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Note

Gas chromatographic characterization of cooking fats with reference to a case of murder

In a recent case of murder the stomach contents of the victim proved to be entirely of newly consumed fried potato chips of unknown origin. Extraction of the stomach contents, by gentle agitation in light petroleum, yielded about 2 g of the (solid) fat in which the chips had been cooked. In order to determine the origin of the meal, samples of fats from all of the fish and chip shops within a distance of six miles from the point where the deceased was last seen alive, shortly before her death, were submitted to the laboratory. Also submitted were fish and chip wrappings and small fragments of chips — in all, over eighty samples representing twenty-three origins. Some of the samples were liquid and, therefore, eliminated immediately. For the others a technique based on the pyrolysis of the tetramethylammonium hydroxide saponification products was found to be appropriate and enabled the problem to be resolved. As cases involving fats seem to occur fairly regularly in the forensic laboratories, the methods used may be of general interest.

The technique essentially is an adaptation of DOWNING AND GREENE'S[1]

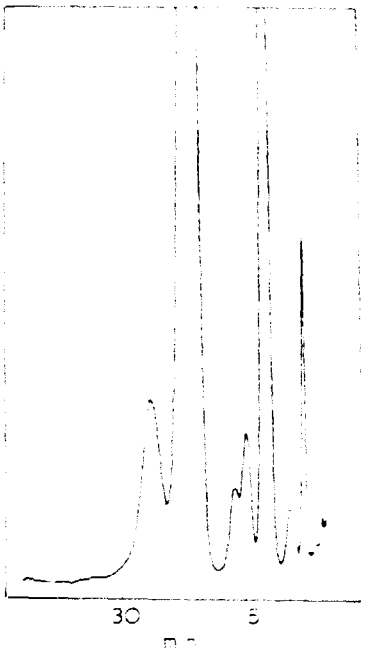

Fig. 1. Gas chromatogram (Porapak P, 250°) of the pyrolysate from a cooking fat hydrolysed

rather lengthy method of saponification in ethanolic potassium hydroxide, acidification, extraction of the liberated fatty acids, and their conversion to tetramethylammonium salts, which are pyrolysed in the heated injection port of a gas chromatograph to yield a chromatogram of the methyl esters. We use tetramethylammonium hydroxide to effect saponification thus forming the salts in a single step. Little manipulation is required and the method is readily applied to small quantities.

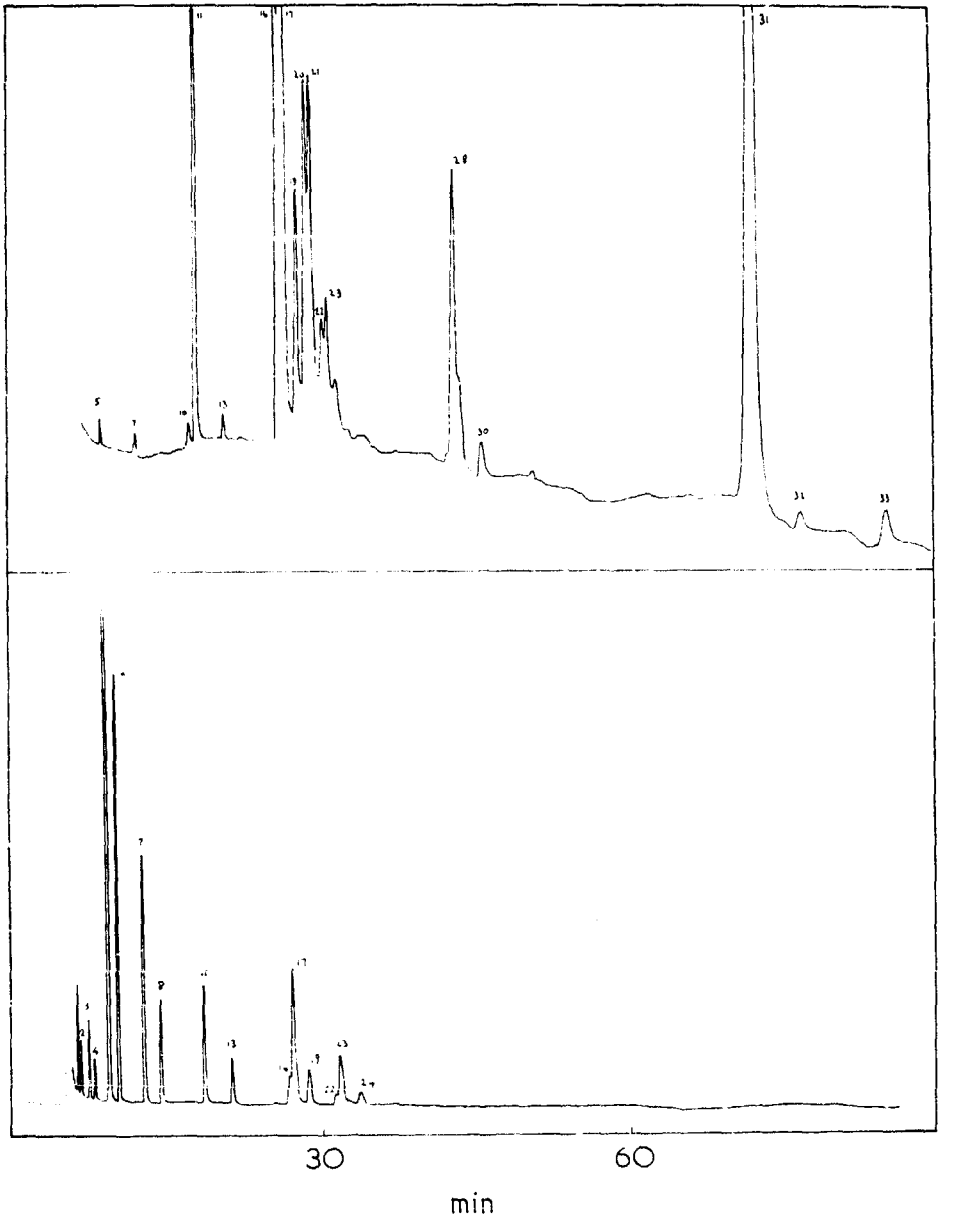

Fig. 2. Gas chromatograms (capillary column) of the pyrolysates from fats hydrolysed in tetramethylammonium hydroxide reagent. The upper trace is from rapeseed oil, the lower is from a partly hardened palm kernel fat. Peak identities are given at the foot of Table I. The

A small sample of each fat was weighed into a polyethylene-capped glass specimen tube. Per milligram of fat taken was added ethanolic tetramethylammonium hydroxide (20 μl, 0.15 M). The mixture, uncapped, was heated over a small flame until dissolution was complete, capped, and then incubated at 50 for 1 h. After this time no further changes occurred in the solution. The loosely coiled end of a 510 Curie point wire (coiled to increase the surface area in the energised zone) was wetted with the hydrolysate, blown dry, and inserted in a Philip's Curie point apparatus connected to a 350 × 4 mm gas chromatography (GC) column of Porapak P operated at 250 and with flame ionization detection. Optimally the nitrogen flow was in the region of 35 ml/min, but the urgency of the situation necessitated a double flow-rate at the expense of some resolution. Such chromatograms (Fig. 1)

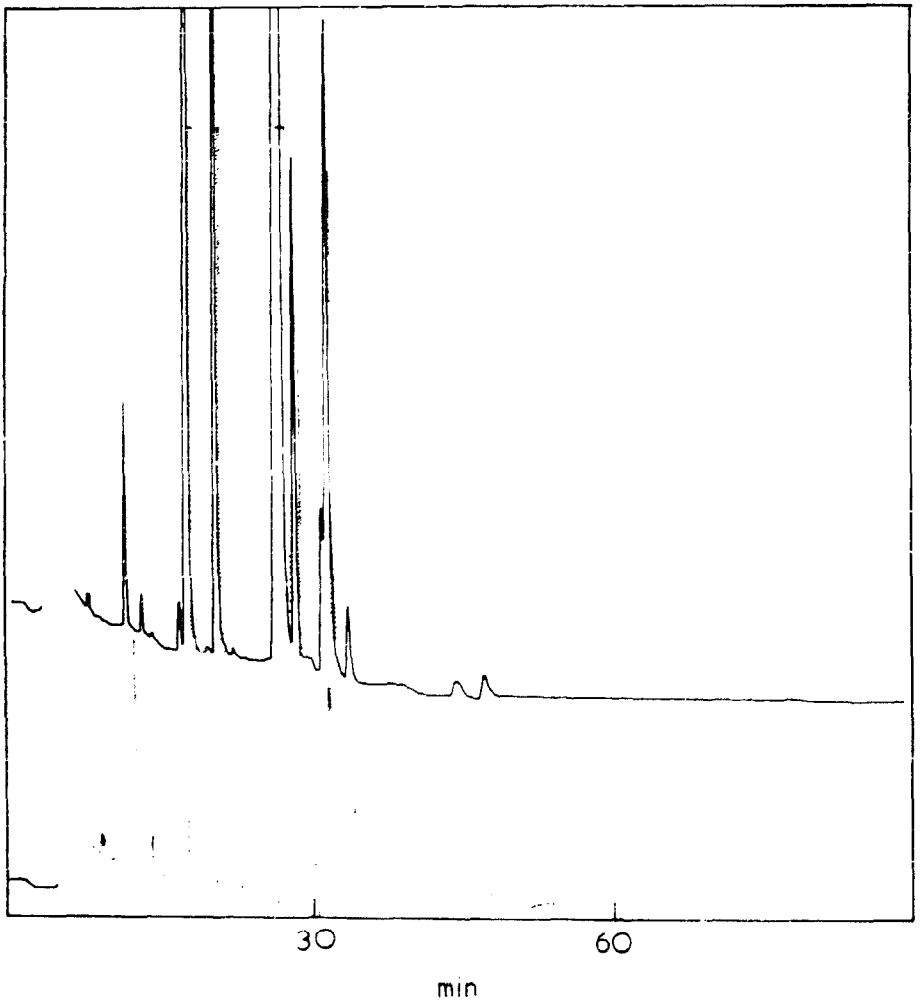

Fig. 3. Gas chromatograms (capillary columns) of the pyrolysates from fats hydrolysed in tetramethylammonium hydroxide reagent. The unbroken trace is from a control sample. The dotted trace is from fat extracted from the stomach contents.

show at least eight components of which methyl palmitate and methyl oleate are usually the most prominent.

The fat samples were found to be of four well defined types differentiated in their oleate/palmitate peak height ratios, which were centered in the regions 0.35, 0.55, 0.75, and 1.1 (± 0.10). Within each type the ratio, as well as the proportions of minor components, varied considerably, and most of the shops could immediately be eliminated from the enquiry as possible origins of the fat. Of the rest (eight shops), two compared closely and one identically to the recovered fat. The others, although of the same type, deviated in composition to an extent that rendered an origin in common with the recovered fat highly improbable.

When sufficient time is available very much more detail can be obtained by open tubular column GC techniques. The fat hydrolysates pyrolyse efficiently when syringed into the heated (*ca.* 350 °) injection port of a gas chromatograph. Separations are made on a 150 ft. × 0.010 in. column coated with the silicone oil OV-101 and operated at 250 ° with a nitrogen flow of 1 ml/min. With a sample splitting ratio of 0.1 a satisfactory chromatogram is given by 0.5 μl of hydrolysate.

TABLE I

RELATIVE GC PEAK HEIGHTS OF METHYL ESTERS FORMED IN THE PYROLYSIS OF TETRAMETHYLAMMONIUM HYDROXIDE HYDROLYSATES OF VARIOUS FATS

Methyl ester[a]	*Sample*										
	Stomach content	*Control*	*Soya bean*	*Maize*	*Rape seed*	*Cotton-seed*	*Sun-flower seed*	*Ground-nut*	*Palm kernel*[b]	*Palm*	*Lard*
1									0.870		0.010
2									0.430		
3						0.0098			0.006		
4									0.311		
5	0.0025	0.0021	0.009		0.036	0.077		0.011	8.08	0.0025	0.0815
6						0.0036			0.361		0.034
7	0.039	0.039	0.011		0.036	0.077		0.009	2.086	0.028	0.003
8			0.016			0.0036			0.870	0.0053	0.019
10			0.015	0.014	0.033	0.030		0.013	0.004	0.004	0.100
11	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
13	0.100	0.172	0.035	0.030	0.030	0.046	0.203	0.119	0.302	0.148	0.125
16	0.140	0.138	2.33	1.055	2.030	1.357	3.881	1.191	0.235	0.117	0.203
17	0.503	0.021	2.22	2.102	3.180	1.071	3.286	5.505	1.130	0.505	1.204
19	0.081	0.084	0.363	0.138	0.281	0.118	0.421	0.303	0.278	0.084	0.373
20			0.105	0.130	0.352	0.428	0.305	0.041			
21			0.105	0.170	0.371	0.0535	0.276	0.000			
22	0.031	0.028	0.000	0.084	0.081	0.070	1.355	0.150	0.070	0.020	0.029
23	0.014	0.011	0.075	0.077	0.110	0.044	0.842	0.575	0.100	0.078	0.152
24							0.002	0.027	0.000	0.011	0.042
25	0.011	0.013									
28	0.0024	0.0026	0.007	0.021	0.395	0.009	0.033	0.064	0.004	0.001	0.015
30			0.000	0.017	0.043	0.001	0.020	0.000	0.008	0.004	0.003
31			0.008	0.050	1.617	0.015	0.002	0.000			

[a] Probable identities of some of the methyl esters are as follows: 1 = caprylic; 2 = capric; 5 = lauric; 7 = myristic; 10 = palmitoleic; 11 = palmitic; 16 = linoleic; 17 = oleic; 19 = stearic; 30 = arachidic; 31 = erucic. Only the more frequently observed components are listed in the table. A blank entry for any particular component signifies that the actual value is less than the smallest listed for the sample concerned.

[b] Partly hardened.

From fats of various types up to thirty-four components can be readily separated by this column. Fig. 2 shows the extent of variation that is possible. Most common vegetable fats lie within the range covered by the chromatograms shown, but all that we have so far examined prove distinguishable from one another.

Fig. 3 compares a capillary chromatogram from the stomach contents with one from the previously indicated point of origin, and Table I compares peak heights, relative to methyl palmitate, from chromatograms of the two samples and from a variety of other fats. The latter are included as an indication of the variation that is detected between differing materials. (Peak identities are given, where possible, at the foot of Table I.) Usually several chromatograms were obtained from each fat. Provided there was an adequate response to methyl palmitate, coefficients of variation are about 4% for peak height ratios greater than 0.2, and increase to about 10% for ratios in the region of 0.01. It is clear both from Fig. 3 and from the tabulated results that the two case work samples are in very close agreement with one another and that the identity of their fatty acid compositions cannot be reasonably doubted.

These last-mentioned results do not, of course, influence in any way the conclusion drawn from the murder investigation, where the lower resolution technique provided a quite unambiguous answer. When there is no circumscribed field of origin, however, and when time seems less desperately short, then the open tubular column technique provides results of increased evidential value.

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1 D. T. DOWNING AND R. S. GREENE, *Anal. Chem.*, 40 (1968) 827.

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