Potential Source of Sterols from a Palm Kernel Oil Methyl Ester Residue of an Oleochemical Plant

T L Ooi, W L Leong and Radzuan Jamaludin *

RINGKASAN

Kandungan dan komposisi satu pecahan baki metil ester minyak isirong yang didapati dari sebuah kilang oleokimia telah dikaji dengan cara TLC, GC dan GC/MS. Bahan-bahan utama yang terkandung adalah ester dan gliserida (lebih kurang 95%) manakala bahan-bahan tak disaponifi terdiri daripada hidrokarbon, triterpene alkohol, beberapa sebatian polar dan sterol. Jumlah tak saponifi didapati lebih kurang 4.2%. Sterol terdiri lebih kurang 1.7% daripada sampel tersebut dan keadaannya ialah dalam bentuk ester. Keputusan ini menunjukkan pecahan baki ini adalah suatu punca sterol yang kaya. Sterol utama ialah β-sitosterol (lebih kurang 78%) dan sterol yang lain adalah campesterol, stigmasterol, cholesterol dan lain-lain. Dengan cara penyulingan molekul, kepekatan sterol dapat ditinggikan ke takat lebih kurang tiga kali lebih pekat daripada sampel asal.

INTRODUCTION

Palm kernel oil is obtained from the kernel of oil palm fruits. *Table 1* shows a typical fatty acid composition of the oil while *Table 2* shows a typical composition of the unsaponifiables of the palm kernel oil. The unsaponifiables constitute about 0.4 % of the oil and consist of mainly hydrocarbons, aliphatic alcohols, triterpene alcohols, methyl sterols and sterols. (Norman, O.V. Sonntag, 1979).

In view of the greater demand for lauric acid methyl esters as compared to the longer chain length methyl esters such as palmitic acid methyl ester or oleic acid methyl ester, the local oleochemical industry uses mainly palm kernel oil as the feedstock for the esterification process to produce lauric acid methyl esters. As a result of this, a residue, which is a by-product from this process is obtained. This fraction constitutes about 6% of the esters produced of which the main bulk is still the methyl esters or the saponifiables. However, it is expected that most of the unsaponifiables of palm kernel oil are concentrated in the residue. Normally, this fraction is discarded unused. So far, no report has been made on the composition of this fraction. Thus, it is important for us to study the composition of this fraction so as to provide a better understanding of the minor components present in palm kernel oil. Furthermore, it will be necessary to determine if any valuable components could be recovered from this unused residue.

This paper describes our studies on the identification of the components present, the composition of the residue and the recovery of sterols from the fraction.

EXPERIMENTAL

Materials

The following materials were used in the experiments.

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TABLE 1. FATTY ACID COMPOSITION OF PALM KERNEL OIL (wt%), GLC

Fatty Acid Composition	wt (%)
Caproic C6	0.3
Caprylic C8	4.4
Capric C10	3.7
Lauric C12	48.3
Myristic C14	15.6
Palmitic C16	7.8
Stearic C18:0	2.0
Oleic C18:1	15.1
Linoleic C18:2	2.7
Others	0.1

TABLE 2. COMPOSITION OF UNSAPONIFIABLES OF PALM KERNEL OIL (separated by preparative TLC)

Fraction	%
Fraction 1 (less polar compounds)	48
Fraction 2 (triterpene alcohols)	18
Fraction 3 (methylsterols)	1
Fraction 4 (sterols)	33

TABLE 3. FATTY ACID COMPOSITION OF R1ME (wt %), GLC

Fatty Acid Composition	wt (%)
Capric C10	-
Lauric C12	6.0
Myristic C14	5.0
Palmitic C16	10.4
Stearic C18:0	11.9
Oleic C18:1	54.6
Linoleic C18:2	7.0
Linolenic C18:3	0.8
Arachidic C20:0	1.1
Others	3.2

Sample : Residue (R1ME) (S1024) was obtained from Henkel Oleo chemicals (M) Sdn. Bhd.
All solvents and chemicals used were of analytical grade.

PROCEDURES

Saponification

5.00 g of homogenized sample R1ME was weighed in a round bottom flask and 30 ml of ethanol(95%) and 5 ml of aqueous potassium hydroxide (MERCK) were added to the sample. A few pieces of anti-bumping granules were added and the mixture was refluxed for one hour. 40 ml of hot distilled water and 40 ml of cold distilled water were added. The mixture was cooled to room temperature before being transferred to a separating funnel. The remaining sample in the flask was washed and transferred completely into the funnel with 50 ml of ethanol (95%). 50 ml of the petroleum ether was added and the flask was shaken vigorously for one minute and was allowed to settle until both layers were clear. The petroleum ether layer(top layer) was collected. The extraction was repeated using 50 ml of petroleum ether every time for five more times.

The combined extract was washed with 25 ml of distilled water containing 10% ethanol. After each wash, the combined extract was shaken vigorously and the water layer (bottom layer) was discarded. The washing was discontinued when the wash was neutral to phenolphthalein. The ether extract was dried with anhydrous sodium sulphate and it was then filtered and the petroleum ether was removed using the rotoevaporator. The unsaponifiable matter was then analysed using thin layer chromatography(TLC).

Preparation of Thin layer Chromatography(TLC)

The unsaponifiables of the sample R1ME are separated on the Kieselgel 60F (Merck) glass plates (20 cm x 20 cm). The developing solvents used were hexane: diethyl ether (4:1 v/v).

One side (about 2 cm wide) of the plate was sprayed with 2,7-dichlorofluorescein and was examined under UV light. Different fractions were marked, scraped and extracted using diethyl ether. Five fractions were obtained and each of these, except Fraction V, were analysed by Gas Liquid Chromatography (GLC) under the following conditions:-

GC Model : Shimadzu GC-9APTF fitted with FID detector.
Column : Packed-column. 3% OV-17, 2.5 M x 3 mm.
INJ/DET Temperature : 330°C
Nitrogen flow rate : 40 ml/min
Column Temperature : 270°C or 280°C

Recovery of sterols from sample RIME by molecular distillation

The sample R1ME was molecular distilled at various temperatures and the conditions were as stated in *Table 9.*

Model of apparatus : Shibata MS-300 (Falling film type molecular still)

Vacuum pressure : 20-40 x 10^{-3} mmHg
Rotation per minute of wiper : 160-280

The various fractions (distillates) collected were analysed for their fatty acid composition by GC. 15.0 g of the residue obtained was crystallized using acetone as the solvent and was analyzed by TLC for the presence of sterols. Another 5.0 g of the residue was saponified and the unsaponifiable matter was analyzed by GC.

RESULTS AND DISCUSSION

The residue (R1ME) is a black liquid with some brownish precipitate settled at the bottom on storage at room temperature. A typical sample is shown in *Figure 1.* The fatty acid composition of the residue (R1ME) was determined and is shown in *Table 3.* It has quite a similar fatty acid composition as palm kernel oil except that R1ME contains higher concentrations of longer chain fatty acids.

Figure 1. A Typical Sample of R1ME

The unsaponifiable matter of the residue obtained was brownish in colour and the total unsaponifiables account for about 4.2% (w/w) as compared to that of the total unsaponifiables of the palm kernel oil, which are usually around 0.4% (w/w).

With this developed solvent system on silica gel, the unsaponifiables were well separated into five fractions including a fraction at the origin. Fraction I consists mainly of less polar compounds. Fraction II consists mainly of hydrocarbons and some triterpene alcohols. Fraction III consists mainly of desmethyl sterols while Fraction IV consists mainly of sterols. Fraction V are mainly polar compounds. *Table 4* summarizes the percentage composition of the residue (R1ME).

Four of the fractions of the unsaponifiables of the residue (R1ME) obtained from preparative TLC were further analysed by GC and GC/MS. Identification of the compounds was based on the mass spectra obtained from GC/MS analysis (F.W. McLafferty, 1966), R_f value from TLC analysis and retention time from GLC analysis. From the interpretation of the mass spectra, it indicates that Fraction I consists of some hydrocarbons and possibly some esterified sterols. Fraction II consists of at least 20 compounds and were mainly hydrocarbons ranging from $C_{23}H_{48}$ to $C_{32}H_{66}$. The relative percentage of these hydrocarbons is shown in *Table 5.* Fraction III consists of at least 25 compounds, which are mainly triterpene alcohols and desmethyl sterols. The sterols present were found in Fraction IV and the relative percentages of the sterols are shown in *Table 6.* As can be seen from *Table 4,* the sterol fraction constitutes about 1/3 of the total unsaponifiables of the residue R1ME. β-sitosterol was the major sterol constituent; same as for most of the other vegetable oils. The other minor sterol components were cholesterol, campesterol,

TABLE 4. PERCENTAGE COMPOSITION OF THE RESIDUE (R1ME), WT %

Methyl Esters and Saponifiables		**95.8%**
Total unsaponifiables [separated by preparative TLC]		
Fraction 1	0.8%	
Fraction 2	0.7%	
Fraction 3	0.4%	4.2%
Fraction 4	1.7%	
Fraction 5	0.6%	

TABLE 5. PERCENTAGE COMPOSITION OF HYDROCARBONS IN FRACTION II

Hydrocarbon	Molecular Weight	% wt [by GLC]
$C_{23}H_{48}$	324	1.7
$C_{24}H_{50}$	338	4.1
$C_{25}H_{52}$	352	7.4
$C_{26}H_{54}$	366	10.4
$C_{27}H_{56}$	380	13.7
$C_{28}H_{58}$	394	13.1
$C_{29}H_{60}$	408	10.4
$C_{30}H_{62}$	422	8.6
$C_{31}H_{64}$	436	5.2
$C_{32}H_{66}$	450	3.0
Others	-	22.4

TABLE 6. PERCENTAGE COMPOSITION OF STEROLS, (FRACTION IV)

Sterols	%
Cholesterol	0.8
Campesterol	9.8
Stigmasterol	14.1
Sitosterol	72.0
Isofucosterol + Others	3.3

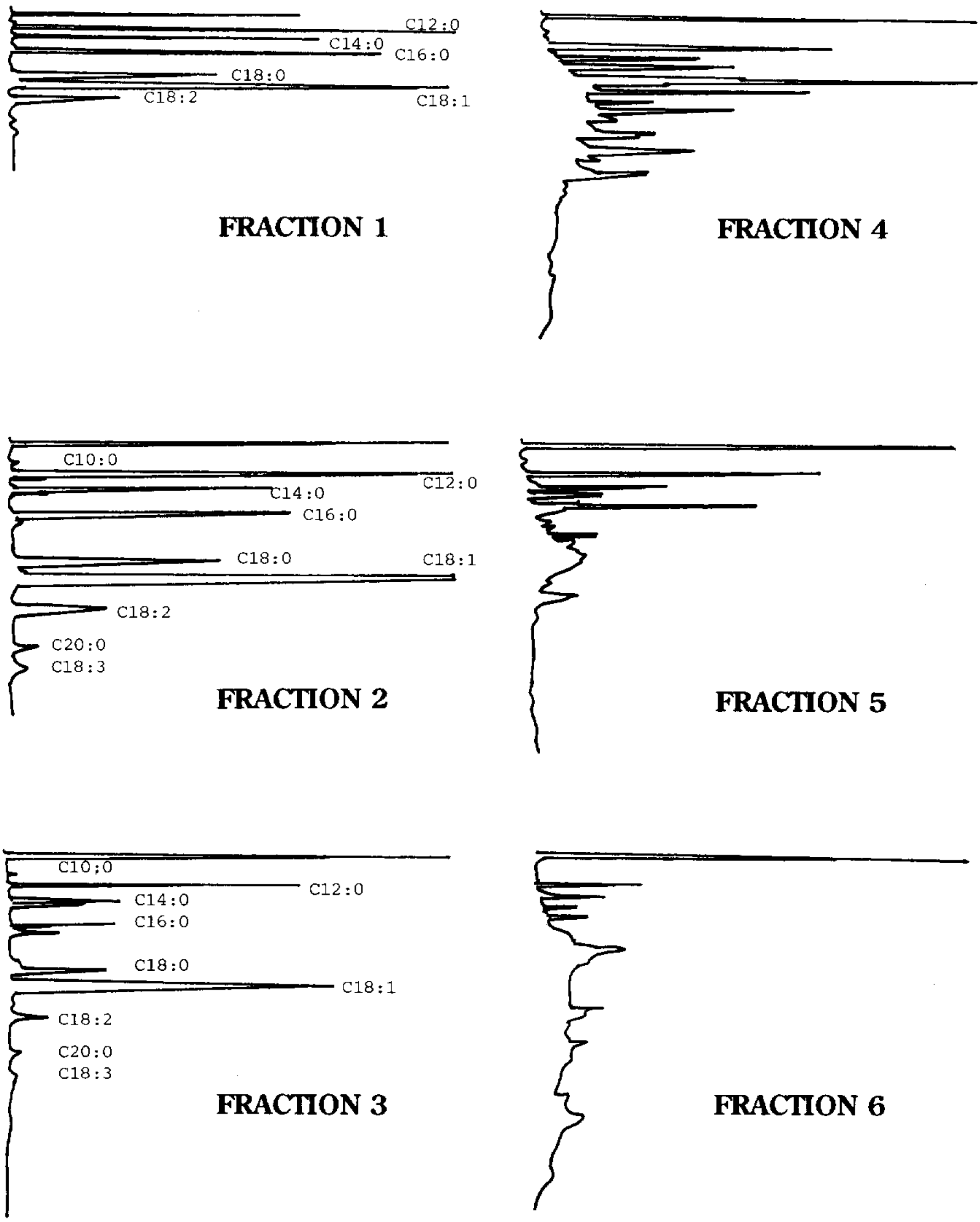


Figure 2. Gas Chromatograms of Fractions 1 to 6 by Molecular Distillation.

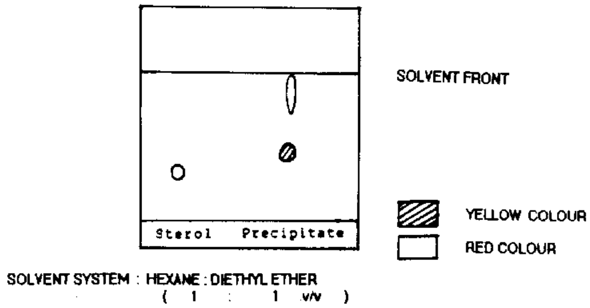


Figure 3. Analytical TLC of the Precipitate of the Residue (Molecular Distillation) Obtained by Solvent Crystallization.

stigmasterol and isofucosterol. The more polar components located at the base line, however, have not been identified.

From our observations, it is interesting to note that the sterols present in the residue R1ME are not present as free form but present as esterified sterols. It is observed that on crystallisation of the R1ME in acetone solvent, a brownish precipitate of 1.2% (w/w) was obtained. This precipitate was suspected to be esterified sterols rather than free sterols from evidence that when the precipitate was subjected to TLC analysis using silica gel G plates with solvent system: hexane : diethyl ether = 1 : 1(v/v), the major band was obtained near the solvent front and had not the same R_f value as the authentic free sterols. On the other hand, not much sterols were present in the mother liquor. Furthermore, when the band near the solvent front was scraped off from the preparative TLC, saponified and acidified, free sterols were released. The GLC analysis showed that the sterols released were the same as that identified in Fraction IV. No significant free sterol could be obtained in the band having same R_f value as the free stigmasterol. The percentage composition of sterols in the crystallised fraction obtained from acetone crystallisation is shown in *Table 7*. Therefore, the results clearly indicate that most of the sterols present in R1ME are in the esterified form. It is not clearly known whether all the esterified sterols were formed during the esterification process or were originally present naturally in the palm kernel oil. It may therefore be interesting to investigate the chemical nature of the sterols present in the palm kernel oil, *i.e.* whether they exist in a free or esterified state. Some studies have been reported in this area (Gordon M.H. and Griffith R.E. 1992). Generally, the composition data of 4-desmethylsterols of palm kernel oil have been obtained by analysis of the sterolic fractions after saponification of the oil. This will also saponify any sterol esters initially present. Esterified α-tocopherol and tocotrienols in palm oils were successfully separated from free tocopherols and tocotrienols by TLC (Kato *et al.*, 1981).

Recovery of sterols from residue, R1ME

From the studies, it was clearly shown that sterols constitute about 1/3 of the total unsaponifiables (about 4.2%) present in R1ME. Therefore, this residue is a good source of sterols particularly ß-sitosterol. Sterols are valuable products and are widely used in synthesis of steroid hormones. *Table 8* shows some of the major applications of some common sterols.

Various methods were attempted to recover sterols from R1ME and these included solvent crystallization, column chromatography and molecular distillation.

Out of the methods attempted till now, molecular distillation was found to be the more promising and is described here. Molecular distillation has been used for the purification of fats, vitamins, food additives, pharmaceuticals, perfumes and other heat sensitive compounds (Klaus. J. Erdeg., 1983). Using this technique, R1ME was subjected to distillation at various temperatures. As a result, six distillated fractions and a black viscous residue (F7), largely having different characteristics, were obtained. The percentage of the residue and distillates which were collected in each distillation step is shown in *Table 9.*

As mentioned earlier, the six fractions of distillates collected had quite different characteristics. The first distillate, being the predominant among the six fractions, had a percentage of weight that amounted to 15.5% of the samples used. It remained in the liquid form at ambient temperature and was slightly yellow to almost colourless. In comparison with the first fraction collected, the percentage of weight of the second fraction was much lower, that is, 3.2% of the sample used. Similar characteristics as for the first fraction were observed for this fraction, except for its colour which was more yellow. The third and fourth fractions existed in solid form at room temperature. Both could be considered as being dark yellow and the amounts collected were relatively small compared to the earlier fractions. The weight of Fraction 3 was only 1.9% of the sample used whereas Fraction 4, being the least collected, amounted to only 1.1%. Fractions 5 and 6 which were collected at 220°C and 240°C respectively were both chromish yellow. They existed in the liquid form at ambient temperature.

The flow rate of each distillation step varied and is presented in *Table 9.* It depended upon the viscosity of the residue which was used as the sample for the subsequent distillation. A hair dryer was used to aid the flow of the viscous sample and thus increased the flow rate a little. The residue and distillates collected were also analysed using GC and TLC.

From the interpretation of the GC, it is noted that Fraction 1 (obtained by molecular distilling the sample R1ME at 120°C) contained merely the methyl esters in which oleic acid constituted the major fatty acid present. The composition of Fraction 2 is basically the same with Fraction 1. As a comparison between the two, percentages of the major fatty acids are shown in *Table 10.* The major difference observed is the higher percentage of linolenic acid (C18:3) in Fraction 2 as the temperature in which distillation was carried out was raised slightly higher. Besides, in the gas chromatogram for Fraction 2, there were a few peaks next to the peaks for C12:0, C14:0 and C16:0 methyl esters which have not been identified. These compounds are not really known, but they may be the monounsaturated methyl esters of the respective methyl esters.

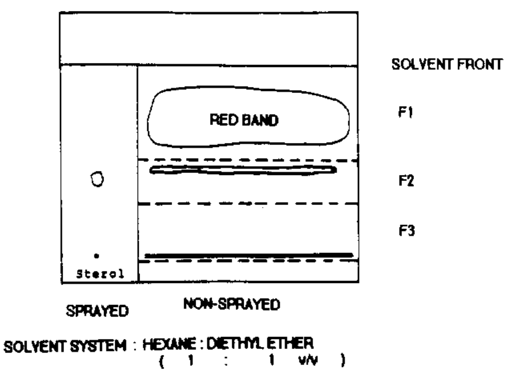
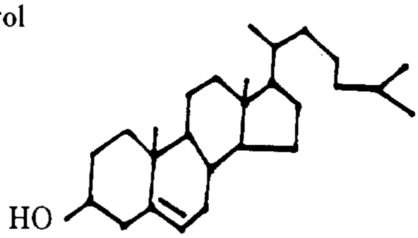
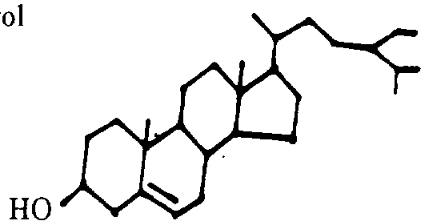
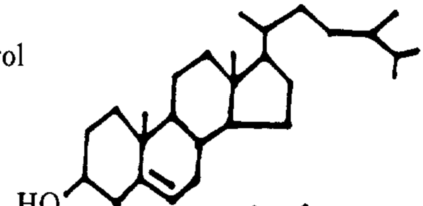
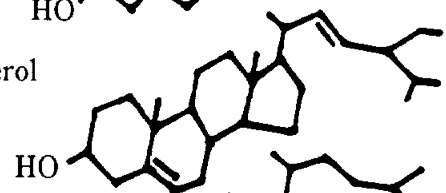
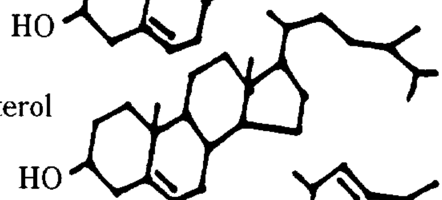
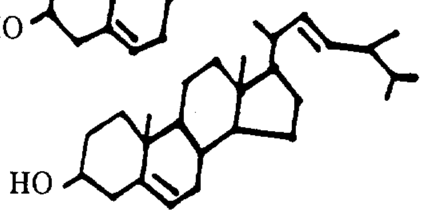


Figure 4. Preparative TLC of the Precipitate Obtained by Solvent Crystallization of the Residue (Molecular Distillation).

TABLE 7. PERCENTAGE COMPOSITION OF STEROLS CRYSTALLIZED FROM R1ME, WT % (by GLC)

Sterols	%
Cholesterol	2.3
Campsterol	2.7
Stigmasterol	6.7
ß-sitosterol	88.3

TABLE 8. MAJOR SOURCES AND APPLICATIONS OF SOME COMMON STEROLS

Sterol Type	Chemical Structure	Source of Raw Materials	Major Applications
1. Cholesterol		Lanolin fish oil Cow's Brain	Vitamin D_3, Steroid hormone, cosmetics, emulsifier, dye dispersing agent
2. Beta-sitosterol		Cottonseed oil, Corn oil, Rapeseed oil, Soyabean oil, Tall oil	Steroid hormone, cosmetics, emulsifier dye dispersing agent
3. Campesterol		Rapeseed oil, Corn oil, Soyabean oil	Steroid hormone
4. Stigmasterol		Soyabean oil, corn oil	Steroid hormone
5. Brassicasterol		Rapeseed oil,	Steroid hormone
6. Ergosterol		Yeast fungus	Vitamin D_2

The composition of Fraction 3 differs a little from the previous two fractions. The few peaks next to C12:0, C14:0 and C16:0 become more distinct and thus more of these unknown compounds were distillated as the temperature was raised to 160°C in this distillation step.

The GC trace of Fraction 4 shows that the compounds in Fraction 4 are not the methyl esters, but rather compounds with higher molecular weight and boiling point. The GC trace of Fraction 5 has a few front peaks which resemble the methyl esters. However, these are believed to be other compounds rather than methyl esters. Compounds with an even higher boiling point were distilled out in Fraction 6 was collected at 240°C. The GC chromatograms of fractions 1 to 6 are shown in *Figure 2.*

The different characteristics observed in Fractions 4, 5 and 6 when compared to the first three fractions are believed to be the methyl esters. Thus Fractions 4 to 6 are believed to have different applications.

15.0 g of the residue (Fraction 7) obtained from the molecular distillation was crystallized using 100ml of acetone. The dark brown precipitate obtained was analysed for its content using TLC. *Figure 3* represents the analytical TLC of the precipitate.

The elongated red spot observed in this chromatogram may be the hydrocarbons or the sterol esters while the yellow spot which had an R_f value quite similar to the free sterols was believed not to be the free sterols since the colour was different when subjected to the 2,7-dichlorofluorescein spray.

The precipitate was also separated on preparative TLC and the three bands obtained were scrapped off. The first two fractions were saponified and were observed by GC. The TLC chromatogram is shown in *Figure 4.* The results obtained after saponification of the two fractions were of great importance as a number of conclusions could be dr
from the analysis of the GC obtained.

The GC analysis showed that the ste
released in Fraction 1 (*Figure 5*) were
same as identified in Fraction IV of R1MI
discussed previously. The relative percent
of the different types of sterols in Fractior
is β-sitosterol (83.8), stigmasterol (9
campesterol (4.6) and cholesterol (1.9).

Therefore, β-sitosterol is the major
stituent in the residue (F7) obtained from
molecular distillation. From the results
tained so far, the elongated red spot obser
in the TLC (*Figure 3*) were the esterified
rols which upon saponification yield the
sterols as shown in *Figure 5.* This a
confirms our previous observations.

The gas chromatogram of saponi
Fraction 2 did not show any significant am
of sterols. The percentage of β-sitosterol
very low when compared to the amount
tained in the saponified Fraction 1. Thus,
only confirms that the yellow spot obse
in the thin layer chromatogram was an
known compound/compounds and not
free sterols as suspected initially. Saponi
tion does not have any effect on the
sterols and thus the GC of Fraction 2 sh
show the presence of sterols too, if
sterols were present.

5.0 g of the residue (Fraction 7) obta
at temperature 240°C was then sapon
and a yellowish precipitate was obtai
From the GC trace, it was observed that
sterols were present in the residue in qu
large amount. The relative % of the ste
are β-sitosterol (78.3), stigmasterol (
campesterol (2.8) and cholesterol (0.7).

Due to the negligible amount of ch
terol present in the residue, cholesterol i
can be used as an internal standard to
mate quantitatively the actual amount
sitosterol, stigmasterol and campesterol w
were actually present in the residue (F7

TABLE 9. THE PERCENTAGE (weight) OF VARIOUS FRACTIONS COLLECTED BY MC LECULAR DISTILLATION

Fraction	Temp. of distil-lation (°C)	Pressure [x10^{-3} torr]	RPM	Flow Rate (g/min)	Wt. of sample [g]	Residue Wt.	Residue %	Distillate Wt.	Distillate %
F1	120	30-40	174	0.47	101.3	82.1	81.0	15.7	15
F2	140	30-40	174	0.69	80.9	77.7	96.0	2.6	3.
F3	160	20-25	162-280	0.22	77.3	75.6	97.8	1.5	1.
F4	180	20-30	177-280	0.57	75.5	74.5	98.7	0.8	1.
F5	220	25-30	178	1.34	73.4	71.8	97.8	1.3	1.
F6	240	25-30	200	0.57	70.1	65.4	93.3	4.6	6.

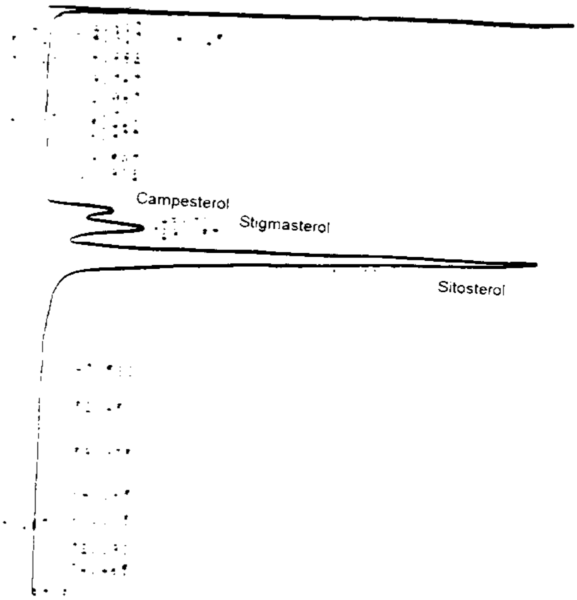
TABLE 10. FATTY ACID COMPOSITION OF FRACTION 1 AND FRACTION 2 FROM M(LECULAR DISTILLATION

Fatty Acid Composition	Fraction 1	Fraction 2
Capric C10	0.1	0.1
Lauric C12	5.4	6.4
Myristic C14	5.3	4.9
Palmitic C16	10.6	8.9
Stearic C18:0	13.1	13.7
Oleic C18:1	54.7	52.4
Linoleic C18:2	8.1	7.6
Linolenic C18:3	0.9	1.8
Arachidic C20:0	1.1	2.6
Others	0.7	1.6

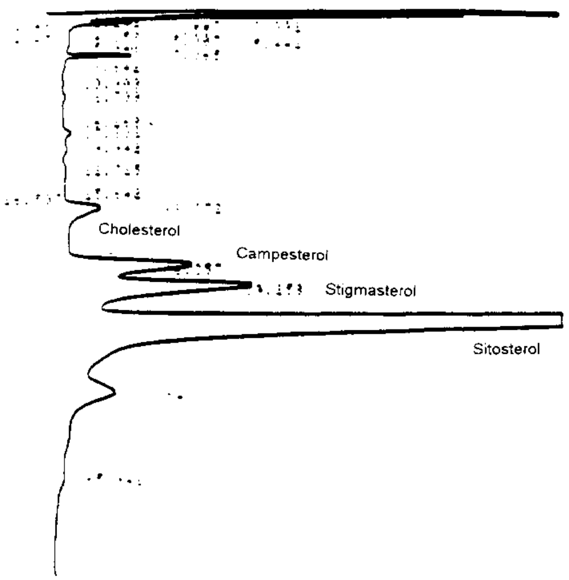
tained from the molecular distillation. 5300 parts per million (ppm) of cholesterol were added to 0.41 g of the unsaponifiable matter obtained from the residue. By GC analysis and calculations, the actual amount of the different sterols in the residue (F7) are as follows:

Type of sterols	Percentage of sterols in the residue (F7)(wt %)
β-sitosterol	4.4
Stigmasterol	0.6
Campesterol	0.3

*Corrections were made for the cholesterol



FRACTION 4 OF THE UNSAPONIFIABLE MATTERS FROM SAMPLE RESIDUE R1ME



SAPONIFIED FRACTION (F1) FROM PREPARATIVE TLC

Figure 5. Trace of Sterols.

Therefore, it is observed that by the molecular distillation method, the sterols contained in the sample R1ME could be concentrated to more than three times the original .

CONCLUSION

From the result of these experiments, it can be concluded that the residue R1ME is a potential source of sterols particularly β-sitosterol. The unsaponifiables present in the residue (RIME) were about 4.2% and the sterol fraction constitutes about 1/3 of the total unsaponifiables. The major sterol present was β-sitosterol (78.0%). The other compounds present are the hydrocarbons, triterpene alcohols and others. The main bulk is still the methyl ester. Most of the sterols present in R1ME are in esterified form.

Of the methods attempted, molecular distillation technique was found to be the most promising for recovery of sterols from R1ME and it was found that in this way that sterols could be concentrated to about three times the original concentration of the residues R1ME. Besides, the different fractions of distillates collected may have different applications.

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