consumption. Hence, CPFA could not be a problem as these are eliminated in the cooking process. But epoxy oleic acid could not be eliminated with any of the processing techniques. It is present in small quantities only, and its level can be reduced further by blending with common cooking oils. Processing of *H. sabdariffa* seed oil thus may render it suitable for human consumption.

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Determination of Mono- and Diglycerides in Palm Oil, Olein and Stearin

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ABSTRACT

Partial glycerides are important constituents of palm oil and can have significant effects on the physical properties of products containing palm oil or on the fractionation of palm oil. A method is described for their routine determination in palm oil. By analysis of 28 weekly composite samples of crude palm oil the following results were obtained: free fatty acids, mean = 3.76%, range 2.4 to 4.5%; monoglycerides, mean = 0.28%, range 0.21 to 0.34%; diglycerides, mean = 6.30%, range 5.3 to 7.7%. During detergent fractionation of palm oil, diglycerides concentrate in the palm olein, but monoglycerides concentrate in the palm stearin. Palm fatty acid distillate was found to contain approximately 3% each of mono- and diglycerides. Because the refining and fractionation processes are continuous in the refinery, it is not possible to follow a single identifiable batch of crude palm oil through the refinery. To circumvent this problem, crude palm oil, stearin and olein from the refinery were bleached and steam refined in the laboratory and the partial glyceride contents determined at each stage of processing. Except for fractionation, the content of glycerides did not change during processing. For oil, olein and stearin, monoglycerides were reduced significantly both after bleaching and after steam refining.

INTRODUCTION

Partial glycerides are important constituents of oils, especially palm oil, and they can have significant effects on physical properties. The lifetimes of α polymorphs in palm oil (1, 2, 3) and in shea fat (4) were influenced significantly by the level of diglycerides in the oils. Palm oil is unusually rich in partial glycerides (5), and their level is probably commercially important since partial glycerides also affect the solid fat contents at all temperatures. Hernqvist and Anjou (6) have used diglycerides successfully to stabilize the β' polymorph in margarines containing hydrogenated rapeseed and soyabean oils. In a margarine stored at 20 C, development of the β polymorph could be delayed from four to 44 weeks by the addition of 5% diglycerides.

Monoglycerides are used at low levels, typically 0.3%, to stabilize the oil/water emulsion in margarine. Palm oil naturally contains this level of monoglycerides (5). Although refining reduces the content of monoglycerides, the surface active properties of monoglycerides also are important in the detergent fractionation of crude palm oil to yield palm olein and stearin.

In this paper we report the use of a gas liquid chromatographic (GLC) method for the study of partial glycerides in crude, fractionated and refined palm oils. Trimethylsilyl (TMS) derivatives are prepared prior to GLC analysis. Similar analytical methods using various silylating and GLC procedures have been reported previously (7, 8, 9, 10, 11).

EXPERIMENTAL

Samples

All samples of palm oil, palm olein and palm stearin were taken from the storage tanks or directly from the refinery of Kempas Edible Oil Sdn. Bhd., Pasir Gudang, Johore, Malaysia.

Fractionation was by detergent fractionation (Alfa-Laval). Refining was by physical refining comprising degumming (0.04–0.07% phosphoric acid at 85 C), bleaching (1–2% earth at 110 C), and steam refining/deodorization at 270 C (EMI).

Analysis of Monoglycerides, Diglycerides and FFA

Preparation of TMS derivatives. 30 mg of the oil are weighed accurately (±0.1 mg) into a 2 ml screw-capped glass vial fitted with a septum (Supelco, Inc., Bellefonte, Pennsylvania, Catalog No. 3-3113). 400 μL of pyridine are added using a syringe and the vial capped and shaken until the oil dissolves. 100 μL of N-trimethylsilyl imidazole (Sigma Chemical Co., Saint Louis, Missouri) are then added through the septum using a syringe and needle. The vial is then shaken well for one min. Finally, 200 μL of the internal standard solution [250.0 mg n-triacontane (Sigma Chemical Co.) in 25 ml of iso-octane] are added, again

TABLE I

Retention Times and Observed and Theoretical Response Factors

	Retention Time (seconds)	Relative Retention Time	Response Factor	
			Theoretical	Observed
Triacontane	700	1	1	1
Palmitic Acid	188	0.27	1.011	1.084
Oleic Acid	276	0.39	1.002	
Monopalmitin	504	0.72	0.977	1.003
Monoolein	589	0.84	0.973	0.998
Dipalmitin	1091	1.56	1.121	1.231
Diolein	1214	1.73	1.102	1.209

through the septum. Solutions were used within four hrs.

Gas liquid chromatography. The TMS derivatives were analyzed on a Hewlett-Packard Model 5790 gas chromatograph. Column: glass, 0.5 m x 4 mm id, packed with 3% OV-1 on Gas Chrom Q (Supelco). Chromatography conditions: detector (FID) and injector at 360 C, oven programmed from 150 C to 355 C at 8 C/min followed by 6.5 min at 355 C, carrier gas nitrogen at 60 ml/min. Peak areas and retention times were determined using a Hewlett-Packard 3390A integrator. For GLC analysis, a 2 μL sample of the solution was injected into the gas chromatograph.

Standard mono- and diglycerides (Nu-Chek Prep. Inc., Elysian, Minnesota) were used to obtain the relative retention times and response factors required to calibrate the method.

Calculation of results. Theoretical response factors for triacontane, monoglycerides, diglycerides and free fatty acids were calculated using the equation:

$$\text{Response factor} = \frac{\text{Molecular Weight}}{W}$$

where W = weight of all carbon atoms in the TMS derivative, excluding the carbonyl carbons.

Actual response factors were obtained using known amounts of the standard partial glycerides which were silylated using the same procedure as for an oil sample. For free fatty acids, a response factor was obtained by comparison of the GLC result with the FFA determined by the standard titration procedure (AOCS Official Method Ca 5a-40). Free fatty acids were calculated as palmitic acid.

Retention times and response factors are given in Table I. Observed response factors were up to 10% higher than theoretical response factors, but the relative magnitudes for acids, mono- and diglycerides were as predicted. For practical purposes it was considered sufficient to use a single response factor for each lipid class, namely: monoglycerides, 1.00; diglycerides, 1.22, and free fatty acids, 1.08. These factors were then used in the following equation to give the percentage by weight of each component:

$$\% = \frac{A(G) \times f \times W(TC) \times 100}{A(TC) \times W(S)}$$

where A(G) = area of glyceride (or FFA) peak from integrator; A(TC) = area of triacontane peak from integrator; W(TC) = weight of triacontane added = 2 mg in above procedure; W(S) = weight of oil sample taken = 30 mg in above procedure, and f = response factor as above.

By 10 replicate analyses of a sample of crude palm stearin, analytical errors for single determinations were estimated as 95% confidence limits (t at P=0.05 x standard deviation) to be: diglycerides ±0.31, monoglycerides ±0.03, free fatty acids ±0.30.

Laboratory Refining

500 g of crude oil were heated to 70 C and mixed with 0.04% of 85% phosphoric acid for 15–20 min. The oil was then bleached with 2% Fulmont AA (Laporte), earth at 110 C for 30 min. After filtering, the bleached oil was steam refined under reduced pressure (2–5 torr) at 265 C for one hr.

RESULTS AND DISCUSSION

A typical chromatogram for the analysis of crude palm oil is shown in Figure 1. Total analysis time is less than 45 min including sample preparation, GLC analysis and cooling of the GLC oven ready for the next analysis. An analysis of triglycerides by carbon number also is obtained, although in our laboratory we prefer to obtain this analysis independently by temperature programming from 295 to 355 C

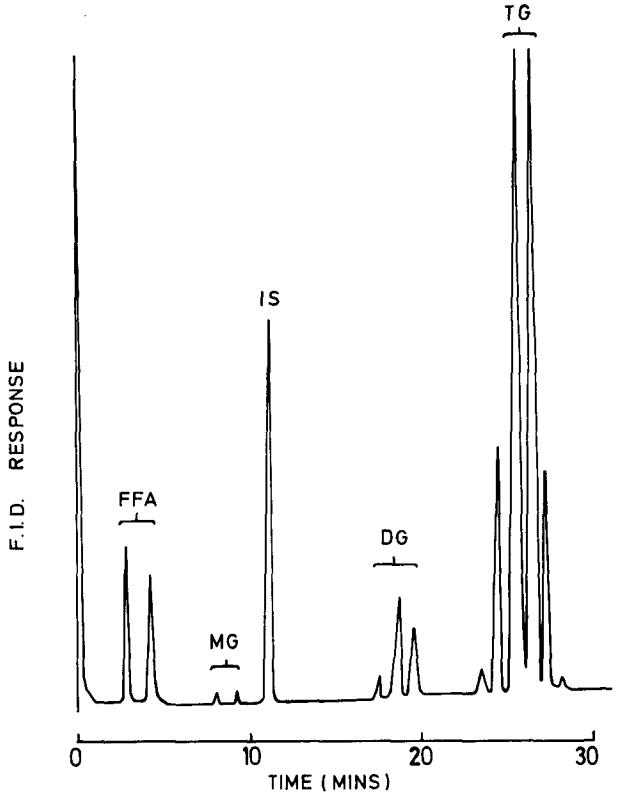

FIG. 1. Chromatogram of crude palm oil containing 0.3% monoglycerides (MG), 5.9% diglycerides (DG) and 3.5% free fatty acids (FFA). TG = triglycerides and IS = internal standard.

TABLE II

Typical Analyses (%) of Crude Palm Oil

Sample No.	Monoglycerides	Diglycerides	Free Fatty Acids	
			This Method	By Titration
1	0.28	6.18	4.35	4.37
2	0.31	6.47	4.56	4.59
3	0.31	6.60	4.39	4.36
4	0.30	7.05	4.32	4.43
5	0.28	6.30	4.26	4.23
6	0.28	6.81	3.46	3.46
7	0.30	6.29	3.47	3.56
8	0.30	6.92	3.72	3.72
9	0.27	6.45	3.40	3.30
10	0.26	5.48	2.50	2.50
Mean	0.289	6.455	3.843	3.852
Minimum	0.26	5.48	2.50	2.50
Maximum	0.31	7.05	4.56	4.59
Range	0.05	1.57	2.06	2.09

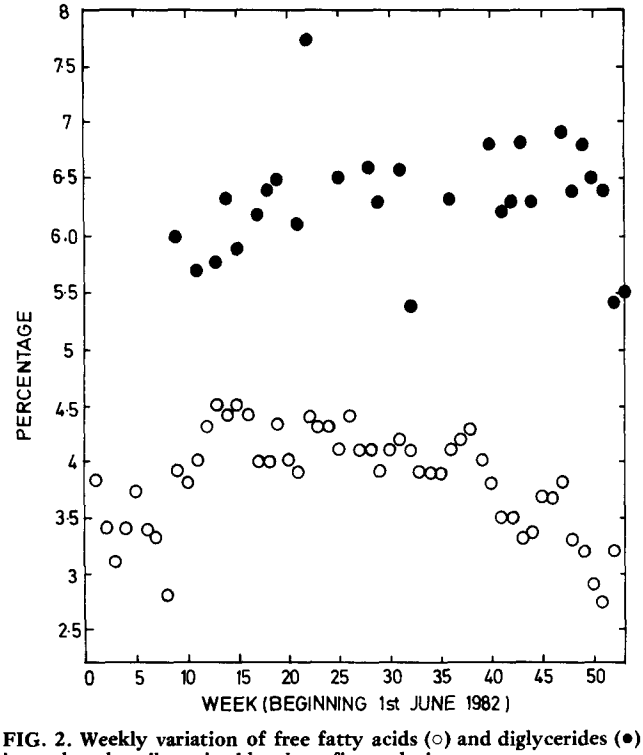

FIG. 2. Weekly variation of free fatty acids (○) and diglycerides (●) in crude palm oil received by the refinery during one year.

at 4 C/min. Because of overlap of diglycerides with low molecular weight triglycerides, the method is not suitable for lauric oils.

In Table II, typical analyses of 10 crude palm oil samples are given. Each sample is a composite of all the palm oil received by the refinery in one week from all suppliers and represents deliveries totalling up to 3000 tons each. Jacobsberg and Oh (5) have reported diglyceride and FFA data for several samples of crude palm oil. Diglyceride values, obtained using thin layer chromatography, ranged from 5.34 to 7.75%, similar to our values.

There was excellent agreement between FFA determined by our GLC method and by the standard titration method. The GLC procedure gives additional information since a quantitative analysis of each fatty acid is obtained.

A more detailed study of crude palm oil was made by analysis of weekly composite samples of crude palm oil over a whole year. 28 out of 53 weekly samples were analyzed for mono- and diglycerides. All 53 samples were analyzed for FFA. The variation of the data with time is shown in Figure 2. There was no significant correlation between FFA and mono- or diglycerides but a highly significant correlation (P=0.001) between mono- and diglycerides. The statistics found were:

	MG	DG	FFA
Mean	0.28	6.30	3.76
Minimum	0.21	5.3	2.4
Maximum	0.34	7.7	4.5

Correlation coefficients: (with 26 degrees of freedom)
MG X DG = 0.782
MG X FFA = −0.066
DG X FFA = 0.141

For 25 degrees of freedom from tables, r=0.323 (P=0.1) and r= 0.597 (P=0.001).

Jacobsberg and Oh (5) also found no correlation between FFA and diglyceride contents. They concluded that most of the diglyceride found in crude palm oil is not formed by hydrolysis of triglycerides, which also would produce free fatty acids, but is a residual by-product of the biosynthesis of the triglycerides. Thus, even an unbruised fruit with FFA only 0.32% contained 5.66% diglyceride with the 1,2 isomer predominating over the 1,3 isomer. Although our GLC method does not distinguish the two isomers, using semi-quantitative thin layer chromatography we always have found more 1,3 than 1,2 isomer (in proportion of about 2 to 1) in all crude and refined palm oils or fractions. The 1,2 isomer is involved in the biosynthesis, but even moderate heating quickly results in the natural equilibrium with the 1,3 isomer predominating.

Typical analyses of refined palm oil, olein and stearin and of palm fatty acid distillate from our refinery are given in Table III. Although all these samples and the crude palm oil samples given in Table II were obtained from the refinery at the same time, it is not possible to correlate them exactly with each other. Nevertheless, the mean diglyceride level in the refined palm oil samples is close to the mean level for the crude palm oil samples, suggesting that the refined and the crude oils were generally similar.

Monoglyceride levels in the refined oils were found to be significantly lower than in the crude palm oil. Monoglycerides are removed by bleaching and steam refining, and the fatty acid distillate shows a high level in consequence.

The results in Table III also show higher levels (relative

TABLE III

Typical Analyses (%) of Fully Refined Palm Oil, Olein and Stearin and of Palm Fatty Acid Distillate (PFAD)

	Palm Oil		Palm Olein		Palm Stearin		PFAD[a]	
	MG	DG	MG	DG	MG	DG	MG	DG
No. of Samples	11	11	10	10	6	6	2	2
Mean	0.078	6.44	0.061	6.83	0.02	5.76	3.3	3.2
Minimum	0.04	5.00	0.05	5.7	n.d.[b]	4.1	2.9	2.8
Maximum	0.15	7.99	0.08	8.3	0.04	6.8	3.8	3.6
Range	0.11	2.99	0.03	2.6	<0.04	2.7	0.9	0.8

[a]Mean FFA = 83.1%.

[b]n.d. = not detected = less than 0.02% and taken as 0.01% for calculation of mean.

TABLE IV

Typical Results (%) for Fractionation and Bleaching of a Single Batch of Crude Palm Oil

Sample	Mono-glyceride	Diglyceride	FFA
Crude Palm Oil	0.27	5.5	3.0
Crude Palm Olein	0.23	6.2	3.4
Crude Palm Stearin	0.44	4.1	2.2
Bleached Palm Olein	0.13	6.0	3.2
Bleached Palm Stearin	0.20	4.3	2.2

to palm oil) of diglyceride in palm olein and lower levels in palm stearin. This is a direct consequence of the fractionation process.

In Table IV a single batch of crude palm oil was traced through the fractionation plant. FFA and diglycerides are concentrated preferentially in the olein while monoglycerides are concentrated in the stearin. The monoglycerides are polar, surface active compounds which move preferentially into the aqueous phase with the stearin crystals. Other polar constituents of palm oil, such as phospholipids and iron, also concentrate in the stearin.

Table IV also shows the effect of bleaching (in the laboratory) the crude palm olein and stearin. After bleaching, monoglycerides were reduced in both fractions, but there was no significant change in diglycerides.

It was not possible in the refinery to follow a single batch of crude palm oil through all the possible fractionation and refining processes. We therefore have taken samples from the fractionation plant and completed the refining in the laboratory. In this way we were able to follow the levels of mono- and diglycerides in a single sample of palm oil right through to the production of fully refined palm oil, olein and stearin. The fractionation and refining scheme and the results are shown in Figure 3.

In Figure 3, the total FFA, mono- and diglyceride are

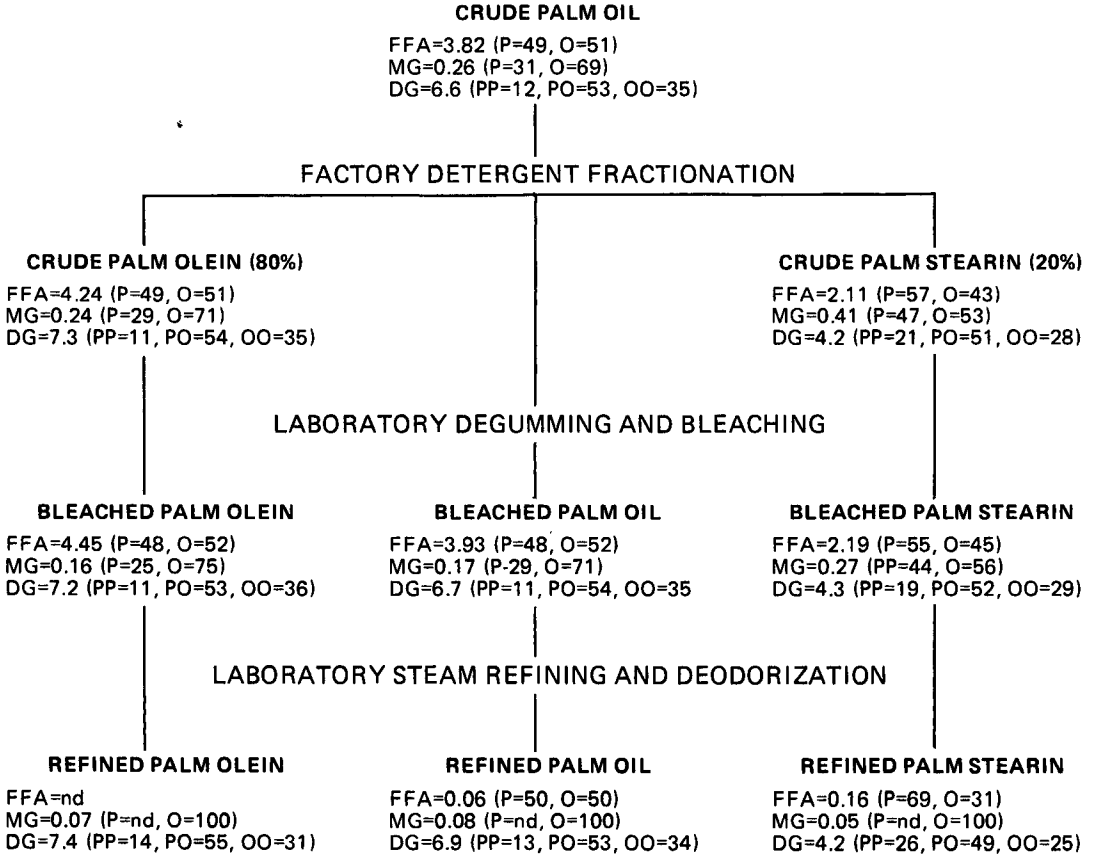

FIG. 3. Fractionation and refining of palm oil (nd = not detected, P = palmitic + myristic, O = stearic + oleic + linoleic; PP, PO and OO indicate diglycerides containing these fatty acid groups; P and O above indicate monoglycerides or fatty acids).

divided into the proportions as percentages of each of the individual components. For crude palm oil it is interesting to compare the observed distribution with what might be expected in a random distribution assuming each component had the same fatty acid composition as the crude palm oil.

	FFA		MG		DG		
	P	O	P	O	PP	PO	OO
Observed	49	51	31	69	12	53	35
Random	46	54	49	51	21	50	29

The free fatty acids do have the approximately random distribution that would be expected by random hydrolysis of the triglycerides. In contrast, the mono- and diglycerides have a distribution which is different from random.

As previously noted, detergent fractionation concentrates FFA and diglyceride in the olein and monoglyceride in the stearin. The results given in Figure 3 also show that the proportion of P and O and of PP, PO and OO change, with the more unsaturated partial glycerides, like the triglycerides, concentrating in the olein.

On bleaching, FFA increases slightly, monoglycerides are reduced and diglycerides are unchanged.

After steam refining and deodorization FFA is reduced almost to zero. For palm stearin, the proportions of P and O seem to have altered, but since P (mainly palmitic acid) would be expected to be more volatile, this may be due to experimental error at these very low levels. Monoglycerides also are substantially reduced, with O (mainly monoolein) being the only detectable monoglyceride. Total diglycerides show no significant change, but the proportions of PP, PO and OO do change in the direction of increased random distribution. This is expected at the high temperatures used in the steam refining. However, it also should be noted that the total amount of P in the diglycerides increases, suggesting that there also may be some interchange of fatty acids between di- and triglycerides or free fatty acids. Percentage of P (mainly palmitic acid) in total diglycerides before and after steam refining:

	Before	After
Olein	37.5	41.5
Oil	38	39.5
Stearin	45	50.5

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♣Studies on Kinetics of Catalytic Isomerization of Methyl Linoleate

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ABSTRACT

Kinetics of isomerization of methyl linoleate are studied on ruthenium (5%) on carbon in the temperature range 200-270 C with different solvents. Some equilibrium experiments also are carried out with rhodium and ruthenium catalysts. The reactions taking place are isomerization, hydrogenation and polymerization. The activities and the selectivities are dependent on the nature of the solvent used. Highly protic solvents like methanol or isopropyl alcohol exhibited very high activity and selectivity for hydrogenation, whereas aprotic solvents like hexane or cyclohexane showed very high selectivities for isomerization reaction. The reaction kinetics were found to be further complicated by polymer formation at low solvent concentrations. The effects of temperature, solvent concentration, catalyst quantity and time of reaction also were investigated.

INTRODUCTION

Isomerization of vegetable oils to conjugated oils is an important reaction which finds applications essentially for making alkyd resins. A number of papers have appeared in the past which describe processes using homogenous metal complexes and hetereogeneous catalysts (1-8). Supported noble metals like palladium, rhodium and ruthenium are well known hydrogenation catalysts (8,10), but their performance for isomerization has been studied very little. Their activity for isomerization is to be expected from the fact that they have vacant d-orbitals which can interact with π bonds of fatty acids as well as activating an adjacent C-H bond, a necessary step for double bond migration. Furthermore, it is likely that the most predominant pathway for the hydrogenation of a dieneic fatty acid could be via conjugation (10). In a recently published French paper (6) and patent (7), results on isomerization of sunflower oil and methyl linoleate on rhodium and ruthenium catalysts were presented. Most of the experiments were carried out up to equilibrium using pure oils or traces of protic solvents. No detailed kinetic data is reported for this system so far.

In the present study, we have investigated detailed kinetics of the network of reactions starting with methyl linoleate as the reactant. We report results of the effects of parameters such as nature of solvent, temperature, catalyst quantity on the kinetic behavior and the product profile.

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