oils have not been corrected for refining loss. They may be however correct within the limits of experimental error.

Conclusions

A procedure for the analysis of cottonseed and its products for Vitamin E has been developed and improved. The present procedure generally gives good checks on duplicate analyses.

The Vitamin E content of whole cottonseed is somewhat more a function of variety than of locality. The 1952 crop of cottonseed contained more Vitamin E than the 1951 crop. The two crops averaged 84 g. per ton and 68.5 g. per ton, respectively.

Most recent results show that there is no loss of Vitamin E in the storage of cottonseed or during the processing of the seed for its oil and other products.

The hexane-soluble portion of a methanol extract of rolled or cooked cottonseed meats contains Vitamin E in concentrations that have exceeded the values reported for wheat germ oil, the present commercial source.

A sample of commercial solvent extracted cottonseed meal was found to contain considerably less Vitamin E than hydraulic- or screw-pressed meals.

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The Component Acids of Kamala Oil (Mallotus Philippinensis, Muell. Arg.)

S. C. GUPTA, S. S. GUPTA, and J. S. AGGARWAL, National Chemical Laboratory of India, Poona

KAMALA oil from the seeds of *Mallotus Philippinensis*, Muell. Arg. (N. O. Euphorbiaceae), which grows fairly abundantly in this country and to some extent in many other parts of the East, has shown promise as a substitute for tung oil (2, 11). Previously some general studies on the drying properties and characteristics of the oil (1, 7) have been made, but no decisive investigation was carried out. While the present study of kamala oil was in progress, Puntambekar (9) reported that the fatty acids of kamala oil consist of isomeric elaeostearic, ketopolyethenoid C_{18}, oleic, linoleic, stearic, and other saturated acids. These results were deduced from his studies on the physical and/or chemical characteristics of the different fractions obtained by the lead salt alcohol method of the total fatty acids. The inadequacy and limitations of lead salt-alcohol method as an analytical procedure for oils containing conjugated polyethenoid acids are now well known. Moreover his contention regarding the nature of the new acids (mixture of keto polyethenoid acid and isomeric elaeostearic acid) with respect to the positions of keto group and double bonds is not very convincing and lacks substantial evidence. Further, the absence of any keto acid in this oil has been definitely established in our earlier communication (2).

Isolation and characterization (2) of a new acid, kamlolenic acid (ω-hydroxy, 9,11,13-octadeca trienoic acid) prompted further studies on the composition of the oil. When separated by means of its insolubility in petroleum ether (40-60°C.), about 5-10% of this acid was always found in the solvent along with other acids. In the absence of any alternative method for the estimation of this typical acid in the oil, the recently developed photometric method (6) as an analytical procedure has been found of considerable importance in assessing the potentialities of this new drying oil.

Extraction of the oil. The presence of 50-60% kamlolenic acid as a major acid renders the extraction of the entire quantity of the oil from the seeds with petroleum ether impossible. It was therefore found advantageous to extract the oil from the seeds in a soxhlet apparatus first by petroleum ether (40-60°C.) and then by ethyl ether. The oils obtained in both cases were kept separate and analyzed as such. The oils from fresh seeds (Bombay variety) and one-year-old seeds (Uttar Pradesh variety) indicated some differences. The analytical data have been recorded in Table I.

Analytical methods and results. The four specimens of the oil referred to above were separately saponified, and the unsaponifiable matter was removed according to the S.P.A. method (12). The mixed fatty acids obtained in each case were then examined for their components as described below.

Estimation of kamlolenic acid. The spectroscopic values for pure α- and β-kamlolenic acids have already been reported (2). These acids have been isomerized according to the method recommended by Hilditch, Morton, and Riley (6) for elaeostearic acid. The complete data are given in Table II.

The amount of conjugated triene acid in the different samples of the fatty acids from the oil was directly calculated from their respective extinction coefficients at 270.5 mμ. These values however would

TABLE II

$E^{1\%}_{1cm}$ Values for α- and β-kamlolenic acids

	270.5 mμ	268 mμ	234 mμ
α-Kamlolenic acid (unisomerized)	1800[a]	....	176
β-Kamlolenic acid (unisomerized)	....	(1990)[a]	(210)
α-Kamlolenic acid (alkali isomerized, 180°C.–60 mins.)	....	(926)	204
β-Kamlolenic acid (alkali isomerized, 180°C.–60 mins.)	....	(1056)	(188)
α-Kamlolenic acid (alkali isomerized, 170°C.–15 mins.)	....	1510	....
β-Kamlolenic acid (alkali isomerized, 170°C.–15 mins.)	....	(1805)	....

[a] Represent the corresponding values at the highest band head.
(Note: Data not actually required in the analysis of Kamala oil are shown in brackets.)

TABLE I

Extraction Data and Characteristics of Kamala Oil

	% of oil	Refractive index (25°C.)	Iodine value (Wijs' ½hr.)	Acid value	Saponification equivalent	Unsaponifiable (%)
I. Fresh seeds						
Oil extracted with petroleum ether (A)	18.3	1.5085	123.2	20.9	287.9	3.7
Oil extracted with ethyl ether (B)	17.0	1.5362	174.3	7.6	285.9	3.7
Total oil content (on whole seeds)	35.3	....	....	....	....	....
II. Seeds from old stock						
Oil extracted with petroleum ether (C)	20.0	1.5072	133.3	23.2	291.6	3.7
Oil extracted with ethyl ether (D)	12.1	1.5375	172.4	5.4	353.5	30.7[a]
Total oil content (on whole seeds)	32.1	....	....	....	....	....

[a] Including a substantial amount of polymerized material, not saponifiable with 0.5 *N* alkali used in the experiment.

also include, along with the kamlolenic acid, any other conjugated trienoic acid of the elaeostearic type if present. This has been easily verified by alternative determination of kamlolenic acid content from the acetyl value of the mixed acids. The results obtained by the two methods (Table V) are in close agreement, and that indicated the virtual absence of elaeostearic or any other conjugated trienoic acid in kamala oil.

Determination of other polyethenoid acids. The spectroscopic examination indicated the presence of a small amount of conjugated diene acid in kamala oil, which could not be isolated.

The mixed acids were isomerized in KOH-glycol at 180°C. for 60 minutes (6), and, after appropriate dilution, $E^{1\%}_{1cm.}$ value was determined at 234 mμ. After allowing for the contribution of kamlolenic acid under these conditions (Table II), the percentage of linoleic acid was calculated in the usual manner. Spectroscopic examination did not reveal the presence of any linolenic acid in kamala oil.

For identification of the non-conjugated diethenoid acid, a rich concentrate of the same was obtained after precipitating most of the kamlolenic acid with petroleum ether. The petroleum ether soluble portion yielded on bromination and crystallization from petroleum ether a crystalline compound melting at 113-114°C., unaltered on admixture with tetrabromostearic aicd (m.p. 113.5-114°C.). The dienoic acid is thus confirmed to be the ordinary 9,12-linoleic acid.

Estimation of saturated acids. The procedure followed for the isolation and estimation of saturated acids was that recommended by Hilditch and Lea (5) for the determination of fully saturated glycerides in natural fats.

Acids from the petroleum ether-soluble and ethyl ether soluble fractions of the oil were mixed and dissolved in four times their weight of absolute methyl alcohol. Dry hydrochloric acid gas was passed to saturate the solution immersed in ice bath. The solution was kept overnight at room temperature (27-30°C.). It was taken in ethyl ether and first washed with cold water and then with dilute sodium bicarbonate solution to remove the mineral acid and unesterified fatty acids. The esters were then isolated in the usual way and oxidized repeatedly with potassium permanganate in acetone solution (3) until the unsaturated portion was almost completely oxidized. From the weights and iodine values of the residual esters the exact proportion of saturated contents in the mixed acids was calculated as shown below:

Sample	Mixed esters oxidized (g)	Residual esters		Saturated acids (%)
		Wt.(g.)	I.V.	
I	36.15	4.58	2.1	12.0
II	45.45	5.39	6.8	10.2

A portion of these esters was fractionally distilled through a micro-fractionation unit under reduced pressure (0.2-1 mm.). The iodine and saponification values and mean molecular weights of all the fractions were determined and the amounts of component esters in each fraction were calculated (Table III).

The composition of saturated acids therefore is as follows:

Acid	Percentage
Lauric	1.25
Myristic	20.86
Palmitic	72.45
Stearic	5.44

The acids from Fractions 1 and 6 were isolated, and these on repeated crystallizations from ethyl acetate gave fairly pure samples of myristic acid (m.p. 53-55°C.) and palmitic acid (m.p. 63-64°C.), respectively.

Component acids of kamala oil. The relevant spectroscopic values for the different samples of oils examined are summarized in Table IV. The percentage

TABLE IV

Spectroscopic Characteristics of Mixed Fatty Acids[a] from Kamala Oil

Extinction coefficients $E^{1\%}_{1cm.}$ at	I. Oil from fresh seeds		II. Oil from stock seeds	
	Petroleum ether soluble, Fraction (A)	Ethyl ether soluble, Fraction (B)	Petroleum ether soluble, Fraction (C)	Ethyl ether soluble, Fraction (D)
234 mμ (unisomerized)	118	200	95	170
270.5 mμ (unisomerized)	674	1460	752	1456
234 mμ (after isomerization, 180°C.–60 mins.)	265	289	244	243
268 mμ (after isomerization, 170°C.–15 mins.)	560	1205	636	1164

[a] Excluding unsaponifiable and polymerized material.

of triene and diene acids was calculated from these data and the saturated acids as mentioned above. The oleic acid was found by the difference. These data are recorded in Table V.

Discussion

Kamala oil has been found to contain a hydroxy conjugated trienoic acid (kamlolenic acid) which appeared to be the only major and active constituent of the oil. Spectroscopic data also indicated the presence of minor proportions of some conjugated diene in both the samples. The non-conjugated diethenoid acid has been indentified as common linoleic acid, which is found to occur in most of the vegetable oils. An unusual feature of the acid composition of kamala oil is the virtual absence of stearic acid in the saturated components, which consist mostly of myristic and palmitic acids while the unsaturated components are almost entirely confined to the C_{18} series.

TABLE III

Fractionation of Methyl Esters of Saturated Acids

Fraction No.	Boiling point (°C.)	Weight of the fraction (g.)	Iodine value (Wijs)	Saponification value	Mean molecular weight	Component esters in the fraction				
						Unsaturated (g.)	Methyl laurate (g.)	Methyl myristate (g.)	Methyl palmitate (g.)	Methyl stearate (g.)
1	60-80	0.381	....	240.98	232.8	....	0.126	0.255	....	....
2	80-85	0.396	....	223.32	251.2	....	....	0.271	0.125	....
3	85-90	1.264	0.5	218.71	256.5	0.004	....	0.630	0.630	....
4	85-90	1.231	0.1	211.29	265.5	....	....	0.215	1.016	....
5	90-100	1.294	0.4	210.27	266.8	0.004	....	0.165	1.125	....
6	100-120	2.186	3.8	205.79	272.5	0.052	....	....	2.019	0.115
7	Residue	0.837	32.7	196.00	286.2	0.167	....	....	0.389	0.281
Total		7.589				0.227	0.126	1.536	5.304	0.396

The two well-known natural fats from *Licania rigida* and *Parinarium laurinum* contain small amounts of elaeostearic acid (8, 10) besides their major components, licanic and Parinaric acids, respectively, whereas kamala oil has been found to contain only one triene conjugated acid (kamlolenic acid) in its acid composition.

Successive extraction of kamala seeds with petroleum ether and ethyl ether has been found to yield two distinct grades of oil, differing appreciably in color, consistency, keeping quality, and composition. The analytical results (Table V) revealed that the

TABLE V

Component Acids (% wt.) of Kamala Oil

Acids	I. Oil from fresh seeds		Total oil	II. Oil from stock seeds		Total oil
	Fraction (A) (51.8%)	Fraction (B) (48.2%)		Fraction (C) (62.3%)	Fraction (D) (37.7%)	
Kamlolenic	37.4 (38.6)[b]	81.8 (80.9)[b]	58.5	41.8 (43.2)[b]	80.9 (80.2)[b]	57.5
Conjugated diene[a]	4.3	4.8	4.5	1.8	2.3	2.0
Linoleic	15.5	7.6	11.7	15.3	5.7	11.7
Oleic (by difference)	13.3		13.3	19.6		19.6
Lauric	0.1		0.1	0.1		0.1
Myristic	2.5		2.5	2.1		2.1
Palmitic	8.7		8.7	7.4		7.4
Stearic	0.7		0.7	0.6		0.6

[a] Assuming that the polymerized portion is not substantially different in composition from the mixed fatty acids.
[b] Calculated from the respective acetyl values.

active constituent, namely kamlolenic acid, was present to almost double the extent in the ethyl ether-extracted fraction of the oil as compared to its content in the petroleum ether soluble portion. Presence of about 40% of kamlolenic acid in the petroleum ether extracted oil would suggest the predominance of mono-kamloleno glycerides in the same, while the ethyl ether-soluble fraction, containing as much as 80% of this acid, would probably consist mostly of glycerides containing two or three kamlolenic acid groups. This is in conformity with the observed insolubility of the latter fraction in petroleum ether because of the increasing concentration of the hydroxy acid in it. Indication of the presence of 15-20% of tri-kamlolenin against a total content of about 60% kamlolenic acid in the oil provides another exception to Hilditch's rule of even distribution (4).

Comparative study of the oils obtained from the fresh and "old stock" seeds has afforded some useful information regarding the extent of deterioration occurring in the seeds during storage. The presence of an abnormally large amount of unsaponifiable matter with a gel-like appearance in the ethyl ether extract from the old seeds clearly showed that a portion of the oil gets polymerized within the seed itself during storage. No such deterioration of the oil was observed in the case of fresh seeds. On the other hand, none of the petroleum ether-extracted fractions appeared to contain any polymerized material, indicating that glycerides richer in kamlolenic acid are more likely to undergo oxidation and polymerization. On the basis of these observations, it is recommended that, as far as possible, the oil should be extracted from the seeds soon after harvesting.

The drying properties of kamala oil will mostly depend upon its kamlolenic acid content. It is mainly for this reason that the ethyl ether fraction of the oil (80% kamlolenic acid) dries very rapidly while the petroleum ether-extracted fraction containing only about 40% kamlolenic acid is very similar to tung oil in this respect (11) and has meen found to keep fairly well. The very high viscosity and extreme gelation tendency of the ethyl ether-soluble fraction of the oil will naturally necessitate its admixture with other drying oils for various coating compositions.

Summary

Kamala oil has been found to contain about 60% of kamlolenic acid as the only major component with minor proportions of common linoleic, oleic, and saturated acids and probably some conjugated diethenoid acid. The saturated components consist mostly of myristic and palmitic acids.

Consecutive extraction of kamala seeds with petroleum ether and ethyl ether yields two fractions of the oil, containing about 40% and 80% of kamlolenic acid, respectively.

During storage a portion of the unsaturated glycerides seems to undergo polymerization within the seed itself and appears as an insoluble gelatinous mass during saponification of the oil.

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