANGEW. CHEM. INT. ED. 37: 1608-1633. 85.SCHUCH, R. AND MUKHERJEE, K. D. 1989. LIPASE-CATALYZED REACTION OF FATTY ACIDS WITH GLY -CEROL AND ACYLGLYCEROLS. APPL. MICROB. BIOTECHNOL. 30: 332-336. 86.SEINO, H. AND UCHIBORI, T. 1984. ENZYMATIC SYNTHESIS OF CARBOHYDRATE ESTERS OF FATTY AC -ID (1) ESTERIFICATION OF SUCROSE, GLUCOSE,

FRUCTOSE AND SORBITOL. J. AM. OIL CHEM. SOC. 61: 1761-1765. 87.SHAW, J. F., WANG, D. L., AND WANG, Y. J. 1991. LIPASE-CATALYZED ETHANOLYSIS AND ISOPRO -PANOLYSIS OF TRIGLYCERIDES WITH LONG-CHAIN FATTY ACIDS. ENZYME MICROB. TECHNOL. 13: 54 4-545. 88.SHIEH, C. J., AKOH, C. C., AND LEE, L. N. 1996. OPTIMIZED ENZYMATIC SYNTHESIS OF GERANY -L BUTYRATE WITH LIPASE FROM CANDIDA RUGOSA. BIOTECHNOL. BIOENG. 51: 371-374. 89.SHIEH, C. J., AKOH, C. C., AND KOEHLER, P. E. 1995. FOUR-FACTOR RESPONSE SURFACE OPTIMI -ZATION OF THE ENZYMATIC MODIFICATION OF TRIOLEIN TO STRUCTURED LIPIDS. J. AM. OIL CHEM. SOC. 72: 619-623. 90.SINGH, C. P., SHAH, D. O., AND HOLMBERG, K. 1994. SYNTHESIS OF MONO- AND DIGLYCERIDES I -N WATER-IN-OIL MICROEMULSIONS. J. AM. OIL CHEM. SOC. 71: 583-587. 91.STEFFEN, B., ZIEMANN, A., LANG, S., AND WAGNER, F. 1992. ENZYMATIC MONOACYLATION OF TRI -HYDROXY COMPOUNDS. BIOTECHNOL. LETT. 14: 773-778. 92.STEVENSON, D. E., STANLEY, R. A., AND FURNEAUX, R. H. 1993. GLYCEROLYSIS OF TALLOW WITH IMMOBILISED LIPASE. BIOTECHNOL. LETT. 15: 1043-1048. 93.STUTZ, R. L., DEL VECCHIO, A. J., AND TENNEY, R. J. 1973. THE ROLE OF EMULSIFIER AND DO -UGH CONDITIONERS IN FOODS. FOOD PROD. DEVELOP. 7: 52-58. 94.TAHOUN, M. K., EL-KADY, M., AND WAHBA, A. 1985. GLYCERIDE SYNTHESIS BY AN INTRACELLULAR LIPASE FROM ASPERGILLUS NIGER. MICROBIOL. LETT. 28: 133-139. 95.TSUJISAKA, Y., OKUMURA, S., AND IWAI, M. 1977. GLYCERIDE SYNTHESIS BY FOUR KINDS OF MIC -ROBIAL LIPASE. BIOCHIM. BIOPHYS. ACTA. 489: 415-422. 96.VALIVETY, R. H., JOHNSTON, G. A., SUCKLING, C. J., AND HALLING, P. J. 1991. SOLVENT EFF -ECTS ON BIOCATALYSIS IN ORGANIC SYSTEMS: EQUILIBRIUM POSITION AND RATES OF LIPASE CATA -LYZED ESTERIFICATION. BIOTECHNOL. BIOENG. 38: 1137-1143. 97.VAN DER PADT, A., KEURENTJ -ES, J. T. F., SEWALT, J. J. W., VAN DAM, E. M., VAN DORP, L. J., AND VAN'T RIET, K. 19 92. ENZYMATIC SYNTHESIS OF MONOGLYCERIDES IN A MEMBRANE BIOREACTOR WITH AN IN-LINE ADSO RPTION COLUMN. J. AM. OIL CHEM. SOC. 69: 748-754. 100.VERGER, R. 1997. INTERFACIAL ACTIVATION OF LIPASE: FACTS AND ARTIFACTS. TRENDS BIOTECH -NOL. 15: 32-38. 101.VULFSON, E. N. 1994. IN: LIPASES, THEIR STRUCTURE, BIOCHEMISTRY AND APPLICATION (PETE -RSEN, S. B. AND WOOLLEY, P., EDS). CAMBRIDGE UNIVERSITY PRESS, CAMBRIDGE. 271- 288. 102.WANDREY, C. AND WICHMANN, R. 1987. PRODUCTION OF L-AMINO ACIDS IN THE MERMBRANE REACT -OR. BIOTECHNOL. 1: 85-92. 103.WEETE, J. D. 1998. MICROBIAL LIPASES. IN "FOOD LIPIDS: CHEMISTRY, NUTRITION, AND BIOTE -CHNOLOGY." ED. C. C. AKOH AND D. B. MIN, MARCEL DEKKER, NEW YORK. 665-698. 104.WEISS, A. 1990. ENZYMATISCHE HERSTELLUNG VON FESTEN FETTSAUREMONOGLYCERIDEN. FAT. SCI. TECHNOL. 92:392-396. 105.YAMAGUCHI, S. AND MASE, T. 1991. HIGH-YIELD SYNTHESIS OF MONOGLYCERIDE BY MONO- AND DI -ACYLGLYCEROL LIPASE FROM PENICILLIUM CAMEMBERTII U-150. J. FERMENT. BIOENG. 72: 162-16 7. 106.YAMANE, Y., SUZUKI, T., SAHASHI, Y., VIKERSVEEN, L., AND HOSHINO, T. 1992. PRODUCTION OF N-3 POLYUNSATURATED FATTY ACID-ENRICHED FISH OIL BY LIPASE-CATALYZED ACIDOLYSIS WITH -OUT SOLVENT. J. AM. OIL CHEM. SOC. 69: 1104-1107. 107.YAMANE, T., HOQ, M. M., AND SHIMIZU, S. 1983. CONTINUOUS SYNTHESIS OF GLYCERIDES BY LI PASE IN A MICROPOROUS MEMBRANE BIOREACTOR. ANN. N. Y. ACAD. SCI. 434: 558-568. 108.YANG, H., CAO, S. G., DING, Z. T., LIU, S. D., AND CHENG, Y. H. 1994. A NEW KIND OF IM -MOBILIZED LIPASE IN ORGANIC SOLVENT AND ITS STRUCTURE MODEL. BIOCHEM. BIOPHYS. RES. CO -MM. 200: 83-88. 109.ZAKS, A. AND KLIBANOV, A. M. 1988. ENZYME CATALYSIS IN NONAQUEOUS SOLVENTS. J. BIO. CH -EM. 236: 3194-3201. 110.ZAKS, A. AND KLIBANOV, A. M. 1985. ENZYME-CATALYZED PROCESSES IN ORGANIC SOLVENTS. PRO -C. NATL. ACAD. SCI. USA. 82: 3192-3196.