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Enzymatic Synthesis of Capric Acid-Rich Structured Lipids (MUM type) Using *Candida antarctica* Lipase

Sumita SilRoy and Mahua Ghosh*

Department of Chemical Technology, University College of Science & Technology, University of Calcutta (92, A.P.C. Road, Kolkata 700009, INDIA)

Abstract: The objective of the work was to produce capric acid rich structured lipids starting from various Indian indigenous vegetable oils, such as rice bran, ground nut and mustard oils. Acidolysis reaction between individual vegetable oils and capric acid in one is to three molar ratios at 45 degree centigrade temperature was carried out using position specific *Candida antarctica* lipase so as to protect the *Sn*-2 position of the oils which are rich in unsaturated fatty acids. The incorporation of capric acid depended on the reaction time showing 6 % within 6 h and 30.8 % in 72 h with rice bran oil. Similarly, in ground nut oil incorporation of capric acid was 34.2 % in 72 h compared to 5.3 % in 6 h. Thus mustard oil showed much lower incorporation than the other two oils, with 3.3 % and 19.5 % in 6 and 72 h respectively. The incorporation of capric acid was influenced by the nature of the fatty acids present in the original oil. The fatty acid composition of *Sn*-2 position of the structured triacylglycerols of the three oils revealed that capric acid was mainly replacing the fatty acids occupying the *Sn*-1 and 3 positions of the triglyceride molecule.

Key words: medium chain fatty acid, *Candida antarctica* lipase, *Sn*-2 position

1 INTRODUCTION

Long chain fatty acids (LCFA) ranging from 12 to 18 carbon atoms are the predominant form of fatty acids present in the dietary oils, where as medium chain fatty acids (MCFAs), by contrast, are composed of only 6 to 10 carbon atoms. Because of their shorter chain length, MCFA have a number of unique properties which give advantages over the more common LCFAs. MCFA containing triglycerides (MCTs) offer numerous benefits—they have a lower calorie content than other fats[1]; are minimally stored as fat[2]; contribute to enhanced metabolism to burn even more calories[3]; MCTs might be advantageous for the ageing of brain[4]. This third property may be due to the fact that MCTs behave metabolically in fashion similar to carbohydrates, as well as their property of promoting the development of ketones. Ketones are a normal and efficient source of fuel and energy for the human body. Ketones are produced in the liver from fatty acids, which result from the breakdown of body fat in response to the absence of glucose/sugar. Ketones are also one of the two substances which the brain can utilize for energy (glucose, being the other).

Subsequently, MCTs have slight cholesterol lowering capacity because it decreases the intestinal absorption of cholesterol and slows its synthesis in liver[4, 5]. MCTs have also been reported to act as antioxidants and reduce tissue requirements for vitamin E[6]. The anti-coagulation effect of MCTs helps to prevent artherosclerosis[7]. The experiment of Kaunitz *et al.* with rabbit indicated that MCTs could have a positive effect on autoimmune reactions characteristic of the ageing process[8]. MCTs have proven useful in treating a number of medical disorders that involve impaired or damaged lipid (fat) metabolism. These include: obstructive jaundice, biliary cirrhosis, pancreatitis, cystic fibrosis, celiac disease, Whipple's disease, Crohn's disease, regional enteritis, and malabsorption in neonates[9]. They have been also reported to be useful for feeding of newborn infants, to both assist their initial growth and to contribute to their physiological development[10]. The absorption of calcium and magnesium appears to be enhanced when the diet contains MCTs, particularly in infants, and the absorption of amino acids also appears to be improved[11]. Thus, MCTs can be a useful addition to the diet of those suffering from any form of malnutrition or tissue wasting. In nature, MCTs are predominantly available only from butter, coconut oil, and palm kernel oil.

There are very few examples of natural fats which have MCFA: UFA: MUFA (MUM) type composition to provide a

*Correspondence to: Mahua Ghosh, Department of Chemical Technology, University College of Science & Technology, University of Calcutta, 92, A.P.C. Road, Kolkata 700009, INDIA
E-mail: mahuag@gmail.com/mgchemtech@caluniv.ac.in
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balanced nutrition and therefore the objective of this study was to develop a suitable process technology for preparing these types of structured lipids. Some natural & indigenous oils such as rice bran, ground nut and mustard were selected as starting material because they have unsaturated fatty acids in the *Sn*-2 position and a position specific lipase, *Candida antarctica*, was used as biocatalyst to protect this unsaturated fatty acid of *Sn*-2 position of the oils.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Mustard oil was extracted from brown mustard seed at laboratory by solvent extraction process. The solvent was evaporated and the oil was bleached and physically refined and stored at 5℃ for further modification. Refined, bleached & deodourised rice bran oil was procured from M/s Sethia Oils Ltd., Burdwan, India and refined, bleached & deodourised ground nut oil was procured from local market and fatty acid composition of the oils were analysed by gas chromatography (GC) and stored. A.R. grade capric acid was procured from Merck India Ltd. and purity analysis was done by GC. Immobilised lipase *Candida antarctica*, used as biocatalyst, was a generous gift from Novozyme India Ltd, Bangalore, India. The original moisture content of the enzyme (2%, w/w) was kept intact to initiate the reaction. Pancreatic lipase (pork pancreatic lipase) was procured from Sigma Chemicals Co., St. Louis, MO. All other chemicals used were of Analytical Grade and procured from SRL, Mumbai, India.

2.2 Method

2.2.1 Acidolysis reaction:

Acidolysis reactions were carried out in a stirred tank batch reactor mixing oil and capric acid in 1:3 molar ratio; temperature was maintained at 45℃ and 10% (w/w on the basis of total substrate weight) of lipase enzyme was used as catalyst. The reaction mixture was stirred with a magnetic stirrer at 300 rpm. All the reaction parameters were optimized in our earlier studies (yet to publish). Samples from reaction mixture were withdrawn intermittently and analysed for MCFA incorporation.

2.2.2 Analysis of fatty acid composition:

Fatty acid composition of original oils and MCFA incorporated oils were analyzed by gas chromatography (GC). Fatty acid methyl esters (FAME) were prepared by the method described by Metcalfe and Schmitz[12]. The GC (make: Agilent, model: 6890 N) instrument used was equipped with FID detector and capillary DB-Wax column (30 mL, 0.32 mm I.D, 0.25 μm FT). N_2, H_2 and airflow rate was maintained at 1 mL/min, 30 mL/min and 300 mL/min respectively. Inlet & detector temperature was kept at 250℃ and the oven temperature was programmed as 150-190-230℃ with increase rate of 15℃/min and 5 min hold up to 150℃ and 4℃/min with 10 min hold up to 230℃.

2.1.3 Analysis of *Sn*-2 position:

All the oil samples were hydrolyzed by pancreatic lipase following the method of Luddy *et al.*[13] and the fatty acid composition of the 2- monoglyceride obtained was analysed by GC after preparing corresponding methyl esters according to the method of Metcalfe and Schmitz[12].

3 RESULTS AND DISCUSSIONS

3.1 Production of capric acid rich rice bran oil

Acidolysis reaction was carried out between rice bran oil and capric acid at molar ratio of 1:3 (oil:acid) at 45℃ using 10% *Candida antarctica* lipase. It was observed from Table 1 that incorporation of capric acid into rice bran oil increased with time and maximized at 72 h. The fatty acid composition of capric acid rich mustard oil showed that capric acid was successfully incorporated into rice bran oil. Another interesting observation was seen from the data that with time capric acid incorporated into rice bran oil by replacing fatty acids in the order of 16: 0, 18: 1 and 18: 2. The selectivity of lipases towards chain length and fatty acids were also reported by Vaysse *et al.* and Ishan *et al.* in their earlier communications[14, 15].

Table 1 Fatty acid composition (% w/w) of triglycerides obtained from acidolysis reaction between rice bran oil and capric acid.

Fatty acid→ / ↓ Reaction time	$C_{10:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Original rice bran oil	0.0	21.7	2.6	46.2	29.5
6 h	6.3	17.8	1.9	46.0	28.0
24 h	26.6	14.0	0.0	41.0	18.4
48 h	26.8	11.6	1.1	35.4	25.0
72 h	31.2	10.8	0.0	34.2	23.8

3.2 Production of capric acid rich groundnut oil

The fatty acid profile of capric acid rich ground nut oil catalyzed by *Candida antarctica* lipase is shown in Table 2. The fatty acid composition of capric acid rich groundnut oil showed that capric acid was successfully incorporated into this oil after the lipase-catalyzed interesterification reaction. The capric acid content of groundnut oil was increased to 34.5% of total fatty acids. Here also the replacement of fatty acids and incorporation of capric acid took place in a similar pattern as in the previous reaction. The Table shows that there was maximum removal of oleic acid with some amount of linoleic acid from ground nut oil within 24 h, whereas in the previous reaction with rice bran oil within 24 h, capric acid mostly replaced linoleic acid. The presence of higher amount of oleic acid in ground nut oil must be the cause in behind.

3.3 Production of capric acid rich mustard oil

The effective production of capric acid rich mustard oil by the exchange of fatty acids at different positions in mustard oil by means acidolysis reaction using *Candida antarctica* lipase is described in Table 3. Following the trend of the previous two reactions in this case too the incorporation of fatty acids increased with time. On acidolysis of mustard oil, capric acid incorporation was brought about firstly by replacing palmitic and oleic acids. As the time of the reaction increased capric acid was increased in mustard oil by removal of erucic acid.

3.4 Pancreatic lipase-catalysed *Sn*-2 positional analysis

Pancreatic lipase catalysed *Sn*-2 positional analysis of the three original vegetable oils and capric acid rich vegetable oils. Tables 4, 5 and 6 show the following fatty acid composition at the *Sn*-2 position of the different original and modified oils. The findings of Table 4 states that *Sn* 2-position of capric acid rich rice bran oil was mainly occupied by oleic and linoleic acid as in original rice bran oil with little acyl migration and capric acid was mainly introduced in *Sn*-1,3 positions. This proved that *Candida antarctica* lipase behaved as 1 and 3-specific in all those reactions. The observation was quite consistent with the observations done by Yomi *et al*. and Li *et al*.[16, 17]. Table 5 shows that the *Sn*-2 position of both groundnut oil and capric acid rich groundnut oil has similar fatty acid composition. From Table 6 it can also be seen that the *Sn*-2 position of capric acid rich mustard oil was mainly occupied by oleic, linoleic and linolenic acid whereas erucic acid and capric acid were mainly present in the *Sn*-1, 3 positions.

3.5 Comparison of amount of incorporation of capric acid into different vegetable oils

Incorporation of capric acid was maximum in the case of capric acid rich groundnut oil as seen from Fig. 1. The capric acid incorporation gradually increased with time till 72 h in the case of groundnut oil. In case of capric acid rich mustard oil the incorporation of capric acid increased up to 48 h and then became constant. Incorporation of capric acid was maximum at 24 h in the production of capric acid rich rice bran oil and then the incorporation was almost

Table 2 Fatty acid composition (%w/w) of triglyceride obtained from acidolysis reaction between ground nut oil and capric acid

Fatty acid→ / ↓ Reaction time	$C_{10:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{22:0}$	$C_{24:0}$
Original ground nut oil	0.0	0.4	10.7	2.4	52.7	30.7	0.9	1.5	0.7
6 h	5.3	0.0	8.9	2.5	51.8	27.3	1.0	2.0	1.2
24 h	21.4	0.0	6.3	1.8	42.8	23.7	0.7	1.4	0.8
48 h	27.9	0.0	4.7	1.1	38.9	25.0	0.6	0.9	0.7
72 h	34.5	0.0	6.2	1.7	35.4	20.4	0.7	0.4	0.7

Table 3 Fatty acid composition (%w/w) triglycerides obtained from acidolysis reaction between mustard oil and capric acid

Fatty acid→ / ↓ Reaction time	$C_{10:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:1}$	$C_{24:0}$
Original ground nut oil	0.0	1.9	0.3	1.0	12.2	17.2	9.3	0.8	5.1	51.1	0.7
6 h	3.3	1.2	0.4	0.4	8.9	13.9	11.4	0.1	5.2	54.1	0.5
24 h	10.6	1.0	0.1	0.3	10.1	17.2	10.5	0.4	3.5	46.0	0.3
48 h	18.8	1.2	0.0	0.7	10.4	14.8	11.1	0.2	3.6	37.5	0.6
72 h	19.5	0.7	0.0	0.1	8.4	14.6	9.9	0.3	4.6	41.4	0.2

Table 4 Fatty acid composition (% w/w) at various places of the triglycerides obtained from acidolysis reaction between rice bran oil and capric acid

Fatty acid→ / ↓ Oil sample	$C_{10:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$
Sn-2 position	0.0	3.5	48.8	47.7
Original rice bran oil				
Sn-1&3 position	0.0	30.8	44.9	20.4
Sn-2 position	1.3	0.0	47.5	51.2
6 h				
Sn-1&3 position	8.8	26.7	45.2	16.4
Sn-2 position	5.5	3.0	45.2	46.3
24 h				
Sn-1& 3 position	37.1	19.5	38.9	4.5
Sn-2 position	0.0	9.1	51.9	39.0
48 h				
Sn-1&3 position	40.2	12.9	27.2	18.0
Sn-2 position	2.9	5.1	47.6	44.4
72 h				
Sn-1&3 position	45.4	13.7	27.5	13.5

Table 5 Fatty acid composition (%w/w) at various places of the triglycerides obtained from acidolysis reaction between Ground nut oil and capric acid

Fatty acid / Oil sample	$C_{10:0}$	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Sn-2 position	0.0	0.8	52.9	45.4	0.8
Original rice bran oil					
Sn-1&3 position	0.0	17.5	58.8	22.5	0.2
Sn-2 position	1.1	2.1	52.9	43.8	0.0
6 h					
Sn-1&3 position	7.4	12.5	52.0	19.5	0.0
Sn-2 position	0.0	0.0	57.5	42.5	0.0
24 h					
Sn-1& 3 position	32.6	9.6	36.5	14.8	0.0
Sn-2 position	2.7	0.0	53.9	42.9	0.0
48 h					
Sn-1&3 position	39.9	6.9	30.7	15.6	0.0
Sn-2 position	0.0	6.3	55.2	38.5	0.0
72 h					
Sn-1&3 position	51.3	6.0	24.9	11.1	0.0

Table 6 Fatty acid composition (%w/w) at various places of the triglycerides obtained from acidolysis reaction between mustard oil and capric acid.

Substrate \ Fatty acid	$C_{10:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{22:1}$
Sn-2 position	0.0	21.2	45.8	32.8	0.0
Original rice bran oil					
Sn-1&3 position	0.0	7.7	2.9	0.0	76.6
Sn-2 position	0.4	21.3	44.0	28.7	5.6
6 h					
Sn-1&3 position	4.8	2.7	0.0	2.8	78.4
Sn-2 position	4.4	27.1	53.7	14.8	0.0
24 h					
Sn-1& 3 position	13.7	1.6	0.0	8.4	69.0
Sn-2 position	0.3	20.9	41.9	33.6	1.0
48 h					
Sn-1&3 position	28.1	5.2	1.3	0.0	55.8
Sn-2 position	3.0	26.4	41.8	27.2	1.5
72 h					
Sn-1&3 position	27.8	0.0	1.0	1.3	61.4

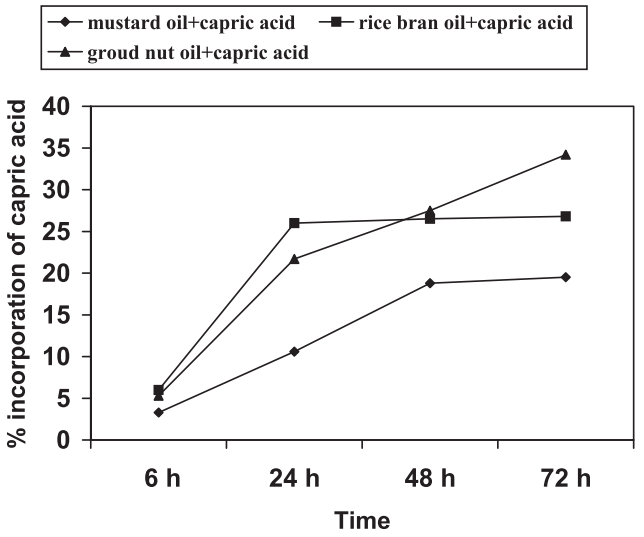

Fig. 1 Comparison of incorporation of capric acid in different vegetable oils.

constant up to 72 h.

4 CONCLUSIONS

From all the findings of the experiments it can be concluded that by using *Candida antarctica* lipase, capric acid can be incorporated into the triacylglycerol back bone of different vegetable oils at *Sn*-1 & 3 positions to produce structured lipid containing medium chain fatty acid though having unsaturated fatty acid at *Sn*-2 position.

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References

1) Kaunitz, H. Dietary use of MCT. in *Bilanzierte Ernaehrung in der Therapie* (Lang, K.; Fekl, W.; Berg, G. ed.) George Thieme Verlag. Stuttgart (1971).
2) Babayan, V. K. Medium chain fatty acid esters and their medical and nutritional applications. *J. Am. Oil Chem. Soc.* **58**, 49A-51A (1981).
3) Baba, N.; Bracco, E. F.; Seylar, J.; Hashim, S. A. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diets containing medium chain triglycerides. *J. Am. Soc. Clin. Nutr.* **34**, 624-633 (1981).
4) Bach, A. C.; Babayan, V.K. Medium-chain triglycerides: an update. Am. *J. Clin. Nutr.* **36**, 950-962 (1982).
5) Von Boonsdorff-Nikander A.; Christiansen, L.; Huikko, L., Lampi, A. M.; Piironen, V.; Yliruusi, J.; Kaukonen, A.

M. A comparison of the effect of medium- vs. Long-chain triglycerides on the *in vitro* solubilisation of cholesterol and/or phytosterol into mixed micelles. *Lipids* **40**, 181-190(2005).
6) Kaunitz, H.; Slanetz, C. A.; Johnson, R. E.; Babayan, V. K.; Garsky, G. Nutritional properties of the triglycerides of medium chain-length. *J. Am. Oil Chem. Soc.* **35**, 10-13(1958).
7) Stewart, J. W.; Wiggers, K. D.; Jacobsen, N. L.; Berger, P.J. Effect of various triglycerides on blood and tissue cholesterol of calves. *J. Nutr.* **108**, 561-566(1978).
8) Kaunitz, H. Medium chain triglycerides in aging and atherosclerosis. in *Advances in Human Nutrition* (Kabara, J. ed.)Vol. 3, Chem-Orbital, POB 134, Park Forest, IL 60466.
9) Jiang, Z. M. A comparison of medium-chain and long-chain triglycerides in surgical patients. *Ann. Surg.* **217**, 175-179(1993).
10) Vaidya, U. V. Vegetable oil fortified feeds in the nutrition of very low birth weight babies. *Indian Pediatric* **29**, 1519(1992).
11) Tantibhedhyangkul, P.; Hashim, S. A. Medium-chain triglyceride feeding in premature infants: effects on calcium and magnesium absorption. *Pediatrics* **61**, 537-540(1978).
12) Metcalfe, L. D.; Schmitz, A. A.; Polka, J. R. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* **38**, 514-515 (1966).
13) Luddy, F. E.; Barford, R. A.; Herb, S. F.; Magidman, P.; Riemenschneider, R. W. Pancreatic lipase hydrolysis of triglycerides by a semimicro technique. *J. Am. Oil Chem. Soc.* **41**, 693-696(1964).
14) Vaysse, L.; Ly, A.; Moulin, G.; Dubreucq, E. Chain-length selectivity of various lipases during hydrolysis, esterification and alcoholysis in biphasic aqueous medium. *Enzyme Microb.Technol.* **31**, 648-655(2002).
15) Ihsan, K; Durmaz, G.; Hayaloglu, A. A. Fatty acid selectivity of lipases during acidolysis reaction between oleic acid and monoacid triacylglycerols. *J. Agric. Food Chem.* **57**, 10466-10470(2009).
16) Yomi W.; Toshihiro, N.; Yuji S. Control of the regio-specificit of *Candida antarctica* lipase by polarity. *New Biotechnol.* **26**, 23-28(2009).
17) Li D.; Hideo, N.; Yugo, I. Direct separation of monoacylglycerol isomers by enantioselective high-performance liquid chromatography. *J. Chromatography A.* **1165**, 67-72(2008).