Technical

Glyceride Analysis of Palm Oil after Solvent Fractionation

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ABSTRACT

A procedure is described for determing the triglyceride composition of palm oil and its fractions by the use of silver nitrate thin layer chromatography (TLC) and gas liquid chromatography (GLC). The triglycerides separated by silver nitrate TLC according to the number of double bonds are quantified using infrared spectroscopy before further analysis by GLC according to carbon number. The results from the two techniques enable the composition of the oil and fractions to be computed on a molecular basis in relation to fatty acid types. The potential application of this procedure is to analyze fractions obtained from the fractionation of oils and fats to which the 1,3-Random-2-Random distribution theory is not applicable.

INTRODUCTION

It has been shown that the 1,3-Random-2-Random distribution theory is invalid for calculating the triglyceride composition of fractions obtained from the fractionation of oils and fats (1-3). One of the advantages in using the distribution theory is that it gives the triglyceride composition according to fatty acid types on a molecular basis. No experimental technique exists which can directly provide such information.

Triglyceride composition of fats determined by analytical methods such as silver nitrate thin layer chromatography (TLC) has been based on unsaturation properties whereas gas liquid chromatography (GLC) results provide information on a molecular weight basis according to carbon number. Both methods are well established and numerous papers on the application of these methods to oils and fats are available (4-9). However, in each case, information on the composition according to fatty acid types is not included.

This paper describes an analytical procedure in which the triglyceride composition of palm oil fractions obtained from solvent fractionation can be determined according to fatty acid types by combining the results obtained separately from silver nitrate TLC and GLC. The precision of the technique is first verified for palm oil before application to the fractions. In each experiment the triglycerides are first separated and quantified according to unsaturation by silver nitrate TLC and infrared (IR) spectroscopy before subsequent analysis by GLC.

EXPERIMENTAL PROCEDURES

Materials

Crude Malaysian palm oil (*Elaeis guineensis* var *tenera*), courtesy of J. Bibby's & Sons Ltd, Liverpool was used. All solvents were of analar grade (unless otherwise stated) and distilled before use. The methyl esters standard mixture was from Nu-Chek-Prep, whereas triglyceride standards were from Sigma Chemical Company with a stated purity of 99%.

Fractionation

Palm oil was completely melted and homogenized at 70 C. To 35 g of the oil was added 350 cm^3 of n-hexane. The solution was transferred to a laboratory-designed fractionation vessel, cooled to 5 C and held at this temperature for 4.5 hr. The solid fraction formed was filtered under vacuum using a precooled Buchner funnel and filter flask and washed with cold hexane (50-70 cm^3). The solvent was removed from the liquid (olein) and solid (stearin) fractions under reduced pressure in a rotary evaporator.

Isolation of Triglycerides by Column Chromatography

Silica gel (Merck 35-70 mesh, 30.0 g) was placed in a glass column (40 x 2 cm) fitted with a sintered disc and a stopcock by means of a slurry in petroleum ether (40-60 C). Palm oil or one of its fractions (1 g) was placed at the top of the column in chloroform (10 cm^3). The triglycerides were eluted from the silica gel in petroleum ether: diethyl ether (95:5, 300 cm^3). The solvent was removed under vacuum and the triglycerides (0.80-0.90 g) were dissolved in n-hexane (1 cm^3) before storage at 3 C in a refrigerator.

Lipolysis

Pancreatin (Sigma Chemical Company, 10.0 g) was stirred with acetone (50 cm^3), filtered and washed with more acetone (50 cm^3) and with diethyl ether (2 x 50 cm^3) to defat it. n-Hexane (0.2 cm^3) and a bile salt solution of 0.037% sodium taurocholate in an ammonia buffer at pH 8.4 (2 cm^3) was added to the 7.5 x 2.5 cm screw-capped vial containing the triglycerides of palm oil or its fractions (30-40 mg). The mixture was shaken in a water bath at 41 C for 5 minutes. A 23% calcium chloride solution (0.25 cm^3) and pancreatic (60 mg) were then added and the whole mixture was further agitated vigorously for 10 min.

Hydrolysis was stopped by adding 1 M HCl (1 cm^3) and the reaction mixture extracted with diethyl ether (5 x 5 cm^3). The ether extract was washed with distilled water and dried over anhydrous sodium sulphate. The extracted lipids were chromatographed on thin layer plates (20 x 20 cm, 0.5 mm thickness silica gel, Merck type 60) using an iso-octane:diethyl ether:formic acid (60:40:1) solvent mixture. The resolved bands of mono-, di- and triglycerides and fatty acids were made visible with 2,7-dichlorofluorescein in ethanol (0.1%). The monoglycerides were scraped off the plates, eluted with chloroform/methanol (1:1, 50 cm^3) and converted to methyl esters by interesterification with 0.5 N sodium methylate (10 cm^3). The methyl esters were extracted with chloroform (2 x 20 cm^3), washed with 0.5 N H_2SO_4 (10 cm^3) and distilled water and dried over anhydrous Na_2SO_4 before concentration for GLC analysis. Triglycerides of palm oil or fractions

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(40 mg) were similarly converted to methyl esters.

GLC of Methyl Esters and Triglycerides

A Pye Unicam 104 gas chromatograph equipped with flame ionization detection was used. Methyl esters were analyzed on a glass column (2 m, 2.5 mm id) of 10% DEGS-PS on 80-100 mesh Supelcoport (Supelco). The separation was done isothermally at 183 C with the injector and detector temperatures at 195 C and 200 C, respectively. The carrier gas was nitrogen at a flow rate of 60 ml/min. The gas chromatograph was calibrated using a standard mixture of methyl esters (Nu-Chek-Prep Reference Mixture 15A). A comparison of the known and determined values from 5 analyses showed a coefficient of variation of less than 1% for the major components (35% and more) and less than 5% for the minor components (6% or less).

Triglycerides were analyzed according to carbon number on a glass column (46 cm x 25 mm id) of 2% Dexsil GC (Phase-Sep) on 100-120 mesh Supelcoport (Supelco). Column temperature was programmed from 250 C to 350 C at 4 C/min with a nitrogen flow rate of 80 ml/min. The detector temperature was set at 350 C. A standard mixture of trilaurin, trimyristin, tripalmitin and tristearin was used to calibrate the gas chromatograph. Typical molar response factors for the triglycerides in the standard mixture were C_{36} (1.00), C_{42} (0.89), C_{48} (0.97) and C_{54} (1.10). Molar response factors for mixed acid triglycerides (C_{46}, C_{50}, C_{52}) were obtained by interpolation from a graph of molar response factor against carbon number for the saturated monoacid triglycerides.

Peak areas in the analyses of methyl esters and triglycerides were measured by multiplying the peak height with the peak width at half height. For each sample, the mean of two separate analyses was taken as the result.

Silver Nitrate TLC

Plates (20 x 40 cm) coated with 0.5 mm thickness of silica gel (Merck Type 60) containing 10% silver nitrate were used. The sample of palm oil or fraction triglycerides were spotted as a band 2 cm from one of the shorter sides. The plates were eluted in the horizontal direction with benzene: petroleum ether:diethyl ether (90:10:3). The separated triglyceride bands were made visible with 2,7-dichlorofluorescein in ethanol (0.1%) under UV light and scraped into beakers. A solution of methanol:water (90:10) containing sodium chloride (1% w/v) was added in portions until the red color of the silver ion:dichlorofluorescein complex was destroyed (3-5 cm^3). A solution of chloroform:methanol (90:01, 10 cm^3) was then added and the mixture was filtered into a flask. The residue was further eluted with the chloroform-methanol solution (20 cm^3). Solvent was removed under pressure in a rotary evaporator, the flask flushed with nitrogen and the triglycerides dissolved accurately in chloroform (2 cm^3) for IR spectroscopy.

Infrared Spectroscopy

A Pye Unicam SP 1200 Spectrophotometer was used. A calibration curve was prepared by using aliquots of a stock solution of tripalmitin in chloroform (894/100 cm^3). The IR spectrum of each solution was recorded from 4000 cm^{-1} to 1200 cm^{-1} in sodium chloride cells (1 mm thickness). The optical density difference between the absorption at 1860 cm^{-1} (taken as baseline) and 1742 cm^{-1} (of the carbonyl stretching) was measured and plotted against the corresponding tripalmitin concentration.

The accuracy of the calibration curve was checked by comparing the known concentrations of three tripalmitin solutions (7.50, 3.84 and 2.54 mmol). The measured values from the curve were 7.50, 4.05 and 2.65 mmol, respectively. The precision of the method was determined using two known palm oil solutions (301 mg/100 cm^3 and 743 mg/100 cm^3); the determined values were 304 mg/100 cm^3 (coefficient of variation 1.0%) and 754 mg/100 cm^3

TABLE I

Fatty Acid Composition of Fractions from Silver Nitrate Thin Layer Chromatography (Mole %)

	Fatty acid	Fractions with number of double bonds				
		0	1	2	3	4 and more
Palm Oil	14:0	3.12	1.87	1.25	0.75	0.98
	16:0	89.92	60.80	39.16	22.86	22.11
	18:0	6.95	5.17	3.77	2.72	2.40
	18:1	—	32.43	47.74	50.11	29.04
	18:2	—	—	8.05	23.53	40.53
	18:3	—	—	—	—	4.90
Calculated number of double bonds		0	0.97	1.90	2.90	3.69
Olein	14:0	3.48	1.56	1.08	0.78	1.41
	16:0	77.72	60.12	40.97	23.42	19.84
	18:0	10.40	6.02	3.70	2.72	2.57
	18:1	8.37	32.27	45.53	49.55	28.80
	18:2	—	—	8.69	23.51	43.20
	18:3	—	—	—	—	4.14
Calculated number of double bonds		0.25	0.96	1.88	2.87	3.80
Stearin	14:0	2.60	1.89	1.47	1.18	0.95
	16:0	91.28	65.10	46.55	25.78	24.58
	18:0	6.10	4.18	3.20	2.45	2.91
	18:1	—	28.81	38.19	47.24	28.27
	18:2	—	—	10.57	23.33	38.67
	18:3	—	—	—	—	4.59
Calculated number of double bonds		0	0.86	1.76	2.79	3.55

(coefficient of variation 1.5%), respectively.

The spectrum of each triglyceride band extracted from silver nitrate TLC was recorded and its concentration determined. The amount of each triglyceride band was expressed as a percentage of the total concentration of all the bands that separated according to 0, 1, 2, 3 and 4 and more double bonds in the triglyceride molecule.

RESULTS AND DISCUSSION

An advantage in using IR spectroscopy to quantify the triglyceride fractions separated by silver nitrate TLC is that it allows subsequent analysis of the fractions for fatty acid and triglyceride composition using GLC. Table I shows the fatty acid composition of each triglyceride fraction separated by silver nitrate TLC for palm oil, olein and stearin. The results enable the efficiency of the TLC separation to be monitored and, as observed, a satisfactory separation is achieved in each case as shown from the calculated number of double bonds per molecule which correspond to the expected 0. 1, 2, 3 and 4. An exception is the trisaturated fraction of the olein, in which a slight contamination from the monounsaturated fraction is observed. Persmark and Toregard (10) have remarked on the occurrence of such contamination in the trisaturated fraction separated by silver nitrate TLC, a problem which is apparently more common than has been reported, especially where the amount of trisaturates analyzed is very small.

Table II provides the detailed results of each triglyceride fraction separated by silver nitrate TLC and quantified by IR spectroscopy for palm oil, olein and stearin. Each triglyceride fraction separated is further analyzed for its

TABLE II

Triglyceride Composition of Palm Oil, Olein and Stearin Analyzed by Silver Nitrate TLC and GLC (Mole %)

	Carbon number	Fractions with number of double bonds				
		0	1	2	3	4 and more
Palm Oil	C 46	1.94	—	—	—	
	48	84.13	5.44	t	—	
	50	13.91	83.58	22.58	5.91	
	52	—	10.97	73.90	67.08	
	54	—	—	3.51	27.00	
Total composition by silver nitrate TLC		8.63	37.86	34.87	13.54	5.09
Olein	C 46	2.72	t	—	—	
	48	49.55	6.57	t	—	
	50	43.90	80.85	24.40	5.69	
	52	3.81	12.57	71.31	66.24	
	54	—	—	4.28	28.06	
Total composition by silver nitrate TLC		4.41	48.24	31.86	11.13	4.36
Stearin	C 46	3.70	0.98	—	—	
	48	84.11	7.42	1.49	t	
	50	12.18	85.31	47.75	14.14	
	52	t	6.27	47.57	62.08	
	54	—	—	3.16	23.77	
Total composition by silver nitrate TLC		55.75	25.62	11.95	4.38	2.30

TABLE III

Triglyceride Composition of Palm Oil Obtained by Silver Nitrate TLC and GLC and That Calculated from the 1,3-Random-2-Random Distribution Theory[a] (Mole %)

Carbon number	Fractions with number of double bonds											
	0			1			2			3		
C 46	MPP[b]	0.17	(0.74)	MOM	—	(—)	MLM	—	(—)			
48	PPP	7.26	(7.42)	MOP	2.06	(1.74)	MLP	t	(—)			
	MPS											
50	PPS			POP			PLP			MLO	0.80	(0.26)
		1.20	(1.87)		31.64	(30.46)		7.87	(8.51)			
	MSS			MOS			MOO					
52	PSS	—	(0.16)	POS	4.15	(4.99)	OOP			PLO	9.08	(9.26)
								25.77	(22.24)			
							PLS					
54	SSS	—	(—)	SOS	—	(0.25)	OOS			OOO		
								1.22	(1.95)		3.66	(4.77)
							SLS			SLO		

[a]Results within brackets are calculated according to the distribution theory from lipolyses data published previously (3).

[b]M = myristic; P = palmitic; S = stearic, O = oleic; and L = linoleic acid.

TABLE IV

Triglyceride Composition of Palm Oil, Olein and Stearin Determined by Silver Nitrate Thin Layer Chromatography and Gas-Liquid Chromatography[a,b] (Mole %)

	Fractions with number of double bonds															
	0				1				2				3			
Carbon number		Palm oil	Olein	Stearin		Palm oil	Olein	Stearin		Palm oil	Olein	Stearin		Palm oil	Olein	Stearin
C 46	MPP	0.17	0.12	2.06	MOM	—	t	0.25	MLM	—	—	—				
C 48	PPP	7.26	2.19	46.89	MOP	2.06	3.17	1.90	MLP	t	t	0.18				
	MPS															
C 50	PPS				POP				PLP				MLO	0.80	0.63	0.62
		1.20	1.94	6.79		31.64	39.00	21.86		7.87	7.77	5.71				
	MSS				MOS				MOO							
C 52	PSS	—	0.17	t	POS	4.15	6.06	1.61	OOP				PLP	9.08	7.37	2.72
										25.77	22.72	5.68				
									PLS							
C 54	SSS	—	—	—	SOS	—	—	—	OOS				OOO			
										1.22	1.36	0.38		3.66	3.12	1.04
									SLS				SLO			

[a]Results are computed from Table II.

[b]Fractions with more than 3 double bonds were not analyzed by GLC.

composition by GLC. For the GLC analyses, only those fractions having 0, 1, 2, and 3 double bonds corresponding to 3, 2 and 1 saturated acid radicals in the triglyceride molecule were analyzed. In each case, the two sets of data obtained enable the triglyceride composition to be computed according to fatty acid types. Five fatty acids, myristic, palmitic, stearic, oleic and linoleic constitute the triglycerides of palm oil and from these, the triglycerides of each fraction separated by silver nitrate TLC and analyzed by GLC can then be deduced as shown in Table III for palm oil. To check the accuracy of the silver nitrate TLC and GLC analyses, we have compared the results obtained with those calculated from the 1,3-Random-2-Random distribution theory since no other analytical technique exists that would provide similar data for comparison. Moreover, it is well established that the distribution theory gives an accurate representation of the triglyceride composition of natural fats having fatty acids of mainly 16 and 18 carbon atoms (4,11). Our results agree fairly well with those calculated from the theory, especially those for the major triglycerides in palm oil such as PPP, POP, PLP, OOP and PLO. Hence, by a combinatin of silver nitrate TLC and GLC techniques, it is now possible to deduce the triglyceride composition of palm oil according to fatty acid types which is similar to that calculated from the distribution theory using lipolysis data.

Two limitations exist for this method of analysis. Isomeric triglycerides are not distinguished and in cases where two triglycerides have the same carbon number such as PPP and MPS (C-48) in the trisaturated fraction, differentiation also is not possible. Differentiation for PPP, although tediuous, is possible after fractionation by pancreatic lipolysis and the Brockerhoff procedure (4,12), whereas for MPS, the limitation can be overcome to some extent if the fatty acid composition of the fat is known. For palm oil that consists predominantly of palmitic and oleic acids, for example it can be assumed that a major proportion of the mixture, PPP and MPS, would be mainly of PPP. Similarly, in the mixture of POP and MOS of the monounsaturated fraction, the bulk of it would be POP rather than MOS. Of the mixtures in the diunsaturated fraction, the predominant triglycerides would consequently be PLP, OOP and OOS, respectively. It should be emphasized that the notation in Table III indicates only the fatty acid radicals present in a triglyceride molecule and does not in any way reflect a positional attachment.

For palm fractions from fractionation where it has been shown that the 1,3-Random-2-Random distribution theory is not applicable (1-3), this method of analysis enables the triglyceride composition of the fractions to be analyzed in relation to fatty acid types. Table IV shows the results of such an analysis for the olein and stearin obtained from the fractionation of palm oil using n-hexane. The results provide a better comparison of individual triglyceride molecules among the oil and fractions that could be obtained by either silver nitrate TLC or GLC alone. By making this comparison, it should now be possible to monitor the crystallization process during fractionation with regard to the triglycerides present in palm oil.

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