

ISOLATION AND IDENTIFICATION OF WAX ESTERS IN PALM OIL MILL FINAL EFFLUENT DISCHARGE

Keywords : Wax esters; Palm oil mill final effluent discharge; Hexadecyl laurate; Hexadecyl myristate.

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Thin layer chromatography of an extract of palm oil mill final effluent discharge revealed that the major components present are quite similar to those of crude palm oil. A band which had not been previously reported and which contributed about 6% (w/w) of the extract was found to consist of long-chain wax esters ranging from molecular weight m/z 396 to m/z 508. Hexadecyl laurate and hexadecyl myristate were found to be the two major constituents; together they accounted for about 45% of the total wax esters detected.

INTRODUCTION

There are various systems for treating palm oil mill liquid effluent, of which ponding is the most commonly practised. In this system, the effluent is progressively degraded in a series of anaerobic and facultative ponds until it meets the required enforcement parameters set by the Malaysian Department of Environment (DOE) under the Environmental Quality Act (1974) (Maheswaran, 1985). It can then be discharged into a river course. *Figure 1* shows schematically a typical ponding system for the treatment of palm oil mill effluent.

'Oil and grease' is one of the enforcement parameters for the final discharge and the liquid/liquid separatory funnel method is the standard analytical procedure set by the DOE (Department of Environment, 1985).

In the present study, organic compounds soluble in hexane were extracted and an attempt was made to characterize them, in order to give a better insight into what is

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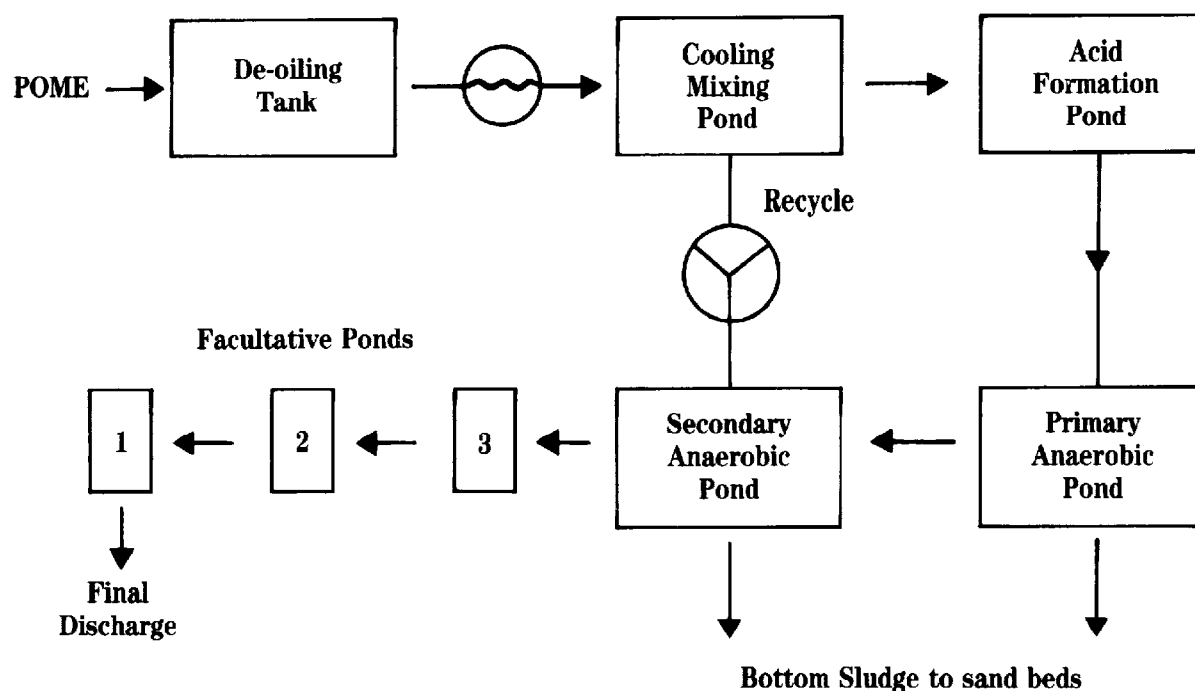


Figure 1.
Ponding System for Treating Palm Oil Mill Effluent (POME)

being discharged into our rivers. It was thought that the knowledge obtained might also contribute to a better understanding of the treatment system. This paper describes the detection and identification of wax esters in the extract of the final effluent discharge by gas chromatography/mass spectrometry.

EXPERIMENTAL

Materials

The samples of palm oil mill final effluent discharge used for extraction were collected from mills which treated the effluent by the ponding system.

All chemicals and solvents used were of analytical grade and were used as received.

Preparation of Effluent Extract

The effluent was acidified to below pH 2 by the addition of dilute sulphuric acid. 500 ml of effluent was extracted consecutively with 4 x 25 ml of hexane using a separatory funnel.

The combined hexane extracts were evaporated in a rotary evaporator and the material obtained was further dried under nitrogen at 30°C.

Preparative Thin Layer Chromatography

The dried extract (0.5 g) was redissolved in chloroform and applied to three thin-layer plates (0.5 mm thick, 17 cm x 17 cm) of silica gel 60 F₂₅₄ (Merck) which had previously been conditioned at 100°C for one hour. A spot of crude palm oil in chloroform was spotted on the side of each plate for comparison. The plates were then developed using hexane/diethyl ether/acetic acid (60:40:1, v:v:v).

A 1% solution of 2,7-dichlorofluorescein in ethanol was used to make the bands visible under UV light. Each band was scraped off and eluted with chloroform. The eluate from each band was filtered into a pre-weighed flask, the solvent was evaporated, and the residue was dried further under nitrogen and its weight determined. The percentage represented by each individual band was calculated.

ed. Identification of the chemical nature of the whole extract and of some of the bands which were not seen clearly in crude palm oil was done by gas chromatography, infrared spectroscopy and gas chromatography-mass spectrometry.

Infrared Spectroscopy

The infrared spectra of the crude palm oil and the whole extract were obtained by running them as thin films in potassium bromide cells using a Hitachi Infrared Spectrophotometer, model 270-30.

Gas Chromatography

Fraction G (see below) was subjected to GC analysis under the following operating conditions :

Instrument	: GC-Shimadzu, model 9APTF equipped with a flame ionization detector (FID)
Column	: 10% SP 2340 glass column, 4.5 m x 3 mm
Column temperature	: 185°C held for 10 min, then programmed to 250°C at 5°C/min
Injector/ Detector temperature	: 260°C
Nitrogen flow rate	: 50 ml/min

Combined Gas Chromatography-Mass Spectrometry of Fraction G

Fraction G was subjected to GC-MS analysis using a JEOL double focusing GC-MS, model DX303, linked to a computer mass data system, model JEOL JMA DA 5000, under the following operating conditions:

Column used	: 3% OV-1 on 1 m x 3 mm glass column
Column temperature	: 170°C to 340°C programmed at 8°C/min
Carrier gas	: Helium 100 ml/min
Injection temperature	: 340°C
GC-MS interphase temperature	: 280°C
Model of ionization	: EI
Accelerating voltage	: 3 kV
Ionization voltage	: 70 eV

RESULTS AND DISCUSSION

The extract of the palm oil mill final effluent discharge was found to represent only 40-50 ppm in the effluent. Since the amount is so small, it is not surprising that there is no information available in the literature on the nature of the extract.

The infrared spectra of the crude palm oil and the extract were identical except that there was an additional C=O stretching at 1715 cm^{-1} in the extract of the effluent. This indicated the presence in the extract of carbonyl compounds which might not be present in crude palm oil in significant amounts. A broad O—H band at 3300-3500 cm^{-1} indicated the presence of long chain carboxylic acids, which could be expected as a result of hydrolysis of the oil in the effluent. Preliminary GC-MS analysis of the crude extract indicated the presence of a complex mixture of compounds. The total ion chromatogram showed that not less than 100 compounds were present. A number of major peaks could be identified from the interpretation of mass spectra: these were methyl palmitate, methyl oleate, methyl stearate and oleic acid. The others could not be identified satisfactorily because of the overlapping of peaks. In view of the difficulty in

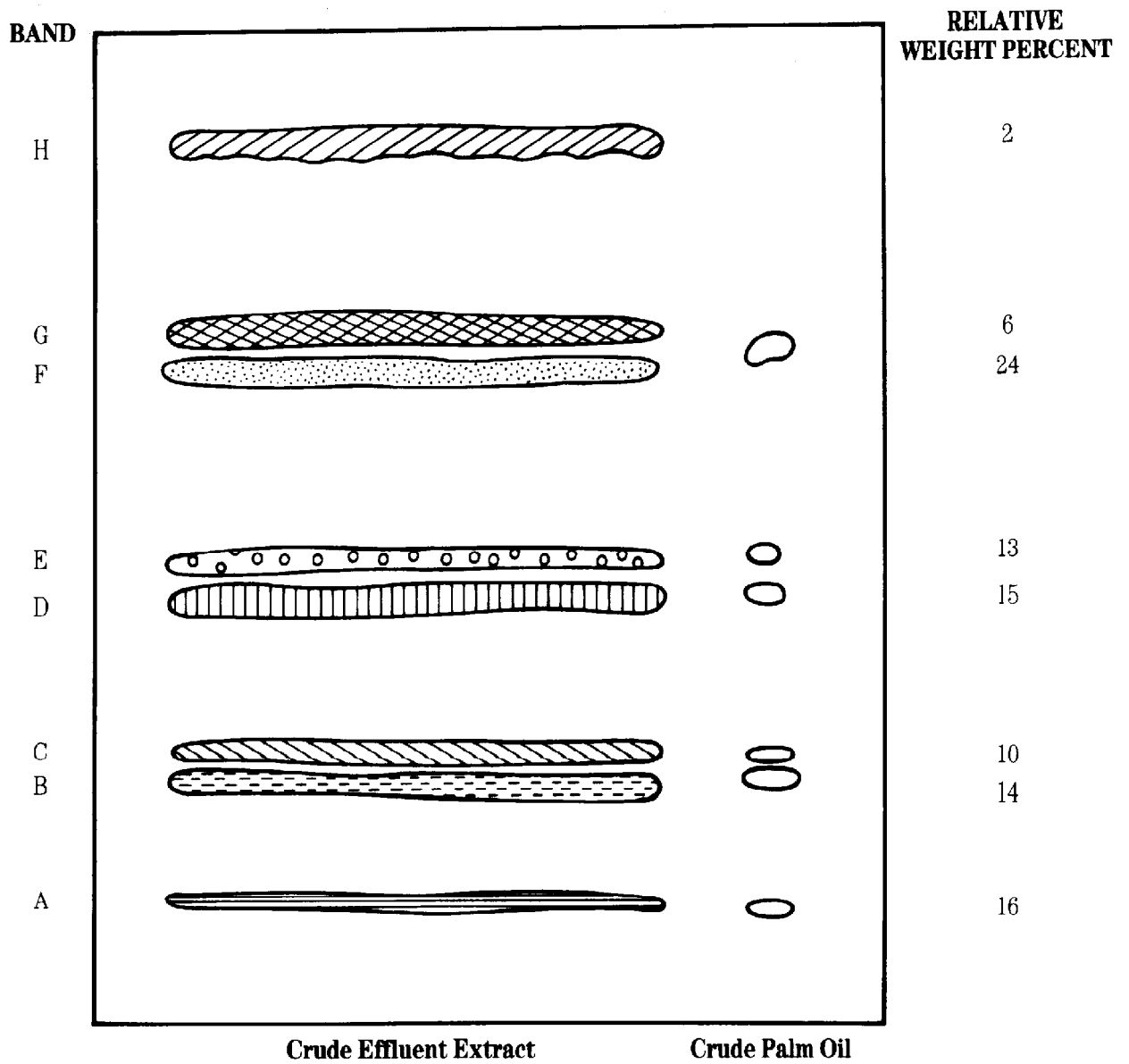


Figure 2.
Thin Layer Chromatography of Crude Extract from
Palm Oil Mill Final Effluent Discharge and of Crude Palm Oil

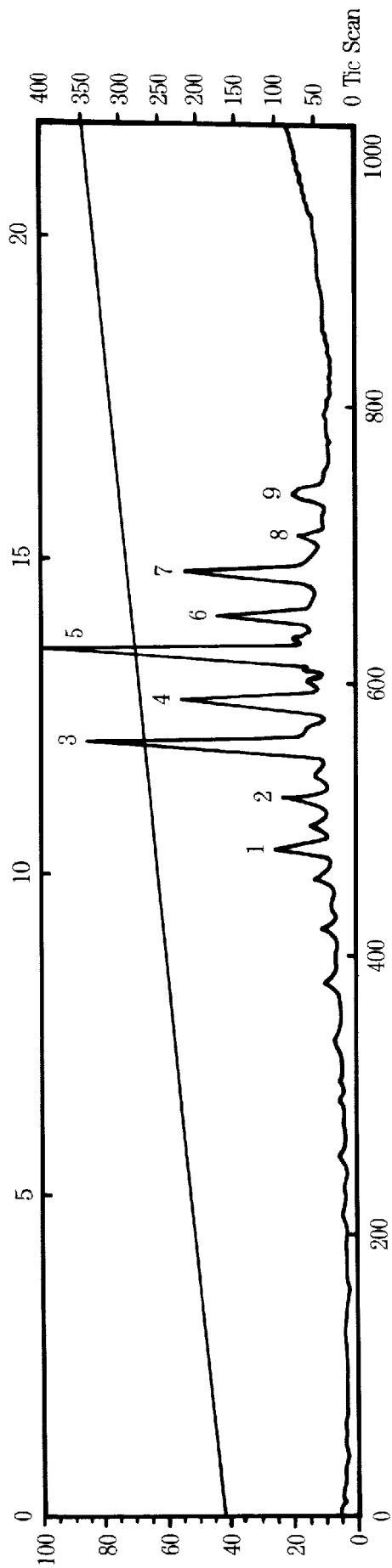


Figure 3.
Total Ion Chromatogram of Band G

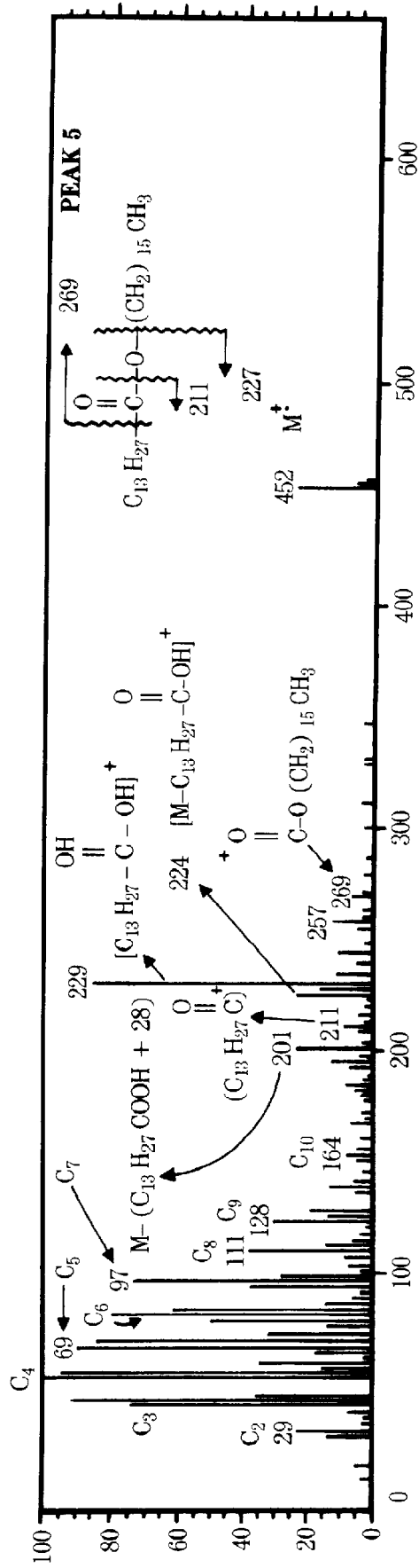
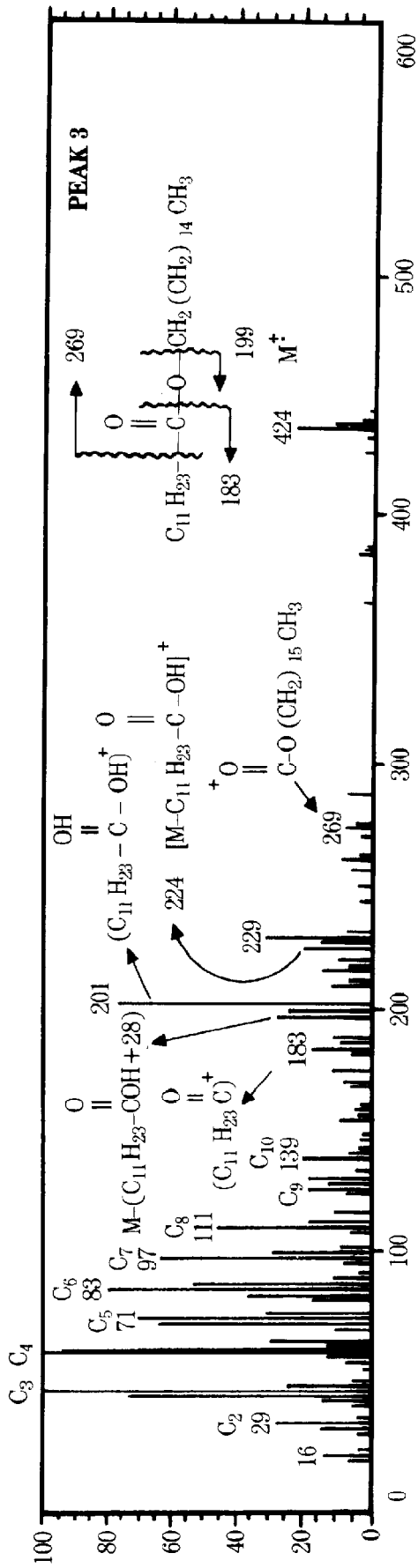
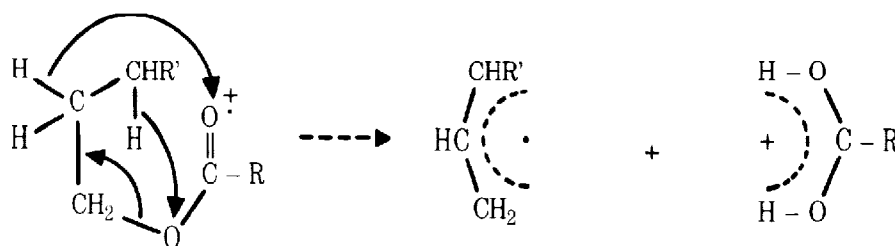


Figure 4.
Mass Spectra of Peaks 3 and 5



R and R' are alkyl groups

Figure 5.

the GC separation of the extract, preliminary separation was done by thin layer chromatography prior to further GC and GC-MS analysis. The thin layer chromatography showed eight clearly separated major bands including material at the origin. On the basis of relative weight percentage, triglycerides were the most abundant components (see *Figure 2*). The chromatograms showed that the crude palm oil effluent extract is quite similar in composition to the crude palm oil in the reference spot. Two separate runs were performed and the chromatograms were identical. The bands from the extract were labelled A to H as shown in *Figure 2*. Each of these bands was subjected to GC analysis, which showed the chromatograms of all the bands were similar and that bands A to F are the same as those from crude palm oil. Since bands G and H were not clearly detected in crude palm oil, these two were studied in greater detail by GC-MS analysis. Major components in band H were identified by GC/MS as methyl palmitate, methyl oleate and methyl stearate. *Figure 3* shows the total ion chromatogram of the material in band G, which was shown to

consist mainly of wax esters. The two largest peaks (3 and 5) have molecular weights of 424 and 452 respectively, and they were identified as hexadecyl laurate and hexadecyl myristate. The mass spectra of these two peaks are shown in *Figure 4*. The molecular ion for peak 3 is at m/z 424. A major peak at m/z 201 may be depicted as a decomposition of the odd electron parent ion with double rearrangement of the hydrogen atoms (*Figure 5*).

The peak at m/z 224 corresponds to a loss of the acid, while the peaks at m/z 269 and m/z 183 correspond to the alkyl/acyl cleavage and the acyl/alkoxyl cleavage respectively, with the

O

formation of fragments $(C_{11}H_{23}-C)^+$ and

O

$(^+)C-O(CH_2)_{15}CH_3$. The peak at m/z 196 may be due to $M-(RCOOH + 28)$.

Thus, peak 3 was identified as hexadecyl laurate. Peak 5 was similarly deduced to be hexadecyl myristate. Similar analysis of the mass spectra clearly showed that the other compounds were also esters of saturated fatty acids with fatty alcohols. The fragmentations

TABLE 1.
MAJOR WAX ESTERS IDENTIFIED IN EFFLUENT EXTRACT

Peak No.	Relative % *	M ⁺	Molecular Formula	Wax Ester Identified $\begin{array}{c} \text{O} \\ \\ \text{R}'-\text{C}-\text{O}-\text{R} \text{ where} \\ \text{R}' \text{ and R are alkyl groups} \end{array}$
1.	9.45	396	C ₂₆ H ₅₂ O ₂	(R'+1) R 10 16
2.	8.35	410	C ₂₇ H ₅₄ O ₂	12 15 11 16
3.	22.14	424	C ₂₈ H ₅₆ O ₂	12 16
4.	15.63	438	C ₂₉ H ₅₈ O ₂	14 15 13 16
5.	22.74	452	C ₃₀ H ₆₀ O ₂	14 16
6.	9.13	466	C ₃₁ H ₆₂ O ₂	16 15 15 16 14 17 17 14
7.	10.08	480	C ₃₂ H ₆₄ O ₂	16 16
8.	1.48	494	C ₃₃ H ₆₆ O ₂	16 17 15 18
9.	1.21	508	C ₃₄ H ₆₈ O ₂	16 18 18 18:1

* by GC analysis

of the ions are explained similarly as in the spectra for peaks 3 and 5. Thus, peaks 1,3,5,7 and 9 were deduced to be saturated wax esters with an even number of carbon atoms. Since the molecular weights of peaks 2,4,6 and 8 were 14 mass units (CH₂) higher than those of peaks 1,3,5 and 7 respectively, it was deduced that they were wax esters with an odd number of carbon atoms, in chains which could be either branched or straight. Each peak of these odd numbered esters was shown to be a

mixture of at least two esters in approximately equal amounts, as can be seen from the equal intensities of the diagnostic ions. These esters were not separated satisfactorily using our present technique. The probable identities of the wax esters in the extract from palm oil mill final effluent discharge are summarized in *Table 1*. The presence of these wax esters was in agreement with the IR spectral data which showed the presence of compounds with the C=O(1715cm⁻¹) functional group.

Also, from the mass spectral analysis, it is evident that the wax esters isolated from the effluent extract were still intact molecules. The relative percentages of peaks 1 to 9 were also determined by GC analysis and are given in *Table 1*. The esters with even numbers of carbons (peaks 1,3,5,7 and 9) contributed about 65% of the total wax esters. The presence of these even numbered wax esters in the effluent extract is not surprising, since these are the acids present in both palm oil and palm kernel oil. However, the presence of wax esters with odd numbers of carbons will be interesting to investigate further, since they are abundant, making up about 30% of the total. Recently, Goh *et al.* (1986, 1988) reported the presence of at least seven major homologous wax esters (C₄₂ to C₅₄) isolated from the exocarp of palm fruits. However, in our studies, no significant amount of wax esters with more than 35 carbon atoms could be detected in the effluent extract. The lack of solubility of longer chain wax esters in palm oil effluent could possibly be one of the reasons for this. However, it is interesting and important to know where these wax esters have gone to. Are they in the crude palm oil produced or still with the palm fibres after the extraction of the oil? There has been no thorough investigation of these questions and it will be fruitful to look into them.

CONCLUSION

The results of this study clearly show that wax esters with both even and odd numbers of carbon atoms are present in palm oil

mill final effluent discharge. The two major wax esters identified are hexadecyl laurate and hexadecyl myristate, which together constitute about 45% of the total wax esters detected.

ACKNOWLEDGEMENT

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