

# Optimization of Lipase-catalyzed Specialty Lipids 1,3-dicapryloyl-2-palmitoyl-sn- Glycerol

陳純敏、謝淳仁

E-mail: 9300282@mail.dyu.edu.tw

## ABSTRACT

Structured lipids have been developed for human milk fat replacer (HMF replacer) substitutes or improved absorption for nutritional application. In this study, transesterification acidolysis of tripalmitin and caprylic acid catalyzed by lipases LipozymeR IM-77 from *Rhizomucor miehei* to produce structured lipids with palmitoyl moieties in the secondary (sn-2) and medium-chain acyl moieties in the primary (sn-1,3) positions (1,3-dicapryloyl-2-palmitoyl-sn-glycerol), which should be helpful products for premature infant nutrient. The reaction stage was simplified to achieve one-stage fractionation. Response surface methodology (RSM) and three-level-four-factor central composite rotatable design (CCRD) were employed to evaluate the effects of HMF replacer substitute synthesis on the parameters: reaction time (1-3 hrs.), temperature (35-55 °C), substrate molar ratio (caprylic acid: tripalmitin =3:1-5:1), and enzyme amount(0.2-1 BAUN). To clarify the relationships between the factors and the response the contour plots analysis was used to determine the optimal conditions for HMF replacer synthesis. Based on the ridge of max analysis, the optimum conditions were: reaction time 2h, synthesis temperature 52.2 °C, enzyme amount 0.75 BAUN, and substrate molar ratio (caprylic acid: tripalmitin) 4.5: 1. At the optimum point, the optimized acid incorporation was 87%.

Keywords : structured lipids, lipase, transesterification acidolysis, human milk fat replacer, response surface methodology, central composite rotatable design, contour plots analysis

## Table of Contents

第一章 緒論 .....	1	第二章 文獻回顧 .....	4
2.1 油脂生物技術-建構脂質 .....	4	2.2 脂解酵素簡介與酵素反應 .....	7
2.3 人類母乳脂質 .....	13	2.4 人類母乳脂肪與市售配方奶粉比較 .....	15
2.5 人類母乳脂肪替代品BetapolR .....	16	2.6 建構脂質BetapolR動物及臨床試驗 .....	17
2.7 早產兒脂質吸收 .....	19	2.8 中鏈脂肪酸簡介 .....	20
2.9 中鏈脂肪酸於早產兒配方應用 .....	21	2.10 中鏈脂肪酸於建構脂質動物及臨床試驗 .....	22
2.11 人類母乳替代品於建構脂質合成與分析之相關研究 .....	25	2.12 反應曲面法之簡介 .....	26
第三章 1,3-二辛酸-2-棕櫚酸甘油酯特化油脂之最優化研究 3.1 前言 .....	33	3.2 實驗材料 .....	34
3.2.1 儀器設備 .....	34	3.2.2 藥品 .....	35
3.3 實驗設計與方法 .....	35	3.3.1 實驗設計 .....	35
3.3.2 1,3-二辛酸-2-棕櫚酸甘油酯特化油脂之合成方法 .....	36	3.3.3 萃取與分析 .....	36
3.3.4 統計分析 .....	37	3.4 結果與討論 .....	37
3.4.1 對合併率的影響 .....	37	3.4.2 溫度對合併率的影響 .....	39
3.4.3 酵素用量對合併率的影響 .....	39	3.4.4 基質莫耳比對合併率的影響 .....	39
3.4.5 最優化合成之研究 .....	40	3.4.6 相關研究之綜合討論 .....	41
第四章 結論 .....	54	參考文獻 .....	54
附錄一 人類母乳替代品於建構脂質合成與分析之相關研究 .....	61	附錄二 商業lipases整理 .....	62

## REFERENCES

1. 田蔚城, 2001 「生物技術的發展與應用」九州圖書文物有限公司。
2. 張曉莉及黃世佑。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。
3. Akoh, C.C. 1995. Structured lipids-enzymatic approach. *INFORM*. 6:1055-1061.
4. Bach, A. and Babayan, V.K. 1982. Medium-chain triglycerides: an update. *Am. J. Clin. Nutr.* 36:950-962.
5. Carnielli, V.P., Luijendijk, I.H.T., Van Goudoever, J.B., Sulkers, E.J., Boerlage, A.A., Degenhart, H.J. and Sauer, P.J.J. 1996. Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. *J. Pediatr. Gastroenterol. Nutr.* 23:553-560.
6. Carnielli, V.P., Rossi, k., Badon, T., Gregori, B., Verlato, G., Orzali, A. and Zacchello, F. 1996, Medium-chain triacylglycerols in formulas for preterm infants: effect on plasma lipids, circulating concentrations of medium-chain fatty acids, and essential fatty acids. *Am. J. Clin. Nutr.* 64:152-158.
7. Deffense, E. 1993. Milk fat fractionation today :a review. *J.*

Am. Oil Chem. Soc. 70:1193-1201. 8. Dotson, K.D. Jerrell, J.P., Picciano, M.F. and Perkins, E.G. 1992. High-performance liquid chromatography of human milk triacylglycerols and gas chromatography of component fatty acids. *Lipids*. 27:933-939. 9. Dunham, E.C. 1955. *Premature infant: A manual for physicians*. Paul B. Hoeber, INC., New York, USA., Chapter 7. 10. Fouw, N.J., Kivits, G.A.A., Qulinlan, P.T., Nielen, W.G.L. 1994. Absorption of isomeric, palmitic acid containing triacylglycerols resembling human milk fat in the adult rat. *Lipids*. 29:765-770. 11. Giovanni, M. 1983. Response surface methodology and product optimization. *Food Technol.* 41-45. 12. Ghazali, H.M., Hamidah, S. and Che Man, Y.B. 1995. Enzymatic transesterification of palm olein with nonspecific and 1,3-specific lipase. *J. Am. Oil Chem. Soc.* 72:633-639. 13. Green, K.D. and Nakajima, M. 1998. Evaluation of immobilized modified lipase: aqueous preparation and reaction studied in n-hexane. *J. Am. Oil Chem. Soc.* 75:1193-15251519. 14. Hamosh, M., Bitman, J., Liao, T.H., Mehta, N.R., Buczek, R.J., Wood, D.L., Grylack, L.J. and Hamosh, P. 1989. Gastric lipolysis and fat absorption in preterm infant: effect of medium-chain triglyceride or long-chain triglyceride-containing formulas. *Pediatrics* 83:86-92. 15. Heim, T. 1985. How to meet the lipid requirements of the premature infant. *Pediatr. Clin.* 32:289-317. 16. Heird, W.C., Grundy, S.M. and Hubbard, V.S. 1986. Structured lipids and their use in clinical nutrition. *Am. J. Clin. Nutr.* 43:320-324. 17. Innis, S.M., Dyer, R. and Nelson, C.M. 1994. Evidence that palmitic acid is absorbed as sn-2 monoacylglycerol from human milk by breast-fed infants. *Lipides*. 29:541-545. 18. Jandacek, R.J., Whiteside, J.A., Holcombe, B.N., Volpenhein, R.A. and Taulbee, J.D. 1987. The rapid hydrolysis and efficient absorption of triglycerides with octanoic acid in the 1 and 3 positions and long-chain fatty acid in the 2 position. *Am. J. Clin. Nutr.* 45:940-945. 19. Jensen, G.L. and Jensen, R.G. 1992. Specialty lipids for infant nutrition. *J. Pediatr. Gastro. Nutr.* 15:382-394. 20. Jensen, R.G. 1996. The lipids in human milk. *Prog. Lipid Res.* 35:53-92. 21. Johnson, R.C., Young, S.K., Cotter, R., Lin, L. and Rowe, W.B. 1990. Medium-chain-triglyceride lipids emulsion: metabolism and tissue distribution. *Am. J. Clin. Nutr.* 52:502-508. 22. Kavanagh, A.R. 1997. A breakthrough in infant formula fats. *Oleagineux, Corps Gras, Lipides*. 4:165-8. 23. Kennedy, T.P. 1991. Structured lipids: fats of the future. *Food Technol.* 76-83. 24. Kubow, S. 1996. The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis. *Nutr. Biochem.* 7:530-541. 25. Ledochowska, E., Jewusiak, A. and Szymczak, M. 2001. Preparation of structured lipids with special functional properties. *Food Lipids* 8:239-250. 26. Lee, K.T. and Akoh, C.C. 1998. Structured lipids: synthesis and applications. *Food Rev. Int.*, 14(1), 17-34. 27. Megremis, C.J. 1991. Medium-chain triglycerides: a nonconventional fat. *Food Technol.* 2:113-114. 28. McLaren, D.S., Burman, D., Belton, N.R. and Williams, A.F. 1991. *Textbook of Paediatric Nutrition*. Churchill Livingstone. New York. Chapter 5. 29. Mrera Pons, S., Castellote Bargallo, A.I. and Lopez Sabater, M.C. 1998. Analysis of human milk triacylglycerols by high-performance liquid chromatography with light-scattering detection. *J. Chromatogr. A.* 823:475-482. 30. Mukherjee, K.D. and Kiewitt, I. 1998. Structured triacylglycerols resembling human milk fat by transesterification catalyzed by papaya (*Carica papaya*) latex. *Biotech. Lett.* 20:613-616. 31. Namal Senanayake, S.P.J. and Shahidi, F. 2002. Enzyme-catalyzed synthesis of structured lipids via acidolysis of seal (*Phoca groenlandica*) blubber oil with capric acid. *Food Res. Int.* 35:745-752. 32. Peng, L., Xu, X., Mu, H., Hoy, C.E. and Adler-Nissen, J. 2002. Production of structured phospholipids by lipase-catalyzed acidolysis: optimization using response surface methodology. *Enz. Micro. Technol.* 31:523-532. 33. Quinlan, P. and Moore, S. 1993. Modification of triglycerides by lipases: process technology and its application to the production of nutritionally improved fats. *INFORM.* 4:580-585. 34. Roig, M.J., Alegria, A., Barbera, R., Farre, R. and Lagarda, M.J. 1999. Calcium bioavailability in human milk, cow milk and infant formulas comparison between dialysis and solubility methods. *Food Chemistry* 65:353-357. 35. Sarda, P., Lepage, G., Roy, C.C. and Chessex, P. 1987. Storage of medium chain triglycerides in adipose tissue of orally fed infants. *Am. J. Clin. Nutr.* 45:399-405. 36. Schmid, U., Bornscheuer, U.T., Soumanou, M.M., McNEIL, G.P. and Schmid, R.D. 1998. Optimization of the reaction conditions in the lipase-catalyzed synthesis of structured triglycerides. *J. Am. Oil Chem. Soc.* 75:1527-1531. 37. Shieh, C.J., Akoh, C.C. and Koehler, P.E. 1995. Four-factor response surface optimization of the enzymatic modification of triolein to structured lipids. *J. Am. Oil Chem Soc.* 72:619-623. 38. Shieh, C.J., and Chang, S.W. 2001. Optimized synthesis of lipase-catalyzed hexyl acetate in n-hexane by response surface methodology. *J. Agric. Food Chem.* 49:1203-1207. 39. Soumanou, M.M., Bornscheuer, U.T. and Schmid, R.D. 1998. Two-step enzymatic reaction for the synthesis of pure structured triacylglycerides. *J. Am. Oil Chem. Soc.* 75:703-710. 40. Villeneuve, P. and Foglia, T.A. 1997. Lipase specificities: potential application in lipid bioconversions. *INFORM.* 8:640-651. 41. Williams, A.F. 1991. *Textbook of paediatric nutrition*. Churchill livingstone INC. New York, USA. Chapter 5. 42. Winter, C.H., Hoving, E.B. and Muskiet, F.A.J. 1993. Fatty acid composition of human milk triglyceride species possible consequences for optimal structured of infant formula triglycerides. *J. Chromatogr.* 616:9-24.