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ENZYMATIC REACTIONS ON THIN-LAYER CHROMATOGRAPHIC PLATES

I. LIPOLYSIS OF TRIGLYCERIDES AND SEPARATION OF PRODUCTS ON A SINGLE PLATE

JYOTIRMOY DUTTA, ARUN KR. DAS and SANDIP SAHA*
Department of Chemistry, Bose Institute, Calcutta 700009 (India)
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SUMMARY

A novel method for the lipase deacylation of triglycerides is described in which 0.1–0.2 ml of the enzyme solution in 1 *M* tris buffer (pH 8.2) containing 2–4 mg of protein is applied as a band on a 0.5-mm thick silica gel thin-layer chromatographic plate. Over this band, 1–4 mg of triglyceride in *n*-hexane is applied evenly and the plate is incubated at 40° for 1–2 min. The reaction is stopped by exposure to hydrogen-chloride vapour and the products are removed from the reaction zone by three consecutive developments in diethyl ether up to 2 cm from the line of application. These are resolved by re-developing the plate in *n*-hexane–diethyl ether–acetic acid (80:20:1.5) up to 14 cm. The *sn*-2-monoglyceride and *sn*-1,2(2,3)-diglyceride bands located by iodine vapour are extracted and the fatty acid compositions evaluated by gas–liquid chromatography for the determination of fatty acid distribution in the glyceride molecule. The method, when applied to groundnut oil, goat depot fat, body fat of shol fish (*Channa striatus*) and yeast (*Rhodotorula glutinis*) fat, produced representative *sn*-1,2(2,3)-diglycerides which can be used for the stereospecific analysis of triglyceride.

INTRODUCTION

Since the postulation by Balls and Matlack[1] in 1938 that mammalian pancreatic lipase catalyses specifically the hydrolysis of the primary ester groups of triglycerides, many workers[2–5] have used this enzyme for the analytical deacylation of triglycerides. The *sn*-2-monoglycerides formed due to lipase hydrolyses have been used for fatty acid analyses in studying the positional distribution of fatty acids in triglycerides of natural fats. The *sn*-1,2(2,3)-diglycerides produced have sometimes been used for stereospecific analysis of the triglycerides.

In a widely used method due to Luddy *et al.*[6] for the deacylation of triglycerides

* Present address: Calcutta Chemical Co. Ltd., 35 Panditia Road, Calcutta 700 029, India.

with pancreatic lipase, a mixture in tris buffer (pH 8.2) of the sample, a solubilizer [bile salt, poly(vinyl alcohol), etc.] and calcium chloride is shaken vigorously for 90 sec and the reaction products are extracted immediately with diethyl ether, washed, dried and resolved into fractions by preparative thin-layer chromatography (TLC). The fatty acid compositions of the glyceride products extracted from the adsorbent are determined by gas–liquid chromatography (GLC).

In this paper, we present a novel method for the lipase deacylation of triglycerides in which the enzymatic reaction, extraction of the reaction products and their resolution are performed on the same TLC plate without any solubilizer or calcium chloride. The fatty acid distribution in the glyceride products can be determined by GLC, from which the composition of the triglycerides can be calculated. By this means, the time required for the entire process has been shortened considerably and the loss of the products due to transfers has been minimized. The dependence of the extent of hydrolysis on reaction parameters such as the amounts of enzyme and substrate, time and temperature have been evaluated, and the fatty acid distributions of the triglycerides from several natural fats have been determined.

EXPERIMENTAL

Solvents

All solvents were of analytical-reagent grade, and were dried and re-distilled.

Reference lipids

Triolein, *sn*-2-monopalmitin, *sn*-1,2(2,3)-dipalmitin, *sn*-1,3-dipalmitin and methyl pentadecanoate, all over 99% pure, and reference quantitative mixtures of fatty acid methyl esters were obtained from Applied Science Labs. (State College, Pa., U.S.A.).

Reagents

Lipase solution. Various amounts of lipase, depending on the activity, were shaken with 1 *M* tris buffer (pH 8.2). The mixture was centrifuged at 167 *g* and the supernatant was used. The amount of protein per 0.1 ml of this solution was determined according to Lowry *et al.*[7]. This amount of the soluble protein is referred to in the text as the amount of lipase. A 30-mg amount of the lipase used (Sigma type II, crude from hog pancreas; Sigma, St. Louis, Mo., U.S.A.) yielded 20 mg/ml of soluble protein. The activity of this enzyme solution, determined according to Luddy *et al.*[6], was 111 units per milligram of protein using olive oil as the substrate. One unit will hydrolyse 1.0 μequiv. of fatty acid from a triglyceride in 1 h at pH 7.7 and 37°.

Bile salt solution (1%)[8]. A 0.1-g amount of dried bile salt (Oxo, London, Great Britain) was mixed with 1 ml of water, autoclaved at 15 p.s.i. for 1.5 h, 10 ml of glycerol were added and the mixture was heated on a water-bath until homogeneous.

Calcium chloride solution (5%). A 5-g amount of $CaCl_2 \cdot 2H_2O$ (E. Merck, Darmstadt, G.F.R.) was dissolved in 100 ml of water.

Preparation of the TLC plate

Glass plates (20 × 14 cm) were coated with 0.5 mm thick layers of silica gel G

(E. Merck, Darmstadt, G.F.R.), in the usual manner, activated at 110° for 1 h and stored in desiccator. Before use the plates, in order to remove any organic contaminants, were pre-developed with diethyl ether to the top of the plate, from where these were removed by scraping off a narrow band.

Lipolysis of triglycerides on the TLC plate, removal of the reaction products from the reaction zone, development of the chromatogram and isolation of the glycerides

A 0.1–0.2-ml volume of lipase solution containing the required amount of lipase (2–4 mg) was applied as a band on the prepared plate. The plate was held in the air stream from an electric fan in order to remove some of the water from the band (until the shine had disappeared). The required amount of triglycerides (1–4 mg) in *n*-hexane was applied as evenly as possible over the enzyme band. The plate was immediately placed, coated side down, on top of a dish of water kept in an incubator at a specific temperature; the dish of water prevents over drying of the reaction zone (the band). After a stipulated time the reaction was stopped by exposing the plate to hydrogen chloride vapour for 1 min in a closed TLC chamber containing some concentrated hydrochloric acid in a basin. The acid fumes were removed from the plate by placing it under a fan for 5–10 min. The products of lipolysis and the unreacted triglycerides were removed from the reaction zone by developing the plate three times with diethyl ether up to 2 cm from the line of application in a saturated chamber. The ether was removed completely from the plate by a stream of air. The plate was then developed up to 14 cm with *n*-hexane–diethyl ether–acetic acid (80:20:1.5) in a saturated chamber to resolve the different reaction products and unreacted triglycerides.

After removal of the solvents from the chromatogram by a stream of air, the different bands were located with iodine vapour (Fig. 1) and marked. The bands were identified by comparison with chromatograms in which reference glycerides were spotted at one side of the plate 2 cm above the line of application of the enzyme solution. After complete evaporation of iodine from the chromatogram, the *sn*-2-monoglyceride, *sn*-1,2(2,3)-diglyceride and unreacted triglyceride bands were scraped off the plate into individual small glass columns fitted with sintered glass discs. The glycerides were extracted from the adsorbent with five 2-ml portions of diethyl ether. The solvents were removed by evaporation under nitrogen and the glycerides were re-dissolved in known volumes of *n*-hexane.

Determination of fatty acid composition of glycerides

The fatty acid compositions of the glyceride products, unreacted triglycerides and initial triglycerides were determined by GLC of the corresponding methyl esters, prepared by potassium hydroxide catalysed methanolysis according to Brockerhoff[9]. A dual-column F & M Model-700R analytical gas chromatograph with flame-ionization detectors was used, equipped with 6 ft. × $\frac{1}{8}$ in. stainless-steel columns packed with 10% EGSS-X coated on 100–120-mesh Gas-Chrom W (Applied Science Labs.) at 160°. The carrier gas (nitrogen) flow-rate was 40 ml/min. The peaks were identified by comparing their retention time with those of reference esters. The compositions were calculated from peak areas obtained by the triangulation method and corrected by multiplication with appropriate calibration factors.

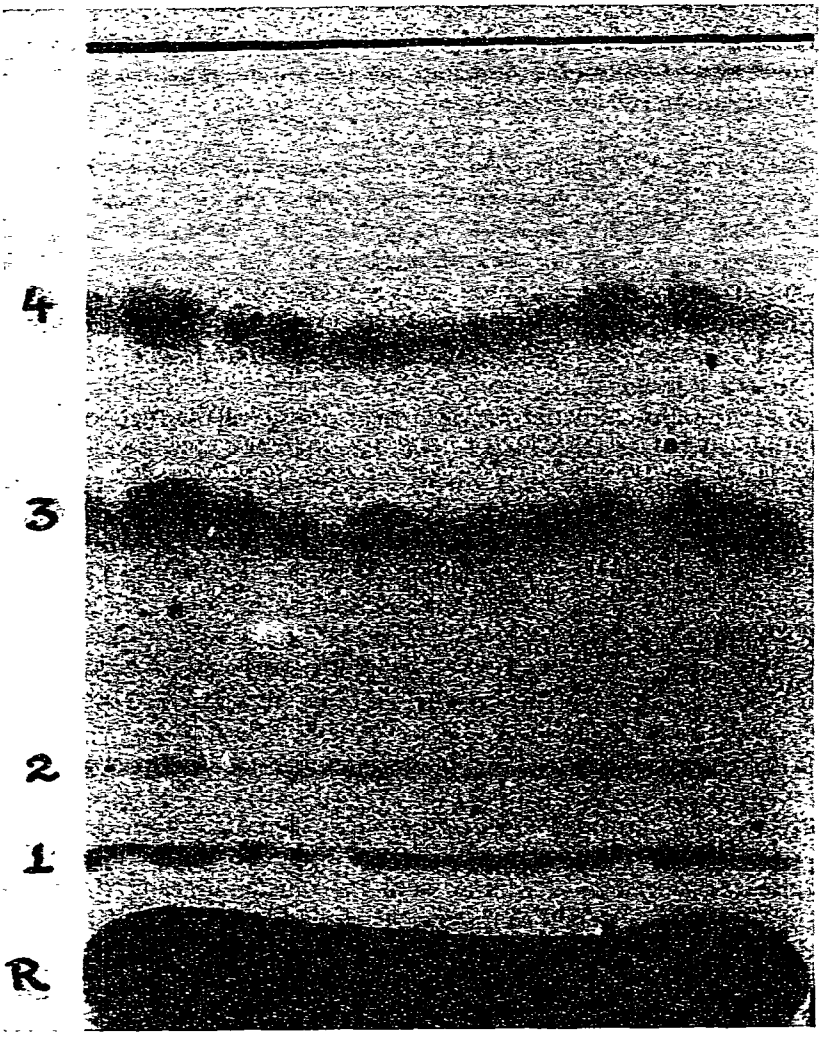

Fig. 1. Lipolysis of triolein and resolution of the reaction products on the same TLC plate. TLC plate: 14 × 20 cm, 0.5 mm thick silica gel layer. Reaction conditions: 4 mg of lipase applied as band over which 2 mg triolein was applied; reaction temperature, 40°; reaction time, 2 min. Removal of the reaction products from the reaction zone and their resolution were carried out as described in the text. Detection: spraying the chromatogram with a solution of ammonium sulphate in dilute sulphuric acid and charring. R = Reaction zone; 1 = *sn*-2-monoolein; 2 = *sn*-1,2(2,3)-diolein; 3 = oleic acid; 4 = unreacted triolein.

Assay of the glyceride products and unreacted triglycerides

The amount of glycerides in each band was assayed by GLC of the methyl esters from an aliquot of the extract with methyl pentadecanoate added as internal standard according to Blank *et al.*[10]. The amount of triglyceride hydrolysed was calculated from that which remained unreacted.

Isolation of triglycerides from natural fat samples

The triglycerides from groundnut oil, goat depot fat, body fat of shol fish (*Channa striatus*) and yeast (*Rhodotorula glutinis*) fat were isolated by preparative TLC on silicic acid plates according to Litchfield[11].

RESULTS

Quantitative removal of the sn-2-monoglyceride from the reaction zone by development in diethyl ether

The number of developments with diethyl ether required for the quantitative removal of reaction products from the reaction zone was established. On each of four TLC plates 0.2 ml of inactive lipase solution (inactivated by boiling for a few minutes) containing 4 mg of protein was applied as band over which 1 mg of *sn*-2-monopalmitin in 0.1 ml of *n*-hexane was applied. Monopalmitin was removed from the bands by developing the first, second, third and fourth plates with diethyl ether, up to 2 cm from the line of application, one, two, three and four times, respectively. The extent of removal was evaluated by extraction and determination of monoglyceride from the bands formed at 2 cm from the line of application.

The results presented in Fig. 2 show that three extractions were sufficient for the quantitative removal of *sn*-2-monoglyceride from the bands. Of all of the reaction products of the lipolysis of triglycerides, the 2-monoglycerides are the most strongly adsorbed owing to the presence of the two hydroxyl groups in the molecule; hence their complete removal from the application zone indicates the quantitative removal of other reaction products and unreacted triglycerides.

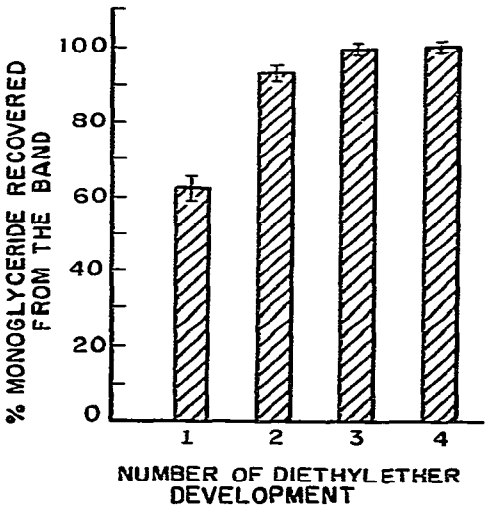

Fig. 2. Histogram showing the number of diethyl ether developments *versus* the extent (wt.-%) of *sn*-2-monopalmitin removed from the reaction zone. On each of four TLC plates 0.2 ml of inactive lipase solution (inactivated by boiling for a few minutes) containing 4 mg of protein was applied as a band over which 1 mg of *sn*-2-monopalmitin in 0.1 ml of *n*-hexane was added. Removal of monopalmitin from the bands was carried out by developing the first, second, third and fourth plates with diethyl ether up to 2 cm from the line of application one, two, three and four times, respectively. The extent of removal was evaluated by determining the content of the monopalmitin bands (average of four experiments).

Effects of bile salt and calcium chloride on on-plate lipolysis of triolein

The results of experiments performed to examine these effects are presented in Table I. The reactants and reagents were applied in sequence, as indicated in the table, on TLC plates coated with silica gel H (Merck). The reaction was carried out at 40° and was stopped after 1 min of substrate addition, the unreacted triolein then being measured. The hydrolysis of triolein to the extent of about 60% irrespective of the presence of bile salt or calcium chloride or both, shows that under the conditions used these reagents have little influence on the on-plate lipolysis of triolein.

TABLE I

EFFECT OF BILE SALT AND CALCIUM CHLORIDE ON ON-PLATE LIPOLYSIS OF TRIOLEIN

Plate No.	*Materials applied in sequence on plate*	*Triolein hydrolysed in 1 min at 40° (wt.-%)* *
1	3.2 mg lipase, 0.04 ml bile salt solution, 0.04 ml $CaCl_2$ solution and 1.0 mg triolein in *n*-hexane	59.0 ± 2.1
2	3.2 mg lipase, 0.04 ml $CaCl_2$ solution and 1.0 mg triolein	60.0 ± 2.0
3	3.2 mg lipase and 1.0 mg triolein	59.0 ± 2.0
4	3.2 mg inactive lipase and 1.0 mg triolein	0

* Average of 4 experiments.

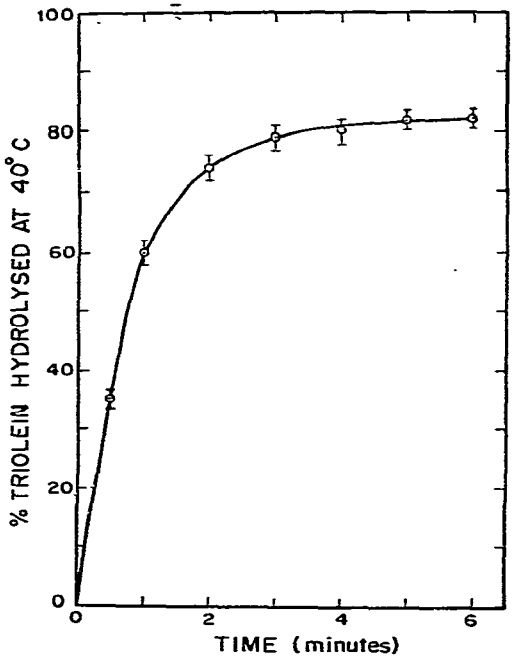

Fig. 3. Time course of on-plate lipolysis of triolein. On each of seven TLC plates 3 mg of lipase was applied as a band over which 1 mg of triolein in 0.1 ml of *n*-hexane was added as quickly as possible. The reaction at 40° was stopped by exposure to HCl vapour at 0.5, 1, 2, 3, 4, 5 and 6 min after the addition was complete. The removal of reaction products from the reaction zone and their resolution was carried out as described in the text. The amounts of triolein hydrolysed (average of four experiments) were evaluated from that which remained unreacted.

Time course of the reaction

Fig. 3 illustrates the time course of the on-plate lipolysis of triolein. It appears that the reaction was rapid up to the first minute, within which *ca.* 60% of triolein was hydrolysed, and then slowed to an equilibrium value of *ca.* 82% hydrolysis within the fifth minute.

Fig. 4 shows chromatograms obtained from lipolyses on a single plate carried out in four different reaction zones containing fixed amounts of lipase and triolein and in which the reaction was allowed to proceed for 4, 3, 2 or 1 min. The

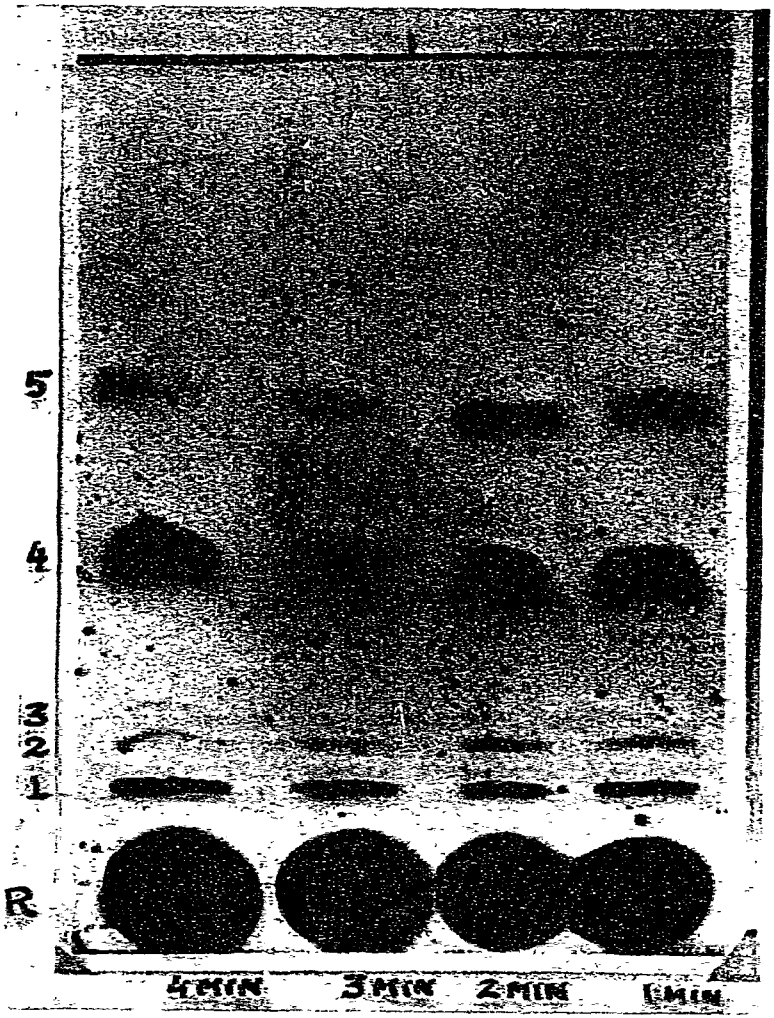

Fig. 4. Photograph of chromatograms due to lipolysis carried out at four different reaction zones on a single TLC plate. Fixed amounts of lipase and triolein were applied in each zone but the reactions were allowed to proceed for 4, 3, 2 and 1 min, for the first, second, third and fourth zones, respectively, from the left. R = Reaction zone; 1 = *sn*-2-monoolein; 2 = *sn*-1,2(2,3)-diolein; 3 = *sn*-1,3-diolein; 4 = oleic acid; 5 = unreacted triolein. Detection: spraying the chromatogram with solution of ammonium sulphate in dilute sulphuric acid and charring.

substrate was added to the successive bands at intervals of 1 min and the reaction was stopped 1 min after the addition to the last band, the time taken for addition of the substrate being kept as short as possible. It can be seen that no detectable acyl migration had occurred within 3 min in *sn*-1,2-(2,3)-diglyceride. The band of *sn*-1,3-diglyceride, the acyl migration product from *sn*-1,2(2,3)-diglyceride, appeared in the chromatogram when the reaction was allowed to proceed for 4 min.

Effect of incubation temperature on the extent of lipolysis

The extents of lipolysis within 1 min using 3 mg of lipase and 1 mg of triolein at different incubation temperatures between 20° and 55° were measured. The results (Fig. 5) show that from 20° to 40° the extent of hydrolysis of triolein increased from 34 ± 1.5 to 60 ± 1.5%. On a further increase in temperature to 55° the extent of hydrolysis decreased steadily.

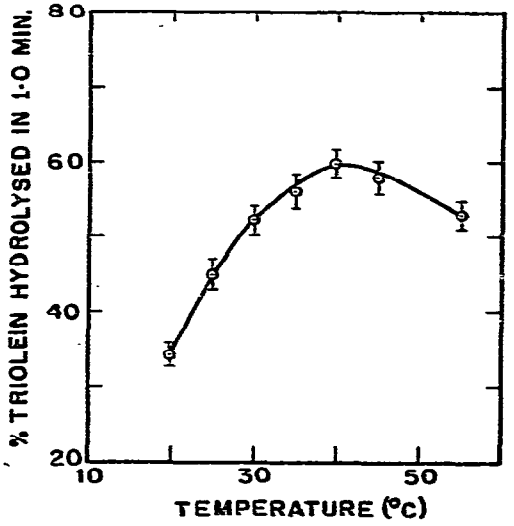

Fig. 5. Relationship between reaction temperature and extent of on-plate lipolysis of triolein: 3 mg of lipase and 1 mg of triolein were applied on each of seven TLC plates. The reaction was carried out for 1 min at 20°, 25°, 30°, 35°, 40°, 45° and 55° from the first to the seventh plate. The amounts of triolein hydrolysed (average of 4 experiments) were calculated from those which remained unreacted.

Enzyme : substrate ratio and extent of lipolysis

Table II presents the results of experiments to find the maximum amount of substrate that can profitably be used per milligram of the enzyme sample (activity = 111 units). On each of six TLC plates 4 mg of lipase were applied, together with 1, 2, 3, 4, 5 or 6 mg of triolein. The reaction was allowed to proceed for 1 min at 40° and the amounts of triolein hydrolysed were measured. The results show that with enzyme:substrate ratios from 4:1 to 1:1 the proportion of triolein hydrolysed was between *ca.* 60 and 65%. With higher substrate concentrations the extent of conversion decreased. This result suggests that the enzyme was saturated when 1 mg of triolein was used per milligram of lipase. Obviously, with enzyme preparations of

TABLE II

EFFECT OF ENZYME:SUBSTRATE RATIO ON THE EXTENT OF ON-PLATE LIPOLYSIS OF TRIOLEIN

Plate No.	*Enzyme:substrate ratio*	*Triolein hydrolysed in 1 min at 40° (wt.-%)* *
1	4:1	59.0 ± 1.5
2	2:1	60.0 ± 2.0
3	4:3	64.0 ± 2.1
4	1:1	65.0 ± 2.0
5	4:5	57.0 ± 1.3
6	2:3	47.0 ± 2.5

* Average of 4 experiments.

higher activity, greater amounts of substrate can be used, provided that the reaction products do not overload the TLC plate. It has also been found (Table III) that using enzyme:substrate ratios such as 4:1, 2:1 and 1:1 the average amounts of *sn*-2-monoglyceride, *sn*-1,2(2,3)-diglyceride and free fatty acids obtained from triolein within 2 min were 25, 12.5 and 38%, respectively.

TABLE III

AMOUNTS OF REACTION PRODUCTS OF ON-PLATE LIPOLYSIS OF TRIOLEIN AT DIFFERENT ENZYME:SUBSTRATE RATIOS

Enzyme:substrate ratio	*Reaction products in 2 min at 40° (wt.-% of triolein applied)* *			
	sn-2-Monoglyceride	*sn-1,2(2,3)-Diglyceride*	*Free fatty acid*	*Total*
4:1	24.0 ± 1.4	12.0 ± 0.7	38.0 ± 1.4	74.0 ± 2.1
2:1	25.0 ± 1.2	13.0 ± 1.2	37.0 ± 1.6	75.0 ± 3.1
1:1	26.0 ± 1.0	13.0 ± 1.6	37.0 ± 1.3	76.0 ± 3.8

* Average of 4 experiments.

Application of the method to triglycerides of some natural fats

Table IV gives the fatty acid compositions (mole-%) of *sn*-2-monoglycerides and *sn*-1,2(2,3)-diglycerides obtained from triglycerides of groundnut oil, goat depot fat, body fat of shol fish and yeast fat by the method described. Table IV also gives the fatty acid compositions of the initial triglycerides and the compositions of *sn*-1,3- and *sn*-1,2(2,3)-diglycerides calculated according to Coleman and Fulton[12]. The most important feature is that, in all instances, the calculated compositions of *sn*-1,2(2,3)-diglycerides agree, within the limits of experimental errors, with those obtained directly.

DISCUSSION

In the lipase hydrolysis of glycerides, the substrate (a hydrophobic substance) and the aqueous enzyme solution form immiscible phases, and intimate contact of these two is of prime importance for the satisfactory progress of the reaction. In the existing methods this is achieved by the addition of emulsifiers[13,14] (bile salt, gum

TABLE IV

FATTY ACID COMPOSITION OF TRIGLYCERIDES FROM SOME NATURAL FATS AND THE GLYCERIDE PRODUCTS OBTAINED FROM THE TRIGLYCERIDES BY ON-PLATE LIPOLYSIS

Source of triglycerides	*Glycerides*	*Fatty acids (mole-%)***												
		12:0	*14:0*	*14:1*	*16:0*	*16:1*	*17:0*	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*	*20:4*	*22:6*
Groundnut (*Arachis hypogaea*) oil	Triglycerides (initial)*	–	tr	–	12.5	tr	–	2.4	56.3	28.1	0.3	0.4	–	–
	sn-2-Monoglycerides	–	tr	–	2.0	tr	–	0.2	54.6	43.0	0.1	0.1	–	–
	sn-1,3-Diglycerides (calculated)	–	tr	–	17.7	–	–	3.5	57.1	20.7	0.4	0.6	–	–
	sn-1,2(2,3)-Di-glycerides Found	–	0.1	–	10.7	tr	–	2.1	54.2	32.2	0.4	0.3	–	–
	sn-1,2(2,3)-Di-glycerides Calculated	–	–	–	9.9	–	–	1.9	55.8	31.8	0.3	0.3	–	–
Goat (*Capra domestica*) depot fat	Triglycerides (initial)	–	2.5	0.2	24.0	–	0.6	45.9	24.0	2.1	0.6	–	–	–
	sn-2-Monoglycerides	–	3.2	–	18.7	–	–	32.5	39.0	4.8	1.8	–	–	–
	sn-1,3-Diglycerides (calculated)	–	2.2	0.3	26.7	–	0.9	52.7	16.5	0.7	–	–	–	–
	sn-1,2(2,3)-Di-glycerides Found	–	2.6	–	21.3	–	0.6	41.9	29.1	3.0	1.5	–	–	–
	sn-1,2(2,3)-Di-glycerides Calculated	–	2.6	0.1	22.7	–	0.5	42.5	27.8	2.8	1.0	–	–	–
Shol fish (*Channa striatus*) body fat	Triglycerides (initial)	1.2	3.2	–	29.2	8.0	–	10.6	36.6	7.5	1.5	–	2.1	0.1
	sn-2-Monoglycerides	0.9	4.4	0.9	38.5	9.8	–	8.6	23.5	10.5	1.7	–	1.2	–
	sn-1,3-Diglycerides (calculated)	1.3	2.6	–	24.4	7.1	–	11.6	43.1	6.0	1.4	–	2.4	0.1
	sn-1,2(2,3)-Di-glycerides Found	0.8	3.4	tr	32.0	7.1	–	11.8	35.1	7.3	1.0	–	1.3	0.2
	sn-1,2(2,3)-Di-glycerides Calculated	1.1	3.5	0.2	31.5	8.5	–	10.1	33.3	8.2	1.6	–	1.8	0.2
Yeast (*Rhodotorula glutinis*) fat	Triglycerides (initial)	–	1.7	–	29.0	–	–	14.2	46.5	7.1	1.5	–	–	–
	sn-2-Monoglycerides	–	1.2	–	7.3	–	–	7.9	69.1	12.6	1.9	–	–	–
	sn-1,3-Diglycerides (calculated)	–	1.9	–	39.8	–	–	17.3	35.3	4.4	1.3	–	–	–
	sn-1,2(2,3)-Di-glycerides Found	–	1.8	–	24.7	–	–	13.1	50.3	8.6	1.5	–	–	–
	sn-1,2(2,3)-Di-glycerides Calculated	–	1.6	–	23.6	–	–	12.6	52.1	8.5	1.6	–	–	–

* The fatty acid compositions of the initial and unreacted triglycerides were found to be identical within the limits of experimental error.

** tr, less than 0.1%.

arabic, etc.) and vigorous shaking of the reaction mixture[6] at pH 8. The addition of calcium chloride, which has been shown to increase the adsorption of the enzyme on the oil–water interface[15], is also necessary. The reaction products are generally isolated by extraction with diethyl ether and resolved by preparative TLC[6]. The glyceride products are isolated from the adsorbent and their fatty acid compositions evaluated by GLC in order to elucidate the fatty acid distribution in the triglyceride molecules.

In the method described here, all of the above steps, from the enzymatic reaction to the separation of the reaction products, are carried out on a single TLC plate. The entire operation does not take more than 1 h and does not require the addition of bile salts or calcium chloride. The function of a solubilizer, *i.e.*, the maximization of the interfacial surface area, is to a large extent fulfilled by the extensive surface area of the TLC adsorbent. This lack of need for a bile salt has an advantage, as the absence of this emulsifier has been shown by Mattson and Volpenhein[16,17] to inactivate a non-specific lipase associated with mammalian pancreatin, and this was confirmed in the case of hog pancreatin by Swell *et al.*[18]. This lipase can produce atypical 1,3-diglycerides and 1(3)-monoglycerides. The effect of calcium ion is probably taken care of by the high enzyme : substrate ratios (1:1 to 4:1) used, because we have found in separate experiments that the rate of lipolysis at these high ratios remains almost constant whether the plates are coated with adsorbent containing calcium sulphate or not, but when a much lower ratio, such as 1:5, was used, calciumion definitely enhanced the rate of on-plate lipolysis.

The most effective temperature for on-plate lipolysis is about 40°, at which *ca.* 60% of the triglycerides were hydrolysed within 1 min. The decrease in the extent of hydrolysis at temperatures above 50° was due to the decrease in activity of the enzyme[19].

The time course of the reaction at 40° shows that *ca.* 35–80% hydrolysis occurs between 0.5 and 3 min. Prolonging the reaction not only fails to increase the hydrolysis, probably owing to the attainment of equilibrium, but also leads to the formation of *sn*-1,3-diglycerides from *sn*-1,2(2,3)-diglycerides through acyl migration.

It is not always possible to obtain *sn*-1,2(2,3)-diglycerides representative of the original triglycerides by lipase hydrolysis[20]. Anderson *et al.*[21] have shown that the production of this representative diglyceride depends both on the sample composition and the reaction conditions. However, the exact conditions that lead to random deacylation of the *sn*-1- and *sn*-3-positions are as yet undefined. Therefore, in order to determine the glyceride composition from calculations[12,22] that assume random deacylation from *sn*-1- and *sn*-3-positions, it is imperative to check whether representative *sn*-1,2(2,3)-diglycerides have been produced. This can be done[23] by comparing the fatty acid composition of the *sn*-1,2(2,3)-diglyceride obtained with that calculated from the *sn*-2-monoglycerides and initial triglyceride compositions. The results should agree within experimental error. The representative *sn*-1,2(2,3)-diglyceride can be used for stereospecific analysis according to Brockerhoff[23]. Our results for the fatty acid compositions of *sn*-1,2(2,3)-diglycerides obtained from triglycerides of groundnut oil, goat depot fat, body fat of shol fish and yeast fat (Table IV) agree with the calculated compositions, within experimental error. This agreement indicates that the method described is adequate for the calculation of triglyceride compositions and the *sn*-1,2(2,3)-diglycerides produced can be used for stereospecific analysis.

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