Applications of GLC in the Analysis of Oils and Fats

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RINGKASAN

Salah satu alat yang digunakan untuk mencirikan minyak dan lemak ialah kromotograf gas. Makalah ini menghuraikan beberapa analisis minyak dan lemak yang melibatkan penggunaan kromotograf gas cecair. Keadaan operasi dan kromotograf yang terdapat untuk analisis yang sering dijalankan seperti komposisi asid lemak, trigliserida dan gliserida separa dalam minyak sawit dan minyak isirong sawit dijelaskan.

INTRODUCTION

Chromatography is a physical separation of two or more compounds based on their differential distribution between two phases, one of which is stationary and the other mobile. In the case of gas chromatography the mobile phase is a gas.

If the stationary phase is a liquid distributed on a solid support material, the process is known as Gas Liquid Chromatography (GLC). The less common Gas Solid Chromatography (GSC) has a solid stationary phase with surface active properties.

The high separating power of GLC has allowed for the analyses of samples which were hitherto regarded as tedious, difficult or even impossible. The speed, accuracy and versatility of the technique is shown by its wide application in both routine and research work in the industrial, medical and educational fields.

The common analyses of oils and fats involving the use of GC techniques are as follows:

I Analysis of Fatty Acid Composition (FAC).
II Analysis of Triglycerides (TG) expressed as carbon numbers.
III Analysis of partial glycerides, also expressed as carbon numbers.
IV Analysis of fatty acid isomers.
V Determination of different combinations of saturated and unsaturated fatty acids in triglycerides.
VI Determination and identification of sterols.
VII Determination of anti-oxidants in oils such as TBHQ, BHA and BHT.
VIII Detection of contaminants (*e.g.* halogenated pesticides).

The first two analyses listed above are the most common usage of GLC in the area of oils and fats. Detection in all the above analyses is by the Flame Ionization Detector (FID) except for halogenated pesticides where the Electron Capture Detector (ECD) is used. This is because halogen-containing compounds have a high electron affinity.

FATTY ACID COMPOSITION (FAC) ANALYSIS

GLC of fatty acid methyl esters (FAME) is the traditional method for analysing the fatty acids in oils and fats as shown in *Figure 1*. The conversion of triglycerides (TG) to FAME can be done through an acid catalyzed (*e.g.* 0.5% HCl in

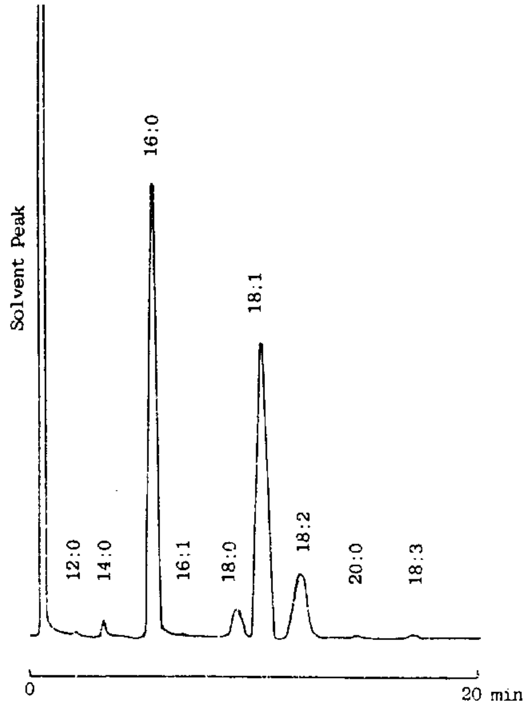
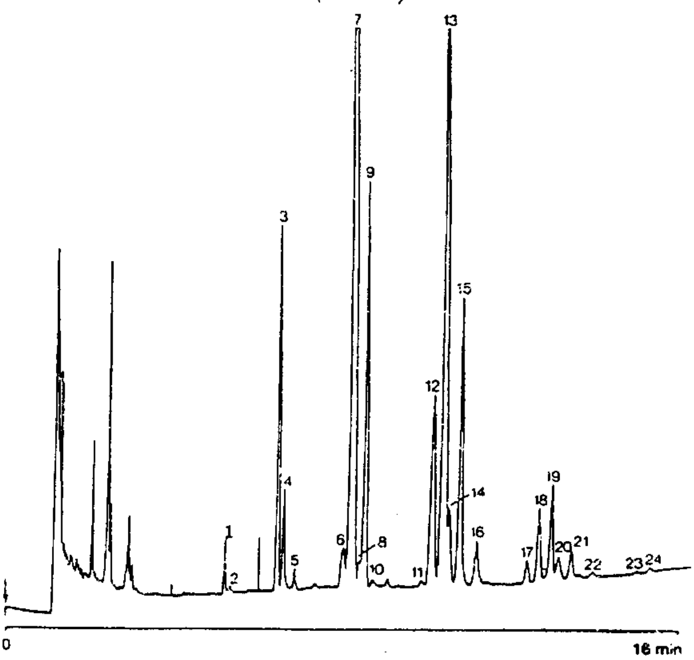


Figure. 1. Chromatographic trace of FAME of Palm Stearin analysed on a 1.8 m x 3 mm glass GLC column packed with 10% SP 2330 (PORIM)



Peak Identification:

		Carbon No.		Carbon No.
1.	MPP	46	13. POO	52
2.	MOM	46	14. PLS	52
3.	PPP	48	15. PLO	52
4.	MOP	48	16. PLL	52
5.	MLP	48	17. SOS	54
6.	PPS	50	18. SOO	54
7.	POP	50	19. OOO	54
8.	MOO	50	20. SLO	54
9.	PLP	50	21. OLO	54
10.	MLO	50	22. OLL	54
11.	PSS	52	23. SOA	56
12.	POS	52	24. AOO	56

Figure 2. Chromatographic trace of TG of Palm Oil analysed on a 25 m armoured fused silica capillary column coated with TAP (chemical bonded polysiloxane, 50% phenyl/50% methyl) (reproduced from Chrompak Application Note - Application 195 - GC)

methanol) or a base catalyzed (*e.g.* 0.5 N sodium methoxide) transesterification reaction. The latter is usually quicker than the former but it does not work if the oil is highly acidic as it may not convert the free fatty acids of the oil to methyl esters. Methods for preparation of FAME have been proposed by the American Oil Chemists' Society (AOCS), the British Standards Institution (BSI), the International Organization for Standardization (ISO), and the International Union of Pure and Applied Chemistry (IUPAC). PORIM laboratory uses a quick method proposed by Timms (1978) for routine palm oil analysis. For acidic oils, the method using boron trifluoride-methanol (ISO 5509) is followed.

The FAME are usually analysed on glass or steel columns containing polar stationary phases such as polyesters (*e.g.* DEGS, EGS) or cyano-silicones (*e.g.* SILAR 5CP, SP 2330). The latter type is preferred because of its greater thermal stability which allows for higher operating temperatures. Both packed as well as capillary columns are used although the trend has been towards the use of the latter. Packed columns vary from 1.8 to 3.0 metres in length and 2 to 4 millimetres in internal diameter. Capillary columns are longer, from 30 to 60 metres, and with internal diameters of 0.25 to 0.30 millimetres.

The identification and quantification of the FAME can be done by the use of commercial standard mixtures or relative retention times. Two of the most frequently used quantification methods are area normalization and area normalized after corrections with response factors. Craske *et al.* (1987) provided a thorough discussion on the maximization of accuracy for GLC analysis of FAC.

TABLE 1. OPERATING CONDITIONS FOR ANALYSIS OF FAME

	Packed Column	Capillary Column
Column	1.8 m x 3 mm i.d. glass or stainless steel	30 - 60 m x 0.25 mm i.d. fused silica
Stationary phase	10% SP 2330 on 100/120 Supelco port	SP 2340, SP 2330
Column Temperature	Isothermal, 180° C	Isothermal, 180° C
Carrier Cas	Nitrogen, 40 ml per min	Helium, Split ratio of 100:1
Injector temperature	220° C	290° C
Detector temperature	220° C	290° C

TABLE 2. OPERATING CONDITIONS FOR PALM OIL AND PALM KERNEL OIL TG ANALYSIS

Column	:	Glass, 0.46 m x 3 mm i.d.
Stationary Phase	:	3% OV-1 on Gas Chrom Q 100/120 mesh size
Programme	:	280°C - 345°C @ 4° C/min for Palm Oil 230°C - 345°C @ 4° C/min for Palm Kernel Oil
Detector Temperature	:	370°C
Injector Temperature	:	370°C
Carrier Gas	:	80 ml/min, Nitrogen

Typical operating conditions for analysis of FAME from palm oil using both packed and capillary columns are shown in *Table 1.*

ANALYSIS OF TRIGLYCERIDES (TG)

This analysis is most often used to monitor processes such as fractionation, transesterification and blending.

GLC separates triglyceride molecules according to the number of carbon atoms in them. Each peak in the chromatogram is referred to by the number of carbon atoms in the acyl chains of that TG molecule. Hence tripalmitin with 3 acyl chains of 16 carbon atoms will have a carbon number abbreviated to C48. Glass or stainless steel columns of non-polar silicone polymers are used for TG analysis. The length of the columns is short, usually from 0.40 to 0.60 metres, with internal diameters from 2 to 4 mm.

In this area of analysis, the trend is also towards capillary columns. Analyses of TG using capillary columns chemically bonded with methyl silicone phases have been carried out for determination of different combinations of saturated and unsaturated TG as seen in *Figure 2* (refer also to section on Analysis of the Fatty Acid Combinations in TG).

Temperature programming is usually essential for GLC analysis of TG. Linear programming rates of 2 to 8°C per minute are normally used. The carrier gas flow rates are set higher than those used for FAME analysis because TGs have low volatility.

Quantification of data requires normalization with response factors in view of losses resulting from the use of high temperatures. The paper by Mares *et al.* (1985) is a useful reference for quantification of capillary GLC of TG. Typical operating conditions for palm oil and palm kernel oil TG analyses, together with their respective chromatographic traces are shown in *Table 2* and *Figures 3* and *4.* A handbook by Tan *et al.* (1984) on TG composition of oils and fats by GLC is also available from PORIM.

The paper by D'Alonzo *et al.* (1981) presented a detailed discussion of the current packed and capillary techniques for TG and FAME analyses.

ANALYSIS OF PARTIAL GLYCERIDES

The amount of mono- and diglycerides (MG and DG) in oils and fats can be determined on the GC using the same column for triglyceride analysis as shown in *Figure 5.* However, the samples must first be silylated. Silylation is the introduction of the silyl group, $-Si(CH)_3$, into a molecule, usually in substitution for active hydrogen, but occasionally in replacement of the metal

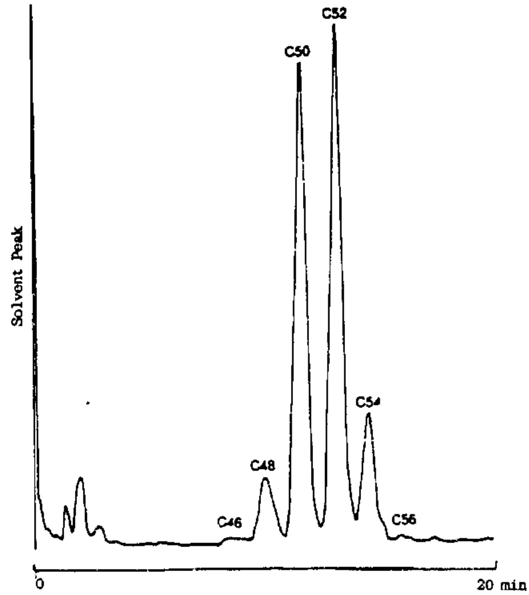


Figure 3. Chromatographic trace of TG of Palm Oil analysed on a 0.46 m x 3 mm glass GLC column packed with 3% OV-1 (PORIM)

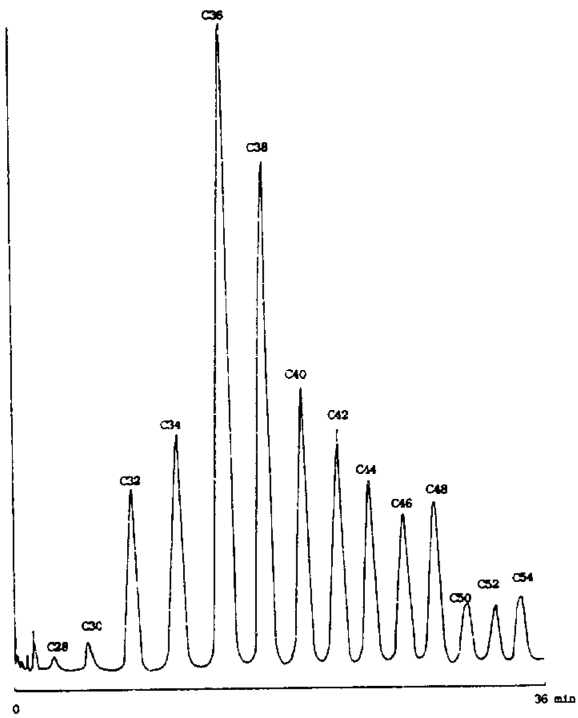


Figure 4. Chromatographic trace of TG of Palm Kernel Oil analysed on a 0.46 m x 3 mm glass GLC column packed with 3% OV-1 (PORIM)

component of a salt. The replacement of the active hydrogen by the silyl group reduces the polarity of the compound and decreases the possibilities of hydrogen bonding. Consequently, where there is marked intermolecular bonding in the parent compound, the silylated derivative is usually more volatile. Further, stability is enhanced upon silylation by reduction of the number of reactive sites with active hydrogen.

Derivatization is necessary for good chromatographing of the fatty acids, mono- and diglycerides, because the hydroxyl groups are prone to adsorption and generally cause tailing problems. Underivatized MG also give rise to additional peaks corresponding to the respective DG and TG. Underivatized DG give rise to peaks corresponding to TG. Only after derivatization of MG and DG do the added peaks not appear. The conclusion drawn is that the MG and DG decompose and rearrange on the column or in the injection port to form higher glycerides.

The silylation of oils for GC analysis is easily carried out by mixing the oil with trimethyl silyl imidazole (TSIM) or N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) at about 40°C. Derivatization of the hydroxyl groups on the MG and DG gives rise to the corresponding trimethyl silyl (TMS) ethers. The fatty acids react with BSTFA to form the respective TMS esters.

A primary standard consisting of palmitic acid, monopalmitin, dipalmitin, distearin, tricaprin, tripalmitin and tristearin is silylated and injected into the GC before analysis of actual oil samples. Quantification and identification are done by comparison with the silylated primary standard and normalization with response factors using tricaprin as the reference peak and internal standard. Tricaprin is chosen as an internal standard as it is a TG not present in most of the samples analysed, and because it does not interfere with other typical glyceride components. It has a retention time approximately midway in the analysis. Goh *et al.* (1985) determined the MG and DG in palm oil products by silylation with TSIM in pyridine using n-triacontane as the internal standard. The operating conditions for the analysis of partial glycerides are shown in *Table 3*.

Serious sources of error in the analysis of MG and DG arise when attempting to analyse low levels (less than 5%). The worst problem in some systems is the decomposition of TG in the injector block, yielding DG and perhaps MG. DG formed in this way can account for up to 2% of the actual peak area detected.

ANALYSIS OF FATTY ACID ISOMERS

The most widely used methods for analysis of isomeric straight chain unsaturated fatty acids are (a) infrared absorption for *trans* unsaturation and (b) oxidative cleavage by ozone or permanganate-periodate followed by analysis of cleavage products for double bond location. However the former is usually applied to oils and the latter to isolated fatty acids, or fractions of total fatty acids. The analysis and the physical properties of isomeric fatty acids are quite thoroughly reviewed by Scholfield (1979).

a) Direct GC Analysis

The determination of *cis-cis* polyunsaturated fatty acid (PUFA)

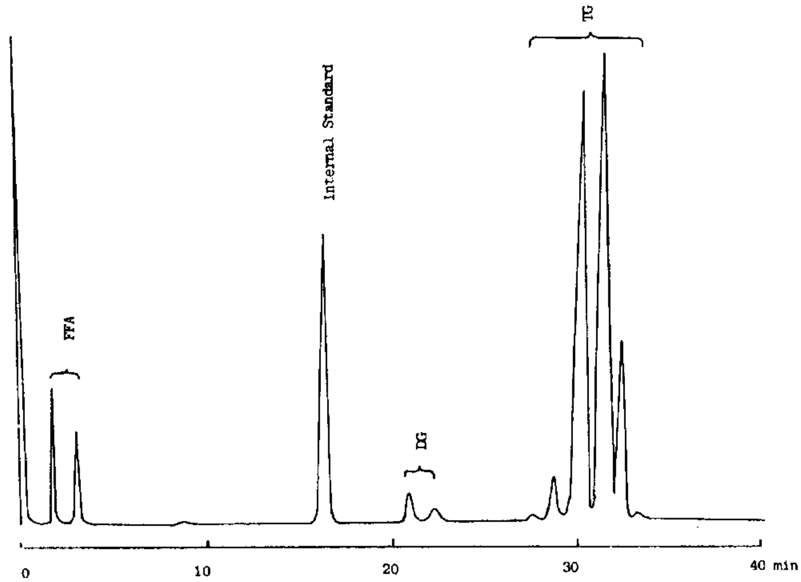


Figure 5. Chromatographic trace of FFA and Glycerides of Palm Olein analysed on a 0.46 m x 3 mm glass GLC column packed with 3% OV-1 (PORIM)

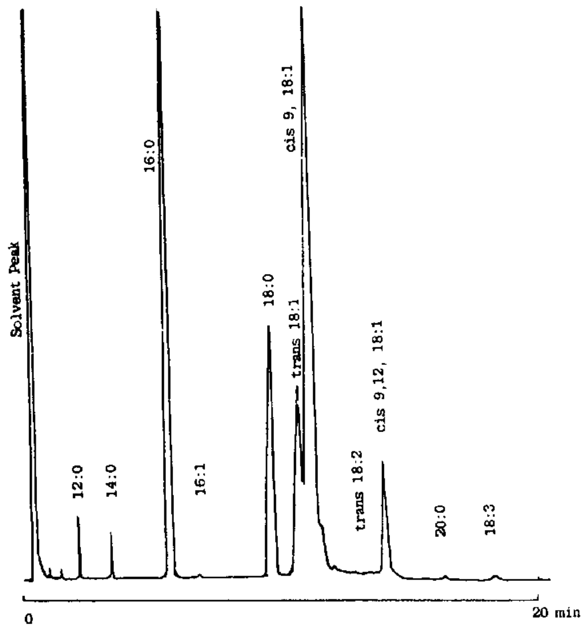


Figure 6. Chromatographic trace of FAME of a Vanaspati analysed on a 60 m fused silica capillary GLC column coated with SP 2340 (PORIM)

TABLE 3. OPERATING CONDITIONS FOR ANALYSIS OF PARTIAL GLYCERIDES

Column	:	Glass, 0.46 m x 3 mm i.d.
Stationary Phase	:	3% OV-1 on Gas Chrom Q 100/120 mesh size
Programme	:	150°C - 350° C @ 6°C/min 350°C for 8°C/min
Detector Temperature	:	370°C
Injector Temperature	:	370°C
Carrier Gas	:	80 ml/min, Nitrogen

content or essential fatty acid (EFA) content is related to the analysis of fatty acid composition. GC can provide information about both geometric and positional isomeric fatty acids, but, in complex mixtures, peaks overlap and individual components are sometimes not separated. It is therefore useful only for analysing simple mixtures for *cis* and *trans* isomers or positional isomers. With more complex mixtures, preliminary separation, for example by thin-layer chromatography (TLC: see below) may be necessary to avoid overlapping peaks. Natural oils are usually simple mixtures, but catalytic hydrogenation as practised in the production of most edible oils moves double bonds up and down the chain in both *cis* and *trans* configuration.

Cyanosilicones are now among the most widely used stationary phases for *cis-trans* isomer separations. On cyanosilicones, *trans* ethylenic fatty acids elute before the corresponding *cis* isomers, hence palmitelaidate before palmitoleate, elaidate before oleate, *etc.* Such separations have been reported by Ottenstein *et al.* (1976, 1977), Perkins *et al.* (1977) and Walters *et al.* (1975); using 6 and 20 foot columns packed with SILAR 10C, SP 2340, OV-225 and OV-275.

Excellent separations have been made on SILAR 10C and SP 2340 capillary columns by Heckers *et al.* (1977) and Enig *et al.* (1983). *Figure 6* shows the separation of *cis* and *trans* fatty acid isomers of Vanaspati on an SP 2340 capillary column. Capillary, or open tubular columns are known to have the high efficiencies required for the separation of closely spaced peaks. The major advantage of capillary columns compared to packed columns is the total number of plates obtainable. Plates per foot of column length are comparable with packed columns, but much longer capillary columns are usable because they have higher permeabilities, *i.e.* they are 'open tubes' with small resistance. Therefore, they are more efficient than packed columns and are recommended for separation of complex mixtures, and the resolution of closely related isomers.

The small sample requirements and low flow rates of capillary columns demand carefully designed instrumentation. Capillary columns are used with a special injection system which splits the injected sample and passes a small percentage (*e.g.* 1%) to the column and vents the rest to the atmosphere. These splitters are necessary because of the physical difficulty of accurately measuring and directly injecting the very small amount of sample which the column can handle.

The detector must have high sensitivity because of the small sample, must be capable of fast response because of narrow peaks, and must have low volume to avoid band broadening. To eliminate or minimize band broadening after the column, a make-up gas is required to increase the linear velocity and decrease the residence time of the components as they are swept into the detector. With a FID, hydrogen gas can serve the dual purpose of fuel and make-up gas.

Capillary columns are generally used under isothermal conditions (see example in *Table 1*). While they can be programmed, this must be done with extreme care and over a narrower range than with a packed column. This is another consequence of the low liquid phase content. Capillary columns are rather expensive and they represent a considerable investment and deserve proper handling.

An alternative to the expensive capillary column is the wide bore (0.75 mm i.d.) capillary column. GLC users with no experience in capillary GLC can easily convert their packed column GLC system to wide bore capillary column use. This can be done quickly and inexpensively. Wide bore capillary columns are capable of providing the resolving ability and inertness of narrow bore capillary columns with the added advantage of a larger sample capacity and packed column-like flow rates. In addition, no further modifications of the injection system, such as the splitters, are required.

Other stationary phases which have been successfully used for FAME isomeric separations include polyphenyl ether (Scholfield *et al.*, 1971), butanediol succinate (BDS) and SILAR 5CP (Ackman, 1974), Carbowax 20M and 1540 (Flanzy *et al.*, 1976 and Panos, 1965), and SE 30 (Holmbom, 1977).

In addition to the methods described for GC of fatty acid isomers, derivatives of the esters may be prepared which are more easily separated on packed columns. Epoxides of esters can be prepared with peracetic acid and then separated on a packed column as studied by Emken (1971, 1972).

b) Indirect GC Analysis

The combination of gas chromatography-mass spectroscopy (GC-MS) is a powerful tool for separation and identification of components. The GC separates the components which are then further analysed and identified by MS. A single isomer will produce a very complex mass spectrum and because of this, MS is more often used to determine double bond location in a single compound than for quantitative analysis of mixtures. Suitable derivatives such as epoxides from methyl esters and methoxy derivatives must be prepared to determine position and configuration of double bonds by GC-MS. The use of GC in conjunction with MS is becoming more popular and GC-MS of lipids has been reviewed by Perkins (1975).

A combination of argentation TLC followed by GLC on a capillary SILAR 10C column is used for the quantitative determination of linolelaidic acid by Leatherhead Food RA. Complexing of silver ions with unsaturated organic compounds has been studied since 1983. *Cis*-double bonds complex more strongly than *trans*, and the strength of the complex is also affected by stearic factors near the double bond. Argentation TLC, in which the plate is coated with a silica-silver nitrate mixture, is especially useful for derivatives of

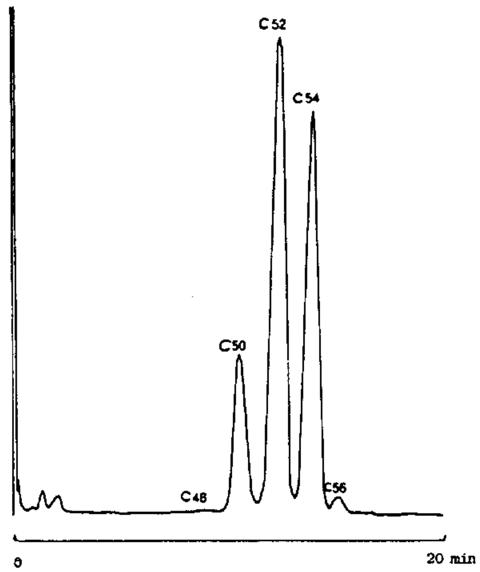


Figure 7. Chromatography trace of a Malaysian Cocoa Butter analysed on a 0.46 m x 3 mm glass GLC column packed with 3% OV-1 (PORIM)

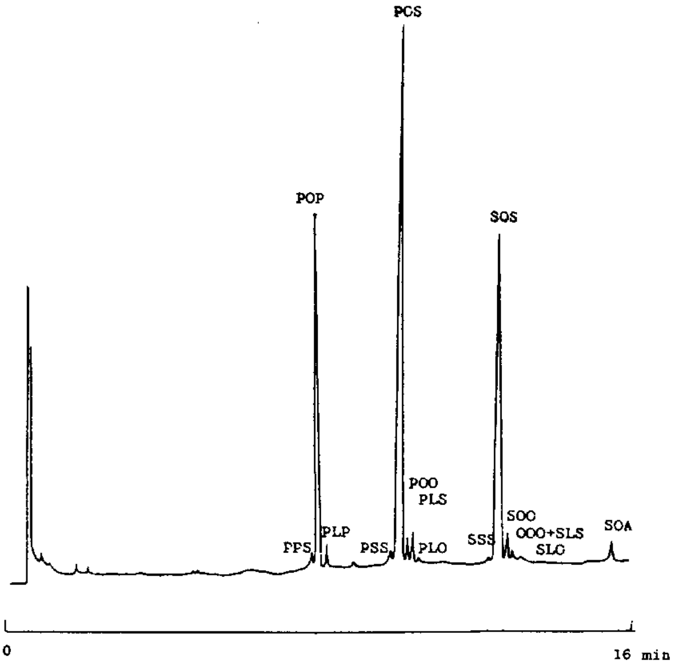


Figure 8. Chromatographic trace of TG of Cocoa Butter analysed on a 25 m x 0.25 mm fused silica capillary GLC column coated with phenylmethylsilicone (reproduced from Geeraert et al. *1987).*

isomeric fatty acids. It depends on the number of double bonds, their geometric configuration and their position. The more unsaturated compounds move more slowly on the plate and separations based on number of double bonds are readily achieved. Separation of *cis* and *trans* isomers is also readily done. The separated isomers can be scraped from the TLC plate and recovered for further separation on the GLC.

ANALYSIS OF THE FATTY ACID COMBINATIONS IN TG

The separation of unsaturated TG with different degrees of unsaturation, unlike that of unsaturated methyl esters, cannot be carried out on a packed column because the polar stationary phases such as diethylene glycol succinate (DEGS) have too high a bleed rate above 250°C. The partial separation of tristearin (S.S.S.) from triolein (O.O.O.) on Apiezon L and JXR phases has been reported by Litchfield (1972) but the type of separation obtained is not satisfactory for the complex mixtures found in nature.

The TG composition of oils can be determined according to fatty acid types by combining the results obtained separately from silver nitrate TLC and GLC. In this analysis, the TG are first isolated from the oil by column chromatography following which they are separated and quantified according to unsaturation by silver nitrate TLC before subsequent analysis by GLC. The results from TLC and GLC enable the composition of the oils to be computed on a molecular basis in relation to fatty acid types.

TG are probably the highest boiling compounds analysed by GLC. The use of capillary columns for TG analysis has been limited partly by poor sample introduction techniques. TG vapour pressures are too low to allow reasonable evaporation in the injector, accounting for insufficient elution from a syringe needle. A study on the evaluation of injection techniques for TG in capillary GLC by Grob (1979) reported that cold-on-column injection gave by far the most reproducible results normalized on internal standards.

TG differing in unsaturation can be resolved on a capillary column coated with a phenylmethylsilicone gum stationary phase as reported by Geeraert *et al.* (1987). Besides a carbon number separation, the TG can be separated according to the different combinations of saturated and unsaturated fatty acids in them. The phenylmethylsilicone gum is stable up to 370°C, which allows for routine analysis of fats and oils. Using this phase, TG such as S.S.S., S.O.S. and O.O.O. are separated but S.O.S., S.L.S and S.Ln.S. elute in one peak. The capillary columns usually used are 25 m x 0.25 mm fused silica. A temperature programme from 340 to 355°C is typical of TG analyses by the GLC capillary technique. *Figures 7* and *8* illustrate the advantage of capillary GLC analysis of TG as compared with packed columns.

Note. The following abbreviations are used: M, myristic acid 14:0; P, palmitic acid 16:0; O, oleic acid 18:1; S, stearic acid 18:0; L, linoleic acid 18:2;' Ln, linolenic acid 18:3; A, arachidic acid 20:0.

DETERMINATION AND IDENTIFICATION OF STEROLS

The standard method for the identification of sterols in animal and vegetable oils and fats by GLC is described in IUPAC Method 2.403. The method can be modified to allow the various sterols present to be determined quantitatively. The procedure involves separation of the unsaponifiable matter in the sample after saponification and isolation of the sterols from the unsaponifiable matter by TLC, followed by analysis of the isolated sterols by GLC. The position of the sterol fraction is identified by comparison with a reference spot of cholesterol. The GLC identification of the sterols is carried out on a column 2 to 4 mm i.d. and 180-200 cm in length, packed with an inert support coated with 2-5% of methyl silicone stationary phase, at an isothermal temperature of about 230 to 240°C. For quantitative analysis pure sterols must be used as reference standards.

Sterol analysis can also be carried out by the method described in Part 1 by Downes (1982), which is actually equivalent to ISO 6799: 1983. In this method, the isolated sterols are determined by GLC as their trimethyl silyl (TMS) ethers on an OV-17 column.

Tocopherols and sterols are commonly determined separately, but it is possible to determine them in the same analysis since they are both found in the unsaponifiable fraction, have a hydroxyl group and are separable by GLC under similar conditions. A method was reported by Slover (1983) for the simultaneous determination of tocopherols and sterols by quantitative capillary GC. The procedure involved saponification of the sample followed by extraction of the unsaponifiable materials with cyclohexane. TMS ethers of both the compounds were obtained by reaction with BSTFA/TMCS mixture before being chromatographed on a 50 m X 0.25 mm capillary column coated with Dexil 400.

DETERMINATION OF ANTI-OXIDANTS IN OILS

Most methods in use for the determination of anti-oxidants such as BHA (mixture of 2- and 3-tert-butyl-4-hydroxyanisole) and BHT (3,5-di-tert-butyl-4-hydroxytoluene) are tedious, involving an extraction step together with a separate detection procedure. The methods described have relied on steam-distillation, solvent extraction, or a partitioning procedure, followed by fluorescence, TLC, gel permeation chromatography (GPC), high performance liquid chromatography (HPLC) and GLC.

A report by Fry *et al.* (1982) described a rapid GLC method for the determination of BHA and BHT. This technique relies on the use of a GLC equipped with a pre-column of silanized glass wool sited in the injector oven. Fat samples, dissolved in ethyl acetate containing an internal standard of biphenyl are directly injected into the pre-column. The fat is entrapped on the glass wool while the anti-oxidants are volatilized quantitatively and carried by the carrier gas into a 3% OV-1 column. The detection limit was reported at about 2ppm for each anti-oxidant but accuracy of quantification was poor below 10 ppm. The results obtained were accurate and precise up to levels of BHT and BHA of 250 ppm.

Min *et al.* (1982a) also described a GC method for determination of BHA, BHT and TBHQ (tert-butyl-hydroxyquinone). The method was reported to be accurate down to 10 ppm.

DETECTION OF CONTAMINATION

Among the possible sources of oil contamination are heat transfer media used during refining. This can result from pin-hole leaks in the heat exchanger or the deodorizer. In 1973, a shipment of rapeseed oil accidentally contaminated with a heat transfer medium containing biphenyl and biphenyl ether was shipped from Japan.

One of the heat transfer media used in refineries for deodorization of palm oil is a mixture of biphenyl (73.5%) and phenyl ether (26.5%), known as Dowtherm. Biphenyl is reported to cause paralysis, central nervous system depression and convulsion in experimental animals. Phenyl ether is also reported to be toxic and exposure of longer than 15 minutes at greater than 2 ppm concentration in air is known to cause irritation and nausea.

Oils containing biphenyl and phenyl ether can be analysed by GLC after extraction of unsaponifiable material, by further column chromatography, steam distillation, or solvent partition. Min *et al.* (1982b) reported a simple GLC method without prior time-consuming steam distillation or extraction.

The method used by Fry *et al.* (1982) for detection of BHT and BHA has been used in the PORIM laboratory for the rapid detection of Dowtherm and xylene contamination in palm oil and palm kernel oil respectively.

The Chick Edema Factor is presumed to be a pesticide residue. It is a mixture of hexa, hepta, and acetachlorodibenzo-p-dioxins. As many pesticides contain organic chlorine, analyses have utilized GLC with electron capture detectors to attain the sensitivity needed for detection of these trace substances. The AOAC (1984) proposes a method where the contaminated oil is first treated with sulphuric acid and then extracted with petroleum ether. The extract is then purified by passing through an aluminium oxide column and further treated with sulphuric acid before GLC analysis using an ECD. The column used is 1.8 to 2.7 m in length x 4 mm i.d., packed with 2.5% SE 52 or other polysiloxane stationary phases on a 60-120 acid washed (AW)-silanized diatomaceous earth. Column temperature is kept at 200° C ± 1° C and carrier gas flow is adjusted to obtain an aldrin peak (internal standard) at 1 to 1.5 minutes. Peaks with retention times relative to aldrin (Ra) between 8 and 45 indicate the presence of Chick Edema Factor.

Other less common uses of GC for analysing oils and fats include the determination of residual hexane in oil (AOCS, 1989; Hirama *et al.* 1977; Dupuy *et al.*, 1975), determination of citric acid and its decomposed products (Miyakoshi *et al.*, 1977) and the determination of free fatty acids (Chapman *et al.*, 1979).

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