it cannot be reversed by acid hydrolysis. Because the amino acid analyses reported here were run on acid hydrolyzates, and also because of good correlation found between lysine destruction and severity of heat treatment, apparently the reaction is irreversible. The preceding data indicate that although some processed meals show extensive amino acid losses, little destruction of basic amino acid occurs if the meals are processed with controlled heat, as illustrated by preparation A. In that method, the moisture reduction step during cooking is not allowed to continue longer than 30 min, and there is no steaming after solvent extraction. The basic amino acid destruction appears to be greatest when meals are dried near the end of the cooking step (13% moisture) and during dry steaming of the spent meal (moisture 7%).

Conclusions

The basic amino acids—lysine, arginine, and histidine—are the most heat-labile in mustard meals. The analytical methods proposed—nitrogen solubility index (NSI), optical density of aqueous extracts, and reducing sugars—provide rapid and simple ways of following amino acid destruction by heat processing.

ACKNOWLEDGMENT

Amino acid analyses by C. H. VanEtten and R. W. Miller.

REFERENCES

1. Altschul, A. M., "Processed Plant Protein Foodstuffs," Academic Press, Inc., New York, 1958, Chapt. 5.
2. Bensabot, L., and V. L. Frampton, J. Agr. Food Chem. *6*, 778 (1958).
3. Ettlinger, M. G., and A. J. Lundeen, J. Am. Chem. Soc. *78*, 4172–73 (1956).
4. Hannan, R. S., and C. H. Lea, Biochim. Biophys. Acta *9*, 293–305 (1952).
5. Hodge, J. E., J. Agr. Food Chem. *1*, 928–943 (1953).
6. Maillard, L. C., Compt. Rend. *154*, 66–68 (1912).
7. Markley, S. K., and A. E. Bailey, "Soybeans and Soybean Products," Vol. I, Interscience Publishers, Inc., New York, 1950, p. 150.
8. Munson, L. S., and P. H. Walker, "A.O.A.C. Official Methods of Analysis," 8th ed., Association of Official Agricultural Chemists, Washington D. C., 1955, p. 545.
9. Mustakas, G. C., E. A. Gastrock, E. L. D'Aquin, E. L. Griffin, Jr. and E. L. Patton, "Pilot-Plant Application of Filtration Extraction of Mustard Seed," Abstract of Papers *17A*, 141st Meeting, ACS, Washington, D. C., March 20–29, 1962.
10. Mustakas, G. C., L. D. Kirk and E. L. Griffin, Jr., JAOCS *39*, 372–377 (1962).
11. Mustakas, G. C., L. D. Kirk and E. L. Griffin, Jr., "Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality," Abstract of Papers *97*, 36th Meeting, AOCS, Toronto, Canada, October 1–3, 1962.
12. Patton, S., J. Dairy Sci. *38*, 457–473 (1955).
13. Smith, A. K., and S. J. Circle, Ind. Eng. Chem. *30*, 1414–1418 (1938).
14. Spackman, D. H., W. H. Stein and S. Moore, Anal. Chem. *30*, 1190–1205 (1958).

[Received November 20, 1963—Accepted January 21, 1964]

Preparation and Analysis of Some Food Fats and Oils for Fatty Acid Content by Gas-Liquid Chromatography

KATHARINE J. HIVON, SUSIE N. HAGAN, and EDITH B. WILE, Human Nutrition Research Division,[1] Beltsville, Maryland

Abstract

The fatty acid compositions of some food fats and oils were determined by gas-liquid chromatography (GLC) before and after application of reagents and conditions of some extraction procedures. The extraction procedures studied had a nonsignificant effect on the fatty acid compositions. Procedures leading to methyl ester formation through a series of room temp reactions were selected over procedures requiring higher temp reactions, on the basis of yield of products, fatty acid compositions of food lipids of simple composition, or both. These procedures were then used to prepare some food fats and oils for analysis by GLC and the fatty acid compositions determined in this manner are presented.

Introduction

THE PRESENT STUDY was undertaken to determine the effects of the environment created by several different extraction procedures on the apparent fatty acid composition of some food fats and oils. Several procedures employing mild conditions have been combined for the quantitative fatty acid analysis of food lipids of relatively simple composition. GLC analysis aided in the selection of these preparative procedures. Following the validation of these procedures, they were applied to some separated food fats and oils and the resulting fatty acid methyl esters were analyzed with GLC.

Experimental

Materials

Fats and Oils. A butter-margarine blend, the covering fat from cured, smoked ham, and a cottonseed oil, No. 2, and a soybean oil, No. 4 (salad or cooking oils from government stocks) were available from other laboratory work. Soybean oil No. 3, a "pure soya salad oil," was purchased from a wholesale dealer in June, 1961. All other samples analyzed were purchased at local retail markets between June and August, 1962.

The fat or oil sample from each container was blended thoroughly under nitrogen before subsamples were taken. The ham fat had been ground, sealed in a coated, tinned container, and stored at −40C for approximately 5 months before analyses were carried out. Replicate analyses were made on material taken from a single container in all cases except the processed soybean oil, values for which are means of 4 analyses on the contents of each of four containers.

Organic Solvents. Ethanol, 95%, refluxed and distilled over KOH.

Methanol, absolute, acetone-free, certified reagent grade.

Benzene, certified reagent grade.

Normal-hexane (purified, bp 65–67C) and petroleum ether (certified reagent grade, bp 30–60C), dried, distilled over KOH.

Mixed ethers: equal volumes of ethyl ether (anhydrous, reagent grade) and petroleum ether.

Apparatus and Procedures

Analysis of Fatty Acids by GLC. The gas chromatographs used were the argon ionization detection systems of the Barber-Colman Co. Some instrumental and operational details appear in Table I. The polar liquid phase was ethylene glycol succinic acid polyester (15%, w/w) on 100–140 mesh Gas-Chrom P. This commercially-prepared packing was used for all quantitative analyses. Apiezon L at a level of 14%

[1] Food Composition Laboratory, ARS, USDA.

TABLE I
Instrumental Components and Conditions for GLC

Item	Model 10	Model 15
Detector:		
Radiation source	56 μc Ra^{226}	100 mc tritiated titanium
Cell volume, ml	5	5
Applied potential, V	750	450–550
Resistance in series, ohm	1×10^9	3×10^8
Electrometer sensitivity, amp	1×10^{-7}	1×10^{-7}
Columns, glass	8 ft x 5 mm (ID)	8 ft x 5 mm (ID)
Temperatures (C):		
Flash vaporizer	295	295
For polar liquid phase—		
Column	185	185
Detector	225	200
For nonpolar liquid phase—		
Column	210	.....
Detector	240	.....

(w/w) on 80–100 mesh acid- and alkali-treated chromosorb W (13) constituted the nonpolar packing and was used as an aid in identification of the methyl esters. The argon gas was dried by passage through a column of molecular sieve. Gas cylinder regulator pressures of 25–40 psi were used and flow rates (determined with a soap film flowmeter) ranged between 50 and 200 ml/min. The lower pressures and flow rates were required by the Model 15 instrument which consistently demonstrated a shorter retention time for a given solute when the two instruments were operated under apparently identical column conditions. Sample size ranged from 50–200 μg fatty acid methyl esters injected in the form of 10–20% (w/w) solutions in *n*-hexane. During these studies each section of data was acquired under instrumental and column conditions maintained as nearly constant as possible to validate comparisons among units of data.

High purity fatty acid methyl esters were used to calibrate the instruments and to provide information on retention times for identification of sample components. Peak areas of an entire chart were either calculated by multiplying the peak height by the width at half-height or were measured with a planimeter. The method employed depended on the contour and height of the peaks.

The tritium-containing detector used for the analyses, reported in Tables V and VI, gave linear response to esters of acids between 12:0 (A "shorthand" designation for fatty acid structure is used throughout the paper. The first numeral represents the number of carbon atoms: the second represents the number of double bonds.) and 20:0 when relative mass sensitivity to saturated esters was plotted against moleculer weights of the esters (17). Correction factors for these esters were obtained from lines fitted by the method of least squares to data obtained from chromatograms of mixed standard esters (20,33). Lines were plotted for saturated and monoene esters and calculated for dienes and trienes. Individual correction factors, based on detector response, were calculated for esters of acids with less than 12 carbon atoms.

Identification of fatty acids was made from the lines obtained by plotting log retention times of known esters, chromatographed on polar and nonpolar columns under the same conditions, against carbon number or degree of unsaturation. Tentative identification of unknowns, the data for which did not fall on the above lines, was made from lines calculated for log retention time against a mol wt ratio.

Extraction Study. Table II lists details of the extraction procedures compared in the initial study. The procedures were essentially those given in the literature references with modifications as follows: samples to yield ca. 0.3 g lipids were used throughout, and the concn and/or amounts of reagents were modified slightly. Solvents were carefully removed from the extracted lipids with a rotary vacuum evaporator at temp not exceeding 40C. The evaporator was returned to atmospheric pressure with nitrogen (9) and the lipids were weighed. Fatty acid methyl esters were prepared from the lipids of each of the samples in the same manner, by procedures described later in this paper. At the time this study was made, the laboratory was extracting the nonsaponifiable fraction by the separatory funnel technique.

TABLE II
Experimental Lipid Extraction Procedures

Procedure	Description			Reference
	Solvents and other reagents	Apparatus	Treatment	
Control	None	None	None	
A	$CHCl_3$ and MeOH	Blender; separatory funnel	RT[a]	(6)
B	Petroleum ether	Soxhlet	Reflux, 16 hr	(2)
C	Ethyl ether	Soxhlet	Reflux, 16 hr	(2)
D	$CHCl_3$	Soxhlet	Reflux, 16 hr	(2)
E	EtOH 1:1 ethers (ethyl and petroleum)	Separatory and Büchner funnels	RT,[a] 1 hr occasional shaking	(12, 14)
F	EtOH 2 N HCl, aq 1:1 ethers (ethyl and petroleum)	Röhrig	RT,[a] 16 hr occasional shaking	(11)
G	EtOH 8.5 N HCl, aq Ethyl ether Petroleum ether	Röhrig	70–80C, 30–40 min occasional shaking	(2)

[a] Room temp.

Saponification. Following the general procedures of Johnston et al. (14) and Willmer and Laughland (29), a 0.25–0.4 g fat or oil sample was saponified with 10 ml 4% KOH in ethanol (w/w) under a nitrogen atmosphere. The mixture was stored in the dark at room temp for 16–18 hr.

Separation of Fatty Acids and Nonsaponifiable Fraction. The nonsaponifiable fraction was removed from the diluted saponification reaction mixture in columns packed with 6 mm nonporous ceramic Berl saddles with the mixed ethers as the extracting solvent. The extraction column assembly consisted of a separatory funnel and 2 chromatographic columns approximately 35 x 2 (ID) cm (all with Teflon stopcocks), and 2 receiving flasks. After the saponified sample had dripped through the columns and sufficient water-washes had produced a neutral effluent, the receivers were exchanged to permit collection of the nonsaponifiable fraction. Solvents were removed from the nonsaponifiable fraction in the same manner as from extracted lipids and the percentage yield was calculated from the weight of the residue.

Separatory funnels were used in the conventional way for the mixed-ether extraction of the nonsaponifiable fraction from the saponification reaction mixture. The organic phase from the funnels was treated in the same manner as the organic phase collected from the columns.

The aqueous soap solution from either separation procedure was brought to ca. pH 4 by the dropwise

TABLE III

The Fatty Acid Composition of Some Fats and an Oil After Exposure to Several Extraction Procedures
[Area Percentage of Total Area of Chromatogram Peaks]

Item	Extraction procedure				
	Control	B	E	F	G
Cottonseed oil:					
Lipids, %[a]	.....	101.4 ± 1.4	94.3 ± 3.6	99.2 ± 3.0	100.9 ± 0.5
Fatty acids[b]					
8:0	trace	trace	trace	trace	trace
12:0	trace	trace	trace	trace	trace
14:0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1
16:0	20.8 ± 0.6	20.8 ± 0.4	20.4 ± 0.3	20.6 ± 0.5	20.4 ± 0.4
16:1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
18:0	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.4	1.9 ± 0.2	2.0 ± 0.2
18:1	16.7 ± 0.2	16.9 ± 0.4	16.7 ± 0.5	16.8 ± 0.7	16.8 ± 0.7
18:2	58.9 ± 0.5	58.4 ± 0.7	59.3 ± 0.5	59.3 ± 0.8	59.3 ± 1.0
18:3	0.3 ± 0.3	0.3 ± 0.3	trace	trace	trace
Shortening, hydrogenated vegetable:					
Lipids, %[a]	.....	101.1 ± 1.0	98.8 ± 3.1	98.4 ± 1.0	100.7 ± 0.8
Fatty acids[b]					
8:0	trace	trace	trace	trace	trace
10:0	trace	trace	trace	trace	trace
12:0	trace	trace	trace	trace	trace
14:0	trace	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	13.0 ± 0.3	13.8 ± 0.6	13.0 ± 0.3	12.9 ± 0.1	12.8 ± 0.2
16:1	trace	trace	trace	trace	trace
18:0	9.8 ± 0.2	10.1 ± 0.3	9.4 ± 0.2	9.7 ± 0.3	9.8 ± 0.3
18:1	52.0 ± 0.3	53.4 ± 0.7	51.2 ± 0.3	51.2 ± 0.4	51.6 ± 1.0
18:2	24.0 ± 0.3	21.2 ± 0.4	25.2 ± 0.2	25.0 ± 0.3	24.2 ± 0.3
18:3	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.2
Ham covering fat[c]					
Lipids, %[a]	.....	69.6 ± 1.5	70.4 ± 2.3	68.4 ± 1.9	69.9 ± 2.0
Fatty acids[b]					
8:0	.....	trace	trace	trace	trace
10:0	.....	trace	trace	trace	trace
12:0	.....	trace	trace	trace	trace
14:0	.....	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.3	1.6 ± 0.2
16:0	.....	26.3 ± 0.3	27.4 ± 1.0	27.7 ± 1.9	27.6 ± 1.7
16:1	.....	4.4 ± 0.3	4.1 ± 0.2	4.1 ± 0.3	4.0 ± 0.4
16:2 (?)	.....	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
18:0	.....	12.1 ± 0.6	11.9 ± 0.4	12.1 ± 1.3	11.5 ± 0.6
18:1	.....	45.5 ± 0.5	45.6 ± 0.5	45.1 ± 1.2	45.7 ± 0.5
18:2	.....	8.5 ± 0.3	7.9 ± 0.4	8.0 ± 0.9	8.1 ± 0.6
18:3	.....	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.9 ± 0.2
20:0	.....	trace	trace	trace	trace
20:2 (?)	.....	trace	trace	trace	trace

[a] Mean ± standard error of the mean of 6 subsamples.
[b] Mean ± standard error of the mean of 4 subsamples.
[c] Additional components, all methods: 11:0-trace; 15:0-trace.

addition of 6N HCl. The solution was then quantitatively transferred to a separatory funnel with the mixed ethers and the fatty acids were extracted with these solvents in the usual manner. During the mixed-ether extraction, cold water was run over the inverted separatory funnel to reduce interior pressure and draw stem contents into the funnel (22). The combined ether extracts were washed and then dried with Na_2SO_4 (granular, anhydrous) for about an hour in the separatory funnel. A thin layer of glass wool inside the separatory funnel prevented obstruction of the stopcock by the drying agent. Excess solvents were removed immediately prior to ester preparation, in the same manner as from the extracted lipids.

Esterification of Fatty Acids. To the fatty acids obtained from a 0.25–0.4 g fat or oil sample were added, under a nitrogen atmosphere, 5 ml benzene, 5 ml 5% anhydrous HCl in methanol (w/w) and 0.3 ml 98% 2,2-dimethoxypropane. The mixture was treated and stored in the same manner as was the saponification reaction mixture. This method is a modified and combined procedure based on those of Johnston et al. (14), Lorette and Brown (16), and Stoffel et al. (26).

Results and Discussion

Fatty Acid Analysis by GLC. The order in which fatty acids appear in Tables III–VI is not entirely the order of elution from the polar chromatographic column. Tentative identification of acids, for which standards were not available at the time the analyses were made, is indicated by a question mark. Fatty acid values of <0.2% are considered trace amounts.

Extraction Study. The conditions of extraction procedures B,E,F, and G (Table II) were applied to an oil, a shortening, and ham fat, a tissue containing lipids of relatively simple composition. The analytical results show in Table III. Soybean oil, a "pure soya salad oil," was subjected to the conditions of extraction procedures A,B,C,D,E,F, and G (Table II) to determine their effects on the octadecenoic acids with 3 as well as 2 double bonds. Data from these analyses appear in Table IV. Comparison of the fatty acid contents of the extracted fats and oils with those of the original products, other than the ham fat, is possible.

The fatty acid compositions of treated oils and fats show only minor differences from the unextracted or control sample for the specific group. These differences apparently are not related to the percentage of lipids extracted, do not follow a pattern, and are not statistically significant (23). Arnold and Choudhury observed similar results from oils extracted from flaked soybeans by 4 hydrocarbon solvents (3). The fatty acid compositions of the ham fat extracted by 4 procedures (Table III) showed good agreement within the group, also.

Saponification Procedures. Yields from the saponification procedure used in this work compared favorably with those from procedures using similar reagents at reflux temp when all were applied to products with simple lipid composition. More complex lipids may require more severe saponification conditions. Daniels and Richmond (8) have separated conjugated and nonconjugated 18:2 and 18:3 acids on a packed chromatographic column with an adipate polyester liquid phase. These authors caution against saponification methods which may result in anomalous peaks on the chromatograms. While unidentified peaks were found on the charts of hydrogenated products and the processed soybean oil, no other oil saponified by the method used in the present work has produced unidentified peaks which might indicate the presence

TABLE IV

The Fatty Acid Composition of Soybean Oil After Exposure to Several Extraction Procedures
[Area Percentage of Total Area of Chromatogram Peaks]

Item	Extraction procedure							
	Control	A	B	C	D	E	F	G
Lipids, % [a]	.....	98.1 ± 4.7	101.0 ± 0.6	100.0 ± 0.7	103.0 ± 1.0	100.3 ± 2.9	98.7 ± 2.9	101.2 ± 2.9
Fatty acids [b]								
8:0	trace	trace	trace	.....	.....	.....	trace	.....
12:0	trace	trace	.....	.....	.....	.....	trace	.....
14:0	0.2 ± 0.0	0.2 ± 0.0	trace	trace	trace	trace	trace	trace
16:0	11.1 ± 0.3	10.9 ± 0.4	10.9 ± 0.3	10.7 ± 0.6	11.1 ± 0.4	11.5 ± 0.3	11.0 ± 0.3	11.2 ± 0.6
16:1	0.2 ± 0.2	0.2 ± 0.1	trace	trace	0.2 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
16:2 (?)	trace	trace	.....	.....	.....	.....	trace	.....
18:0	3.8 ± 0.3	3.6 ± 0.5	4.2 ± 0.3	4.0 ± 0.4	4.1 ± 0.3	4.4 ± 0.1	4.1 ± 0.2	4.0 ± 0.3
18:1	28.2 ± 0.3	28.5 ± 1.0	28.5 ± 0.3	28.5 ± 1.5	28.7 ± 0.1	28.9 ± 0.4	28.2 ± 0.7	28.6 ± 0.6
18:2	50.3 ± 0.7	50.6 ± 1.6	49.0 ± 0.6	49.4 ± 2.0	48.6 ± 1.0	48.3 ± 0.2	49.1 ± 0.6	49.4 ± 1.0
18:3	6.2 ± 0.7	6.0 ± 0.5	7.2 ± 0.3	7.1 ± 0.4	7.1 ± 0.5	6.5 ± 0.2	7.1 ± 0.3	6.4 ± 0.9
20:4	trace	.....	.....	trace	trace	trace	trace	trace
22:0	trace	trace	trace	trace	trace	trace	trace	trace

[a] Mean ± standard error of the mean of 6 subsamples.
[b] Mean ± standard error of the mean of 4 subsamples.

of conjugated 18:2 or 18:3 acids. Large samples were chromatographed to aid in the detection of minor components.

Separation of Fatty Acids and Nonsaponifiable Fraction. A comparative study was made of the separation of fatty acids and the nonsaponifiable fraction by the separatory funnel and the porcelain saddle-packed column techniques. A shortening consisting of pork and beef fats and a butter-margarine blend were used in these comparisons because these samples formed troublesome emulsions during the separatory funnel extraction of the nonsaponifiable fraction, and they contained a fairly broad range of fatty acids. The samples were saponified and esterified by the previously described methods. The percentage yields of fatty acid methyl esters plus the nonsaponifiable fraction and of the nonsaponifiable fraction alone, from the column extraction and the separatory funnel techniques, were shown to be statistically equivalent (23). The fatty acid composition of the samples obtained by the 2 techniques were so similar that statistical analysis was not applied. The analyses did not include fatty acids below 6 carbon atoms. The principal advantage of the columns was the avoidance of long waiting periods for separation of emulsions. This permitted a more predictable time schedule for the analytical scheme.

Esterification Procedure. The esterification procedure described in this paper was selected over a number of common procedures on the basis of yield of methyl esters of natural color, refractive indices of oil and esters, and yield of glycerol (27). Some fatty acid determinations also were made. The procedure was applied to the fatty acids obtained from 52 subsamples of 17 products including meat, vegetables, baked products, and a few oils and shortenings, and to 114 subsamples of the 19 products in Tables V and VI. The average prcentage yield and standard error of the mean of the first group were 98.4 ± 5.3 and of the second group were 96.2 ± 5.5 where percentage yield = 100 (g fatty acid methyl ester + g nonsaponifiable fraction)/g lipid. Since yields are based on weight of the lipid sample, both the saponification and the esterification procedures contribute to the final value.

Determination of Fatty Acid Content. Room-temp preparative procedures, including separation of fatty acids from the nonsaponifiable fraction on a packed extraction column, were applied to some separated food fats and oils. The methyl esters so prepared were analyzed by GLC. Saponification and subsequent removal of the nonsaponifiable fraction from the fatty acids of natural products decreases the possibility of extraneous peaks on the chromatograms (21). The nonsaponifiable fractions of the food fats and oils in Table V, VI averaged $<0.8\%$. The mild saponification reaction made removal of these fractions possible without evidence of fatty acid alteration or destruction. The component oils of the vegetable oil and the fats and oils of the shortenings were not specified by the manufacturers. In addition to the fatty acids listed, corn oil contained a trace of 20:1 (Table V), lard, brand F, contained a trace of 12:1 (?), and lard, brand H, contained a trace of 14:1 (Table VI).

Differences in the fatty acid patterns of products from the same natural source are probably due to a combination of natural variation and the manu-

TABLE V

Fatty Acid Composition of Salad or Cooking Oils by Gas-Liquid Chromatographic Analysis
[Weight Percentage of Total Fatty Acids]

Fatty acids [a]	Corn	Cottonseed		Olive	Peanut [b]	Safflower		Soybean		Processed soybean [c]	Vegetable
		1	2			Brands		3	4		
						A	B				
8:0	0.2	trace	0.2	trace	trace	0.2	trace	trace	0.2	0.3	trace
10:0	trace	trace	trace	.....	trace	.....	trace	.....	trace	trace	trace
12:0	trace	trace	trace	.....	.....	.....	trace	trace	trace	trace	trace
14:0	trace	0.7	0.6	trace	trace	trace	trace	0.2	trace	trace	0.6
16:0	11.5	22.6	20.8	12.4	10.1	6.5	6.9	11.5	10.3	10.0	21.6
16:1	0.2	0.5	0.4	0.8	trace	trace	trace	0.2	trace	trace	0.4
16:2 (?) and/or 17:1 (?)	.....	.....	trace	trace	trace	.....	trace	trace	trace	trace	trace
17:0	trace	.....	trace	trace	trace	.....	.....	.....	trace	trace	trace
18:0	1.6	1.8	1.6	1.8	2.1	1.8	2.2	4.0	3.6	3.9	1.6
18:1	26.8	14.5	13.8	74.8	51.4	10.6	13.4	26.7	23.5	39.5	14.7
18:2	57.7	59.8	61.9	8.1	32.1	79.4	76.1	51.4	55.9	36.6	61.2
18:3	1.4	trace	0.4	1.4	0.9	1.1	0.9	6.0	5.7	2.2	trace
20:0	0.2	trace	0.3	0.4	1.0	0.2	0.3	.....	0.2	0.4	trace

[a] Mean of 4 subsamples except 4 x 4 subsamples of processed soybean oil.
[b] Also: 1.8% of 22:0 and 0.7% of 24:0.
[c] Also: traces of 6:0, 11:0, and 15:0; 0.5% of an unknown eluted between 18:1 and 18:2; 4.4 and 1.4% of unknowns in peaks following and merged with 18:1 and 18:2; and 0.3% of an unknown leading and merged with 18:3.

TABLE VI

Fatty Acid Composition of Cooking Fats by Gas-Liquid Chromatographic Analysis
[Weight Percentage of Total Fatty Acids]

Fatty acids[a]	Shortenings							
	Hydrogenated vegetable			Animal and vegetable	Pork and beef[c]	Lards		
	Brands					Brands		
	C	D	E[b]			F	G	H
8:0	trace	trace	0.2	trace	0.2	trace	0.2	0.2
9:0	.....	.....	.....	.....	trace	trace	trace	.....
10:0	trace	trace	.....	trace	0.2	trace	trace	trace
12:0	0.4	trace	trace	trace	0.2	trace	trace	trace
14:0	0.3	0.2	0.4	1.1	1.5	1.3	1.3	1.3
15:0	.....	.....	.....	trace	.....	trace	trace	.....
16:0	15.2	14.2	17.3	25.3	26.4	27.8	26.3	27.2
16:1	trace	trace	0.5	1.7	1.7	2.1	1.8	2.0
16:2 (?) and/or 17:1 (?)	trace	.....	trace	trace	0.4	0.2	0.2	trace
17:0	trace	trace	trace	0.2	0.7	0.3	0.4	0.3
18:0	11.7	12.6	10.0	11.8	17.8	12.9	20.7	17.9
18:1	43.8	43.2	61.4	51.4	40.8	44.6	39.2	41.4
18:2	26.4	26.9	6.9	7.5	6.8	9.4	8.5	8.5
(?)[d]	0.2	0.6	2.4	.....	.....	.....	.....	.....
18:3	1.8	2.0	0.2	0.5	1.0	1.2	0.8	0.6
19:0	.....	.....	.....	0.3	0.4	.....	.....	trace
20:0	.....	0.2	trace	trace	0.4	trace	0.4	0.2
20:2 (?)	.....	.....	.....	.....	0.2	trace	trace	trace

[a] Mean of 4 subsamples.
[b] Also: 0.8% of an unknown eluted just before 18:2.
[c] Also: trace of 13:1 (?), 0.4% of 14:1, and 0.2% of (14:2 and 15:1) (?).
[d] Unknown peak following and merged with 18:2 peak.

facturing processes. When the fatty acid data on salad or cooking oils (Table V) are compared with data from similar products analyzed by other laboratories (5,7,10,19,24,28) this variation is observed, yet there is general agreement among major fatty acid components of refined commercial oils from the same plant source. With the exception of the soybean oils in columns 9, 10 the 18:2 acid contents were somewhat higher and the 18:1 acid contents lower than those of similar products in the cited references. The 18:2 acid content of refined commercial corn oil is an average 103% of the 18:2 acid content of similar oils analyzed by 4 other laboratories, and the 18:2 acid content of refined commercial cottonseed oil is an average 105% of the 18:2 acid content of similar oils analyzed by 3 other laboratories. While products similar to the shortenings in Table VI were analyzed by other laboratories (4,5,18,19), comparisons are more difficult since the component fats and oils of the shortenings are largely unidentified or proportions of each unknown, and the composition may be varied by the manufacturers. There was closer agreement in the 18:2 acid content of one hydrogenated vegetable oil shortening analyzed by 2 other laboratories than occurred in the salad or cooking oil analyses. With one exception (19) the fatty acid values from the cited references appear to be only area percentages of total area of the chromatogram peaks although comparative analyses of known synthetic mixtures are given in some cases.

The production of various fatty acid isomers by the hydrogenation process has recently been summarized by Sreenivasan et al. (25). These workers separated some of the isomers on an Apiezon L-coated capillary column after previous fractionations. Some degree of separation of positional (8) and geometric (15) isomers of the unsaturated fatty acid methyl esters has been achieved on packed columns with ethylene glycol adipate polyester as the liquid phase. The unidentified components of the processed soybean oil (Table V) and of the hydrogenated shortenings (Table VI) which elute near the 18:1, 18:2, and 18:3 acids appear to be isomers of these acids, but little supporting evidence is available. The processed soybean oil had an iodine value (I.V.) of 110 as determined by AOCS procedure (1). Some degree of hydrogenation in the processing of this oil is indicated since soybean oil I.V. usually range between 127 and 138 (1).

Summary

Exposure of some food lipids to the reagents and conditions of several lipid extraction procedures did not significantly change the fatty acid compositions. Saponification and esterification reactions at room temp produced satisfactory yields of fatty acid methyl esters when applied to food fats and oils of relatively simple lipid composition. Following the application of these procedures, the fatty acid compositions of some separated fats and oils were determined by GLC.

REFERENCES

1. AOCS "Official and Tentative Methods," 2nd ed. rev. to 1962, Chicago, Cd 1–25; Table—I 1–46.
2. Assoc. Official Agr. Chemists, "Official Methods of Analysis," 9th ed., Washington, 1960, p. 297, 287, 174.
3. Arnold, L. K., and R. B. R. Choudhury, JAOCS *39*, 378 (1962).
4. Beare, J. L., J. Agr. Food Chem. *10*, 120–123 (1962).
5. Bernfeld, P., F. Homburger, and T. F. Kelley, Am. J. Clin. Nutri. *11*, 554–558 (1962).
6. Bligh, E. G., and W. J. Dyer, Can. J. Biochem. Physiol. *37*, 911–917 (1959).
7. Craig, B. M., and N. L. Murty, JAOCS *36*, 549–552 (1959).
8. Daniels, N. W. R., and J. W. Richmond, Nature *187*, 55–56 (1960).
9. Entenman, C., JAOCS *38*, 534–538 (1961).
10. French, R. B., *Ibid.* *39*, 176–178 (1962).
11. Grande, F., J. T. Anderson, and A. Keys, J. Nutri. *74*, 420–428 (1961).
12. Herb, S. F., R. W. Riemenschneider, and J. Donaldson, JAOCS *28*, 55–58 (1951).
13. James, A. T., in "Methods of Biochemical Analysis," edited by D. Glick, Vol. 8, Interscience Publishers, New York, 1960, p. 10.
14. Johnston, P. V., F. A. Kummerow, and C. H. Walton, Proc. Soc. Exp. Biol. Med. *99*, 735–736 (1958).
15. Landowne, R. A., and S. R. Lipsky, Biochim. Biophys. Acta *46*, 1–6 (1962).
16. Lorette, N. B., and J. H. Brown, Jr., J. Org. Chem. *24*, 261–262 (1959).
17. Lovelock, J. E., J. Chromatog. *1*, 35–46 (1958).
18. Magidman, P., S. F. Herb, F. E. Luddy, and R. W. Riemenschneider, JAOCS *40*, 86–88 (1963).
19. McOsker, D. E., F. H. Mattson, H. B. Sweringen, and A. M. Kligman, J. Am. Med. Assoc. *180*, 380–385 (1962).
20. Messner, A. E., D. M. Rosie, and P. A. Argabright, Anal. Chem. *31*, 230–233 (1959).
21. Privett, O. S., J. D. Nadenicek, R. P. Weber, and F. J. Pusch, JAOCS *40*, 28–30 (1963).
22. Radell, J., Chemist-Analyst *46*, 73–74 (1957).
23. Snedecor, G. W., "Statistical Methods," 4th ed., Iowa State College Press, Ames, 1946.
24. Sreenivasan, B., J. B. Brown, E. P. Jones, V. L. Davison, and J. Nowakowska, JAOCS *39*, 255–259 (1962).
25. Sreenivasan, B., J. Nowakowska, E. P. Jones, E. Selke, C. R. Scholfield, and H. J. Dutton, JAOCS *40*, 45–51 (1963).
26. Stoffel, W., F. Chu, and E. H. Ahrens, Jr., Anal. Chem. *31*, 307–308 (1959).
27. Whyte, L. K., Oil & Soap *23*, 323–326 (1946).
28. Wilding, M. D., E. E. Rice, and K. F. Mattil, JAOCS *40*, 55–57. (1963).
29. Willmer, J. S., and D. H. Laughland, Can. J. Biochem. Physiol. *35*, 819–826 (1957).

[Received September 11, 1963—Accepted December 30, 1963]