TABLE II
Nitrogen Analyses of Pure Ammonium Salts

Sample	Theoretical % N	% N found by Kjeldahl method	% N found formaldehyde titration A.O.A.C. 2.26 (1)	pH of 5% solution
Fisher reagent ammonium sulfate	21.20	21.18	21.19	5.2
A.O.C.S. standard ammonium sulfate	21.20	21.17	21.18	5.5
N.B.S. monoammonium phosphate	12.18	12.17	.....	.....

tion. The A.O.C.S. standard ammonium sulfate is marked "lot No. 42848, 25.70% NH_3." This corresponds to 21.14% of nitrogen. A 5% solution of pure ammonium sulfate should have a pH of 5.2 to 5.3 at room temperature.

Table III presents our analyses of a number of reference chemicals. These analyses were obtained over a period of several months (during our participation in the Smalley Series), and the results were collected and compiled.

TABLE III
Nitrogen Analyses of Reference Chemicals

Substance	Theory	No. of analyses	Mean	Average deviation
Ammonium sulfate reagent	21.20	8	21.18	0.009
Ammonium oxalate reagent	19.71	4	19.69	0.007
l-Cystine reagent	11.66	3	11.59	0.000
Acetanilide reagent	10.36	8	10.31	0.005
Benzidine reagent	15.21	4	15.09	0.008
U.S.P. nicotinic acid	11.38	3	11.37	0.007

NOTE: Identical nitrogen analyses were obtained on three specimens of acetanilide. These were sample 141 from the U. S. National Bureau of Standards, the micro-analytical standard of the British Drug Houses, and an Eastman Kodak sample.

Discussion

Our rapid Kjeldahl method (4) has been modified to use sample weights up to 5 g., new one-piece distilling apparatus, and weight burette technique in the titrations. In the distillation step, zinc was eliminated and a minimum of excess alkali was used. These improvements, along with other refinements described above, resulted in improved precision.

This special investigation (which included analyses of reference chemicals, precipitation of ammonium chloroplatinate, demonstration that the alkali isolated was completely volatile, intercomparisons of standards, and standardized solutions and other checks) proves that the Smalley Oilseed Meal Samples contain about 0.05% units more of nitrogen than the average analyses of the participating laboratories which used the official A.O.C.S. procedure (2). Had these laboratories used the latest modification of the A.O.A.C. procedure (1), it is likely that somewhat higher values would have been obtained. We believe that the A.O.A.C. procedure is superior to the A.O.C.S. procedure because the digestion temperature is higher, the digestion heating intensity is specified, and the acid used in measuring the ammonia is standardized directly and then checked against the standardized sodium hydroxide solution.

The report of R. C. Berry (3) compared high and low digestion temperatures on cottonseed meal, meat scrap, and fish meal. The increase in protein recovery was 0.937, 0.57, and 0.713% units, respectively. It is not surprising therefore that we should obtain slightly higher nitrogen values in using our very intense digestion conditions, wherein complete mineralization of the nitrogen is obtained in about 15 min. on 1-g. samples. Insufficient sample was available to us to investigate the relative importance of the two factors likely responsible for our higher nitrogen recoveries. These are a more intense digestion and a one-piece distilling apparatus, which is purged at the end of each distillation. Insufficient sample also made it impossible for us adequately to estimate the importance of the errors resulting from segregation of the particles during the filling of the containers and during the weighing out for analysis. It is felt that better agreement among laboratories and better precision within laboratories would result if samples could be issued which have a lesser tendency to become heterogeneous on handling.

Acknowledgment

Edward Wichers of the U. S. National Bureau of Standards gave helpful advice on reference standards and kindly donated the pure single crystal of monoammonium phosphate. The author is also grateful to Phillip Ferguson of Canada Packers' Research Laboratories for performing many of the analyses presented.

REFERENCES

1. Association of Official Agricultural Chemists, Methods of Analysis.
2. American Oil Chemists' Society, Official and Tentative Methods, Ba 4-38.
3. R. C. Berry, J. Assoc. Offic. Agr. Chemists, *31*, p. 617.
4. C. H. Perrin, Analytical Chem., *25*, p. 968.

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Separation of the Oxidation Products of Fatty Acids by Means of Gas-Liquid Partition Chromatography[1]

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A. T. JAMES AND A. J. P. MARTIN (2) have shown that gas-liquid partition chromatography can be successfully used for the analysis of natural fats and applied the method to goat's milk, olive oil, and fatty extracts from bacterial culture. The work reported here is a preliminary study of the oxidation products of soybean-oil fatty acids by means of gas-liquid chromatography.

Apparatus and Procedure

A stainless steel column, 6 ft. 3 in. long, and 8-mm. I.D., was packed with Celite [3] impregnated with D.C.

[1] Presented at the fall meeting, American Oil Chemists' Society, Chicago, Ill., September 24–26, 1956.
[2] One of the divisions of the Agricultural Research Service, U. S. Department of Agriculture.
[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

TABLE I
Methyl Esters of Monocarboxylic Acids

Compound	Retention time, minutes, flow rate of helium 13 ml./min. Temperature, °C.								
	150	160	170	200	210	215	230	240	250
C_6 Methyl caproate	7:40	6:45	5:51	4:25					
C_7 Methyl enanthate	11:19	9:24	8:21	5:11					
C_8 Methyl caprylate	16:41	13:05	11:18	6:38					
C_9 Methyl pelargonate	28:59	20:20	16:23	8:03					
C_{10} Methyl caprate			24:38	11:07	8:15	8:03	6:31	5:28	5:16
C_{12} Methyl laurate				21:05		14:34	10:10	8:15	7:47
C_{14} Methyl myristate				42:10		27:13	17:38	13:42	11:53
C_{16} Methyl palmitate							31:26	23:52	20:00
C_{18} Methyl stearate								42:22	34:07

silicon, high-vacuum grease in the ratio of 10:1 by weight. Celite No. 545 was size-graded by screening to a particle size 40–120 mesh, acid-washed, and dried according to the method suggested by James and Martin (3). The column was suspended in an electrically heated, well-insulated, air bath kept to within ±1°C. The sample was introduced into the column with a calibrated, micrometer pipette and hypodermic syringe through a rubber serum cap. At higher temperatures the rubber cap was kept cool by water circulating through a small copper condenser soldered to the steel tube just below the cap. The components of the sample were eluted with helium, and the gas-vapor mixture was passed through a thermal conductivity Gow-Mac TR-II detector for analysis. The detector consists of two thermal conductivity cells, a reference and an indicating cell, which form two arms of a Wheatstone bridge circuit.

The presence of vapor changed the thermal conductivity of helium in the indicating cell and affected the temperature of the heated tungsten filament and consequently its resistance. The change of resistance was registered with an automatic, 10-millivolt Leeds and Northrup recorder. The detector housed in a thermostat was mounted outside of the constant-temperature, air bath containing the column. The short connecting tube between the column and the detector was enclosed in a small separate heater to prevent condensation of vapor.

This arrangement permitted the variation of the column temperature from room temperature to 250° C. while the detector temperature was kept constant in all determinations at 197°C., *i.e.*, 3 degrees below its safe maximum operating temperature. A constant helium flow rate 12.7 to 13 ml./min. was maintained in all determinations. The outlet end of the column was open to the atmosphere.

Results and Discussion

In the analytical study of the oxidation of fatty acids from soybean oil it was necessary first to investigate the best conditions for the separation of known mixtures of compounds most likely to occur in the oxidized product. Since the methyl esters have lower boiling points than their parent fatty acids, they can be resolved at a correspondingly lower column-temperature. Moreover C_{18} and also some lower acids are not stable at column temperatures necessary for their separation. The methyl esters were therefore used throughout this study. The oxidized products were converted to their methyl esters, using a semi-micro adaptation of the method described by Clinton and Laskowski (1).

The optimum conditions for the separation of a mixture of esters of monocarboxylic fatty acids, as a function of retention time, are represented in Table I. The results show that a compromise must be made between temperature and spacing of individual components. At 200°C., for example, it would take 42 min. to complete the separation of a mixture of esters of capric, lauric, and myristic acids while at 240°C. the total time is reduced to about 14 min. with sufficiently large spacing between the components for easy identification. A mixture of nearly equal volumes of methyl esters of monocarboxylic acids with an even number of carbon atoms from capric to stearic acids was prepared. Figure 1 [4] illustrates the resolution of this mixture at 250°C.

A similar relationship between temperature and retention time was found for mixtures of methyl esters of dicarboxylic fatty acids (Table II). It was noticed however that the resolution temperatures for these esters are lower than would be expected from a consideration of vapor pressures of the pure esters and indicate the influence of the stationary liquid on partial pressures. A chromatogram of the dicarboxylic esters from pimelate to sebacate (C_7–C_{10}), Figure 2, shows that a good separation for this mixture can be obtained at 215°C.

Difficulties were experienced in resolving some mixtures of esters of mono- and dicarboxylic acids.

TABLE II
Methyl Esters of Dicarboxylic Acids

Compound	Retention time, minutes, flow rate of helium 13 ml./min. Temperature, °C.					
	150	170	180	200	220	240
C_3 Dimethyl malonate	8:46	5:34	5:00	3:27		
C_4 Dimethyl succinate	13:25	8:38	7:29	5:22		
C_5 Dimethyl glutarate	20:42	12:05	9:58			
C_6 Dimethyl adipate	31:49	17:50	13:48	12:39	7:17	
C_7 Dimethyl pimalate	52:08	26:39	19:33	18:24	11:19	8:26
C_8 Dimethyl subarate				24:32	14:46	9:35
C_9 Dimethyl azelate				33:44	19:45	14:00
C_{10} Dimethyl sebacate						

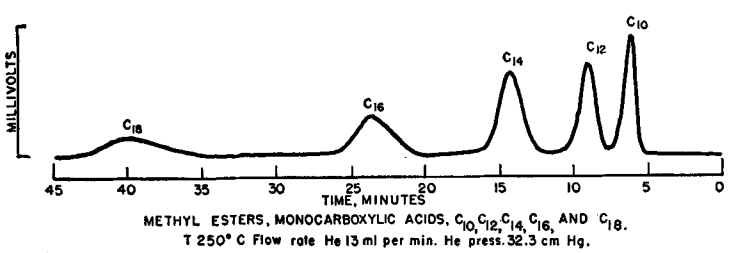

FIG. 1

[4] All figures were prepared from actual tracings of the recorder with time ordinate moving from right to left.

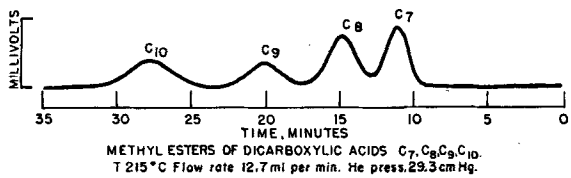

FIG. 2

It was found that those with three carbon atoms apart, like malonate and caproate or adipate and pelargonate, have retention times lying too close for good separation. A liquid phase with different separating characteristics is being tried and may solve this difficulty.

The oxidation was carried out according to the procedure of Lemieux and von Rudloff (4), which is specific for olefinic linkages. In accordance with the method, oxidation of oleic acid should result theoretically in pelargonic and azelaic; oxidation of linoleic in malonic, caproic, and azelaic; and oxidation of linolenic in propionic, malonic, and azelaic acid. The individual C_{18} unsaturated fatty acids, oleic, linoleic, and linolenic, were investigated first. These acids were of the highest purity obtainable commercially.

The chromatogram of the oxidized products of oleic acid in Figure 3 shows the two prominent peaks for the methyl esters of pelargonic and azelaic acids in nearly quantitative ratio of 1:1. There is however an intermediate peak, or possibly two peaks, which have not been as yet identified.

Figures 4 and 5 give the results for the oxidation of linolenic acid at column temperatures of 200° and

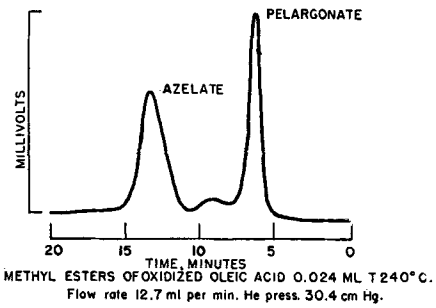

FIG. 3

240°C. The multiplicity of peaks was unexpected. It may indicate the presence of impurities in the original linolenic acid and/or of the possible migration of double bonds or of other chemical changes taking place during oxidation. The prominent peak in either diagram is methyl azelate. Tentative identification of the other peaks was attempted from the standard plots of log retention volume against the number of carbon atoms and from log retention volume against 1/T for mono- and dicarboxylic acids.

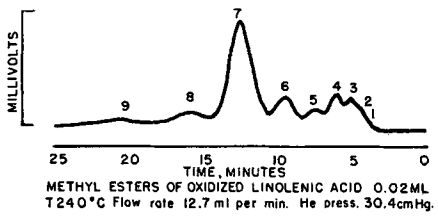

FIG. 5

From these plots there appeared to be present methyl malonate or caproate, or both, pelargonate, myristate, and possibly methyl esters of C_{11} and C_{15} carbon atoms. Considerable study will be needed to clarify the results. Since the oxidation product of methyl linoleate gave similar chromatograms, these are not shown.

The identification of flat small peaks in oxidized products in oleic, linoleic, and linolenic acids by retention time only (or volume) presents difficulties. However their chromatograms indicate that these acids contain, in addition to saturated components, a mixture of one, two, and three double-bond C_{18} acids. This is confirmed by the fact that the peak for methyl azelate is always too large to originate from one source only.

Figure 6 is a chromatogram of methyl esters of

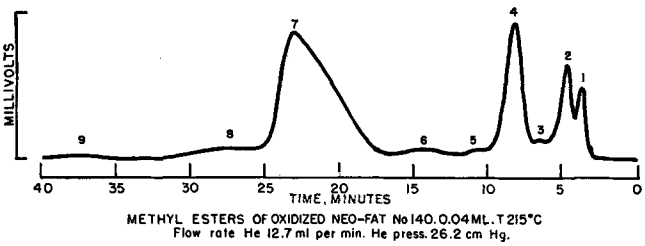

FIG. 6

oxidized Neo-Fat No. 140, a commercial product consisting of approximately 45% oleic, 55% linoleic, and a trace of linolenic acids. Figure 7 is a chromatogram of an oxidized mixture of saturated and unsaturated fatty acids derived from the hydrolysis of soybean oil. These chromatograms and others, taken at different temperatures, indicate again the presence of many additional compounds besides those expected by a simple oxidation of the fatty acids. Neo-Fat, similar to linolenic acid, gave nine discernible peaks when only three or four were expected. It is possible that columns with still better resolving power and better detecting methods might reveal an even greater number of components in these acids.

The peaks in Figure 6 from right to left appear to fall in the position of monocarboxylic C_2, C_6, C_8, C_9, C_{10}, C_{11}, C_9 dicarboxylic, and C_{14} and C_{15} monocarboxylic acids. The first peak at the extreme right may result from the incomplete removal of dichloro-

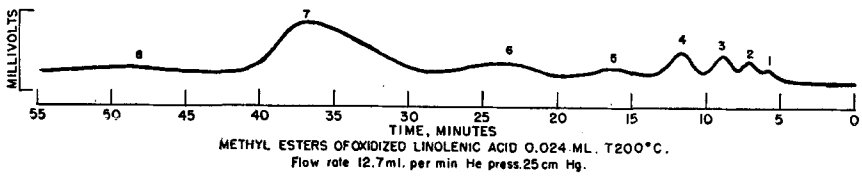

FIG. 4

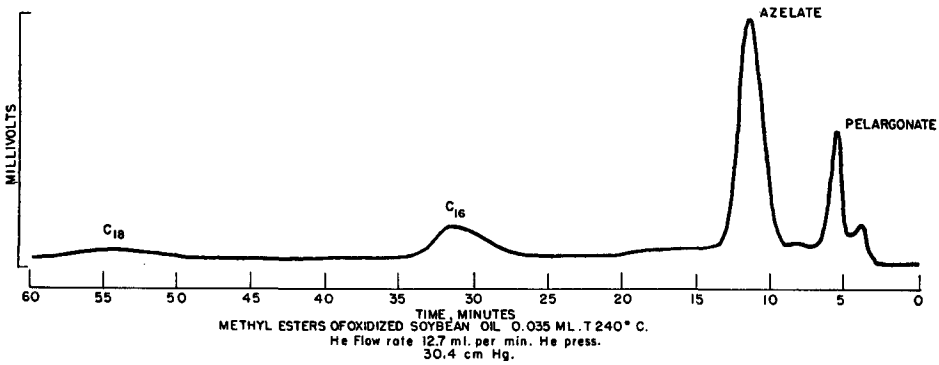

FIG. 7

ethane from the methylation process while the second peak may contain also malonate since retention volume of these two esters are practically the same.

The main oxidation products from soybean oil, Figure 7, are similar to those of Neo-Fat. The last two peaks, reading from right to left, are methyl esters of palmitic and stearic acids originally present in the saponified oil.

Conclusions

The oxidation of oleic, and particularly of linoleic and linolenic acids, indicated the presence of a considerable number of unexpected products in addition to those foreseen from a simple severance of the double bonds. The chromatograms of the oxidized pure C_{18} acids, of Neo-Fat No. 140, and of a mixture of fatty acids from hydrolyzed soybean oil showed a striking similarity in many respects. It was not possible to identify all the peaks, especially those of trace amounts, in the chromatograms and to obtain a quantitative estimate of the oxidation products.

Further work on this is in progress. The results indicate that gas-liquid partition chromatography is a sensitive method for the study of the oxidation of vegetable oils.

Acknowledgment

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REFERENCES

1. Clinton, R. O., and Laskowski, S. C. J., J. Am. Chem. Soc., *70*, 3135 (1948).
2. James, A. T., and Martin, A. J. P., Biochem. J., *63*, 144 (1956).
3. James, A. T., and Martin, A. J. P., Biochem. J., *50*, 679 (1952).
4. Lemieux, R. U., and von Rudloff, E., Can. J. Chem., *33*, 1701 (1955).

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ABSTRACTS . . . R. A. REINERS, Editor

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• Oils and Fats

Melting point diagrams of saturated fatty acids. A. Kofler. *Z. Elektrochem.* **60**, 1014–7(1956). Melting point diagrams of the 2-component systems palmitic acid-stearic acid, and margaric acid-stearic acid were investigated by micro-thermal analysis. Comparison with previously published data leads to the generalization that systems in which both acids have an even number of carbon atoms show 3 mixed-crystal phases, separated by a eutectic and peritectic, and the central phase is considered as a stabilized intermediate phase rather than a compound. Systems of adjacent even- and odd-numbered acids form continuous series of mixed crystals, which are complicated by a separate intermediate phase. When the acids differ by three carbon atoms, two intermediate phases may occur. (*C. A.* **51**, 6304)

Refractometric fat determination with alpha-bromonaphthalene in oil seeds. H. Grynberg and T. Patzek. *Przemysl Spozywczy* **6**, 455–9(1952). The refractometric method for fat determination is accurate and reproducible. It gives results which are higher than those obtained with the extraction method. (*C. A.* **51**, 7039)

Pale-colored fatty oil from kamala seeds. V. N. Ojha, P. G. Sharma, and J. S. Aggarwal(Natl. Chem. Lab., Poona). *J. Sci. Ind. Research(India)* **15B**, 551–2(1956). Kamala oil extracted with petroleum ether is too dark for use in pale-colored varnishes and paints. Washing to obtain a pale oil is uneconomical for industrial purposes. (*C. A.* **51**, 7040)

Examination of the fixed oil of Adhatoda vasica seeds. K. L. Handa, Ishwar Chandra and Vasudev(Drug Research Lab., Jammu). *J. Sci. Ind. Research(India)* **15B**, 612–3(1956). Dry seeds crushed and extracted with petroleum ether yielded 25.8% of a clear yellow oil: n^{24} 1.468, saponification number 169.2, acetyl number 3.4, iodine number 71.7 and unsaponifiable 3.2%. The over-all analysis of the acid components of the oil is arachidic 3.1, behenic 11.2, lignoceric 10.7, cerotic 5.0, oleic 49.9 and linoleic 12.3%. The unsaponifiable fraction is *beta*-sitosterol. (*C. A.* **51**, 7040)

Hydrophilic and sorptive properties of oil cake. A. V. Dumanskii, P. A. Demchenko, I. K. Girman and L. G. Demchenko. *Zhur Priklad. Khim.* **29**, 1555–61(1956). Hydrophilic and sorptive properties of sunflower cake, ground and dried at 105–110° for 16 hours in vacuo, were determined. From the experimental heat of sorption, 16.3–16.8 cal./g., the calculated amount of water absorbed was 20.5–21.0%(dry basis). This was identical with the values determined by absorption of water from xylene saturated with water and subsequent distillation with an excess of xylene. The rates of sorption and desorption as a function of humidity of the atmosphere *(loc.*