

Production of Medium Chain Glycerides and Monolaurin from Coconut Acid Oil by Lipase-Catalyzed Reactions

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Abstract: Medium chain glycerides (MCGs) containing C_{8:0} and C_{10:0} fatty acids have got commercial importance by considering their medicinal and nutritional applications. Monolaurin is also a value added commercial product. Coconut acid oil, which is mainly utilized in soap manufacturing process, can be effectively utilized to produce MCGs and monolaurin by a combination of lipase-catalyzed hydrolysis and esterification. Coconut acid oil containing 68% free fatty acid (as lauric acid), and 31.4% neutral glycerides are hydrolyzed by *Candida rugosa* lipase. The hydrolyzed acid oil was then subjected to steam distillation under atmospheric pressure to get a fraction (yield, 5%) rich in medium chain fatty acids (C_{8:0}, 46.2%, C_{10:0}, 19.2%), and the residual fraction was fractionally distilled under vacuum to get a fraction rich in lauric acid (68.4%). These two fractions were esterified with glycerol with *Rhizomucor miehei* (Lipozyme RM IM) lipase to produce MCGs from the first fraction and monolaurin from the second fraction.

Key words: medium chain glyceride, monolaurin, *Rhizomucor miehei* lipase, *Candida rugosa*

1 Introduction

Short chain fatty acids (SCFAs) and medium chain fatty acids (MCFAs) have got some special importance, as SCFAs and MCFAs provide a quick source of energy for infants and stressed adults (1). Medium chain triglycerides (MCTs) containing C_{8:0} and C_{10:0} fatty acids have also got commercial importance because MCTs go directly into the portal vein and are rapidly converted to energy (2). MCFAs have several distinguished characteristics, such as high oxidative stability, low melting point and viscosity, and high solubility in water. MCTs are mainly utilized as a nutritional supplement for patients suffering from malabsorption caused by intestinal resection and as a component in infant formula (3-5). In addition, they also used as a solvent or a

carrier of lipophilic nutrients or drugs, such as vitamin K (6) and phospholipid (7).

Medium chain glycerides (MCGs) which is mainly a mixture of mono-, di- and triglycerides have specific application in the field of foods, pharmaceuticals and cosmetics. Medium chain monoglycerides are ideal solvent for aromatics, steroids, dyes and perfume bases. A mixture of medium chain monoglyceride and diglyceride was found to be an effective solvent for dissolving cholesterol gallstones in humans (8).

Commercially MCGs are manufactured by glycerolysis of MCFAs at high temperature and high pressure, followed by alkali refining, washing, molecular distillation and activated carbon treatment for the products.

Recently structured lipids that contain mixture of short or medium chain fatty acids or both and long

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chain fatty acids are beneficial in human nutrition because they can be tailor-made to metabolic conditions. Lipase-catalyzed modification of triglycerides (TGs) to produce various structured TGs containing MCFAs and long chain essential fatty acids have got tremendous commercial importance due to several advantages over chemical catalyzed process. Structured TGs have been produced by exchanging fatty acids in natural fats and oils with MCFAs using an immobilized 1,3-specific lipase by several workers (9-13). MCFA rich glycerides have been prepared from coconut oil and MCFA methyl esters by lipase catalyzed polyester-monoester interchange reaction (14).

Coconut acid oil, a by-product of coconut oil refinery industry, is mainly utilized in soap manufacturing process. Different value added products are produced from commercial acid oils by lipase catalyzed reactions (15). But no one has utilized the coconut acid oil as a source of cheap raw material to produce MCFAs. The aim of the present work is to utilize coconut acid oil to produce MCFAs and lauric acid, and subsequent production of MCGs and monolaurin by an enzymatic esterification with glycerol.

2 Experimental

2.1 Material

Coconut acid oil was obtained from Edible Products (I) Pvt. Ltd., Kolkata, India. The immobilized lipase *Rhizomucor miehei* (Lipozyme RM IM) was a gift of Novozyme South Asia Pvt. Ltd. Bangalore, India, *Candida rugosa* lipase was purchased from Sigma Chemical Co. USA, "Amano" 30 (*Candida rugosa*) lipase was a kind gift of AMANO ENZYME Inc., Nagoya, Japan.

2.2 Enzymatic Hydrolysis of Coconut Acid Oil

Acid oil (100 g) was taken in a 250 ml stoppered Erlenmeyer flask and water (60% by weight of neutral glycerides) containing lipase powder (0.4% *Candida rugosa* lipase or 1.0% "Amano" 30 lipase) was added. The reaction mixture was magnetically stirred with a 1 inch teflon coated stir bar at $35 \pm 2^\circ\text{C}$ in a controlled bath. The degree of hydrolysis was determined by the content of free fatty acid in the sample periodically withdrawn. After complete reaction, the oil layer and water layer containing enzyme and glycerol were sepa-

rated by centrifugation.

2.3 Atmospheric Steam Distillation

The hydrolyzed coconut acid oil was subjected to steam distillation for 2 h under atmospheric pressure in an all glass distillation equipment fitted with steam inlet, steam outlet and thermometer pocket adapter. The steam volatile fatty acids were collected in the distillate.

2.4 Fractional Distillation

The residual fatty acids from the steam distillation were fractionally distilled in a Claisen-Vigroux (250 ml) flask. The flask was connected to a vacuum pump through a distillation head, an air condenser and pre-weighed 100 ml round bottom collecting flask. The volatile fraction from 100°C to 140°C at 4 mmHg pressure was collected for 30 min. The feed, distillate and residue fractions were weighed and analyzed.

2.5 Enzymatic Esterification Reaction

Fatty acids and glycerol in an appropriate proportion were taken in a round bottom flask and stirred by a magnetic stirrer at $60 \pm 2^\circ\text{C}$ for 8 h using 10% (by weight of fatty acids) RM IM lipase. The stopper of the flask was kept open to eliminate the water formed during esterification reaction. The esterification reaction was monitored by estimating the free fatty acid content in the samples periodically withdrawn. After complete reaction, the product mixture was isolated and mono-, di- and triglycerides, and their amounts were determined by column chromatographic method.

Fatty acid composition was determined by a gas-liquid chromatographic (GLC) method after converting the fatty acids into methyl esters. The HP-5890A GLC was connected with a HP-3390A data integrator. The GLC was fitted with a glass column ($6' \times 1/8''$ i.d.), packed with 10% DEGS supported on Chromosorb-WHP (100/200 mesh), of HP make. The oven temperature was programmed from 100°C to 190°C at $5^\circ/\text{min}$. The injector, and detector block temperatures were maintained at 230°C , and 240°C , respectively. IOLAR-2 nitrogen was used as the carrier gas (flow rate, 30 mL/min). The fatty acid ester peaks were identified and calibrated with standard methyl esters. Data are averages of three determinations.

3 Results and Discussion

Table 1 shows the total fatty acid composition, unsaponifiable matter, and neutral glycerides content of the coconut acid oil. Neutral glycerides present in the acid oil is a mixture of mono-, di- and triglycerides. The acid oil contains 4.9% caprylic acid (C_{8:0}) and 6.2% capric acid (C_{10:0}). So about 11% MCFAs are present.

The neutral glycerides present in acid oil were hydrolyzed by *Candida rugosa* lipase as shown in **Fig. 1**. After 12 h, the degree of hydrolysis reached about 96%. The hydrolyzed acid oil was then subjected to steam distillation to get a fraction (fraction I) rich in MCFAs (C_{8:0} and C_{10:0}). The residual fraction was then fractionally distilled under vacuum to get a fraction (fraction II) rich in lauric acid. **Table 2** shows the yield and fatty acid composition of the two fractions and the residue. The yield of fraction I was 5%, which contained 8.2% caproic acid (C_{6:0}), 46.2% caprylic acid (C_{8:0}) and 19.2% capric acid (C_{10:0}). So fraction I can be used as a source of MCFAs. Fraction II (yield 63%) contained mainly 68.4% lauric acid (C_{12:0}) and 17.1% myristic acid (C_{14:0}). So fraction II can be used as a source of lauric acid.

Table 1 Analytical Characteristics and Fatty Acid Composition of Coconut Acid Oil.

Properties	(%w/w)
FFA(as lauric acid)	68.5
Unsaponifiable matter	0.3
Neutral glycerides	
Monoglycerides	4.3
Diglycerides	3.6
Triglycerides	23.3
Fatty acid Composition (total)	
C _{6:0}	0.9
C _{8:0}	4.9
C _{10:0}	6.2
C _{12:0}	50.3
C _{14:0}	19.2
C _{16:0}	8.3
C _{18:0}	1.3
C _{18:1}	6.3
C _{18:2}	2.6

Fraction I and II were then esterified with glycerol using 1,3-specific RM IM lipase to get MCGs and monolaurin, respectively. The fraction I fatty acids were esterified with glycerol to produce a mixture of MCGs and the reaction was monitored by determining free fatty acid (FFA) with time (**Fig. 2**). After 8 h, the FFA in the mixture was only 6.5%. So most of the fatty acids were esterified with glycerol after 8 h. The final product contained 18.9% monoglycerides (MGs), 62.4% diglycerides (DGs) and 12.2% TGs (**Table 3**). The higher amount of DGs in the reaction mixture was due to the specificity of the lipase (1,3-position specific). TGs were produced due to some acyl migration from the 1,3-positions to 2 position.

Fraction II was also esterified with glycerol by taking larger amount of glycerol to efficiently produce MGs

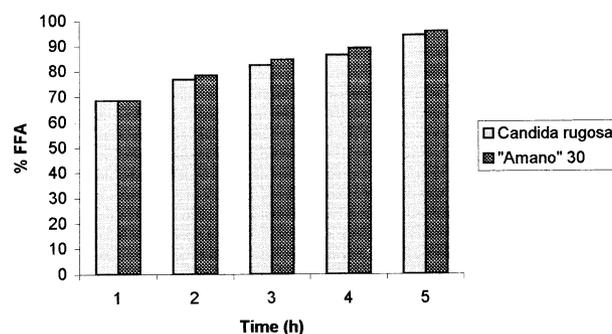


Fig. 1 Hydrolysis of Coconut Acid Oil.

Table 2 Yield and Fatty Acid Composition of the Fractions.

	Fraction I (by steam distillation)	Fraction II (by fractional distillation)	Residue
Yield (% w/w)	5	63	32
Fatty acid composition			
C _{6:0}	8.2	0.7	0
C _{8:0}	46.2	3.2	1.8
C _{10:0}	19.2	5.0	6.5
C _{12:0}	23.0	68.4	20.5
C _{14:0}	3.4	17.1	24.4
C _{16:0}	-	4.5	17.0
C _{18:0}	-	0.4	3.1
C _{18:1}	-	0.7	18.6
C _{18:2}	-	0	8.1

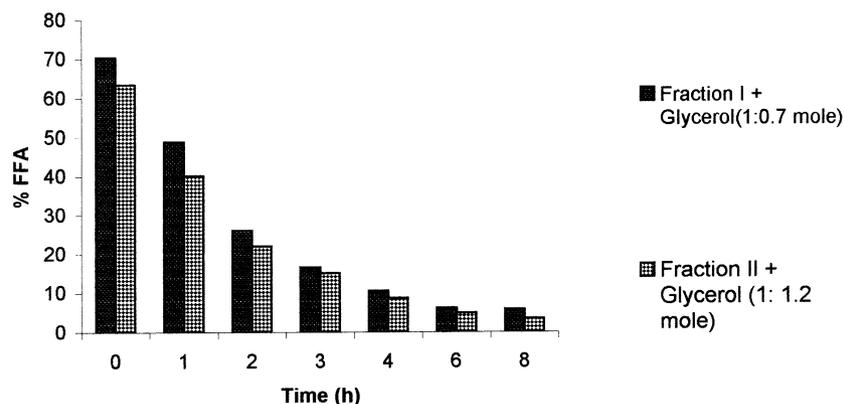


Fig. 2 Esterification of the Distilled Fatty Acids with Glycerol.

Table 3 Composition of the Medium Chain Glycerides and Monolaurin.

	Composition (%w/w)			
	FFA	MG	DG	TG
Medium Chain Glycerides from Fraction I	6.5	18.9	62.4	12.2
Monolaurin from Fraction II	4.2	55.3	36.9	3.6

(Table 2 and 3). The product (monolaurin) contained 4.2% FFAs, 55.3% MGs, 36.9% DGs and 3.6% TGs.

4 Conclusion

It can be noted that the microbial lipase technology with a non-specific lipase offers excellent means of producing fatty acids from coconut acid oil. The valuable MCFAs were produced from coconut acid oil. MCFAs and lauric acid were readily converted into MCGs and monolaurin by lipase-catalyzed esterification. Microbial lipase may be preferred to the chemical catalyst process in producing better quality of the concerned products from the relatively inferior grade raw material.

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