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The triacylglycerols from microbial lipase-catalyzed reaction of coconut oil and oleic acid

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The product triacylglycerols from the reaction of coconut oil and oleic acid using microbial lipase from *Candida cylindracea* as catalyst were analyzed by reversed phase HPLC on C18 column. A peak by peak analysis of the HPLC chromatogram of the triacylglycerol product from lipase-catalyzed interesterification reaction between coconut oil and oleic acid is herein presented. Analysis of the column fractions revealed that oleic acid has been introduced by substitution into the triacylglycerols of coconut oil. Individual column fractions after their saponification and determination of their fatty acid composition showed the presence of mixed tiacylglycerols. The retention times of these mixed triacylglycerols were determined by comparison with those of triacylglycerols microbially synthesized by acidolysis of simple triacylglycerols with oleic acid or by esterification of fatty acids (present in coconut oil) with oleic acid and glycerol. From correlation of carbon numbers with retention times of simple triacylglycerols, the retention times of the mixed triacylglycerols were extrapolated. It was deduced from this correlation that oleyl acylglycerols coeluted with saturated triacylglycerols of lower carbon numbers.

Key Words: Triacylglycerols, oleyl acylglycerols, coconut oil, oleic acid, microbial lipase interesterification, transesterification, acidolysis, *Candida cylindracea.*

A NUMBER OF RESEARCH INVESTIGATIONS ARE CURRENTLY BEING undertaken by local scientists in order to diversify the industrial utilization of coconut oil. One area of research in this field is the structural modification of coconut oil to alter its properties. Properties of interest are the traditional fat constants, e.g., melting point, saponification value, iodine value, which in turn may affect the nutritional value of the oil.

The restructuring of triacylglycerol mixtures involves different interesterification or transesterification techniques traditionally employing chemical catalysts [1-4]. Recently, however, the use of microbial lipase as catalyst [5-14] for interesterification has gained interest for the following reasons:

1) it entails an easy work-up since reactions are carried under mild conditions, and

2) the production of the desired product can be controlled by choosing the right enzyme. Side reactions are minimized due to the high specificity of enzyme action.

The applications of products of lipase-catalyzed interesterification reactions in the industry have been investigated. The production of carbohydrate esters through lipase-catalyzed reaction of simple sugars with fatty acids [14] or coconut oil [10] has been made possible. In the food industry, a valuable cocoa butter equivalent was produced from cheap starting materials [12]. This was accomplished by reacting palm oil with either stearic acid or tristearylglycerol in the presence of 1,3-specific lipase. The product was rich in 1(3)-palmityl-3(1)-stearyl-2-oleylglycerol and 1,3-distearyl-2-oleylglycerol. These two triacylglycerols are the main components of cocoa butter. Vanaspati, a major processed food fat in India, was recently produced by lipase catalyzed interesterification reaction in leiu of the traditional hydrogenation process [7]. Coconut oil has also been shown to hold great potential as a resource in the production of tailor-made fats by interesterification with olive oil using a lipase from *Candida cylindracea* [12]. In this connection, oleic acid, being an unsaturated fatty acid and one of the major fatty acids of olive oil may be used to improve the unsaturation of coconut oil triglycerides and consequently its by-product's acceptability to foreign consumers in the world market.

Local studies devoted to lipase-catalyzed structural modification of coconut oil have been carried out in the past [10,11]. These include the three classes of reactions: ester-ester exchange, acidolysis and alcoholysis. While apparently new products were obtained from these reactions, no attempt has been made to identify them.

The basic limitation already encountered by previous workers in the determination of triacylglycerols in coconut oil by HPLC [15] and GC [16] is the unavailability of standard triacylglycerols of mixed fatty acid composition.

This limitation, however, can be overcome by the application of computerized calibration based on the linear interpolation of relative response factor (RRF) values of pure components provided that these compounds are ho-

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mologues. A study of the data presented by Drusshel et al. [17], reveals that a linear relationship with a high degree of correlation is obtained when the relative response factor (RRF) values of alcohol homologs are plotted against the logarithm of their carbon numbers.

This technique has been applied in the determination of triacylglycerols in plasma lipids [18], and in lipase catalysed interesterification reaction product from butterfat solid fraction and hydrogenated rapeseed oil [19] by gas-liquid chromatography. This technique was also applied by Mares and Husek [20] in their study on quantitative capillary GC of triacylglycerols with carbon numbers of 30-60. These quantitative determinations commonly employ simple triacylglycerols standards (C30, C36, C42, C48 and C45) to interpolate the response factors of mixed triacylglycerols for their evaluation in oil samples.

However, species containing polyunsaturated fatty acids tend to decompose at high temperatures such that several authors used reversed phase C-18 column chromatography [15, 21 -24]. The main concern of this investigation is therefore the analysis of the triacylglycerols in the acidolysis of coconut oil with oleic acid by HPLC. Reaction parameters [11] already established will be applied to the synthetic process to produce a substantial amount of product needed for this investigation. The authors believe that the fat and oil industry will need some knowledge on the absolute structures of the synthesized triacylglycerols, and that these informations will be valuable for the specialty use of coconut oil.

Experimental

Lipase from *C. cylindracea* (Kansai Corporation, Japan) was immobilized by adsorption onto kieselguhr. The crystalline lipase (600 mg) was dissolved in 100 mL of phosphate buffer of pH 8. Kieselguhr (35 g) was slowly added until a slurry was formed. The adsorption of the enzyme on the inorganic particles was induced by slow additon of 95% ethanol. The immobilized lipase was then separated by filtration, air-dried and stored at 0°C to 5°C.

The coconut oil used in this study was either a commercial oil (Magnolia) or an extract of coconut meat. Finely ground coconut meat was steeped for five minutes in hot water (70° - 80°C). The hot water extract was obtained from the combined squeezing and sieving of the coconut meat. This extract was boiled to evaporate the water. After cooling, the crude oil was then separated from the solids through a coarse sieve.

In order to eliminate the unwanted color and odor, the crude extract (50 g) was dissolved in 50 mL hexane. This solution was passed through a column of Kieselgel S (10 cm x 1 cm I.D.) previously slurried and conditioned with hexane. The eluate was collected together with the hexane effluent (100 mL). The purified oil was purged of the solvent with a stream of nitrogen at 80°C.

The fatty acid profile and triacylglycerol content of the two oil samples were compared. The oleic substrate was obtained from Merck (product no. 471). This was assayed by GC as methyl ester derivative.

Pure fatty acid standards (caproic, caprylic, capric, lauric, myristic, pentadecanoic, palmitic, palmitoleic, stearic, oleic and linoleic) and simple triacylglycerols (tricaprylyl-, tricapryl-, trilauryl-, trimyristyl-, and tripalmityl- glycerols) were purchased from Sigma Chemicals, U.S.A.

The analysis of fatty acids as methyl esters was performed on Shimadzu GC-B equipped with a flame ionization detector and a wide bore glass capillary column coated with 1.0 μm film Supelcowax 10 (30 mm x 0,75 mm I.D.). The GC instrument used for the analysis of mono-, di- and triacylglycerols was a Varian GC M-2700 equipped with a flame ionization detector and 1% Dexsil 300 coated on Supelcoport, 100/120 mesh (0.5 m x 2 mm I.D.). The HPLC system consisted of Waters Associates refractive index detector R401, U6K universal injector, M-6000 pump, 3 pieces of Novapak-C18 columns (5 μm, 15 cm x 3.9 mm I.D., ss) and a column heater with automatic control unit. The detector outputs were monitored, processed and stored using either a Shimadzu Chromatopac CR-4A or an IMI Chromatochart PC data processor.

I. Interesterification Reaction of Coconut Oil and Oleic Acid

The acidolysis reaction was performed according to the procedure of Lorica et al. [11] using the following mixture:

Coconut oil	100	g
Oleic acid	100	g
Immobilized lipase	10	g
Petroleum ether	50	mL

Prior to incubation, 0.5 mL distilled water was slowly added to the mixture through a 500 μL syringe. The reaction mixture, contained in a stoppered flask, was agitated in a Lab-line Orbit environmental shaker at the predetermined incubation temperature (37°C) and time (24 hrs.). At the end of the reaction period, the catalyst was removed by filtration. The combined filtrate and petroleum ether washing were purged of the solvent with a stream of nitrogen at 80°C to give the crude extract.

The preliminary purification of the crude extract was carried out either by solvent extraction or by solid phase adsorption essentially to separate the lipids from the nonlipids. The solvent extraction technique was done without modification of the previously described method [11]. As an alternative to solvent extraction, solid phase adsorption was accomplished by the passage of the solution of the crude product in hexane (1 g in 25 mL) through a florisil cartridge (Waters Sep-Pak florisil). The eluate containing the lipids was collected and the cartridge was washed with 5 mL hexane. The combined eluate and washing was purged of the solvent with a stream of nitrogen at 50°C.

II. Separation of the Product Triacylglycerols

A. HPLC Analysis

Using a modified procedure by Kondoh and Takano [22], 2 μL of the product were injected into the HPLC. The

analytical column consisted of 3 of Novapak C18 columns connected in series (5mm, 15 cm x 3.9 mm I.D., ss). The column temperature was set at 35°C. The solvent system consisted of ethanol/acetonitrile (50/50) with a flow rate of 1.0 mL/min.

B. HPLC Fractionation of Triacylglycerols from the Product

This HPLC fractionation technique was based on the procedure by Bezard and Ouedraogo [21] when they determined the triacylglycerol composition of peanut and coconut seed oils, except that a volume of 5 μL was injected into the liquid chromatograph. The triacylglycerols of interest were collected individually through a 10 x 0.1 mm I.D. ss tube emerging from a 3-way valve into 5 mL test tubes. The solvent in each fraction was evaporated at 50°C under a stream of nitrogen. Proper timing was observed on the collection of fractions to avoid contamination from the neighboring peaks. Refractionation of the collected isolates was done when there was more than 3% contamination due to the preceeding peak. Quantitation was based on peak areas. The inject-collect-evaporate-fractionate cycle was repeated until a sufficient amount of chromatographically pure isolate was obtained for fatty acid determination.

III. Determination of Fatty Acid components of Substrate and Product

Analysis of fatty acids, as methyl esters, was carried out after saponification of the triacylglycerols with potassium hydroxide solution in methanol (0.2 N). The methyl esters were prepared according to procedure by Firestone and Hortwitz [25]. A volume of 5 μL of the derivatized solution was injected into the Shimadzu GC. The column used was a wide bore glass capillary coated with Supelcowax 10 (30 m x 0.75 mm I.D.). The initial column temperature was initially set at 100°C and then raised to 270°C at the rate of 10°C/min. The detector and injector temperature were set at 290°C. The carrier gas was helium with a flow rate of 12 mL/min. The detector make-up gas was also helium at 30 mL/min.

IV. Lipase-Catalyzed Acidolysis and Esterification Reactions of Known Starting Materials

Based on previous studies [13, 14], reactions were carried out under the same conditions used in the coconut oil/oleic acid system in order to produce acylglycerols of oleic acid among others.

1. Acidolysis

Each reaction mixture consisted of 5 grams of triacylglycerol, 5 grams of analytical grade (AR) oleic acid, 0.5 gram of hydrated lipase, where the triacylglycerol is either tricaprylglycerol (GC grade) or trilaurylglycerol (GC grade).

2. Esterification

Each reaction mixture consisted of 5 grams of a fatty acid, 5 grams of analytical grade (AR) oleic acid, 5 grams of glycerol and 0.5 gram anhydrous lipase; where the fatty acid is myristic acid (GC grade), palmitic acid (GC grade), palmitoleic acid (GC grade), stearic acid (GC grade), or analytical grade (AR) oleic acid.

The product of each reaction mixture was fractionated by HPLC and the identity of the isolates were confirmed after analysis of the fatty acids by GC.

Discussion

Fatty Acid Analysis of the HPLC Fractions

HPLC chromatograms of the reaction product and the coconut oil substrate are shown in Fig. 1. The overlaid chromatograms of product and substrate reveal that the product contains triacylglycerols with different structures from those of coconut oil, since there is peak-to-peak variation in retention times between those in the substrate and the product. A comparison of the fatty acid compositions of the two fractions (fraction 1 and fraction 2 of Fig. 1) from the substrate coconut oil and the product is shown on Table 1. The data indicate that oleic acid is indeed introduced by substitution into the triacylglycerols of coconut oil through the reversed-lipase catalyzed reaction (see composition of product on Table 1).

To determine whether oleic acid is present in each fraction, individual peaks were collected from the product (Fig. 2) saponified and analyzed for fatty acid composition by GC (Table 2). It is noteworthy that oleic acid is present in high amounts in all fractions which could consist of mixed or mixture of triacylglycerols containing oleic acid. A complex substrate, like coconut oil, in interesterification reaction naturally leads to formation of a product of complex composition. Intermediates (diacylglycerols, monoacylglycerols, glycerol and fatty acids) are also present in the product. Since oleic acid is added in large amount, the expected

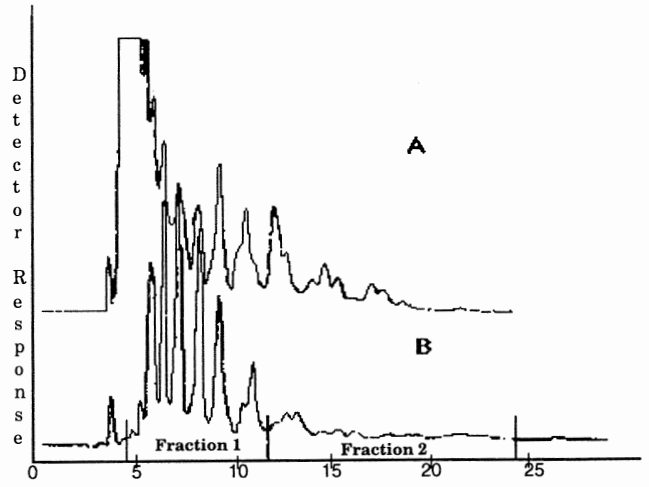


Figure 1. Overlaid chromatograms of product (A) and coconut oil (B) from lipase-catalyzed interesterification reactions between coconut oil and oleic acid.

Table 1. Fatty Acid Composition of HPLC Fractions (Fig. 1) of Coconut Oil and Reaction Product

	Coconut Oil		Product	
Fatty Acids, mole %	Fraction 1	Fraction 2	Fraction 1	Fraction 2
CO6:0	4.2	–	–	–
CO8:10	10.8	–	5.4	–
C10:0	6.8	–	4.5	0.3
C12:0	46.5	17.6	35.5	13.7
C14:0	18.8	20.2	13.3	13.2
C16:0	7.0	27.6	5.8	12.9
C16:1	–	–	3.7	3.3
C18:0	1.6	8.1	–	2.0
C18:1	3.6	23.3	28.5	51.6
C18:2	0.8	3.2	3.3	3.0

Table 2. Fatty Acids in Isolated Fractions (Fig. 2) from the Reaction Product

Fraction	Retn. Time sec.	Collect Time sec.	Fatty Acids, mole % C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
1	471	510-530	–	9.6	43.4	11.5	2.1	4.1	2.3	22.6	4.4
2	540	580-610	3.3	2.7	46.4	9.1	3.1	2.9	–	30.8	1.7
3	620	650-700	–	3.3	30	21.1	4.5	6.1	–	30.3	4.7
4	707	750-780	–	–	28.6	7.4	2.3	3.4	–	54.5	3.8
5	737	790-825	–	0.3	26.9	14.6	17.1	2.9	–	39.1	2
6	818	850-870	–	0.3	17.0	19.4	14.4	4.1	3.1	36.7	5
7	853	890-915	–	–	7.4	18.2	7.3	7.3	–	53.9	5.9
8	895	925-960	–	–	9.2	21.9	13.7	3.8	7.3	40	4.0
9	992	1025-1060	–	–	5.9	10.3	10.8	–	3	68.7	1.3
10	10[illegible]	1080-1110	–	–	1.2	2.8	19.2	–	1.4	73	1.4
11	1095	1135-1175	–	–	2.6	7.4	26.7	–	6.2	55.7	1.4
12	1288	1330-1365	–	–	3.8	7.8	6.1	–	28.3	54.7	–

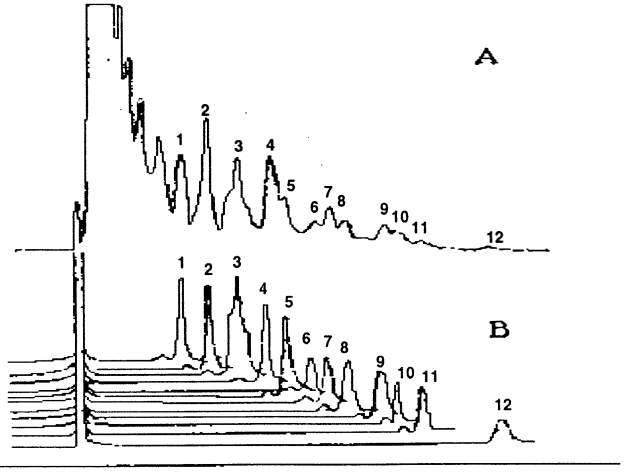


Figure 2: Fractionation of triacylglycerols in the product: A) typical HPLC chromatogram of the product B) HPLC chromatograms of the collected fractions

major triacylglycerols are mono- and dioleyl triacylglycerols. This, in turn, makes their analysis difficult because commercial standards of mixed triacylglycerols are not available.

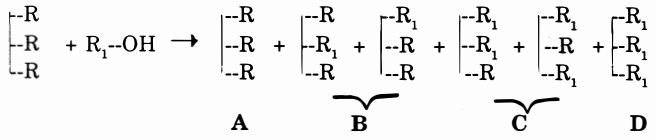
Mixed Triacylglycerols from Selected Microbial Synthesis

Due to the unavailability of commercial standards of mixed triacylglycerols, microbial lipase synthesis of these were done by acidolysis and esterification of simple triacylglycerols and fatty acis. For a typical acidolysis reaction involving a simple triacylglycerol and a fatty acid catalyzed by a non-specific lipase, the product components can be separated by reversed phase HPLC and their retention times noted and compared with those of the product from the reaction of coconut oil and oleic acid. For example a study was made on the interesterification of equal amounts of trilaurylglycerol and oleic acid using *C.*

Table 3. Identification of HPLC Fractions from Products of Lipase Catalyzed Interesterification Reactions.

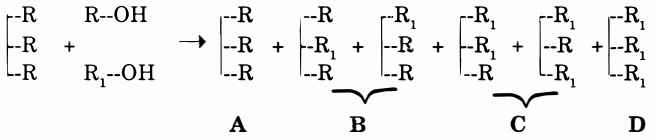
Reaction Mixture	R. T., min	Fatty Acids (mole %)	Identity glycerol
Tricaprylglycerol/	6.25	C 10:0 (2%), C 16:0 (1%), C 16:1 (2%)	
Oleic Acid (AR)/		C 18:1 (95%)	Dioleyl
Hydrated Lipase	6.80	C 10:0 (65%), C 18:1 (35%)	Dicapryl-oleyl
	8.60	C 10:0 (33%), C 16:1 (34%), C 18:1 (33%)	Capryl-palmitoleyl-oleyl
	10.02	C 10:0 (33%), C 18:1 (67%)	Dioleyl-capryl
Trilauryglycerol	6.15	C 12:0 (1%), C 16:0 (1%), C 16:1 (2%)	
Oleic Acid (AR)/		C 18:1 (96%)	Dioleyl
Hydrated Lipase	6.91	C 12:0 (98%), C 18:1 (2%)	Trilauryl
	7.64	C 12:0 (66%), C 16:1 (34%)	Dilauryl-palmitoleyl
	8.79	C 12:0 (67%), C 18:1 (33%)	Dilauryl-palmitoleyl
	9.96	C 12:0 (33%), C 16:1 (67%)	Dilauryl-palmitoleyl
	11.74	C 12:0 (33%), C 18:1 (67%)	Dioleyl-lauryl
	16.25	C 18:1 (100%)	Trioleyl
Myristic Acid/	5.13	C 14:0 (48%), C 18:1 (52%)	Myristyl-oleyl
Oleic Acid (AR)	10.74	C 14:0 (100%)	Trimyristyl
Glycerol/	12.00	C 14:0 (64%), C 16:1 (3%), C 18:1 (33%)	Dimyristyl-oleyl
Anhydrous Lipase	14.78	C 14:0 (30%), C 16:1 (6%), C 18:1 (61%)	Dioleyl-myristyl
	16.25	C 18:1 (100%)	Trioleyl
Palmitic Acid/	16.25	C 18:1 (100%)	Trioleyl
Oleic Acid (GC)/	16.62	C 16:0 (28%), C 18:1 (72%)	Dioleyl-palmityl
Glycerol/	17.60	C 16:0 (61%), C 18:1 (39%)	Dipalmityl-oleyl
Anhydrous Lipase	19.16	C 16:0 (100%)	Tripalmityl
PalmitoleicAcid/	5.13	C 16:1 (97%), C 18:1 (3%)	Dipalmitoleyl
Oleyl Acid (GC)/	5.59	C 16:1 (55%), C 18:1 (45%)	Oleyl-palmitoleyl
Glycerol/	6.18	C16:1 (1%), C 18:1 (99%)	Dioleyl
Anhydrous Lipase	9.60	C 16:1 (100%)	Tripalmitoleyl
	11.05	C 16:1 (66%), C 18:1 (34%)	Dipalmitoleyl-oleyl
	13.13	C 16:1 (33%), C 18:1 (67%)	Dioleyl-palmitoleyl
	16.20	C 18:1 (100%)	Trioleyl
Stearic Acid	16.20	C 18:1 (100%)	Trioleyl
Oleic Acid (GC)/	21.76	C 18:0 (33%), C 18:1 (67%)	Dioleyl-stearyl
Glycerol/			
Anhydrous Lipase			
Oleic Acid (AR)/	5.59	C 16:1 (45%), C 18:1 (55%)	Oleyl-palmitoleyl
Glycerol/	6.15	C 18:1 (100%)	Dioleyl
Anhydrous Lipase	11.10	C 16:1 (66%), C 18:1 (34%)	Dipalmitoleyl-oleyl
	13.13	C 16:1 (35%), C 18:1 (65%)	Dioleyl-palmitoleyl

cylindricea lipase. HPLC analysis of the product revealed that triacylglycerols found are trioleylglycerol, dioleyl-laurylglycerol, dilauryl-oleylglycerol, and trilaurylglycerol at about the ratio of 1/2/2/1, respectively (see Fig. 3B). The positional isomers of dioleyl-laurylglycerol and dilauryloleylglycerol however were not resolved on the reverse phase C18 column. This acidolysis reaction is represented by the equation:



where, -- = CH_2--O- / CH--O- / CH_2--O- and R and R_1 = lauryl and oleyl groups, respectively. Both products B and C consist of two positional isomers while products A and D are simple triacylglycerols.

Furthermore, esterification involving fatty acids RCOOH and R_1COOH and glycerol as in the following reaction leads to the same set of products as in acidolysis:



where R- = fatty acyl group and R_1-= oleyl group.

If these representations are applied in the acidolysis and esterification reactions, R can come from any of the fatty acids (C10, C12, C14, C16:1, C18:0) and R_1 from oleic acid. Hence, product A is a simple triacylglycerol of fatty acid RCOOH, product B is a monooleyltriacylglycerol, product C is a dioleyltriacylglycerol and product D is trioleylglycerol.

An HPLC study of the above products of acidolysis and esterification reactions in the presence of lipase as catalyst is shown on Table 3. Fatty acid composition of the saponified HPLC fraction was used to identify any of the triacylglycerol (TAG) peak for a particular reaction product and later for coconut oil and oleic acid reaction product. In addition, diacylglycerols were also positively identified in some of the reaction systems studied.

Retention time of mixed triacylglycerols (TAG) from Correlation Curve

The identity of simple and mixed triacylglycerols produced from the acidolysis and esterification reaction can also be confirmed by comparison of retention times with those of standard triacylglycerols (Fig. 4A). A third order regression analysis of the experimentally determined etention time (t_R) and carbon number (C.N.) of these saturated triacylglycerols, gives the following correlation equation:

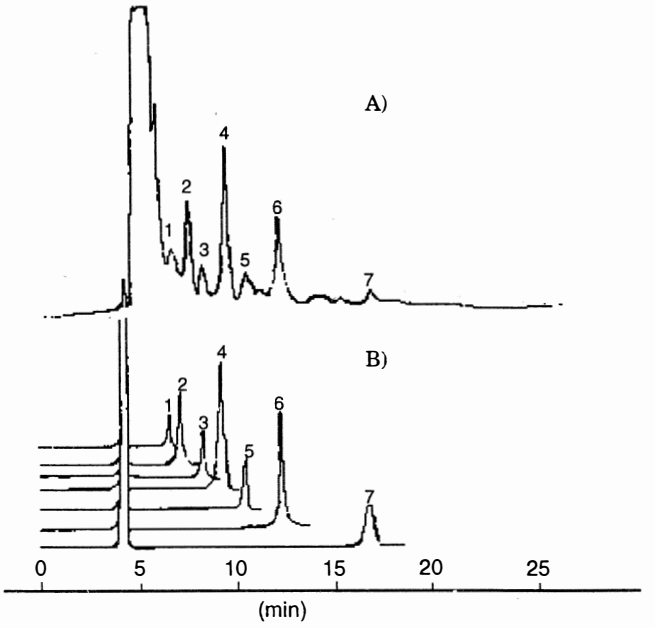


Figure 3: HPLC chromatograms of: A) product from trilaurylglycerol/oelic acid lipase-catalyzed interesterification reaction, and B) isolated fraction from the product.
Peaks: 1. dioleylglycedrol (6.15 min): 2. trilaurylglycerol (6.91 min.): 4. dilauryl-oleylglycerol (8.79 min). 6. dioleyl-laurylglycerol (11.74 min), and 7. trioleylglycerol (16.20). Other product components due to oleic acid contaminant – Peaks: 3. dilauryl-palmitoleylglycerol (7.64 min) and 5. dipalmitoleyl-laurylglycerol (9.96).

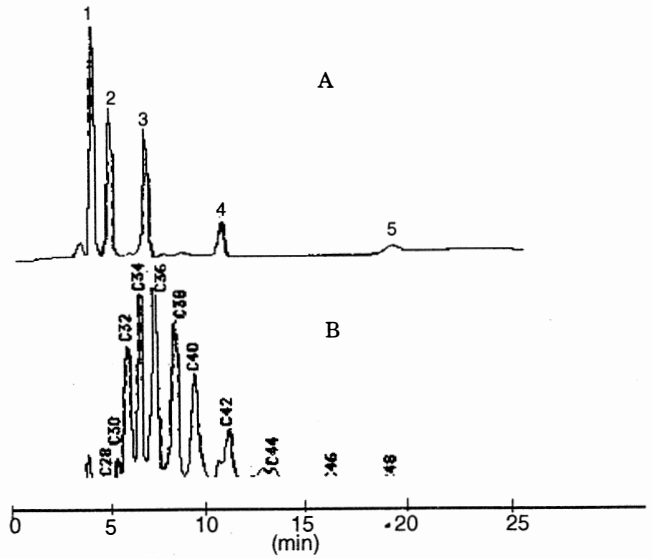


Figure 4: HPLC chromatograms of saturated triacylglycerols in: A) standard mixture; B) coconut oil.
Peaks: 1. tricaprylylglycerol (4.09 min); 2. tricaprylglycerol (5.23 min); 3. trilaurylglycerol (6.91 min)/ 4. trimyristyl glycerol (10.74 min), and 5. tripalmitylglycerol (19.20 min).

t_R = - 32.1 + 3.59 (C.N.) - 0.12 (C.N.)^2 + 0.0014 (C.N.)^3
Coefficient of Correlation = 1.00
Standard Error of Estimate = 0.03

From this correlation equation, retention times of saturated triacylglycerols with mixed fatty acid composition in coconut oil can be extrapolated (Fig. 4B). Comparison of retention times between the actual data obtained from the HPLC run of coconut oil and the inter- or extrapolated values, shown on Table 4, implies that this is an excellent technique for predicting the retention behavior of components in a mixture. A plot of retention times and carbon numbers of saturated triacylglycerols is exhibited in Fig. 5. This relationship between carbon numbers and retention times of simple saturated triacylglycerols has been observed previously in reversed phase HPLC [21, 23].

Studies made on the retention behavior of triacylglycerols on a non-aqueous reversed phase liquid chromatography also reveal that triacylglycerols with unsaturated fatty acids are less retained than corresponding saturated triacylglycerols [26]. The higher the degree of unsaturation, the larger is the difference in retention time. Once double bond is said to be almost equivalent to a reduction of two carbon atoms. To some extent, this observation is evident in the data presented in Table IV. For example, dicapryl-oleyl-glycerol (C38), t_R = 6.8 min) will tend to co-elute with trilaurylglycerol (C36, t_R = 6.90 min).

Separate regression analysis is performed on experimental retention times pertaining to monooleyl TAG's reported in Tables III. The plot of retention times versus carbon numbers is illustrated in Fig. 5. The correlation obtained is

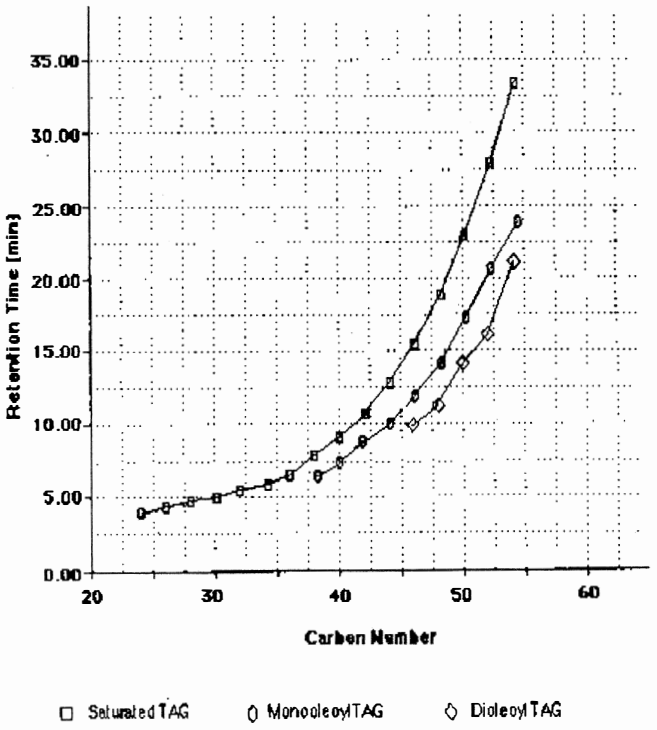


Figure 5: Relationships Between Retention Times on RP-C18 Column and Carbon Numbers of Triacylglycerols.

t_R = 78.87 - 4.09 (C.N.) + 0.0056 (C.N.)^2
Coefficient of correlation = 0.999
Standard Error of Estimate = 0.25

From this equation, the retention times of the following monooleyl diacylglycerol can be calculated:

C.N.		Rt (min)
C40	C10C12C18:1	7.92
C44	C10C16C18:1	10.19
	C12C14C18:1	
C48	C12C18C18:1	14.64
	C14C16C18:1	
C52	C16C18C18:1	20.90
C54	C18C18C18:1	24.20

Based on the equivalent number of carbons and double bond, the following retention behaviour can also be deduced:

- capryl-myristyl-oleylglycerol co-elutes with dilauryl-oleyl-glycerol (C42)

- capryl-stearyl-oleylglycerol and lauryl-palmityl-oleyl-glycerol co-elute with dimyristyl-oleylglycerol (C46), and

- myristyl-stearyl-oleylglycerol co-elutes with dipalmityl-oleylglycerol (C50).

The results of this regression analysis are also reported in Table 4.

Figure 6 shows a typical HPLC chromatogram of the product. The list of peak identities results from the fatty acid analysis of the fractional peaks, comparison of product retention times with those of reference standards and a study of retention behavior of triacylglycerols on the RP - C18 column.

The identities of triacylglycerols in the major HPLC fractions of the product (Fig. 6) can be obtained by correlation of data presented in Tables II, III, and IV. The result of this correlation, accounts for the triacylglycerols produced from the interesterification reaction of the major faty acid constitutents of coconut oil and oleic acid.

Conclusion

The result of the coconut oil - oleic lipase-catalyzed reaction was a broad spectrum of triacylglycerol products which certainly included more than what is presented in Fig. 6. Separate regression analyses on the HPLC retention behavior of saturated triacylglycerols, monooleyldiacylglycerols and dioleylacylglycerols have made possible the positive identification of oleic acid-containing triacyl glycerol components of the product. The oleyl acylglycerols consist mainly of the major fatty acid found in coconut oil; namely, lauric, myristic, and palmitic acids. In the HPLC

Table 4. Retention Behavior of Triacylglycerols on a RP-C18 Column (15 μm. 3x15 cm x 3.9 mm I.D.).

No. of Carbons	Retention Time, min.			
	Coconut Oil TAG	Saturated TAG	Monooleyl TAG	Dioleyl TAG
24	-	4.09	-	-
	C8C8C8			
26	4.46	4.53*	-	-
28	4.84	4.89*	-	-
30	5.23	5.23	-	-
	C10C10C10			
32	5.67	5.64*	-	-
34	6.91	6.90	-	-
36	6.91	6.90	-	-
38	7.93	7.90*	6.80	-
40	9.21	9.22*	7.52*	-
42	10.94	10.94	8.79	-
44	13.16	13.14*	10.19*	-
46	16.02	15.86*	12.00	10.00
			C14C14C18:1	C18:1C18:1C10
48	19.2	19.20	14.64*	11.74
				C18:1C18:1C12
50	-	23.21*	17.60	14.78
			C16C16C18:1	C18:1C18:1C14
52	-	28.00*	20.90	16.62
				C18:1C18:1C16
54	-	33.52*	24.20*	21.76
				C18:1C18:1C18

analysis, co-elution of triacylglycerols from the product of this lipase-catalyzed reaction was further revealed by HPLC fractionation and subsequent fatty acid analysis of the saponified fraction by GC.

This work has established workable instrumentation techniques for monitoring and assessing the triacyl glycerols from lipase-catalysed reactions of coconut oil. Through the application of these techniques, the change in concentration of product components like the oleyl-triacylglycerols, can be used as guide for the optimazation of reaction parameters. A similar approach can be applied to assess other reaction systems currently being studied for the structural modification of coconut oil.

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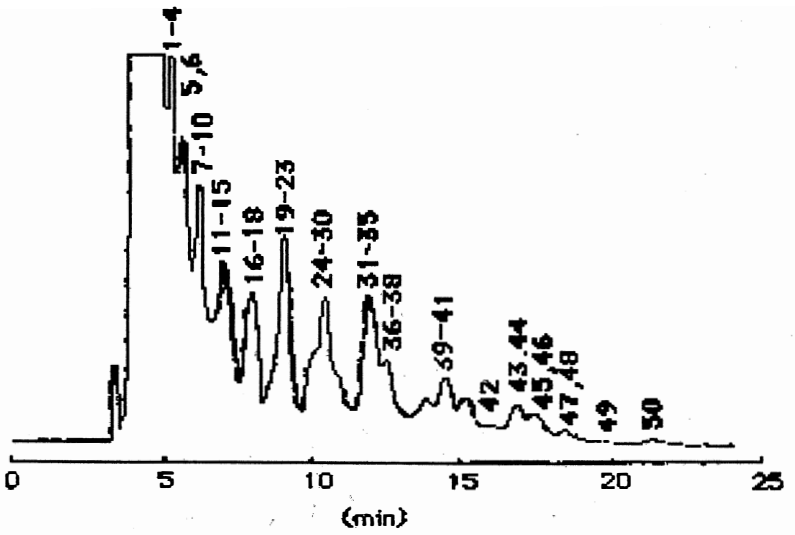

Figure 6: HPLC analysis of the product from lipase-catalyzed interesterification reaction between coconut oin and oleic acid.

Peaks and retention times (minutes):

1.	*Myristy-oleylglycerol*	*5.13*	*26.*	*Capryl-palmityl-oleylglycerol*	*10.19*
2.	*Dipalmitoleylglycerol*	*5.13*	*27.*	*Lauryl-myristyl-oleylglycerol*	*10.19*
3.	*Tricaprylglycerol*	*5.23*	*28.*	*Trimyristylglycerol*	*10.74*
4.	*C30*	*5.23*	*29.*	*C42*	*10.74*
5.	*Oleyl-palmitoleylglycerol*	*5.59*	*30.*	*Dipalmitoleyl-oleylglycerol*	*11.05*
6.	*C32*	*5.67*	*31.*	*Dioley-laurylglycerol*	*11.74*
7.	*Dioleylglycerol*	*6.20*	*32.*	*Lauryl-palmityl-oleylglycerol*	*12.00*
8.	*C34*	*6.26*	*33.*	*Capryl-stearyl-oleylglycerol*	*12.00*
9.	*2.3-dipalmitylglycerol*	*6.37*	*34.*	*Dimyristyl-oleylglycerol*	*12.00*
10.	*1.3-dipalmitylglycerol*	*6.60*	*35.*	*Myrityl-palmitoleyl oleylglycerol*	*12.00*
11.	*Dicapryl-oleylglycerol*	*6.80*	*36.*	*Dioleyl-palmitoleylglycerol*	*13.13*
12.	*Trilaurylglycerol*	*6.91*	*37.*	*C44*	*13.16*
13.	*C36*	*6.91*	*38.*	*Laury-steary-oleylglycerol*	*13.16*
14.	*Capryl-lauryl-oleylglycerol*	*7.52*	*39.*	*Myristyl-palmityl-oleylglycerol*	*14.64*
15.	*Dilauryl-palmitoleyl-oleylglycerol*	*7.64*	*40.*	*Palmitoleyl-palmityl-oleylglycerol*	*14.78*
16.	*C38*	*7.93*	*41.*	*Dioleyl-myristylglycerol*	*14.78*
17.	*2.3-Distearyglycerol*	*8.39*	*42.*	*C46*	*16.02*
18.	*Capryl-palmitoleyl-oleylglycerol*	*8.60*	*43.*	*Trioleylglycerol*	*16.20*
19.	*Dilauryl-oleylglycerol*	*8.80*	*44.*	*Dioleyl-palmitylglycerol*	*16.62*
20.	*Capryl-mirystyl-oleylglycerol*	*8.80*	*45.*	*Dipalmityl-oleylglycerol*	*17.60*
21.	*1.3-Disterylglycerol*	*8.91*	*46.*	*Myristyl-stearyl-oleylglycerol*	*17.60*
22.	*C40*	*9.21*	*47.*	*Tripalmitylglycerol*	*19.20*
23.	*Tripalmitoleylglycerol*	*9.60*	*48.*	*C48*	*19.20*
24.	*Dipalmitoleyl-laurylglycerol*	*9.96*	*49.*	*Palmity-steary-oleylglycerol*	*20.90*
25.	*Dioleyl-caprylglycerol*	*10.02*	*50.*	*Dioleyl-stearylglycerol*	*21.76*

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