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A RAPID METHOD FOR PREPARATION OF FATTY ACID METHYL ESTERS OF PALM OIL

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RINGKASAN

Satu kaedah mudah untuk penyediaan metil ester asid lemak dari minyak sawit *(E. guineensis)* telah ditentukan. Minyak yang terdapat di mesokap telah dijadikan metil ester dengan secara terus dengan menggunakan 2% H_2SO_4 – metanol atau 5% HCl-metanol (v/v) di dalam oven bersuhu 80°C masing-masing selama 1 atau 2½ jam. Kaedah ini didapati cepat, tepat dan boleh diulangi prosesnya.

INTRODUCTION

Breeding programmes aimed at changing the fatty acid composition of palm oil require screening a large number of samples. Conventional methods of preparing the fatty acid methyl esters from oil for gas liquid chromatographic (GLC) analysis of the composition involve several lengthy and tedious procedures in which the oil has first to be extracted from the mesocarp before esterification of the oil.

The extraction of the oil usually involves a sampling procedure of the fruits from the bunch, sterilisation of the fruits, chopping of the mesocarp, and solvent extraction of the oil from the chopped mesocarp.

Esterification of the extracted oil is normally carried out using base-catalysed reagents such as 0.5N sodium or potassium methanolate, (LUDDY, *et al.*, 1968) or acid catalysed reagents such as sulphuric acid-methanol (AOCS methods) and boron trifluoride-methanol (METCALFE *et al.*, 1966).

The present paper evaluates the use of a rapid esterification procedure for determining the fatty acid composition of palm oil in which the use of sulphuric acid-methanol and hydrochloric acid-methanol as reagents for the direct esterification of the oil in the mesocarp is examined. The method studied avoids the lengthy process of sterilisation and oil extraction. The method is rapid, accurate and allows a large number of samples to be analysed.

MATERIALS AND METHODS

Sampling

Fresh fruit bunches of the Tenera variety were chopped to remove the spikelets and samples were taken. A sample consisted of six spikelets selected randomly from the apical, middle and basal sections of the bunch (the bunch being arbitrarily sub-divided into 3 regions).

Method A

Method A involves the extraction of oil from the mesocarp of the fruits using n-hexane followed by esterification of the oil.

The fruits were steam-sterilised for an hour at 10psi (0.7 kg/cm^2). After separating the mesocarp from the nuts of the sterilised fruits, the oil was extracted using approximately 600–800 ml of n-hexane in a food blender. The hexane was removed under vacuum using a rotary evaporator. Moisture and fine residue from the extraction procedure was removed from the oil by centrifugation. The purified oil was then dried under vacuum in a rotary evaporator.

The extracted oil may be esterified using four reagents as indicated below:–

A1 a solution of 2% (v/v) sulphuric acid in methanol, refluxing for 2½ hours, OR

A2 a solution of 2% (v/v) sulphuric acid in methanol – benzene (3:1) as according to AOCS method (2), refluxing for 2½ hours, OR

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A3 a solution of 5% (v/v) hydrochloric acid in methanol, refluxing for 2½ hours, OR

A4 a solution of 0.5N sodium methanolate, refluxing for 5 minutes.

Method B

The method does not involve the time consuming procedure of oil extraction as described in method A. The mesocarp from unsterilised fruits were chopped finely using a food grinder and dried overnight at 103°C in an oven. Direct esterification of the oil in the mesocarp was conducted by adding to the chopped mesocarp (100–200mg), the following reagents in a screw cap flask(25ml):–

B1 a 10ml solution of 2% (v/v) sulphuric acid in methanol, heating for 2½ hours, OR

B2 as against B1 above but heating for 1 hour, OR

B3 a 10ml solution of 5% (v/v) hydrochloric acid in methanol, heating for 2½ hours.

The flask was left in the oven at 80°C. The screw cap of the flask was lined with neoprene to prevent any loss of solution through evaporation. The methyl esters formed were extracted with 20 ml petroleum spirit (40° – 60°C), washed with distilled water (2x20ml) till neutral (tested with litmus paper) and filtered over anhydrous sodium sulphate.

Gas – Liquid Chromatography

The methyl esters were separated isothermally at 180°C in a glass column of 10% DEGS on 100–200 mesh Diatomite C, AW using a Pye 104 instrument equipped with a flame ionisation detector. Peak area measurement was done using a DP 88 computing integrator. Weight percent of methyl esters was done by area normalisation.

Operating Conditions

Column	: 10% DEGS on 100–200 mesh Diatomite C, AW, glass 2m x 4mm i.d.
Column temperature	: 180° C
Carrier gas	: Nitrogen at 40 ml/min.
Detector	: Flame ionisation detector, 200°C
Hydrogen gas	: 40ml/min.
Air	: 500 ml/min.
Sample size	: 0.1 microlitre

RESULTS AND DISCUSSION

Table 1 outlines the various steps and the time taken for each step in the sample preparation of the two methods leading to the preparation of methyl esters for GC analysis.

In the case of method A, various sample preparation steps are involved in the extraction of oil from the mesocarp while method B involves only three sample preparation steps – chopping the spikelets from oil palm bunch, chopping the fruit mesocarp off the spikelets samples, and the drying of chopped mesocarp. It is evident that the time consuming and tedious part of method A is in the oil extraction which limits the number of samples that can be analysed for fatty acid composition. Method B, in avoiding the tedious parts of sample preparation in method A, enables the number of samples prepared to be tripled. An added advantage is that the sample of dried mesocarp for methylation is directly available from the bunch analysis usually carried out in oil palm breeding and thus one avoids doing double work.

Use of Sulphuric Acid – Methanol (2½ hrs)

The precision and accuracy of methods A and B using the various reagents are examined from the fatty acid composition of the sample analysed by gas-liquid chroma-

TABLE 1: APPROXIMATE TIME INVOLVED IN THE EXTRACTION AND ESTERIFICATION BETWEEN METHOD A AND B PER OPERATOR

Steps involved in the method	Method A	Method B
1. Chopping of spikelets from bunch and sampling	10 minutes	10 minutes
2. Sterilisation and cooling	1 hour 30 mins.	None
3. Removal of mesocarp	10 minutes	50 minutes (includes chopping of fresh mesocarp)
4. Solvent Extraction	30 minutes	None
5. Removal of solvent	1 hour	None
6. Drying time	1 hour (oil)	Overnight[a] (mesocarp)
7. Centrifugation to remove impurities	10 minutes	None
8. Esterification	½ – 3 hours[b]	1 – 3 hours[b]
Total time taken	5 – 8 hours[b]	2 – 4 hours[b] (not including drying time)

a – Optional. It was found from experiment that there was no significant variance between fatty acid composition of fresh mesocarp and dried mesocarp. Overnight drying is only necessary for samples to be stored.

b – The duration depends on the esterification method selected.

tography. *(Table 2a)*. The fatty acid composition of the samples obtained by the three procedures of esterification (A1, A2 and A4) of the extracted oil within method A are in good agreement. A similar result is obtained for B1 of method B, the fatty acid composition of which is comparable to those determined by the procedures of method A.

The accuracy of each esterification procedure in Method A and B and the overall accuracy of the gas-liquid chromatography analysis have been determined according to the method proposed by HERB and MARTIN (1970). The following calculations were done on the data in *Table 2a*.

1) Normalize the results from all analyses.

2) For each component fatty acid, calculate the mean, deviation from the mean, and the standard deviation.

3) Add the standard deviations for each component fatty acid to give a value similar to a standard deviation of the total sample. Analysis of each acid is dependent on every other acid in the oil and the errors are therefore additive. The sum of standard deviations indicates the minimum accuracy expected for an analysis.

4) Calculate the total deviation of each method by summing the deviation from the mean of each fatty acid component of the sample.

5) Grade each method by subtracting the total deviation from 100. The value obtained gives the accuracy of the method.

The results calculated as described are shown in *Table 2b*. The sum of the standard

TABLE 2a: COMPARISON OF METHODS A AND B USING A 2% (v/v) SULPHURIC – ACID METHANOL SOLUTION

	Method	Fatty Acids (%)[a] C 14:0	C 16:0	C 18:0	C 18:1	C 18:2
	Method A					
A1.	Oil refluxed with 2% H_2SO_4 – Methanol for 2½ hours.	1.0	45.5	6.5	35.4	11.7
A2.	AOCS method (Ce 2–66) oil refluxed with 2% H_2SO_4 – Methanol/Benzene Mixture (3:1) for 2½ hours.	1.1	45.4	6.4	35.5	11.7
A4.	Oil refluxed with 0.5 N sodium methanolate solution for 5 mins.	1.1	45.5	6.3	35.3	11.8
	Method B					
B1.	Mesocarp heated with 2% H_2SO_4 /Methanol for 2½ hours at 80°C.	1.0	45.8	6.7	35.3	11.2

a – Mean of 5 complete analysis.

deviations of each fatty acid component measures the overall precision of the methods employed in this study. When the value is subtracted from 100, a measure of the minimum accuracy to be achieved in an analysis is obtained, which in this case is 99.2%. The accuracy grade of each procedure can be determined by subtracting the total deviation of the results of each esterification procedure from 100. The results in *Table 2b.* showed that except for procedure B1 of method B, the minimum accuracy is achieved and exceeded by all the other esterification procedures.

A classification is arbitrarily adopted whereby esterification procedures which yield a total deviation less or equal to the sum of standard deviations of each fatty acid component, are considered good. Procedures which give a total deviation more than, but not greater than twice the sum of the standard deviations are satisfactory while those with a total deviation greater than twice the sum of standard deviations are considered poor. Accordingly, all the esterification procedures of method A give good accuracy but procedure B1 of method B is considered satisfactory.

Use of 5% Hydrochloric Acid – Methanol as Esterification Reagent

The possibility of using a 5% (v/v) hydrochloric acid-methanol solution for esterifying the oil in the mesocarp was also investigated. The results are compared to methods A4 and A3 in which the extracted oil is refluxed with a solution of 0.5N sodium methanolate and a 5% (v/v) solution of hydrochloric acid-methanol and method B1 in which a solution of 2% (v/v) sulphuric acid-methanol is used. *(Table 3a).* The accuracy of each esterification procedure is similarly determined as described before and the results shown in *Table 3b.* A greater accuracy is achieved with the 5% HCl–methanol than 2% H_2SO_4–methanol. The lower accuracy obtained from the use of the latter reagent could perhaps be due to a

TABLE 2b: ACCURACY OF METHODS A AND B USING A 2% (v/v) SULPHURIC ACID – METHANOL SOLUTION

Fatty Acid	Deviation from mean				Mean	s.d.	C.V.(%)
	Method A			Method B			
	A1	A2	A4	B1			
C 14:0	0.1	0.0	0.0	0.1	1.1	0.05	4.5
C 16:0	0.1	0.2	0.1	0.2	45.6	0.17	0.4
C 18:0	0.0	0.1	0.2	0.2	6.5	0.17	2.6
C 18:1	0.0	0.1	0.1	0.1	35.4	0.09	0.3
C 18:2	0.1	0.1	0.2	0.4	11.6	0.27	2.3
Total deviation from mean	0.3	0.5	0.6	1.0		0.75	
Accuracy of Procedure (100 – T.d)	99.7	99.5	99.0				
Minimum Accuracy of Analysis (100 – s.d.)	99.2						

TABLE 3a: COMPARISON OF METHODS A AND B USING 5% HYDROCHORIC ACID – METHANOL SOLUTION

	Methods	Fatty Acids (%)[a]				
		C 14:0	C 16:0	C 18:0	C 18:1	C 18:2
	Method A					
A3.	Oil refluxed with 5% HCL – Methanol for 2½ hours.	1.0	40.0	4.1	45.1	9.7
A4.	Oil refluxed with 0.5N sodium methanolate for 5 minutes.	0.8	39.5	4.4	45.9	9.5
	Method B					
B3.	Mesocarp heated with 5% HCl – Methanol for 2½ hours at 80°C	0.8	40.0	4.1	45.8	9.2
B1.	Mesocarp heated with 2% H_2SO_4 – Methanol for 2½ hours at 80°C.	0.8	40.4	4.5	45.8	8.5

a – Mean of 5 complete analysis.

TABLE 3b: ACCURACY OF METHODS A AND B USING A 5% HCl – METHANOL SOLUTION

Fatty Acid	Deviation from mean				Mean	s.d.	C V.(%)
	Method A		Method B				
	A3	A4	B3	B1			
C 14:0	0.1	0.1	0.1	0.1	0.9	0.10	11.1
C 16:0	0.0	0.5	0.0	0.4	40.0	0.36	0.9
C 18:0	0.2	0.1	0.2	0.2	4.3	0.20	4.7
C 18:1	0.6	0.2	0.1	0.1	45.7	0.36	0.8
C 18:2	0.5	0.3	0.0	0.7	9.2	0.52	5.7
Total deviation from mean	1.4	1.2	0.4	1.5		1.54	
Accuracy of Procedure (100 – T.d)	98.6	98.8	99.6	98.5			
Minimum Accuracy of Analysis (100 – s.d.)	98.5						

partial decomposition of fatty acids, in particular of the unsaturated types. The results obtained by the use of 5% HCl–methanol on the mesocarp (B3) also showed a greater accuracy than the conventional methods of refluxing the extracted oil using a similar solution or 0.5N sodium methanolate. In all cases however, the minimum degree of accuracy in analysis is achieved by all the esterification procedures. The accuracy of each esterification procedure, is good according to the arbitrary classification defined.

Shorter Reaction Time for 2% H_2SO_4–Methanol

A study was also conducted to determine whether the accuracy of method B1 could be enchanced by reducing the reaction time from 2½ hours to 1 hour. The results obtained are shown in *Table 4a* and *4b*.

A comparison of the above procedure of 1 hour reaction time (B2) is made with that of the same reagent but of a 2½ hours reaction time (B1), and with method A using 0.5N sodium methanolate (A4). It can be observed from *Table 4b*, that reducing the reaction time, enchances the degree of accuracy of the esterification procedure involving 2% H_2SO_4–methanol. In addition the minimum degree of accuracy is again attained by all.

Precision of Each Procedure

The precision of the direct esterification procedures involving solutions of 2% H_2SO_4–methanol and 5% HCl–methanol

TABLE 4a: COMPARISON OF METHOD B OF DIFFERENT REACTION TIME USING 5% H_2SO_4 – METHANOL SOLUTION

	Method	Fatty Acids (%)[a]				
		C 14:0	C 16:0	C 18:0	C 18:1	C 18:2
	Method A					
A4.	Reflux oil with 0.5 N sodium methanolate for 5 minutes.	0.7	39.1	4.5	46.0	9.7
	Method B					
B2.	Mesocarp heated with 2% H_2SO_4 – methanol for 1 hour, at 80°C.	0.8	39.6	4.2	46.4	9.0
B1.	Mesocarp heated with 2% H_2SO_4 – methanol for 2½ hours, at 80°C.	0.8	40.4	4.5	45.8	8.5

a – Mean of 5 complete analysis.

TABLE 4b: ACCURACY OF METHOD B OF DIFFERENT REACTION TIME USING a 2½ H_2SO_4 – METHANOL SOLUTION

Fatty Acid	Deviation from mean			Mean	s.d.	C.V.(%)
	Method A	Method B				
	A4	B2	B1			
C 14:0	0.1	0.0	0.0	0.8	0.05	6.3
C 16:0	0.6	0.1	0.7	39.7	0.05	1.6
C 18:0	0.1	0.2	0.1	4.4	0.17	3.9
C 18:1	0.1	0.3	0.3	46.1	0.30	0.7
C 18:2	0.6	0.1	0.6	9.1	0.60	6.6
Total deviation from mean	1.5	0.7	1.7		1.17	
Accuracy of Procedure (100 – T.d)	98.5	99.3	98.3			
Minimum Accuracy of Analysis (100 – s.d.)	98.2					

has been determined and compared to those obtained by AOCS method and the use of 0.5N sodium methanolate *(Table 5)*.

The results indicate a coefficient of variation (C.V) of less than 2% for major acids and a C.V of less than 5% for the minor acid, which are well within the limits of accuracy of gas chromatographic analysis as reported by KUKSIS (1972).

CONCLUSION

The present study has shown that palm oil in the fruit mesocarp can be directly esterified by heating the mesocarp at 80°C for 2½ hours using either a solution of 2% H_2SO_4–methanol or a 5% HCl–methanol. The accuracy obtained by these methods ranges from satisfactory to good and compares well with that obtained by conventional methods of trans-esterification of extracted oil. In the case of H_2SO_4–methanol, greater accuracy is obtained if the reaction time is reduced to 1 hour to minimise partial decomposition of fatty acids. The proposed methods are rapid, reproducible and accurate and should provide a useful method for fatty acid composition in the breeding programmes of oil palms of *E. guineensis*.

TABLE 5: PRECISION OF ESTERIFICATION PROCEDURES[a]

	Method	Statistical parameter	Fatty Acid (%) C 14:0	C 16:0	C 18:0	C 18:1	C 18:2
A4.	Oil reflux with 0.5 N sodium methanolate for 5 minutes	s.d	0.08	0.40	0.22	0.08	0.15
		$\bar{x}$	1.1	45.3	6.4	35.4	11.9
		CV(%)	7.3	0.9	3.4	0.2	1.3
A2.	AOCS method	s.d	0.05	0.53	0.21	0.38	0.17
		$\bar{x}$	1.1	45.4	6.4	35.5	11.7
		CV(%)	4.5	1.2	3.3	1.1	1.5
B2.	Mesocarp heated with 2% H_2SO_4 – methanol for 1 hour at 80°C.	s.d	0.04	0.36	0.19	0.25	0.22
		$\bar{x}$	1.1	46.0	6.3	35.0	11.3
		CV(%)	3.6	0.8	3.0	0.7	1.9
B3.	Mesocarp heated with 5% HCl – methanol for 2½ hours at 80°C.	s.d	0.05	0.49	0.22	0.31	0.39
		$\bar{x}$	1.3	46.3	5.5	35.0	11.9
		CV(%)	3.8	1.1	4.0	0.9	3.3

a – Determined from 5 complete analysis.

ABSTRACT

A simple method for the quantitative preparation of fatty acids methyl esters from palm oil *(E. guineensis)* is determined. The oil in the mesocarp is directly methylated using either 2% H_2SO_4–methanol or 5% HCl–methanol (v/v) at 80°C in an oven for 1 or 2½ hours respectively. The methods are found to be rapid, accurate and reproducible.

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