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Determination of Cyclopropenoic and Cyclopropanoic Fatty Acids in Cottonseed and Kapok Seed Oils by Gas–Liquid Chromatography

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This paper describes a new method for direct quantitative analysis of cyclopropenoic fatty acids (CPEFA) and cyclopropanoic fatty acids (CPAFA) methyl esters by gas–liquid chromatography (GLC) with glass capillary columns coated with BDS and Carbowax 20M. Results obtained by this method are compared with those given by glass capillary GLC after derivatization with silver nitrate in anhydrous methanol of CPEFA methyl esters, by hydrogen bromide titration, and by nuclear magnetic resonance (NMR). Equivalent chain lengths (ECL) of dihydromalvalic, dihydrosterculic, malvalic, and sterculic acids and the methoxy esters and enonic derivatives resulting from the silver nitrate reaction were determined on these two capillary columns. A temperature effect study upon CPEFA stability shows that, at 170 °C with Carbowax 20M or 150 °C with BDS, a slight decomposition occurs. This method is applied to samples of cottonseed and kapok seed oils for determination of CPEFA and CPAFA. Samples of peanut oils adulterated by cottonseed and kapok seed oils are also examined.

The presence of cyclopropenoic fatty acids (CPEFA) in higher plant families has been described over the past 20 years. These papers were summarized in recent reviews (*1–5*). Recent work shows that CPEFA such as sterculic (9,10-methylene-9-octadecenoic) and malvalic (8,9-methylene-8-heptadecenoic) acids occur together with cyclopropanoic fatty acids (CPAFA) such as dihydrosterculic and dihydromalvalic acids in many other plant families (*6, 7*). These fatty acids having a small ring structure are responsible for physiological disorders in fowl (*2*) and rainbow trout (*8*). Cottonseed accounts for approximately one-tenth of global production of edible vegetable oil, and protein meal for feed, with the United States production about one-fifth of this amount (*9*). Cottonseed oils contain CPEFA (*10*) which are revealed by the Halphen color test (*11*) which is characteristic of cyclopropenic groups (*12*). The amounts of CPEFA are relatively small in cottonseed oils (*13*) but are more important in kapok seed oils (*14*) which are also used as edible oils. As it would be unwise to consume such oils, procedures have been developed for removing cyclopropenoids from the oils (*9, 14*). The most desirable procedure for reducing or eliminating these compounds would be a genetic approach (*9*), but otherwise this quality factor involves the development of methodology and instrumentation for measuring both CPEFA and CPAFA. At present, the principal methods which permit assays of CPEFA are the Durbetaki method (*15*), the Halphen reaction (*11, 16*), the nuclear magnetic resonance (NMR) method (*17*), and the gas–liquid chromatography (GLC) analysis of the derivatives of CPEFA obtained after reaction with silver nitrate in anhydrous methanol (*18, 19*).

The purpose of this work is to describe a new rapid assay of CPEFA, without previous derivatization, and CPAFA by GLC using glass capillary columns coated with Carbowax 20M or BDS. Results obtained are compared with those from the methods given above. These methods were applied to samples of cottonseed and kapok seed oils. We have studied three samples of cottonseed oils 1–3, two samples kapok seed oils 4 and 5, and two samples of peanut oils 6 and 7 adulterated with 10% and 5% (by wt) of cottonseed and kapok seed oils, respectively.

EXPERIMENTAL SECTION

Samples of Cottonseed, Kapok Seed, and Peanut Oils. Two samples of cottonseed (*Gossypium hirsutum*, Acala 1517 BR variety) were obtained from the Compagnie Française pour le Développement des Fibres Textiles (CFDT, Mahajanga, Madagascar). Sample **1** was collected in the year 1978 and sample **2** in the year 1980. Sample **3** (year 1980) is a raw industrial cottonseed oil given by the Société Industrielle du Boina (SIB, Mahajanga, Madagascar). Two samples of kapok (*Ceiba pentandra*, Gaertn or *Eroidendron enfractuosum*) seeds were collected in the Diégo-Suarez area (**4**, 1979) and Ambanja area (**5**, 1980) of Madagascar. A sample of peanut seeds (*Arachis hypogea*; Valencia variety) was purchased in a local market of Antananarivo (Madagascar).

The seeds were decorticated, crushed into coarse particles, and dried at 103 ± 2 °C. Oil was extracted from the crushed seeds with light petroleum (40–60 °C) using a Soxhlet apparatus and the solvent was removed by vacuum distillation at low temperature according to NFV 0-3-905 Norm (*20*). The seeds were found to contain about 31% oil for cottonseed, 24% for kapok, and 47% for peanut.

Halphen Color Test on Oils. The original method (*11*) was used for characterization of CPEFA. Equal volumes (about 1–3 mL) of oil, 1-pentanol, and carbon disulfide containing 1% sulfur were placed in a test tube and warmed on a steam bath for 10–15 min. All the samples gave the characteristic red-pink color.

Preparation of the Methyl Esters. Methyl esters were prepared from oils by base-catalyzed transmethylation (*21*). Approximately 5 g of oils was refluxed with a solution obtained by reacting 0.2 g of sodium in 40 mL of anhydrous methanol. After 20 min, the solution was cooled with 100 mL of distilled water, and the methyl esters were extracted twice with 100 mL of hexane. The methyl ester solution was dried over anhydrous sodium sulfate and evaporated to dryness in a stream of nitrogen. For direct analysis by gas–liquid chromatography (GLC), hexane was added to obtain a 5% solution.

Argentation of Methyl Esters. The methyl esters (100 mg) containing CPEFA were reacted with 15 mL of anhydrous methanol saturated with silver nitrate (*18*). The reaction was carried out for 2 h at 30 °C. All the esters (normal and reaction products from cyclopropenes) were recovered from the reaction mixture by adding 30 mL of distilled water and by extracting twice with 100 mL of hexane. The combined hexane fractions were dried over anhydrous sodium sulfate and evaporated in a stream of

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nitrogen to obtain a 5% solution of methyl esters. The mixture of products was used for direct analysis of GLC.

Preparative Silica Gel Chromatography. Normal methyl esters were separated from methyl esters of CPEFA derivatives using preparative thin-layer chromatography (TLC) with silica gel (F 254 chromatoplate, 0.1 mm gel thickness, 10 × 20 cm, E. Merck, West Germany) according to Bohannon and Kleiman (*6*); 150 μL of the 5% methyl ester solution was deposited on the plate and developed using hexane ether (70:30, v/v) as eluting solvent. The plate was examined under UV light at 366 nm after spraying with Rhodamine-B solution. Two bands (R_f = 0.79 and 0.63) corresponding to normal methyl esters and methyl ester derivatives (methoxy ethers and enones) of CPEFA were seen. These bands were scraped off and the silica gel extracted with hexane or dichloromethane. After solvent evaporation, the residues were diluted to obtain 5% solutions in hexane. The mixtures were then used for direct analysis by GLC.

Normal methyl esters were separated according to their degree of unsaturation using a silica gel F 254 chromatoplate (E. Merck) impregnated with aqueous silver nitrate solution (20% of silver nitrate) and activated in an oven at 105 °C for 1 h and 30 min. After depositing normal methyl esters as indicated above, the plate was developed with benzene. After Rhodamine-B spray and examination under UV light at 360 nm, three bands (R_f = 0.77, 0.53, and 0.36) were seen corresponding to saturated, monounsaturated, and diunsaturated acids methyl esters, respectively. These bands were scraped off and extracted for examination by GLC.

Gas–Liquid Chromatography. An Intersmat IG 12 DFL (93 Pavillon-sous-Bois, France) gas chromatograph equipped with a flame ionization detector and a glass injector was used for the analysis. Two glass capillary columns were prepared according to Grob et al. (*22*), 45 m long, 0.35 mm i.d. coated with Carbowax 20M (0.15 μm phase thickness) and 25 m long, 0.40 mm i.d. coated with BDS (0.20 μm phase thickness). Temperatures used were 190 °C for the Carbowax 20M column and 150 °C for the BDS column, 200 °C for the detector, and 220 °C for the inlet. The flow rate of hydrogen used as carrier gas was 5 mL/min with a split ratio of 5/100. The injections averaged about 0.5–1 μL with a 5% solution of methyl esters. A Perkin-Elmer Model 56 recorder was used, and peak areas were integrated by an Spectra-Physics Minigrator integrator.

The gas chromatographic sensitivity has been compared with the AOAC methods (*23*). The lower limit of detection of malvalic and sterculic acids was less than 0.02% with our glass capillary columns.

Hydrogen Bromide Titration of CPEFA. The Durbetaki method (*15*, *24*) was used for direct quantitative titration of the cyclopropenic function of CPEFA using hydrobromic acid. Anhydrous hydrogen bromide (Fluka, Switzerland) was bubbled through 1 L of glacial acetic acid until 0.1 N normality was attained. The reagent was standardized with 0.1 g of sodium carbonate, dissolved in 5 mL of glacial acetic acid, and titrated to the blue-green end point of the crystal violet indicator. Accurately weighed samples of oils (~0.4 g) were dissolved in benzene, and 5 drops of 0.1% crystal violet in glacial acetic acid was added. A Teflon stopper was placed to lower the buret tip to a point just above the solution. The oils were titrated, using a magnetic stirrer, to the blue-green end point. Results were expressed in sterculic acid (mol wt 294).

Nuclear Magnetic Resonance Titration of CPEFA. The oils were dissolved (dilution 1:20) in a carbon tetrachloride–chloroform (90:10, v/v) mixture according to Pawlowski's method (*17*). A Perkin-Elmer Model R 32 NMR spectrometer (Norwalk, CT) or a Varian Model XL 100 A NMR spectrometer (Palo Alto, CA) was used for the analysis. Tetramethylsilane (Me_4Si) was used as internal standard. The high field region (δ 0.5–1.5) of NMR spectra showed a singlet peak at δ 0.8 due to the two hydrogens on the cyclopropene ring and a triplet at δ 1.0 due to the terminal methyl groups of all fatty acids. The percent CPEFA was calculated by dividing the area of cyclopropene absorption by the area of the methyl absorption and multiplying by 150 (*17*). Areas were measured with a planimeter because the two peaks of interest were too close together to allow satisfactory use of either instrument's integrator. Scanning of samples was done in duplicate using a 250-Hz sweep width.

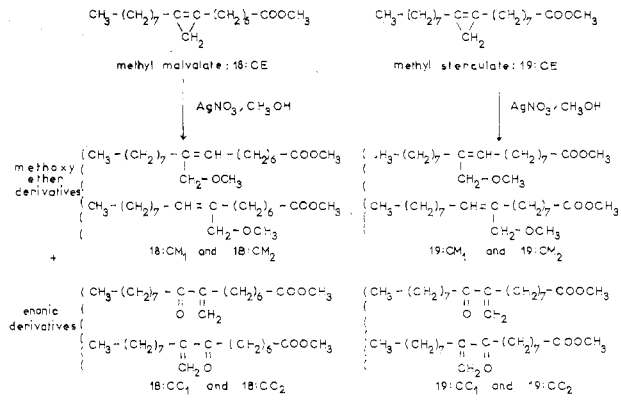

Figure 1. Methoxy ether and enonic derivatives formation of methyl malvalate and methyl sterculate by silver nitrate reaction in anhydrous methanol.

RESULTS AND DISCUSSION

Cottonseed oils being slightly poorer in CPEFA than kapok seed oils, we first made a complete analysis of the kapok seed oil 4 in order to characterize the main fatty acids, CPAFA, CPEFA, and their derivatives after reaction with silver nitrate in anhydrous methanol. Then we analyzed the different samples of oils investigated.

Complete Analysis of the Kapok Seed Oil 4. The CPEFA are considered as unstable compounds. Many authors have taken particular caution for the preparation of methyl esters, such as cold saponification of the oil with ethanolic potash (*25*, *26*), the fatty acids being then treated with diazomethane. Various methods used have been reviewed by Kleiman et al. (*27*). We chose transmethylation of the oils in methanol containing 1% sodium methylate as catalyst, at ambient temperature during 16–24 h or at reflux of methanol during 20 min (*21*). The methyl esters obtained can be analyzed directly by GLC; however, Recourt et al. (*3*) showed that in many cases isomerization or total or partial decomposition of CPEFA occurs. For this reason, most authors preferred to transform CPEFA into more stable derivatives (*10*, *18*, *19*). We have chosen the derivative preparation method, with silver nitrate in the presence of anhydrous methanol (*18*). For each CPEFA, two methoxy ether compounds and two enonic compounds are formed, as shown in Figure 1, in the case of sterculic and malvalic acids. The methyl ester mixture obtained can be fractionated into two parts using preparative thin-layer chromatography (TLC), in order to separate CPEFA methyl esters derivatives from normal methyl esters. Normal methyl esters are then fractionated into three parts using preparative TLC in order to separate saturated monounsaturated and diunsaturated fatty acid methyl esters. The scheme for these operations is given in Figure 2. The four fractions obtained are analyzed by GLC using two glass capillary columns coated respectively with Carbowax 20M and BDS. Equivalent chain length (ECL) values were calculated according to the James and Martin method (*28*, *29*). The ECL obtained are in good agreement with those found by Flanzy et al. (*30*) and Mordret et al. (*31*) for usual fatty acids in the case of the Carbowax 20M phase. The ECL values obtained with BDS are comparable with those given by Ackman (*32*) and Jamieson (*33*). The use of a glass capillary column for the derivatives of CPEFA methyl esters separation permitted the separation of the two methoxy ethers of methyl malvalate (18:CM_1 and 18:CM_2) on the chromatograms and also of the two methoxy ethers of methyl sterculate (19:CM_1 and 19:CM_2). The resolution of the two isomers of the corresponding enones (18:CC_1 and 18:CC_2 for malvalic acid and 19:CC_1 and 19:CC_2 for sterculic acid) was also observed. The separation pattern of kapok seed oil 4 with Carbowax 20M is given in Figure 3. The normal methyl esters separate from

Table I. Equivalent Chain Lengths of Fatty Acid Methyl Esters on Various Phases, Fatty Acid Analysis of Kapok Oil 4[a]

	equivalent chain lengths (ECL)[b]				kapok oil 4[c,i]			
					normal methyl esters		CPEFA derivs	
fatty acid[g]	BDS (this work)[e]	Carbowax 20M (this work)[f]	Carbowax 20M (*30*)[d]	Carbowax 20M (*31*)	BDS[e]	Carbowax 20M[f]	BDS[e]	Carbowax 20M[f]
14:O	14.00	14.00	14.00	14.00	0.2	0.2		
15:O	15.00	15.00	15.00	15.00	tr	tr		
16:O	16.00	16.00	16.00	16.00	29.0	27.9		
16:1ω7	16.35	16.29	16.27	16.24	0.4	0.3		
17:O	17.00	17.00	17.00	17.00	0.2	0.3		
17:1	17.25	17.21	17.23	17.20	0.7	0.7		
17:2	17.80	17.66			0.5	0.5		
18:O	18.00	18.00	18.00	18.00	3.7	3.7		
18:1ω9	18.27	18.21	18.20	18.20	24.5	25.1		
18:1ω + 18:CA[h]	18.32	18.27	18.26	18.28	1.5	1.5		
18:2ω6	18.82	18.66	18.63	18.58	36.0	36.3		
19:CA[h]	19.27	19.23			1.9	2.1		
18:3ω3	19.50	19.29	19.25	19.23	0.1	0.1		
20:O	20.00	20.00	20.00	20.00	0.8	0.8		
20:1ω9	20.16	20.13	20.77	20.15	0.1	0.2		
22:O	22.00	22.00	22.00	22.00	0.4	0.3		
18:CM_1	20.70	20.37					29.0	28.4
18:CM_2	20.77	20.42					36.4	37.4
19:CM_1	21.65	21.34					15.6	15.1
19:CM_2	21.72	21.39					15.0	14.9
18:CC_1	22.33	21.70					4.0	2.8
18:CC_2	22.67	21.82					tr	1.4
19:CC_1	23.50	22.70					tr	tr
19:CC_2	23.56	22.80					tr	tr

[a] After reaction with anhydrous methanol saturated with silver nitrate. [b] Results obtained with glass capillary columns. [c] After separation of normal and CPEFA derivatives methyl esters using preparative TLC with silica gel F 254. [d] Steel capillary column. [e] Temperature column 150 °C. [f] Temperature column 190 °C. [g] The corresponding formula of CPEFA derivatives is given in Figure 1. [h] 18:CA:dihydromalvalic acid; 19:CA:dihydrosterculic acid. [i] Percent by weight.

Table II. Fatty Acid Analysis[a] of Various Samples of Seed Oils after Reaction with Anhydrous Methanol Saturated with Silver Nitrate

	cotton				kapok				peanut[c]			
	1		2		4		5		6		7	
fatty acid[b]	BDS[d]	Carbowax 20M[a]	BDS	Carbowax 20M[e]	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M
14:O	0.8	0.8	0.9	0.9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
15:O	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
16:O	24.4	23.4	24.1	24.0	21.6	20.4	22.1	21.6	13.2	12.9	14.5	14.0
16:1	0.6	0.6	0.6	0.6	0.3	0.2	0.3	0.3	0.1	0.1	0.1	0.1
17:O	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
17:1	0.1	0.1	0.1	0.1	0.7	0.6	0.7	0.6	0.1	0.1	tr	tr
17:2	0.1	tr	0.1	0.1	0.6	0.6	0.6	0.7	tr	tr	tr	tr
18:O	2.3	2.4	2.4	2.4	2.8	2.8	2.7	2.7	2.7	2.8	2.9	2.9
18:1ω9	15.4	15.6	15.3	15.3	21.2	21.5	21.1	21.0	36.8	37.2	35.7	35.8
18:1ω7 + 18:CA[f]	0.8	0.8	0.7	0.8	1.2	1.3	1.1	1.3				
18:2ω6	53.8	54.4	53.6	53.6	37.0	38.8	37.2	27.6	39.9	40.2	40.1	40.0

19:CA[f]	0.3	0.3	0.3	0.3	1.6	1.6	1.4	1.6	tr	tr	tr	tr
18:3ω3	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1
20:O	0.2	0.3	0.3	0.3	0.7	0.6	0.7	0.6	1.2	1.2	1.2	1.2
20:1ω9	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	1.2	1.2	1.2	1.1
22:O	0.1	tr	0.1	0.1	0.3	0.3	0.3	0.5	3.3	3.1	3.2	3.2
not identified[g]	0.5	0.6	0.6	0.6								
$18:CM_1$	0.2	0.2	0.2	0.2	3.4	3.7	3.5	3.5	tr	tr	tr	tr
$18:CM_2$	0.1	0.2	0.2	0.2	3.5	3.6	3.5	3.5	tr	tr	tr	tr
$19:CM_1$	0.1	0.1	0.1	0.1	1.5	1.6	1.5	1.6	tr	tr	tr	tr
$19:CM_2$	tr	tr	0.1	0.1	1.5	1.7	1.5	1.6	tr	tr	tr	tr
$18:CC_1$	tr	tr	tr	tr	0.7	0.3	0.7	0.9	tr	tr	tr	tr
$18:CC_2$	tr	tr	tr	tr					tr	tr	tr	tr
$19:CC_1$	tr	tr	tr	tr	0.1	tr	0.6	tr	tr	tr	tr	tr
$19:CC_2$	tr	tr	tr	tr		tr		tr	tr	tr	tr	tr
24:O									1.2	0.9	0.8	1.4
total CPEFA[a]	0.4	0.5	0.6	0.6	10.7	10.9	11.3	11.1	tr	tr	tr	tr

[a] Percent by weight. [b] The corresponding formula of CPEFA derivatives is given in Figure 1. [c] Peanut oils adulterated with 10% of Cottonseed oil (6) and with 5% of Kapok oil (7). [d] BDS glass capillary column at 150 °C. [e] Carbowax 20M glass capillary column at 190 °C. [f] 18:CA, dihydromalvalic acid; 19:CA, dihydrosterculic acid. [g] ECL:22.30 on BDS and 22.25 on Carbowax 20M.

Table III. Fatty Acid Analysis[a] of Various Samples of Seed Oils Obtained by Direct GLC Analysis of Their Methyl Esters

	cotton						kapok				peanut[c]			
	1		2		3[i]		4		5		6		7	
fatty acid[b]	BDS[d]	Carbowax 20M[e]	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M	BDS	Carbowax 20M
14:O	1.1	0.9	1.0	0.8	1.8	1.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
15:O	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
16:O	26.6	24.9	25.0	23.5	22.9	22.1	21.0	22.0	22.0	22.4	14.3	12.9	14.4	13.5
16:1	0.6	0.7	0.6	0.6	0.5	0.5	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
17:O	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
17:1	0.1	0.1	0.1	0.1	0.1	0.1	0.6	0.6	0.6	0.8	tr	0.1	tr	tr
17:2	0.1	tr	tr	tr	tr	tr	0.6	0.7	0.6	0.6	tr	tr	tr	tr
18:CE[f]	0.5	2.7	0.4	2.7	0.3	2.5	8.3	5.2	8.3	4.4	0.1	3.0	tr	tr
18:O	2.4		2.4		2.3		2.6	2.7	2.7	2.7	3.0		2.7	2.8
18:1ω9	15.6	15.9	15.2	15.6	17.7	18.1	21.4	22.5	21.2	22.6	36.7	36.8	35.4	35.3
18:1ω7 + 18:CA	0.8	0.9	0.8	0.8	0.7	0.8	1.2	1.3	1.1	1.2	0.4	0.2	0.5	0.1
18:2ω6	49.7	51.0	52.3	54.3	47.5	48.9	37.3	39.8	36.9	40.4	39.9	39.8	41.6	41.2
19:CE[g]	0.3	0.1	0.3	0.1	0.2	0.1	3.8	4.8	3.6	1.6	tr	tr	tr	tr
19:CA	0.3	0.4	0.3	0.3	0.3	0.3	1.5	1.7	1.5	1.7	tr	tr	tr	tr
18:3ω3	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
20:O	0.2	0.3	0.2	0.3	0.3	0.4	0.5	0.6	0.5	0.6	1.3	1.3	1.1	1.2
20:1ω9	tr	tr	tr	tr	0.1	0.2	0.1	0.1	0.1	0.1	1.0	1.2	1.0	1.1
22:O	tr	0.1	0.1	0.1	0.3	0.5	0.3	0.3	0.2	0.2	2.6	3.2	2.5	3.1
not identified[h]	1.5	1.8	0.3	0.5	0.3	0.5								
24:O											0.3	1.1	0.4	1.3
total CPEFA[a]	0.8		0.7		0.5		12.1	7.0	11.9	6.0	0.1		tr	tr

[a] Percent by weight. [b] 18:CE, malvalic acid (8,9-methylene-8-heptadecenoic); 19:CE, sterculic acid (9,10-methylene-9-octadecenoic); 18:CA, dihydromalvalic acid; 19:CA, dihydrosterculic acid. [c] Peanut oils adulterated with 10% cottonseed oil (6) and with 5% kapok oil (7). [d] BDS glass capillary column at 150 °C. [e] Carbowax 20M glass capillary column at 190 °C. [f] ECL for 18:CE, 17.94 with BDS, 17.92 with Carbowax. [g] ECL for 19:CE: 18.90 for BDS, 18.97 for Carbowax. [h] ECL: 22.70 for BDS, 22.25 for Carbowax. [i] Industrial cottonseed oil contained 1.1% C8, 0.5% of C10, and 2.9% of C12 with BDS; 0.5%, 0.3%, and 2.4%, respectively, with Carbowax 20M.

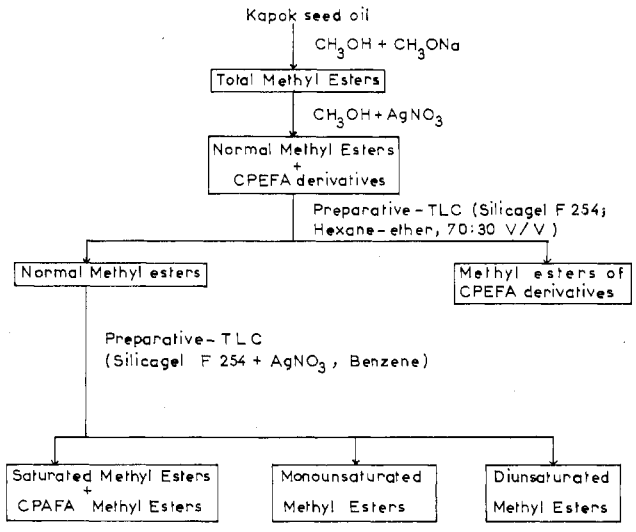

Figure 2. Scheme used for the separation and characterization of methyl esters of kapok seed oil.

the CPEFA methyl ester derivatives, permitting determination of the ECL values of these derivatives. In the case of normal methyl esters the peak of ECL 19.27 on BDS and 19.23 on Carbowax 20M remaining in the saturated fatty acid fraction has been attributed to dihydrosterculic acid (19:CA). It should be noted that all the fatty acid methyl esters are resolved on these two columns except in the case of the *cis*-vaccenic acid (18:1ω7) and dihydromalvalic acid (18:CA). We have demonstrated the presence of these two compounds, of ECL 18.32 on BDS and 18.27 on Carbowax 20M, after having achieved the separation of the normal methyl esters using preparative TLC on silica gel impregnated with silver nitrate. The saturated methyl ester fraction presents a peak of ECL 18.27 on Carbowax 20M which is methyl dihydromalvalate (18:CA) and the monounsaturated fraction involves the same ECL peak which is the *cis*-vaccenic acid (18:1ω7). We have observed that with Carbowax 20M the separation of methyl dihydrosterculate (19:CA) and methyl linolenate (18:3ω3) depends on the columns used. This phenomenon was never observed in the case of BDS columns. The two enonic isomers of methyl malvalate ($18{:}CC_1$ and $18{:}CC_2$) and of methyl sterculate ($19{:}CC_1$ and $19{:}CC_2$) are also not as well separated with some of the Carbowax 20M columns used. The results obtained are summarized in Table I.

Quantitative Analysis of Various Oil Samples after Reaction with Silver Nitrate in Anhydrous Methanol. The different fatty acids having been identified, we could verify that no ECL value of CPEFA methyl ester was identical with those of normal fatty acid methyl esters (Table I). It was therefore possible to analyze total methyl esters, after reaction with silver nitrate in anhydrous methanol without previous fractionation. We have made a quantitative analysis of two samples of cottonseed oils 1 and 2, two of kapok seed oils 4 and 5, and two samples, 6 and 7 of peanut oils adulterated with 5% kapok seed oil and 10% of cottonseed oil, respectively. The results obtained on BDS at 150 °C and Carbowax 20M at 190 °C are given in Table II. In the case of cottonseed oils, it should be noticed that there is a high percentage of palmitic (23–24%), oleic (15%), and linoleic (53–54%) acids. The CPEFA content is low and varies between 0.4 and 0.6%. The CPAFA content is low since we have found 0.3% for dihydrosterculic acid and the sum of the *cis*-vaccenic acid plus malvalic acid is about 0.7–0.8%. In the case of kapok seed oils 4 and 5 the more important fatty acids are palmitic (20–22%), oleic (21%), and linoleic (37–38%) acids. The malvalic acid content (7.6–7.9%) and sterculic acid content (3.1–3.6%) are in good agreement with some results

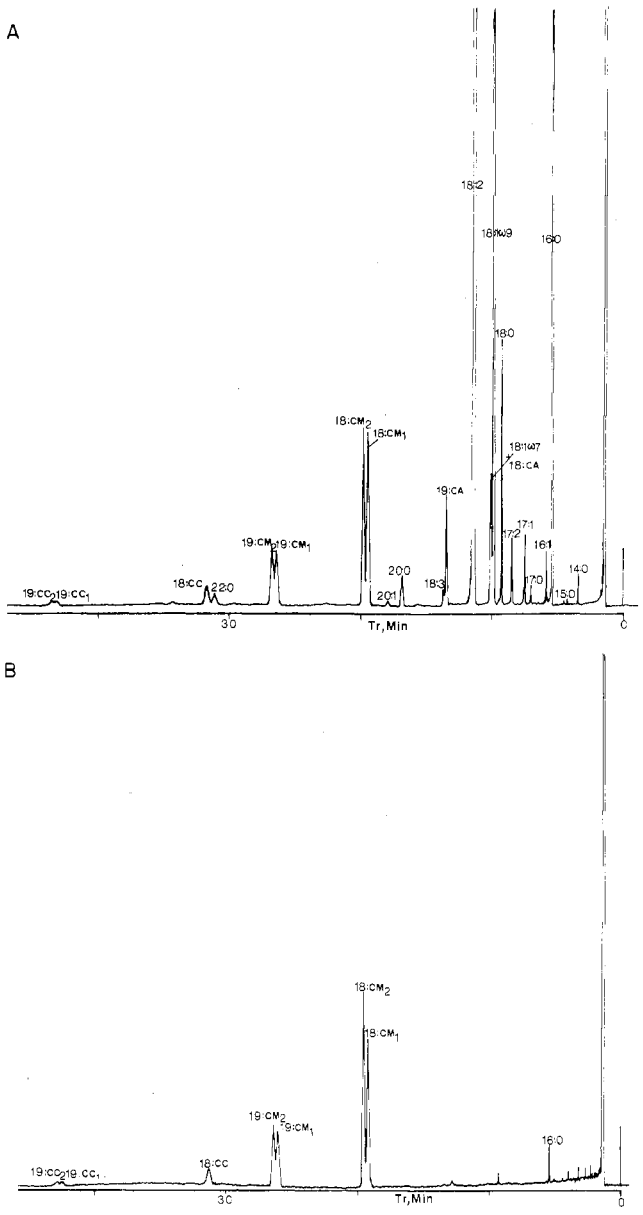

Figure 3. Capillary GLC analysis of kapok seed oil (4) methyl esters, after silver nitrate reaction in anhydrous methanol. Column, Carbowax 20M at 190 °C; pressure, 0.9 bar; carrier gas, H_2; split 5/100; sample volume, 1 μL. Peak identifications are as in Figure 1:18:CA, dihydromalvalic acid; 19:CA, dihydrosterculic acid; A (top), total methyl esters; B (bottom), methyl esters of CPEFA derivatives obtained by preparative TLC (Silica gel F 254; solvent, hexane–ether, (70:30 v/v)).

obtained recently (*14*). The dihydrosterculic acid content (1.5–1.7%) is in the same range than those obtained by Recourt et al. (*3*). It should be noted that in the two cases of peanut oils adulterated with kapok seed oil 6 and cottonseed oil 7 it was possible to characterize and measure CPEFA by titration.

Direct Analysis of Total Methyl Esters by GLC. Direct GLC analysis of total methyl esters on glass capillary columns, without previous CPEFA transformation, gives chromatograms with two new peaks with ECL 17.94 and 18.90 on BDS and 17.92 and 18.98 on Carbowax 20M. They are attributed in increasing elution order to methyl malvalate (18:CE) and methyl sterculate (19:CE). Chromatogram patterns obtained on BDS and Carbowax 20M are given in Figure 4. Quantitative analysis results obtained for the seven samples investigated are presented in Table III. It should be noted that for low CPEFA content (cottonseed oils), the Carbowax 20M column at 190 °C gives a poor separation of stearic acid (18:0)

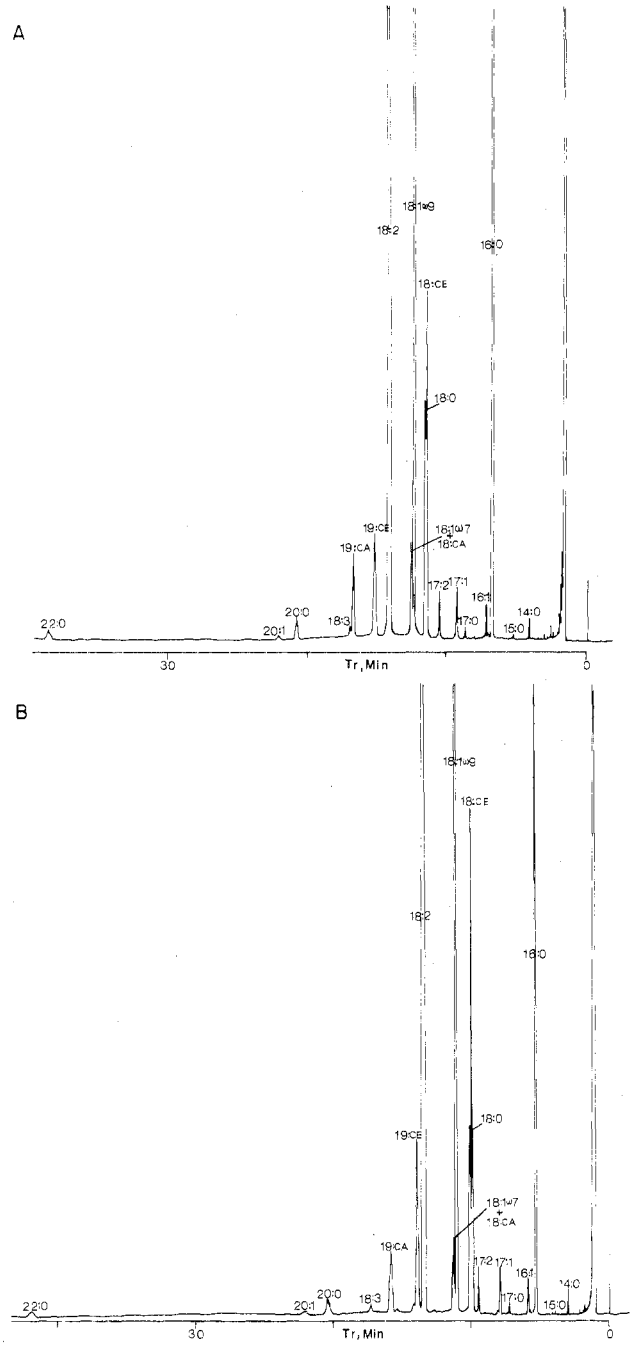

Figure 4. Capillary GLC chromatogram of total methyl esters of kapok seed oil 4. Direct analysis without derivatization: 18:CA, dihydromalvalic acid; 19:CA, dihydrosterculic acid. (A top), Column, 1 Carbowax 20M; conditions as in Figure 3. (B, bottom) column: BDS at 150 °C; pressure, 0.5 bar; carrier gas, H_2; split 5/100; sample volume, 1 μL.

and malvalic acid (18:CE). In all the cases, it can be observed that the CPEFA content on Carbowax 20M is always lower than on BDS. Indeed, for kapok seed oil CPEFA vary from 12% on BDS to 6–7% on Carbowax 20M. This phenomenon is due to the temperature influence on the CPEFA stability and has been shown by a study of the kapok oil compounds at three temperatures (190, 180, and 170 °C) on Carbowax 20M (Table IV). The CPEFA content increases from 7.0 to 9.3% when temperature decreases. On an other hand, at lower temperatures (180 °C), malvalic acid and stearic acid are separated, as shown in Figure 5, and a quantitative analysis may be effected even at lower content of CPEFA. This direct analysis method therefore permits the assay of CPEFA and CPAFA contained in oils. The results are comparable to those

Table IV. Temperature Influence upon CPEFA Stability of Kapok Oil 4 on Carbowax 20M Glass Capillary Column

	composition[a]		
fatty acid[b]	190 °C	180 °C	170 °C
16:O	22.0	21.5	22.2
18:CE[c]	5.2	6.0	6.7
18:O	2.7	2.8	3.0
18:19	22.5	22.4	22.3
18:17 + 18:CA	1.3	1.3	1.0
18:26	39.8	39.3	39.3
19:CE[d]	1.8	2.3	2.6
19:CA[e]	1.7	1.7	1.4
18:33	0.2	0.1	tr
others[f]	2.8	2.6	1.6
total CPEFA[a]	7.0	8.3	9.3

[a] Percent by weight. [b] 18:CE, malvalic acid; 19:CE, sterculic acid; 18:CA, dihydromalvalic acid; 19:CA, dihydrosterculic acid. [c] ECL: 17.97 at 190 °C, 17.95 at 180 °C, 17.94 at 170 °C. [d] ECL: 18.92 at 190 °C, 18.91 at 180 °C, 18.90 at 170 °C. [e] ECL: 19.23 at 190 °C, 19.22 at 180 °C, 19.21 at 170 °C. [f] Others: 14:O, 15:O, 16:1, 17:O, 17:1, 17:2, 20:O, 20:O, 20:1, and 22:O.

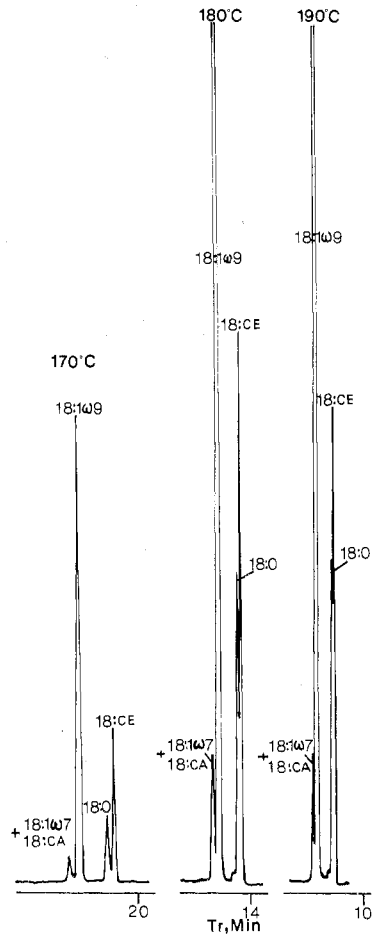

Figure 5. Temperature influence upon total methyl esters separation of kapok seed oil 4, direct analysis without derivatization: 18:CA, dihydromalvalic acid: 19:CA, dihydrosterculic acid; 18:CE, malvalic acid; 19:CE, sterculic acid. Column was Carbowax 20M. Conditions were as in Figure 3.

obtained with the CPEFA derivatives analysis in the case of BDS column and with Carbowax 20M column at lower temperature (170 °C). It was also possible to detect the presence of CPEFA and CPAFA in adulterated peanut oils 6 and 7.

Comparison of Different Methods of CPEFA Determination. Quantitation of cyclopropenoic lipids has long been a problem because of unstability of their methyl esters on the

Table V. Comparison of Different Methods for CPEFA Quantitation[a] in Various Oil Samples

			cotton			kapok		peanut[b]	
method	column phase	temp, °C	1	2	3	4	5	6	7
NMR[f]		ambient	⩽1.0	⩽1.0	⩽1.0	10.2	9.7		
HBr		55	2.8[c]	1.3[c]	*d*	11.9	11.9	0.9	*d*
GLC	BDS	150	0.4	0.6	*d*	10.7	11.3	tr[e]	tr
after derivatization	Carbowax 20M	190	0.5	0.6	*d*	10.9	11.1	tr	tr
direct GLC	BDS	150	0.8	0.7	0.5	12.1	11.9	0.1	tr
	Carbowax 20M	170				9.3			
		180	0.5	0.5	0.3	8.3			
		190	*d*	*d*	*d*	7.0	6.0	tr	tr

[a] Percent by weight. [b] Peanut oils adulterated with 10% cotton oil (6) and with 5% kapok oil (7). [c] Higher values because the cotton oils were green and titration to the blue-green end point of the crystal violet indicator was difficult. [d] Not detected. [e] Trace. [f] Instrument used is Perkin-Elmer R 32, sweep width 250 Hz.

column of GLC (*18*). Five methods for the determination of cyclopropenoic fatty acids were reviewed by Coleman (*16*). This author recommends the method of Schneider et al. (*18*), involving reaction of transesterified lipids with silver nitrate in anhydrous methanol to form methoxy ether and enonic derivatives (see Figure 1), which then are analyzed by GLC. The results obtained by using this method (vide supra) are summarized for CPEFA in Table IV. The Halphen color test is generally used for CPEFA detection. The absorbance of Halphen reaction products is not accurate (*34*), but Hammonds et al. (*35*) have since reported a modification of the Bailey method (*34*) which appears to be a good routine procedure. All the samples that we have investigated gave a positive Halphen color test. Pawlowski et al. (*17*) presented the use of nuclear magnetic resonance (NMR) spectrometry as a rapid, simple and quantitative method of analysis for the cyclopropenoic function in lipids. By this method, samples of 5–30 mg are sufficient and lipids with as low as 1% cyclopropenoic can be measured. The accuracy is about 0.5% for 10% cyclopropenoic concentration. In our case, for the three samples of cottonseed oils, the high field region of NMR spectra showed the cyclopropenoic peak, but accurate results could not be worked out below 1% levels with the particular spectrometers used. In the cases of kapok seed oils 4 and 5, 9.7 and 10.2% were obtained. Coleman (*16*) reviewed also several methods for the quantitative titration of CPEFA using hydrobromic acid. Using Durbetaki's method (*15, 23*), we have found that end points of the crystal violet indicator were poorly defined, especially when colored oils, such as raw cottonseed oils were used. The results are too high, as shown in Table V. The comparison between the direct GLC analysis of oil samples containing CPEFA and NMR analysis, hydrobromic acid titration, and GLC analysis after derivatization is presented in Table V. In the case of cottonseed oils, where CPEFA content is low, the NMR method is not accurate, and hydrobromic acid titration method is unsuitable in the case of unrefined oils. It can be noted that results obtained after derivatization are very near to those of direct GLC. For kapok seed oils a good agreement of results, with different methods used, may be observed. Therefore, the two chromatographic methods permit CPEFA and CPAFA titration separately, whereas NMR and hydrobromic acid methods do not permit the CPAFA content determination. In the case of peanut oils adulterated with cottonseed or kapok seed oils, the lower limit of detection of CPEFA can be extended to less than 0.02%.

GLC method after derivatization gives results slightly higher than those with the NMR method. The direct GLC method thus appears to be an accurate and rapid method to simultaneously determine CPEFA and CPAFA methyl esters. Results obtained with BDS glass capillary column seem to give results higher than those obtained with the other methods. A Carbowax 20M column, which is now very widely used for fatty acid analyses, gave good results for CPEFA when the oven temperature is about 170 °C.

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Stray Light Ratio Measurements

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Two new methods of expressing and measuring the stray light ratio in spectrophotometers are described. The first of these methods involves a convolution of the detected radiant power spectrum of the instrument with the slit function. The convolution integral is performed over wavelength intervals dictated by limits that distinguish stray from primary light. The second simpler method utilizes the optical properties of the blocking filter both to reduce and to measure the remote stray light. The latter method has been automated in two new microprocessor controlled spectrophotometers. These methods are capable of measuring the stray light ratio at any wavelength and in the presence or absence of any sample and, consequently, are far superior to conventional stray light tests when applied to instruments using moderately narrow band blocking filters.

Stray light is a well-known source of error in spectrophotometer measurements. Its significance increases with sample absorbance. Unfortunately, the accurate measurement of stray light has been nearly impossible. The presence of any test material in the beam always affects the level of stray light. If the spectrophotometer is equipped with efficient blocking filters, even relatively narrow band or sharp cutoff materials conventionally used to test for stray light may absorb most of the stray light and lead to a gross error. This situation was encountered in developing the Beckman DU-5 spectrophotometer. When testing this instrument for stray light using a $NaNO_2$ solution following the ASTM (*1*) procedure, an unbelievably low level of stray light was measured. The reasons are now obvious. The $NaNO_2$ test solution absorbed nearly all of the potential stray light and led to an underestimation of stray light by a factor of 500×. Different methods had to be developed and are described below. The new methods proved to be more accurate than the ASTM test and moreover measure the stray light in the presence of any sample.

A major difficulty resides in the definition of stray light. The most widely accepted definition is that given by ASTM (*1*) which expresses stray light as "radiant energy of wavelengths remote from those of the nominal pass band transmitted through the monochromator ...". The term "remote" is imprecise and, in practice, varies with the material used to measure the stray light. In this paper, stray light is defined as detected radiant power of wavelengths more than L units away from the center, $\bar{\lambda}$, of the monochromator pass band. Under this definition, stray light must always be qualified by the parameters L and $\bar{\lambda}$. The parameter L is called the "limit" distinguishing stray from primary radiation. This limit may have any specified value greater than the spectral slit width (SSW). The smaller the L, the more nearby stray light is incorporated in the measurement.

This refinement in definition is practical only when using a test method that incorporates the "L" parameter. Such a test method is described here. It involves the convolution of the detected radiant power (DRP) spectrum (sometimes called the single-beam energy spectrum or relative instrument spectral function) with the slit function of the monochromator. The L value determines the limits of the convolution integration.

The stray light by either of the above definitions is seldom of interest. Rather one desires the ratio of the stray light to the total DRP. The quantity measured by the ASTM test is such a ratio and is called the stray radiant energy (SRE) or sometimes the stray radiant power ratio. The ratio measured by the new test described here is called the stray light ratio (SLR).

The concept of qualifying a stray light measurement by a limit is not new. It was first suggested by Poulson (*2*), but no way of incorporating the limit in an experimental test was then known. Later a convolution method similar to that described here was attempted, but the measurement of the slit function was difficult and confined to wavelenghts provided by intense laser sources (*3*). With the introduction of improved instrumentation of very high signal-to-noise ratio, it is now possible to obtain slit functions with more convenient sources (*4*).

In addition to a rigorous convolution test for the SLR, a simplified test is also described. It does not require a convolution or knowledge of the slit function. It has been automated and incorporated in two new microprocessor-controlled spectrophotometers. While it measures only remote stray light, it can be applied in the presence or absence of any sample and hence avoids the larger error of conventional tests.

THEORY

The dark current corrected signal from a detector monitoring the output of a monochromator set to wavelength dial setting $\bar{\lambda}$ can be expressed as

$$P(\bar{\lambda}) = \int_{-\infty}^{+\infty} P(\lambda)F(\bar{\lambda} - \lambda)\, d\bar{\lambda} \qquad (1)$$

where $P(\lambda)$ describes the spectrum displayed when scanning the source spectrum in the single-beam mode. $F(\bar{\lambda} - \lambda)$ is the slit function of the monochromator. Convolution integrals of this type have been widely employed to study the interaction between instrument resolution and sample bandwidth (*5*). A modification of this integral is used here to provide

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