A Further Study of the Tetrabromide Method of Estimating Linoleic Acid in Fatty Acid Mixtures With Pentane and Heptane as Solvents[1]

MARY F. WHITE and J. B. BROWN, Laboratory of Physiological Chemistry and the Institute of Nutrition and Food Technology, Ohio State University, Columbus, Ohio

WE have previously reported (1) a procedure for the estimation of the linoleic acid content of fatty acid mixtures, based on the yield of insoluble tetrabromides following bromination of the mixture in petroleum ether under precisely controlled conditions. The yield of tetrabromides was shown to be empirical, depending on the conditions of bromination. One of the factors affecting the yield of bromides is the petroleum ether used during bromination and subsequent washings. Several pure hydrocarbons have become available recently at a cost which makes them economically feasible for use in the procedure. Accordingly it seemed to us to be worthwhile to re-evaluate our data with n-pentane and n-heptane as solvents for bromination and as wash liquids. These two hydrocarbons are available from the Phillips Petroleum Corporation, Bartlesville, Oklahoma, in a purity of not less than 99 moles per cent at a cost comparable with that of analytical grade petroleum ether. Hexane of this purity is still too expensive to be considered for this purpose in routine work.

Our procedure, as before, is based on data for a curve obtained by brominating 1.0-g. specimens of linoleic-oleic acid mixtures and plotting the yields of tetrabromides from 1 g. of mixture against the known percentages of linoleic acid in the mixtures. In the method it is not necessary to brominate exactly 1-g. specimens. However the sample should approximate this amount after the yield of bromides is corrected to 1 g. of the unknown mixture. This yield is applied to the curve and the content of linoleic acid estimated by interpolation.

We have found (Tables I and II) that the tetrabromide yields in n-pentane are slightly (1-2%) higher than those previously reported for Mallinckrodt petroleum ether (30-60°). Another difference is the fact that a significant yield of bromides was obtained in pentane from a mixture containing only 10% linoleic acid. In petroleum ether 20% of linoleic acid is about the minimum that can be detected by the method. With heptane as solvent for bromination and washing, the yields of bromides can be superimposed on the pentane curve. There are no important differences down to 25% of linoleic acid. However the heptane curve shows a sharp break below 25%, indicating that the lower limit of sensitivity with this solvent is about 16%. From these results it is concluded that pentane is the preferred solvent.

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TABLE I

The Tetrabromide Yields of Known Mixtures of Linoleic and Oleic Acids in Pentane and Heptane

Composition of Mixture			Tetrabromides from 1.0 g. Mixture, g.
Oleic Acid, g.	Linoleic Acid, g.	Linoleic Acid, %	
n-Pentane			
0	1.0000	100.0	1.0069
0	1.0000	100.0	1.0065
0.2535	0.7468	74.7	0.7274
0.2534	0.7473	74.0	0.7220
0.4015	0.6034	60.0	0.5670
0.4021	0.5966	59.7	0.5561
0.6010	0.4083	40.5	0.3640
0.6014	0.3968	39.8	0.3250
0.7551	0.2534	25.1	0.1777
0.7488	0.2512	25.1	0.1728
0.8433	0.1591	15.9	0.0708
0.8508	0.1500	15.0	0.0806
0.9041	0.1007	10.0	0.0122
0.8995	0.1011	10.1	0.0116
n-Heptane			
0	1.0000	100.0	0.9944
0	1.0000	100.0	0.9987
0.2499	0.7530	75.1	0.7375
0.2521	0.7559	75.0	0.7336
0.4018	0.5986	59.8	0.5560
0.4040	0.5981	59.7	0.5623
0.5942	0.4039	40.5	0.3497
0.5966	0.4032	40.3	0.3491
0.7525	0.2498	24.9	0.1750
0.7503	0.2526	25.2	0.1816
0.8460	0.1509	15.1	0.0024
0.8546	0.1496	14.9	0.0028
0.9003	0.0997	10.0	0
0.9012	0.1024	10.2	0

TABLE II

Simplified Data for Drawing Interpolation Curves for the Estimation of Linoleic Acid in Fatty Acid Mixtures

Linoleic Acid, %	Tetrabromide Yield from 1.0 g.		
	MPE[a]	n-Pentane	n-Heptane
100	0.985	1.007	0.9966
75	0.702	0.731	0.731
50	0.437	0.455	0.455
25	0.152	0.178	0.178
20	0.028	0.124	0.092
10	0	0.012	0

[a] From data reported in (1).

Several specimens of fatty acids were analyzed for linoleic acid by the use of the pentane curve and results shown to agree closely with values obtained by thiocyanometry.

Experimental

Preparation of Linoleic Acid. A specimen of linoleic acid was purchased from the Hormel Foundation, Austin, Minnesota. The iodine value of this acid, 180.7, indicated it to be practically 100% dienoic acid, but its melting point (−7.1° to −6.8°) was about 2° lower than that of pure cis,cis-9,12-octadecadienoic acid. This acid contained 0.2% of diene and triene conjugation. Since the Hormel acid was prepared by debromination, we believed it to contain iso-acids in line with a previous report from this laboratory (2). We found 1.03 g. of the acid to give 0.891 g. of tetrabromide, corresponding to a linoleic acid content of 89.7%. Accordingly 21 g. of the acid was crystallized six times from petroleum ether (430 cc.) at −65°. The final crystal fraction was distilled at 4-mm. pressure to give 14.2 g. of linoleic acid of theoretical iodine value and melting at −5.3° to −5.0°, the reported value for the pure cis-acid (2). The filtrates were combined and distilled to give 6.2 g. of acids melting

TABLE III

Estimation of the Linoleic Acid Contents of the Fatty Acids of Several Oils by Bromination in Pentane and by Thiocyanometry

Fatty Acids of	Bromides from 1.0 g. of Acids, g.	Linoleic Acid, %	
		From Pentane Curve	From SCN No.
Olive Oil[a]	0.0091	9.7	12.2
Corn Oil	0.5564	59.7	59.2
	0.5675	60.7	
	0.5419	58.4	
	0.5613	59.9	
Peanut Oil	0.1463	21.7	21.0
	0.1412	21.2	
	0.1213	19.3	
	0.1371	20.7	
Tobacco Seed Oil	0.7403	75.5	75.5
	0.7049	72.5	
	0.7415	75.8	
	0.7433	75.6	
Almond Oil	0.1580	22.0	21.7
	0.1574	21.9	
	0.1566	21.8	
	0.1639	22.4	
Poppyseed Oil	0.6208	65.0	64.6
	0.6211	65.0	
	0.6125	64.0	
	0.6192	64.6	
Sesame Oil	0.4073	44.8	44.7
	0.4063	44.6	
	0.4020	44.4	
	0.4099	45.1	

[a] It is to be noted here that in petroleum ether no tetrabromides would have been obtained. The significantly lower result here by the tetrabromide method is an indication of the presence of isomeric linoleic acids in this oil, as previously reported from this laboratory (3).

at −12.9° to −12.1°. The tetrabromide number of the filtrate acids in heptane, 61.7, show the composition to be 35% iso-acids and 65% linoleic acid.

Tetrabromide Yields

Linoleic acid and known mixtures of linoleic and oleic acids were brominated in n-pentane and n-heptane according to the procedure described by White and Brown (1). The linoleic acid used in this work was the recrystallized specimen described above; the oleic acid was a very pure specimen isolated from corn oil. The results are found in Table I.

In order to compare the data in Table I with the previously reported data (1) in which Mallinckrodt petroleum ether was employed in bromination and washing of the bromides, the percentages of linoleic acid in Table I were plotted against tetrabromide yields and the results at certain round number percentages were estimated by interpolation. These are summarized in Table II.

The linoleic acid contents of the fatty acid mixtures from several oils were next determined from the tetrabromide-pentane curve and the results compared with those obtained by analysis of these mixtures by thiocyanometry. Admittedly this comparison would have been the more valuable if spectrophotometric analyses were included, but this was not done. The oils selected for these comparisons were those which contain no appreciable amounts of linolenic acid. The results are shown in Table III.

Summary

Known mixtures of linoleic and oleic acids were brominated in n-pentane and n-heptane and the yields of insoluble tetrabromides determined under precise conditions. In general, the yields were somewhat higher in both hydrocarbons than in Mallinckrodt petroleum ether. Tetrabromides were obtained in detectable amounts in mixtures containing as little as 10% linoleic acid when pentane is used. Therefore it is recommended that this solvent be employed in applying the procedure in the analysis of fatty acid mixtures. The linoleic acid contents of the fatty acids of several oils were estimated from the tetrabromide-pentane curve and the results found to agree favorably with those obtained by thiocyanometry.

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Carbohydrate Constituents of Soybean "Lecithin"[1]

C. R. SCHOLFIELD, HERBERT J. DUTTON, and ROBERT J. DIMLER,
Northern Regional Research Laboratory,[2] Peoria, Illinois

THE status of carbohydrate material in plant phosphatides, whether bound or free, has been a point of discussion for many years. As long ago as 1906 Winterstein and Hiestand reported that some of the carbohydrates in various plant phosphatides studied could be removed by washing with water, but that part appeared to be in firm chemical combination, and that it was necessary to boil for several hours with 5% sulfuric acid to split off all carbohydrates (24, 25). In a series of publications beginning in 1929 Rewald has maintained however that the carbohydrates in soybean "lecithin" were free, and he believed that they could be removed from the phosphatides by physical means (18, 19). The work of McKinney, Jamieson, and Holton (15) supported the earlier concept of Winterstein and Hiestand and indicated that some sugar in soybean phosphatides was bound to lecithin by a glycosidic linkage. More recently Woolley (26) and Folch (7) have reported the presence of galactose-containing phosphoinositides. The presence of the free sugars, sucrose and stachyose, has been reported in the whole soybean many times (14). Celmer and Carter (3, 4) have shown that these free sugars, as well as a sugar-containing phosphoinositide, are present in commercial lecithin.

This paper reports the identification of both free and bound sugars by means of paper chromatography along with an estimate of the amounts of each sugar in one sample of phosphatides.

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[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.