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Quick regiospecific analysis of fatty acids in triacylglycerols with GC using 1,3-specific lipase in butanol

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A new micro-procedure for the quick regiospecific analysis of triacylglycerols (TAG) with a 1,3-specific lipase, Lipozyme IM 20 (produced from strains of *Muccor miehei*) is described. After dissolution of triacylglycerols in butan-1-ol or butan-2-ol 250 mg of Lipozyme IM 20 were added and the whole mixture was agitated. This operation was repeated 5–10 times and the esterified fatty acids in the form of butyl esters (FABE) were dissolved in pentane and washed with water. The pentane layer was dried over sodium sulfate and the solvent was removed under vacuum to a constant weight. The residue was analysed using GC-MS. The same triacylglycerols were converted to fatty acid methyl esters (FAME). The FAME were analysed by gas chromatography (GC).

Introduction

The topic of stereospecific analyses of triacylglycerols (TAG) has received much attention in recent years. One general approach is the partial degradation of TAG with Grignard reagents to produce diacylglycerols (DG) and monoacylglycerols (MG) that are then derivatised with a chiral isocyanate to produce diastereomeric carbamates (separable by chromatographic methods).[1–3] Because acyl migration is quite facile during cleavage caused by the organometallic reagent, the conditions for this procedure are critical. Moreover, reaction readily occurs at the 2-position of the TAG, further complicating the positional analyses of the TAG in question. The Grignard method has been modified and adapted for the regiospecific analyses of specific TAG and fats and oils in general.[4,5] Recently, a procedure for determining lipase regioselectivity in reactions with homogeneous TAG was reported[6] that also enables the determination of triacylglycerol structures. A second method based on lipase selectivity requires the partial hydrolysis of TAG with highly 1,3-specific lipases, followed by analyses of the partial acylglycerols formed.[7] Examples of lipases that are reported as being highly 1,3-specific include those from the genus *Rhizopus*.[8,9] Other lipases also generally referred to as 1,3-specific are those from *Mucor miehei* and *Pseudamonas fluorescens*, but recent evidence indicates that the latter lipases should be regarded as being regioselective and not regiospecific.[9]

In this study, we report an evaluation of the 1,3-specific lipase Lipozyme IM 20 approach to the regiospecific analysis of oil triacylglycerols. The micro-procedure consists of dissolution of the oils in butan-1-ol or butan-2-ol and addition of Lipozyme and agitation of the whole mixture. The oil was allowed to react in butanol followed by washing with pentane (controlled by TLC), isolation of butyl esters and analysis by gas chromatography (GC). Confirmation of the structures of butyl esters was made using GC-MS. The method is quick, and with an experienced operator it is possible to analyse many samples per day. The results of this micro-method have been examined for their accuracy by using classical methods.

Experimental

Reference compounds and solvents

1,3-Specific lipase from *Muccor miehei* (Lipozyme IM 20, Batch: LM7 0753 and Lipozyme RM IM, Batch: Q LUX 00112) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Both batches revealed the same activity against triacylglycerol when they were used in an organic medium.

Tristearine was purchased from Sigma (St. Louis, MO, USA). All solvents (analytical grade) were purchased from Merck Ltd. (Darmstadt, Germany).

TLC was conducted on 0.25 mm Silica gel 60 plates F254 (5 × 20 cm) purchased from Merck.

Vegetable oils (corn, soybean, sunflower, cottonseed, almond and olive oil) were purchased from a local market in Greece.

TLC plates were developed with hexane–ether–acetic acid (80 + 20 + 2), dried and revealed by phosphomolybdate solution or exposure to I_2 vapour.

Instrumentation

GC-MS analysis was performed using a Hewlett-Packard 6890 gas chromatograph connected to a Hewlett-Packard 5973 series mass selective detector. The fused-silica capillary column HP1 (25 m × 0.2 mm id) was coated with cross-linked, 100% methyl siloxane (0.33 μm film) (Hewlett-Packard UK). Samples (1 μL) were injected using a split mode. The injector temperature was 200 °C. The helium carrier gas was set at a pressure of 119 kPa at 50 °C (flow rate: 1 mL min^{-1}). The temperature program was 120 °C for 2 min, followed by a ramp rate of 1 °C min^{-1} to 250 °C, and a hold at 250 °C for 10 min. Data acquisition and analysis were performed using the HP Chemstation G170BA version B.01.00 data system (Hewlett-Packard). Mass spectra were recorded in the electron-impact (EI) mode, with an ionization voltage of 70 eV and an ionization current of 50 μA. A source temperature of 175 °C and a scan range of 33–400 *m*/*z*,

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with a scan time of 0.69 s, were used. Mass spectra were interpreted by spectral matching, using either the mass spectrometer data system library (NBS/NIH) or other collections of reference spectra. Linear retention indices (LRI values) of compounds were obtained by reference to a series of standard *n*-alkanes, run under the same conditions. Compound identifications were confirmed, when possible, by comparing experimental LRI values with those of authentic compounds.

The results displayed in Tables 1 and 2 were determined using GC analysis carried out with a Varian 3800 gas chromatograph (Varian, California, USA) equipped with a Supelco (Supelco, Bellefonte, PA, USA) capillary SPB-TM1 column (30 m × 0.32 mm id; 1.0 μm). Helium flow rate was set at 15 psi.

For the determination of methyl esters the following program for the column temperature was used: 50 °C to 250 °C (held for 50 min) at 4 °C min^{-1}. For the injector temperature the program used was: 40 °C (held for 1 min) to 200 °C (held for 100 min) at 180 °C min^{-1}. The detector was maintained at 300 °C.

Butyl and iso-butyl esters were determined using the following program for the column temperature: 50 °C (held for 1 min) to 250 °C (held for 60 mn) at 10 °C min^{-1}. For the injector temperature the program was: 40 °C (held for 1 min) to 200 °C (held for 100 min) at 180 °C min^{-1}.

Methyl esters formation

Fatty acid methyl esters (FAME) were prepared by reaction of the oil (20 mg) with 0.5 M sodium methoxide in methanol (2 mL) at 80 °C for 1 h, diluting with saturated NaCl solution (2 mL), addition of hexane (1 mL) and agitation. The upper hexane layer was separated and dried over anhydrous Na_2SO_4.

Butyl and iso-butyl esters formation

Samples were treated as follows: a mixture containing 200 μL of sample (oil) and 200–600 μL of butan-1-ol or butan-2-ol were placed into a test tube. Lipozyme IM (250 mg) was added and the whole mixture was agitated. The oil was allowed to react with the above alcohols for 2 min and the mixture was agitated gently using a vortex mixer. This procedure was repeated 5 to 10 times. The sample in the test tube was rinsed with 20 mL of pentane. In the rinsing liquid 5 mL of a saturated solution of NaCl were added. After gentle agitation and separation of the two phases, the water phase was removed. The organic phase was extracted twice with a saturated solution of NaCl and then with water. The organic phase was dried over Na_2SO_4. The reaction was monitored by TLC until the oil spot with R_f 0.5 was minimised. The butyl or iso-butyl esters spot R_f 0.8 was followed by two other spots with R_f 0.2 and 0.1 due to mono- and diglycerides. During washings with water the two last spots practically disappeared. The solvent was removed in a rotary evaporator. Finally, the products were subjected to gas chromatography analysis.

Results and discussion

For natural products, the fatty acid composition of oils can vary depending on the geographic origin, the local climatic conditions under which the plant is grown, the stage of development, the particular variety of the plant, and the processing conditions to which it has been subjected, such as extent of pressing, fractionating, and refining.[10] Genetic variability of the plant material leads to a different fatty acid at *sn*-1,3 distribution in triacylglycerols, and due to that to a great difference among their physical or chemical properties, such as the behaviour against oxidation or the melting point.

As it was mentioned previously, a very quick and simple method for the determination of the fatty acids in the 1,3 position was more than necessary. Previous methods were in some cases time consuming with multiple steps that induce possible sources of errors. The analysis using the method described in this manuscript can be finished within a few minutes without any requirement of specific equipment except classical gas chromatography.

The new method described is based on two facts. Lipozyme IM 20 is an immobilised enzyme from *Muccor miehei* exhibiting high selectivity for hydrolysis of acyl moieties located at the *sn*-1,3 position in triacylglycerols of fats and oils in aqueous solutions. The second is that the same enzyme remains active in organic media carrying out transesterifications with a total selectivity. It has also been shown that the same enzyme does not catalyse transesterification reactions between secondary or tertiary alcohols and other esters.[11] Based on that a new method has been developed for the analysis of fatty acids at *sn*-1,3 positions in triacylglycerols. The reaction medium is the mixture of alcohol and oil and the catalyst is the immobilised enzyme. To achieve high rates of reaction an overdose of the enzyme has been used and the reaction products are fatty acid butyl esters and monoacylglycerides at the *sn*-2 position (see Fig. 1). The latter is removed by extraction and the final composition of butyl esters is obtained by GC analysis.

The regioselectivity of the *Muccor miehei* lipase in aqueous solution has been shown from previous works.[12] The above enzyme also exhibits a high activity in transesterification reactions using organic esters both as solvents and acyl donors (ethyl acetate, propyl acetate, butyl acetate, butyl butyrate, *etc*.) and primary alcohols as nucleophiles.[11] In this type of enzyme catalysed reaction the acyl donor is the acid moiety of the triacylglycerol, and it has been proved that only primary alcohols react while secondary alcohols do not. In our case there is a specific reaction between primary alcohol esters of fatty acids at *sn*-1,3 triacylglycerols and primary alcohols such as butan-1-ol or butan-2-ol. Other alcohols (such as ethanol or propanol) have been tested, but the results were less applicable because of the low reaction yield. With butan-1-ol or butan-2-ol the results have been proved to be quantitative. The highest activities were also recorded when butanol was used as nucleophile in esterification reactions between oleic acid and alcohols in organic solvents using an immobilised lipase from *Candida rugosa*.[13] In the same study the optimal temperature for esterification was 40 °C. Reports by Hills *et al.*[14] (1990) showed that butanol and pentanol were esterified most efficiently, and alcohols with longer chains were esterified more slowly by lipase from oilseed rape.

Migration of the fatty acid residues does not appear to be a problem with this technique. Migration of the fatty acid residue from position 2 to position 3 is mainly affected by the temperature of the reaction, the polarity of the solvent and the type of fatty acid residue.

From the beginning of this work care was taken to ensure that by the end of the reaction only minor amounts of unreacted diglycerides were formed from the above transesterification. The end of the reaction was checked by TLC.

Butyl esters of fatty acids at positions 1,3 of triacylglycerols were obtained from different oils using the above described method. All the results are given in the Tables 1 and 2.

To validate the aforementioned analytical procedure, analysis of 1 mmol of tristearin with butanol yielded 0.67 mmol of butyl stearate.

The total mole percentage of the various fatty acid residues present in triacylglycerols was determined by their transformation to methyl esters, using sodium methoxide in anhydrous methanol under a nitrogen atmosphere. Quantification of the obtained methyl esters was obtained through gas chromatography analysis. Fatty acid methyl esters preparation was necessary in order to identify the type of the oil because fatty

acid distribution is strongly related (as mentioned before) to genetic variability, cultivation type, climatic conditions, and sometimes the procedure of extraction.

The technique described previously was used to carry out regiospecific analysis of triacylglycerols of 13 edible oils, namely, corn (2), soybean (2), sunflower (2), cottonseed (1),

Table 1 Comparative fatty acid compositions (mol %) and positional distribution of fatty acid and residues of triacylglycerols using butan-1-ol[a]

Mol %	C16:0	C18:0	C20:0	C16:1	C18:3	C18:2	C18:1	C20:1	Total
Corn oil (A)—									
Fatty acids 1,2,3	11.54	1.98	0.00	0.00	0.00[b]	56.29	30.18	0.00	100.00
	(0.99)	(0.05)	(0)	(0)	(0)	(3.28)	(1.61)	(0)	
Fatty acids 1,3	18.43	2.88	0.47	0.14	0.00[b]	49.99	27.84	0.28	100.00
	(0.35)	(0.30)	(0.11)	(0.01)	(0)	(0.62)	(0.04)	(0.11)	
Corn oil (B)—									
Fatty acid 1,2,3	10.91	2.00	0.00	0.00	0.00[b]	54.72	32.27	0.00	99.92
	(0.31)	(0.03)	(0)	(0)	(0)	(4.01)	(0.10)	(0)	
Fatty acids 1,3	16.80	2.42	0.45	0.13	0.00[b]	51.18	28.80	0.24	100.00
	(0.30)	(0.05)	(0.08)	(0.01)	(0)	(0.27)	(0.08)	(0.06)	
Literature	17.4, 14.5	3.3, 2.7	0.6	0	0.7, 0.4	52.4, 54.5	25.6, 27.7	—	
Soybean oil (A)—									
Fatty acids 1,2,3	11.69	3.96	0.00	0.00	0.00[b]	60.45	23.89	0.00	100.00
	(0.90)	(0.33)	(0)	(0)	(0)	(1.25)	(0.11)	(0)	
Fatty acids 1,3	18.44	6.10	0.43	0.12	0.00[b]	47.23	27.53	0.16	100.01
	(0.43)	(0.29)	(0.05)	(0.02)	(0)	(1.30)	(2.02)	(0.01)	
Soybean oil (B)—									
Fatty acids 1,2,3	12.66	4.03	0.00	0.00	0.00[b]	60.01	23.31	0.00	100.00
	(0.86)	(0.21)	(0)	(0)	(0)	(2.36)	(1.36)	(0)	
Fatty acids 1,3	16.42	6.61	0.45	0.12	0.00[b]	47.67	28.53	0.22	100.01
	(1.29)	(0.27)	(0.02)	(0.01)	(0)	(0.18)	(0.78)	(0.06)	
Literature	17.7, 16.5	6.8, 6.9	0.4	0.2	6.5, 4.3	44.8, 42.5	23.8,29.5	—	
Sunflower oil (A)—									
Fatty acids 1,2,3	6.35	3.31	0.00	0.00	0.00[b]	58.98	31.35	0.00	100.00
	(0.23)	(0.11)	(0)	(0)	(0)	(2.31)	(1.16)	(0)	
Fatty acids 1,3	10.85	4.92	0.41	0.30	0.00[b]	52.88	30.53	0.13	100.00
	(2.64)	(0.30)	(0.03)	(0.04)	(0)	(4.20)	(0.69)	(0.05)	
Sunflower oil (B)–									
Fatty acid 1,2,3	7.35	4.01	0.00	0.00	0.00[b]	56.72	34.66	0.00	100.00
	(0.41)	(0.09)	(0)	(0)	(0)	(2.06)	(1.35)	(0)	
Fatty acids 1,3	10.03	5.14	0.41	0.16	0.00[b]	55.78	28.39	0.16	99.98
	(0.42)	(0.13)	(0.30)	(0.10)	(0)	(0.12)	(0.06)	(0.03)	
Cottonseed oil—									
Fatty acids 1,2,3	23.41	2.12	0.00	0.00	0.00[b]	56.78	17.69	0.00	100.00
	(1.19)	(0.03)	(0)	(0)	(0)	(2.01)	(0.06)	(0)	
Fatty acids 1,3	36.18	3.09	0.28	0.65	0.00[b]	45.02	14.69	0.11	100.01
	(4.78)	(0.12)	(0.05)	(0.05)	(0)	(4.53)	(0.30)	(0.06)	
Almond oil—									
Fatty acids 1,2,3	6.93	1.31	0.00	0.00	0.00[b]	26.08	65.68	0.00	100.00
	(0.10)	(0.02)	(0)	(0)	(0)	(1.33) (2.21)	(0)		
Fatty acids 1,3	12.75	2.10	0.08	0.62	0.00[b]	18.80	65.58	0.08	100.00
	(0.52)	(0.22)	(0.02)	(0.01)	(0)	(1.13)	(0.84)	(0.01)	
Olive oil (A)—									
Fatty acids 1,2,3	9.58	2.63	0.00	0.00	0.00[b]	9.07	78.71	0.00	100.00
	(0.12)	(0.02)	(0)	(0)	(0)	(0.52)	(2.21)	(0)	
Fatty acids 1,3	18.46	3.40	0.46	1.10	0.00[b]	7.22	69.09	0.30	100.00
	(1.17)	(0.13)	(0.04)	(0.04)	(0)	(0.35)	(0.81)	(0.04)	
Literature	16.1, 13.0	5.1, 4.1	0.6	0.8, 0.6	0.4	4.2, 4.8	73.2, 74.0	—	
Olive oil (B)—									
Fatty acids 1,2,3	12.26	2.87	0.00	0.00	0.00[b]	8.64	76.23	0.00	100.00
	(2.93)	(0.03)	(0)	(0)	(0)	(0.27)	(2.18)	(0)	
Fatty acids 1,3	18.61	3.87	0.43	0.99	0.00[b]	6.64	69.28	0.25	100.00
	(3.74)	(0.43)	(0.08)	(0.19)	(0)	(0.56)	(3.66)	(0.07)	
Olive oil (C)—									
Fatty acids 1,2,3	11.90	2.75	0.00	0.00[b]	12.20	73.16	0.00	100.00	
	(1.63)	(0.36)	(0)	(0)	(2.73)	(3.61)	(0)		
Fatty acids 1,3	17.75	3.99	0.54	1.01	0.00[b]	8.81	67.56	0.36	100.00
	(1.07)	(0.46)	(0.06)	(0.11)	(0)	(0.62)	(1.13)	(0.06)	
Olive oil (D)—									
Fatty acids 1,2,3	11.26	2.59	0.00	0.00	0.00[b]	11.72	74.43	0.00	100.00
	(1.54)	(0.46)	(0)	(0)	(0)	(0.90)	(3.11)	(0)	
Fatty acids 1,3	19.33	3.29	0.43	1.19	0.00[b]	9.31	66.47	0.30	100.31
	(0.12)	(0.11)	(0.00)	(0.02)	(0)	(0.46)	(1.21)	(0.04)	
Olive oil (E)—									
Fatty acids 1,2,3	11.91	2.72	0.00	0.00	0.00[b]	9.18	76.19	0.00	100.00
	(1.94)	(0.12)	(0)	(0)	(0)	(1.12)	(1.85)	(0)	
Fatty acids 1,3	18.04	3.51	0.46	0.96	0.00[b]	7.17	69.57	0.30	100.00
	(1.55)	(0.11)	(0.02)	(0.10)	(0)	(0.12)	(1.36)	(0.02)	

[a] Values are means of triplicate determinations. Standard deviation is given in parentheses. [b] It was not possible to distinguish the percentage of C18:3 from C18:2 with GC.

almond (1) and olive oils (5) (see Tables 1 and 2). The experimental results obtained with our procedures were compared with the results obtained by Arcos *et al*., Williams *et al*. and Brockerhoff.[10,15,16]

Inspection of the entries in Tables 1 and 2 indicates a very good agreement between our results and those reported by the above authors.

In Fig. 2 a typical chromatogram of GC-MS analysis of isobutyl esters of a mixture oil made with olive and soybean oil (95 + 5, w/w) is presented. Minor fatty acid iso-butyl esters are detected confirming that the enzymatic transesterification is well performed independently of the quantity of the fatty acid in the triacylglycerol.

The methods that have been used previously (see Introduction) are different than the one described in this work. First, Brockerhoff[16] reported a stereospecific analysis using a pancreatic lipase or Grignard reagent to effect partial diacylation of triacylglycerol to *sn*-1, *sn*-2 and *sn*-3 diacylglycerols. Then, the diacylglycerols were purified *via* TLC and converted to phosphatides. The fatty acid residues located at the *sn*-2 position were liberated and quantified using a phospholipase. The method has been subject to several criticisms due to multiple steps that could induce experimental errors. Christie *et al.*[1] used ethyl magnesium bromide to make a partial hydrolysis of triacyl-*sn*-glycerols. After this partial hydrolysis under controlled conditions, to avoid acyl migrations, 1,3-, 1,2- and 2,3-*sn*-diacylglycerols have been isolated by thin layer chromatography on boric acid impregnated plates and then converted to stable derivatives of (*S*)-(+)-1-(1-naphthyl)ethyl urethane which have been separated by HPLC and analysed with GC for determination of their fatty acids. The results of common oils presented in this method are in agreement with those of our work. Certainly, with the method of Christie *et al.*[1] it is possible to separately determine the identity of fatty acids present in position *sn*-1 and *sn*-3 which is not possible with our method. However, the overall technique is much more time consuming

Table 2 Comparative fatty acid compositions (mol %) and positional distribution of fatty acid and residues of triacylglycerols using butan-2-ol[a]

Mol %	C16:0	C18:0	C20:0	C16:1	C18:3	C18:2	C18:1	C20:1	Total
Corn oil (A)—									
Fatty acids 1,2,3	11.54	1.98	0.00	0.00	0.00[b]	56.29	30.18	0.00	100.00
	(0.99)	(0.05)	(0)	(0)	(0)	(3.28)	(1.61)	(0)	
Fatty acids 1,3	17.08	2.81	0.44	0.12	0.00[b]	51.23	28.14	0.19	100.01
	(1.64)	(0.02)	(0.10)	(0.02)	(0)	(1.05)	(0.75)	(0.02)	
Soybean oil (A)—									
Fatty acids 1,2,3	11.69	3.96	0.00	0.00	0.00[b]	60.45	23.89	0.00	100.00
	(0.90)	(0.33)	(0)	(0)	(0)	(1.25)	(0.11)	(0)	
Fatty acids 1,3	17.61)	6.13	0.43	0.13	0.00[b]	48.43	27.01	0.25	99.97
	(0.52	(0.08)	(0.01)	(0.05)	(0)	(0.25)	(0.42)	(0.02)	
Sunflower oil (A)—									
Fatty acids 1,2,3	6.35	3.31	0.00	0.00	0.00[b]	58.98	31.35	0.00	100.00
	(0.23)	(0.11)	(0)	(0)	(0)	(2.31)	(1.16)	(0)	
Fatty acids 1,3	9.63	5.07	0.29	0.39	0.00[b]	53.81	30.67	0.15	99.99
	(0.70)	(0.04)	(0.01)	(0.06)	(0)	(1.39)	(0.18)	(0.01)	
Cottonseed oil—									
Fatty acids 1,2,3	23.41	2.12	0.00	0.00	0.00[b]	56.78	17.69	0.00	100.00
	(1.19)	(0.03)	(0)	(0)	(0)	(2.01)	(0.06)	(0)	
Fatty acids 1,3	35.15	3.07	0.27	0.65	0.00[b]	45.40	15.36	0.12	100.00
	(1.33)	(0.08)	(0.05)	(0.02)	(0)	(1.46)	(0.01)	(0.03)	
Almond oil—									
Fatty acids 1,2,3	6.93	1.31	0.00	0.00	0.00[b]	26.08	65.68	0.00	100.00
	(0.10)	(0.02)	(0)	(0)	(0)	(1.33)	(2.21)	(0)	
Fatty acids 1,3	10.82	1.91	0.09	0.72	0.00[b])	18.63	67.77	0.07	100.00
	(0.06)	(0.12)	(0.02)	(0.12)	(0)	(0.12)	(0.04)	(0.00)	
Olive oil (A)—									
Fatty acids 1,2,3	9.58)	2.63	0.00	0.00	0.00[b]	9.07	78.71	0.00	100.00
	(0.12)	(0.02)	(0)	(0)	(0)	(0.52)	(2.21)	(0)	
Fatty acids 1,3	19.34	3.22	0.53	1.09	0.00[b]	6.76	68.75	0.33	100.01
	(2.21)	(0.02)	(0.06)	(0.13)	(0)	(0.41)	(1.55)	(0.10)	
Literature	16.1, 13.0	5.1, 4.1	0.6	0.8, 0.6	0.4	4.2, 4.8	73.2, 74.0	—	
Olive oil (B)—									
Fatty acids 1,2,3	12.26	2.87	0.00	0.00	0.00[b]	8.64	76.23	0.00	100.00
	(2.93)	(0.03)	(0)	(0)	(0)	(0.27)	(2.18)	(0)	
Fatty acids 1,3	20.06	3.78	0.52	1.08	0.00[b]	6.49	67.81	0.28	100.00
	(0.88)	(0.08)	(0.10)	(0.10)	(0)	(0.04)	(0.87)	(0.04)	
Olive oil (C)—									
Fatty acids1,2,3	11.90	2.75	0.00	0.00	0.00[b]	12.20	73.16	0.00	100.00
	(1.63)	(0.36)	(0)	(0)	(0)	(2.73)	(3.61)	(0)	
Fatty acids 1,3	17.93	3.93	0.58	1.02	0.00[b]	9.05	67.17	0.34	100.00
	(2.22)	(0.46)	(0.00)	(0.04)	(0)	(0.39)	(2.81)	(0.08)	
Olive oil (D)—									
Fatty acids 1,2,3	11.26	2.59	0.00	0.00	0.00[b]	11.72	74.43	0.00	100.00
	(1.54)	(0.46)	(0)	(0)	(0)	(0.90)	(3.11)	(0)	
Fatty acids 1,3	19.57	3.36	0.49	1.13	0.00[b]	9.12	66.02	0.33	100.00
	(1.10)	(0.11)	(0.04)	(0.01)	(0)	(0.80)	(0.17)	(0.00)	
Olive oil (E)—									
Fatty acids 1,2,3	11.91	2.72	0.00	0.00	0.00[b]	9.18	76.19	0.00	100.00
	(1.94)	(0.12)	(0)	(0)	(0)	(1.12)	(1.85)	(0)	
Fatty acids 1,3	17.27	3.53	0.47	0.94	0.00[b]	6.89	70.62	0.30	100.00
	(2.16)	(0.40)	(0.08)	(0.08)	(0)	(0.94)	(1.06)	(0.05)	

[a] Values are means of triplicate determinations. Standard deviation is given in parentheses. [b] It was not possible to distinguish the percentage of C18:3 from C18:2 with GC.

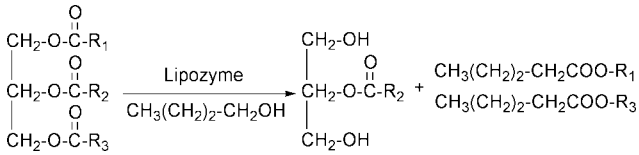

Fig. 1 The overall enzymatic transesterification of triacylglycerol in butanol.

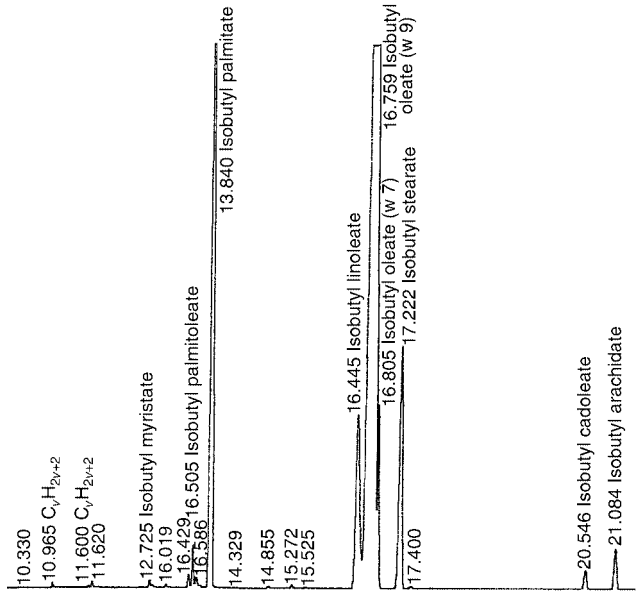

Fig. 2 Fatty acid iso-butyl esters GC-MS chromatogram of a mixture of olive and soybean oil (95 + 5, w/w).

and not applied in triacylglycerols in which HPLC separation of diasteroisomers does not appear to be possible. Ando and Takagi[3] reported a micro-method for stereospecific analysis of TAG. They were partially hydrolysed with ethyl magnesium bromide followed by conversion to 3,5-dinitrophenyl urethane derivatives. 1- and 2-monoacyl glycerols were isolated by HPLC on a silica column. The 1-monoacyl glycerol was further resolved into *sn*-1 and *sn*-3 monoacyl glycerol fractions by HPLC and derivatised to give fatty acid methyl esters which were analysed by GC on a capillary column. The method was applied for the stereospecific analysis of Jujube pulp and soybean triacylglycerols. The procedure used by Arcos *et al.*[10] utilised an *sn*-1,3-specific lipase from *Rhizopus arrhizus* to diacylate the fatty acid residues located at the *sn*-1 and *sn*-3 positions. The fatty acid residues located at the *sn*-1,2 position were esterified and determined by subtraction of the results of the *sn*-1,3 analysis from an overall compositional analysis based on complete saponification of the original sample. As the authors describe, the fatty acid mixtures were converted to *p*-bromophenacyl esters and analysed using conventional HPLC techniques. In our work, the determination of positional distribution of fatty acids in *sn*-1,3 was obtained in one step by transesterification using a specific lipase in butanol followed by GC analysis while Arcos *et al.*[10] obtained the same results using one specific lipase digestion followed by derivatisation before the HPLC analysis.

The direct mole percentage determination of fatty acids in position *sn*-1,3 in triacylglycerols is not possible by the aforementioned methods (except for Arcos *et al.*[10] In addition, the engagement of multiple steps induces sources of errors and these methods cannot be applied for quick routine determinations. The chemicals used and the reaction conditions described in this work make it suitable for rapid positional determinations in fat analysis.

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