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Direct hydrogenation of unsaturated fatty acids on the support of the gas–liquid chromatographic column

When qualitatively identifying fatty acids separated by GLC analysis, extrapolations from plots of relationships between the logarithms of specific elution volumes and the number of carbon atoms, polar and nonpolar phases, the number of double bonds, Kovats' indices, etc. can be used. To supplement data on qualitative identification of unsaturated and saturated fatty acids in a mixture, the so-called discrimination chromatogram, *i.e.* a new GLC analysis of the sample after its chemical treatment by bromination, oxidation or hydrogenation, can be used.

LANDOWNE AND LIPSKI[1] analyzed mixtures of unsaturated and saturated fatty acids using bromination. They first carried out a GLC analysis of an untreated sample and then compared the results with those of a GLC analysis of derivatives of unsaturated fatty acids after bromination. CARTONI *et al.* used bromination for the separation of stereoisomers of 9:10-octadecenoic acid on an open tubular capillary GLC column[2]. JAMES AND WEBB[3] used an oxidative method for distinguishing unsaturated and saturated fatty acids, and GUNSTONE AND SYTER[4] used the oxidative method for determination of the structure of some unsaturated fatty acids. FARQUHAR *et al.* introduced GLC separation of unsaturated and saturated fatty acids before and after hydrogenation in a microdevice using platinum as catalyst[5]. SMITH[6] applied this method for qualitative and quantitative analyses of fatty acid in milk fat by GLC, for example. Bromination and/or hydrogenation in combination with GLC analysis of fatty acids as described above are introduced by BURCHFIELD AND STORRS[7] in the chapter *Subtraction methods* of their monograph. Microhydrogenation of unsaturated fatty acids on filter paper with the use of palladium as catalyst was described by KAUFMANN AND CHOWDHURY[8]; the combination of this method with GLC analysis of fatty acid mixtures was published by KOMAN[9].

In the present paper, direct hydrogenation of unsaturated fatty acids by filling the GLC column with palladium as catalyst precipitated on Celite is described, and the separations are compared with results achieved on the column without catalyst.

Experimental

Preparation of columns. Two identical aluminium columns 2 m long and 4 mm in diameter were packed with 12.4 g of Celite having a particulate diameter of 0.12–0.15 mm. Celite was floated, acid washed[10], silanized with dichlorodimethylsilane[11] and coated with 20 % DEGS which was prepared according to ref. 12. At the inlet of one column, 250 mg of Celite with freshly precipitated palladium were added.

Preparation of the catalyst. Palladium was precipitated and applied as described in refs. 8, 13 and 14, dealing with distinguishing "critical pairs" of unsaturated and saturated fatty acids by partition paper chromatography. In our case, unsilanized Celite was used as a support and promotor of the catalyst. The precipitation of palladium was carried out under the following conditions. 6.4 ml of a 10 % solution of palladium dichloride and 10 ml of a freshly prepared mixture of 80 ml of 20 % KOH and 20 ml of 40 % formaldehyde were added to 1.0 g of unsilanized Celite. Then the Celite was washed for 10 min with a 5 % solution of acetic acid, and the acid was

removed by a five-fold washing with water. The Celite with palladium precipitated in this way was dried for 30 min at 90°, and 250 mg were applied to the inlet of one column.

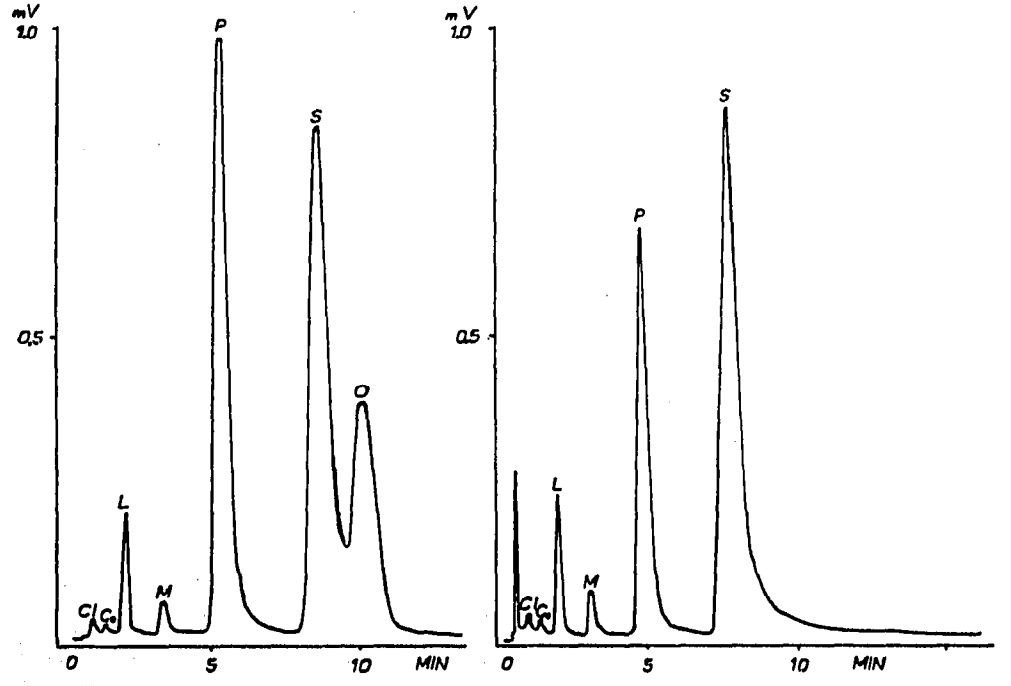

Fig. 1. Chromatograms of mixtures of fatty acids separated on columns without (left-hand side) and with palladium (right-hand side). L = lauric ($C_{12:0}$); M = myristic ($C_{14:0}$); P = palmitic ($C_{16:0}$); S = stearic ($C_{18:0}$); and O = oleic ($C_{18:1}$) acid.

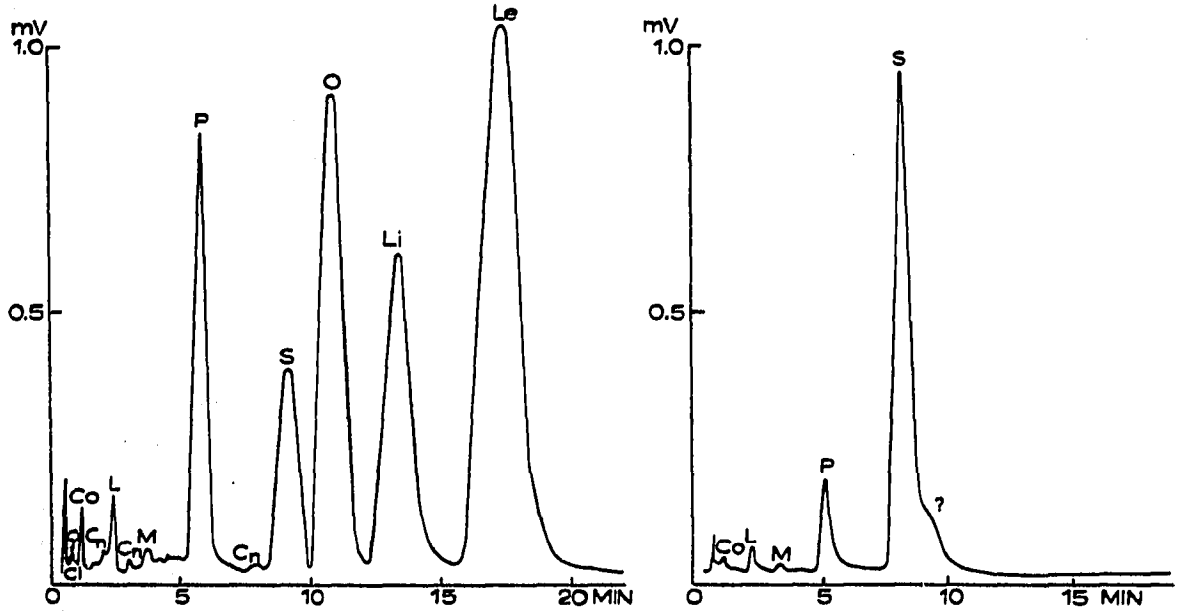

Fig. 2. Chromatograms of mixtures of fatty acids separated on columns without (left-hand side) and with palladium (right-hand side). P = palmitic ($C_{16:0}$); S = stearic ($C_{18:0}$); O = oleic ($C_{18:1}$); Li = linoleic ($C_{18:2}$); and Le = linolenic ($C_{18:3}$) acid.

Chromatographic conditions. Before use both prepared columns were conditioned for 24 h at 220°. For conditioning, as well as for the GLC analysis of fatty acid mixtures, hydrogen as carrier gas was used. Optimal values of the hydrogen flow (65 ml/min) and the optimal column temperature (197°) were determined experimentally. For the assay 0.1–0.35 μl of fatty acid mixtures, a temperature of 295° at the injection port of the gas chromatograph (type CHROM 3*) and isothermic conditions were used.

Results and discussion

The results of the GLC separations of some natural fatty acid mixtures on the columns with and without palladium, presented in Figs. 1–3, indicate a complete

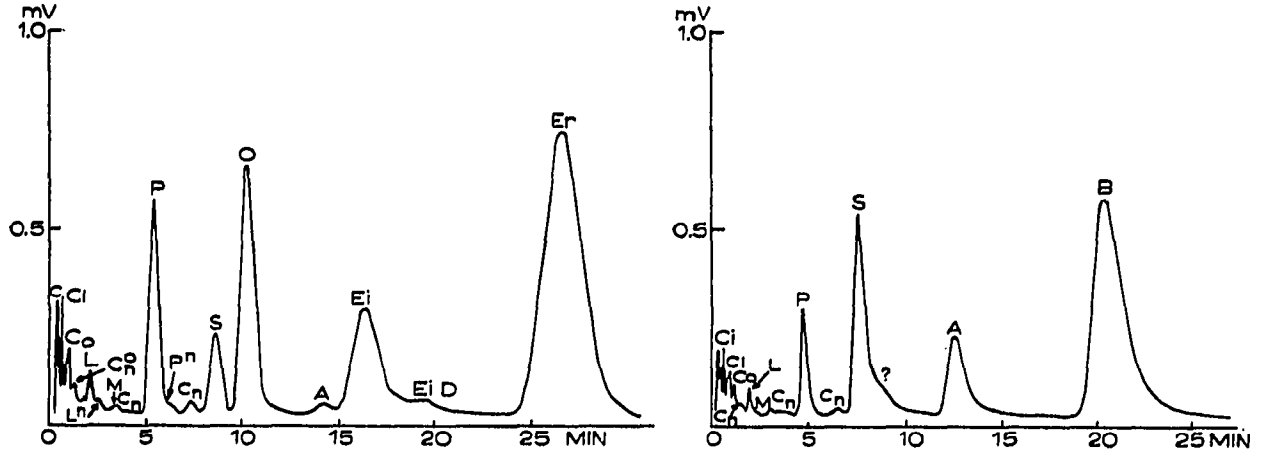

Fig. 3. Chromatograms of mixtures of fatty acids separated on columns without (left-hand side) and with palladium (right-hand side). P = palmitic ($C_{16:0}$); S = stearic ($C_{18:0}$); O = oleic ($C_{18:1}$); A = arachidic ($C_{20:0}$); Ei = eicosenoic ($C_{20:1}$); EiD = eicosadienoic ($C_{20:2}$); Er = erucic ($C_{22:1}$); and B = behenic ($C_{22:0}$) acid.

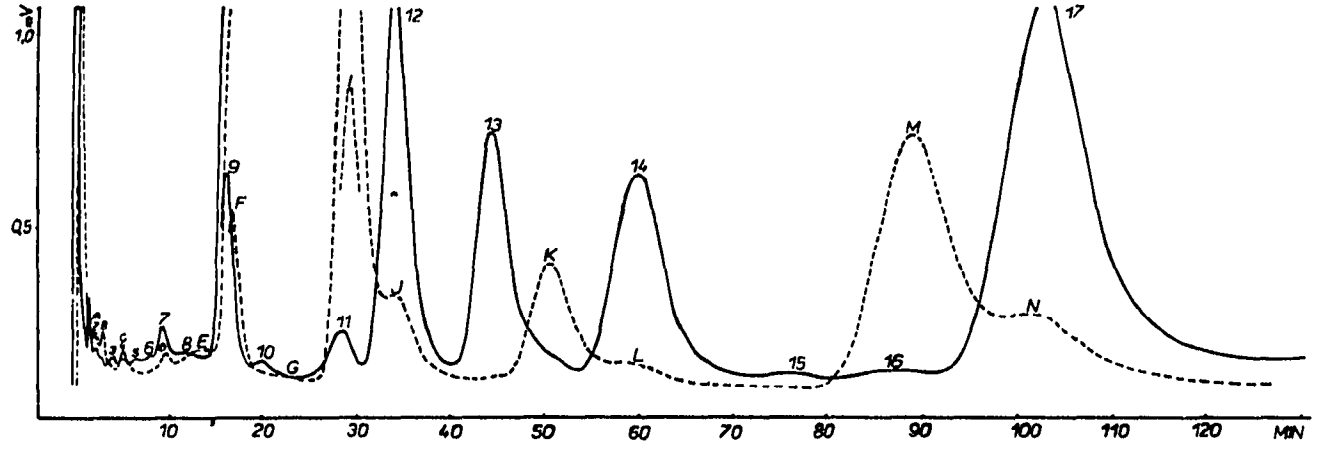

Fig. 4. Chromatograms of a mixture of fatty acids: part of sample unhydrogenated (full line) and part pre-hydrogenated on paper before GLC analysis according to ref. 9 (dotted line). Individual peaks: F ≡ 9 = palmitic acid ($C_{16:0}$); I ≡ 11 = stearic acid ($C_{18:0}$); J ≡ 12 = oleic acid ($C_{18:1}$); 13 = linoleic acid ($C_{18:2}$); K = arachidic acid ($C_{20:0}$); L ≡ 14 = eicosenoic acid ($C_{20:1}$); 15 = eicosadienoic acid ($C_{20:2}$); M ≡ 16 = behenic acid ($C_{22:0}$); N ≡ 17 = erucic acid ($C_{22:1}$)**.

* Manufactured by Laboratorní přístroje n.p. Praha, Czechoslovakia.

** In all figures the peaks in front of palmitic acid belong to short-chain minority fatty acids of the analysed mixtures.

conversion of unsaturated fatty acids into the corresponding saturated ones. An example of a GLC analysis of a fatty acid mixture before and after microhydrogenation, according to ref. 9, is presented in Fig. 4. Comparison of Figs. 3 and 4 shows that the hydrogenation of unsaturated fatty acids is quantitative on the column with the catalyst, while the unsaturated fatty acids from the same mixture hydrogenated on filter paper prior to the GLC analysis did not react completely.

It is apparent from the results that the application of the described direct hydrogenation of unsaturated fatty acids on the GLC column with palladium can be recommended as a supplement for qualitative identification of unsaturated and saturated fatty acids in mixtures.

When the column with palladium is already prepared and GC equipment with a dual column system is used, the time required for obtaining a discrimination chromatogram under the conditions described for hydrogenation is reduced to the time required for injection of the sample only.

The direct hydrogenation of unsaturated fatty acids on the GLC column was verified by numerous examples using known natural fatty acid mixtures and gave a good reproducibility. It was successfully used for qualitative and quantitative determinations of fatty acids, *e.g.* in the citrinin producer *Penicillium notatum* during cultivation[15].

The activity of palladium which was prepared and used at the beginning of these experiments has remained unchanged for two years.

Note

Recently we have found that the hydrogenation presented herein can be simplified further by placing 250 mg of the catalyst prepared as described above into a "microreactor" which can be connected to the front of any GC column.

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