Quantitative semimicro analysis of triglyceride fatty acid distribution in a Congo palm oil

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SUMMARY The triglycerides of a Congo palm oil were separated into fractions with 0, 1, 2, 3, and 4 double bonds by thin-layer chromatography using silica gel impregnated with silver nitrate as adsorbent. The amounts and fatty acid compositions of these fractions were determined. In addition, the fatty acid compositions at the 2-positions of the palm oil and of the triglyceride fractions were determined by lipase splitting. The analytical results allow an accurate determination of the triglyceride composition. The results confirm the correctness of Vander Wal's theory on the distribution of fatty acids in triglycerides of natural fats.

THE TRIGLYCERIDE composition of palm oil has already been investigated by means of fractional crystallization (1–3). After it had been possible to determine, besides the over-all fatty acid composition, the composition at the 2-position, Vander Wal (4) was the first to give a method for calculating the triglyceride composition of natural fats. Independently of Vander Wal, Coleman and Fulton (5) developed a method for calculating the triglyceride composition from the fatty acid compositions, starting from similar assumption. This method of calculation has been applied to various fats, including a palm oil (6).

The present paper describes the determination of the glyceride composition of a Congo palm oil with the aid of thin-layer chromatography (TLC) on silica gel impregnated with silver nitrate (7), combined with a micro lipase-splitting method and determination of fatty acid compositions by gas–liquid chromatography (GLC).

METHODS

The triglycerides of the crude Congo palm oil[1] were obtained by column chromatography over silica (8). They were separated into fractions having different degrees of unsaturation by TLC on silica gel impregnated with siliver nitrate (9) using the horizontal technique of Brenner and Niederwieser (10) and petroleum ether (bp 40–60°)–benzene 1:4 (v/v) as eluent (7, 9).

After extraction of the glycerides from the adsorbent the amount of each fraction was determined by saponification and determination of the glycerol formed with periodic acid (7). The fatty acids were converted into methyl esters and analyzed by GLC.

The lipase splitting of the triglyceride fractions was carried out at 40° by emulsifying 10–20 mg of sample in 2 ml of 1.2 M NH_4Cl/NH_4OH buffer pH 8.5 containing 50 μg of bile salts, after which 8 mg of $CaCl_2 \cdot 6H_2O$ and 4 mg of purified pancreas lipase were added. The hydrolysis was stopped after 15 min by adding 1 ml of 1 N HCl solution. The fatty acids and glycerides were extracted from the water layer. The monoglycerides were isolated from this mixture by TLC on silica gel (7) and subsequently converted into methyl esters, which were analyzed by GLC.

When the amount of triglycerides available was less than 10 mg, half the amount of the reagents was used and the hydrolysis was stopped after 10 min. Trisaturated glycerides, which are difficult to emulsify, were mixed with about the same amount of triolein prior to the analytical procedure.

The accuracy of the method was determined by analysis of a mixture of the oleoyl–dipalmitoyl isomers

TABLE 1 PERCENTAGES OF TRIGLYCERIDES WITH 0, 1, 2, 3, AND 4 DOUBLE BONDS

Number of Double Bonds of Triglyceride Fractions	Mole %	% by Weight
0	6.6	6.3
1	38.1	37.8
2	35.0	35.2
3	13.3	13.5
4	7.0	7.2

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[1] Saponification value, 197; iodine value, 52.2; unsaponifiable 0.3%; monoglycerides, 1.4%; diglycerides, 8.1%; triglycerides 84.3%; and free fatty acids, 5.9%.

TABLE 2 Fatty Acid Composition (Mole %) of the Triglycerides of Palm Oil and Its Fractions

Fatty Acid	Palm Oil		Fractions									
			0 Double Bonds		1 Double Bonds		2 Double Bonds		3 Double Bonds		4 Double Bonds	
	Overall	2-Position	Overall	2-Position	Overall	2-Position	Overall	2-Position	Overall	2-Position	Overall	2-Position
12:0	0.1	0.1	0.2	0.3	0.2	0.2	tr.	0.1	0.1	tr.	tr.	0.2
14:0	1.7	0.7	3.3	6.1	1.5	1.1	1.2	0.5	1.2	0.3	1.3	2.5
16:0	44.2	11.9	86.9	86.5	56.2	11.0	35.6	4.2	22.1	1.9	15.1	8.4
18:0	5.2	0.9	9.6	7.1	8.0	1.2	5.3	0.5	3.1	0.4	2.0	1.6
20:0	tr.	tr.	tr.	—	tr.	tr.	—	—	—	tr.	—	tr.
16:1	—	tr.	—	—	—	0.2	0.1	—	—	0.2	—	0.2
18:1	36.7	62.3	tr.	—	34.1	86.3	46.6	63.8	47.1	56.5	33.3	28.6
18:2	12.0	23.9	—	—	tr.	—	11.2	30.9	26.4	40.6	44.9	55.1
18:3	0.1	0.2	—	—	—	—	—	—	tr.	0.1	3.4	3.4

PPO and POP in the ratio 68.5:31.5, using 14, 7, and 3 mg of substrate. The mean value for palmitic acid found was 68.5 mole %, with a standard deviation of 1.5.

RESULTS

The percentages of triglycerides with 0, 1, 2, 3, and 4 double bonds are given in Table 1. The over-all fatty acid composition and that at the 2-position of the total palm oil triglycerides and of the five fractions are shown in Table 2.

DISCUSSION

For all calculations the fatty acids were classified as follows:

12:0 to 20:0	Saturated fatty acids	S
16:1 + 18:1 + 20:1	Mainly oleic acid	O
18:2	Linoleic acid	L
18:3	Linolenic acid	Le

As can be calculated from Table 2, the triglycerides of the fractions separated on silica gel impregnated with silver nitrate have on the average 0, 1.02, 2.08, 3.00, and 4.00 double bonds per molecule. For the calculation of the triglyceride components the only assumption necessary is that each fraction is composed of triglycerides of the same degree of unsaturation. For the group with 0 double bonds this means SSS, for that with one double SOS, SSO, and for that with two double bonds SOO, OSO, SSL, etc.

Together with the results from Table 2, this supposition enables the composition of each fraction to be calculated in a simple way. In principle this calculation is capable of one solution only. There are, however, two difficulties.

(*a*) The number of double bonds found per molecule in fractions with one and two double bonds is not exactly a whole number. The fatty acid composition of these fractions is in contradiction to our assumption, so that only approximate solutions are possible. To bring the fatty acid composition into agreement with this supposition a minor correction was applied to the total fatty acid composition of the fractions "1" and "2." In fraction "1," 18:1 was reduced from 34.1 to 33.3%, 18:2 in fraction "2" from 11.2 to 10.8, and 18:1 in fraction "2" from 46.6 to 45.1. The percentages of saturated fatty acids were proportionally increased.

(*b*) For a complete calculation of the fraction with four double bonds, an additional datum was required. Since the triglycerides containing linolenic acid constitute only 10.2% of this fraction, we chose for this purpose SLeO = SOLe = OSLe. This assumption introduces only a slight uncertainty. The triglyceride compositions of the fractions given in Table 3 are deduced from the fatty acid compositions found experimentally, both for the overall and for the 2-positions. By using the per-

TABLE 3 Triglyceride Composition (Mole %) of the Fractions with 0, 1, 2, 3, and 4 Double Bonds

Fraction with O Double Bonds		Fraction with 1 Double Bond		Fraction with 2 Double Bonds		Fraction with 3 Double Bonds		Fraction with 4 Double Bonds	
Glyceride	Mole % of Fraction	Glyceride	Mole % of Fraction	Glyceride	Mole % of Fraction	Glyceride	Mole % of Fraction	Glyceride	Mole % of Fraction
SSS	100	SOS	86.5	SOO	63.8	OOO	20.6	OOL	25.4
		SSO	13.5	OSO	3.8	SOL	36.1	OLO	19.5
				SSL	1.5	SLO	40.7	SLL	35.6
				SLS	30.9	OSL	2.6	LSL	9.3
								SOLe SLeO OSLe	10.2

TABLE 4 TRIGLYCERIDE COMPOSITION (MOLE %) OF CONGO PALM OIL

Triglycerides with O Double Bonds			Triglycerides with 1 Double Bond			Triglycerides with 2 Double Bonds			Triglycerides with 3 Double Bonds			Triglycerides with 4 and More Double Bonds		
	A*	B*		A	B		A	B		A	B		A	B
SSS	6.6	6.7	SOS	33.0	30.7	SOO	22.4	20.9	OOO	2.7	3.5	OOL	1.8	1.8
			SSO	5.1	4.5	OSO	1.3	0.8	SOL	4.8	5.2	OLO	1.4	1.4
						SSL	0.5	1.1	SLO	5.4	8.0	SLL	2.5	2.0
						SLS	10.8	11.8	OSL	0.4	0.4	LSL	0.6	0.1
									SLeS	—	0.1	SOLe SLeO OSLe	0.7	—
												LOL	—	0.2
												OLL	—	0.7
												LLL	—	0.1
Totals	6.6	6.7		38.1	35.2		35.0	34.6		13.3	17.2		7.0	6.3

* A, deduced from results in Tables 1 and 2. B, calculation (from data of columns 1 and 2, Table 2) according to the method of Coleman and Fulton (5).

centages of the fractions (Table 1) the over-all triglyceride composition of the palm oil was calculated and is shown in Table 4. This table also shows the triglyceride composition calculated according to the method of Coleman and Fulton from the fatty acid composition of the unfractionated triglycerides (over-all and at the 2-position).

There is a striking resemblance between the triglyceride composition thus determined and that calculated according to Coleman and Fulton. The results obtained by other investigators (11–13) have already shown the distribution theory of Vander Wal (4) to be in all probability correct, although the conclusions are based on less well-defined facts. Our results substantiate the correctness of this theory for palm oil.

The analysis of various other natural fats, such as olive oil, Sumatra palm oil, lard, groundnut oil, and soybean oil (unpublished results) show that the triglycerides completely conform to the same distribution pattern.

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