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Number of Double Bonds in Fatty Acids from Fats and Oils by HPLC Using Pentafluorobenzyl Esters

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A simple, one-pot procedure for the saponification of fats and oils and the subsequent esterification of the resulting fatty acid salts with pentafluorobenzyl bromide is presented. A normal phase high pressure liquid chromatographic procedure for the separation of these pentafluorobenzyl esters, primarily on the degree of unsaturation, is also given. Chromatographic detection of the esters can be carried out conveniently at 254 nm, and the procedure should therefore find application in the routine determination of the number of double bonds in fatty acids from fats and oils.

In a recent publication (1), we examined the chromatographic properties of fatty acid pentafluorobenzyl (PFB) esters in both normal and reversed phase systems. We found a normal phase system that essentially separates the fatty acid PFB esters on the number of double bonds in the fatty acid moiety. Although there is a small separation based on the number of carbon atoms, we felt this technique offered considerable promise for rapidly monitoring the degree of unsaturation of various commercial fats and oils. This note, then, presents a simple method for achieving this aim and gives the results of its application to some domestically used fats and oils.

EXPERIMENTAL

The following fats and oils were obtained from local retail outlets: Tandaco suet mix, 40% rendered beef suet, 60% aerated flour, Cerebos (Aust.) Ltd., Seven Hills, New South Wales Australia; Allowrie Lard, PDS Rural Products Ltd., Sydney, New South Wales, Australia; No Frills Butter, Franklins P/L, Chullora, New South Wales, Australia; San Giorgio olive oil, San Giorgio Sez. Agric SpA, Pomezia, Italy; No Frills Sunflower Oil, Polyunsaturated, Franklins P/L, Chullora, New South Wales, Australia; Meadow Lea polyunsaturated margarine (P/S>2:1), Vegetable Oils P/L, Mascot, New South Wales, Australia, and Glendale raw linseed oil, Glendale Chemical Products P/L, Alexandria, New South Wales, Australia. Approximately 20 mg (54 mg of suet, to compensate for flour content) of each fat or oil was added to a small screwcap tube followed by 1 ml of 0.2 M KOH in methanol. The tubes were then incubated at 90 C for 25 min. As Kihara et al. (2) have pointed out, derivatization procedures that utilize fatty acid salts can be performed in the same reaction vessel as the saponification. Accordingly, the methanol was evaporated from each tube under a stream of N_2. In the case of suet only, the white solid material, which was presumably the flour, was removed by filtration using a Gelman Sciences Inc. (Ann Arbor, Michigan, USA) Acro LC13 0.45 micron disposable filter. The addition of 1 ml of 0.1 M tetrabutylammonium hydrogen sulphate (Fluka, Buchs, Switzerland), 1 ml dichloromethane and 20 µl (ca. 2-fold XS) pentafluorobenzyl bromide (Fluka) followed. The mixtures were then incubated at room temperature with vigorous shaking for 40 min. These conditions essentially reproduce those given by Ehrsson (3), and therefore all

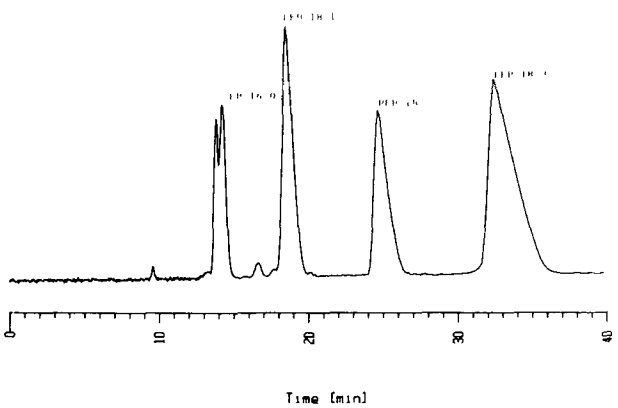

FIG. 1. PFB esters from linseed oil. Contains PFB 16:0, PFB 18:0, PFB 18:1 (0.069 AU), PFB 18:2, PFB 18:3. Chromatograms (Figs. 1 and 3-8) and log retention data (Fig. 2) for the PFB esters derived from various fats and oils. In all cases the column was 5 µm silica eluted with 10% dry dichloromethane, 90% half water saturated hexane. The chromatograms were monitored at 263 nm and are normalized to the largest peak (absolute absorbance given for each chromatogram).

fatty acids longer than C_8 should be quantitatively derivatized. The dichloromethane layers were extracted and evaporated. These were then taken up in three aliquots of hexane and applied to silica "Sep-Paks" (Waters Associates, Milford, Massachusetts, USA).

Elution with 10 ml hexane removed excess PFB bromide and unwanted by-products. This was followed by 10 ml 15% dichloromethane in hexane which, upon evaporation, gave the desired PFB esters. Following the addition of 200 µl hexane, 10 µl was injected into the HPLC. The system consisted of Waters Associates' M6000A pump and U6K Injector with a Brownlee Labs Inc. (Santa Clara, California, USA) Silica Spheri-5 column. The detection system was a Hewlett-Packard (Palo Alto, California, USA) diode array detector (HP1040A) fitted with an HP85B Computer, HP7470A Plotter and HP9121 disc drive. This detection system is not necessary for PFB esters, because they have an adequate absorption at 254 nm (1), but it does have the advantage that chromatographic signals at both 263 and 216 nm (the absorption maxima) and ratio plots of these two wavelengths can be made. The ratio plot can be helpful in determining the number of double bonds in an unknown peak. Quantitation of the chromatographic peaks may be performed by standard integration techniques utilizing the UV absorption at 263 nm (log ε = 2.78) or 254 nm (log ε = 2.67) (1). The solvent system for all of the separations reported here was 10% dry dichloromethane, 45% dry hexane, 45% water-saturated hexane at 2 ml/min. The water content is absolutely critical; minute variations in it will cause marked changes in retention time and long re-equilibration times.

RESULTS AND DISCUSSION

A cursory examination of the figures reveals the advantage of the techniques reported here. For example, the chromatogram for linseed oil (Fig. 1) shows four major peaks, the first of which is double. Comparison with standards shows that these, in order of elution, are fatty acid esters corresponding to PFB 18:0 and PFB 16:0, PFB 18:1, PFB 18:2 and PFB 18:3. Further a plot of log retention time against number of double bonds (Fig. 2) gives a straight line for PFB esters with a given number of carbon atoms. One can, therefore, read off the number of double bonds in the PFB esters from an unknown fat provided the chain length does not vary too widely. Figure 3 shows a chromatogram for the PFB esters from butter which illustrates this point. It is clear from the spectra obtained by the diode array detector that all the peaks eluting between 10 and 20 min are PFB esters. Further, from the log retention data the major peak eluting just after 17 min is PFB 18:1. Plots of log retention time against log No. of carbon atoms (1) suggest that the peaks eluting between 12 and 17 min are the PFB esters of 20:0 (minor), 18:0, 16:0, 14:0 (shoulder) 12:0, 10:0 and 8:0 with PFB 4:0 possibly eluting at 20 min. Thus, the peak designated as PFB 18:1 probably also contains some PFB 6:0.

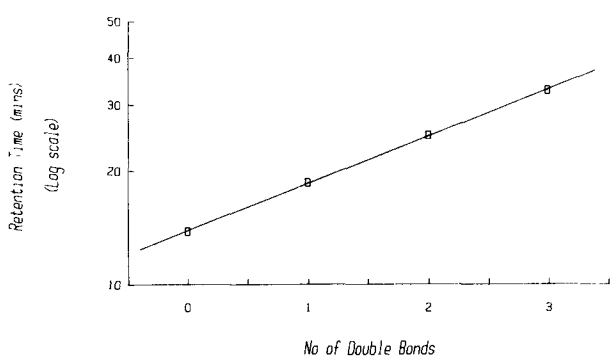

FIG. 2. Plot of retention time (log scale) against number of double bonds for the C_{18} PFB esters of the fatty acids from linseed oil. For details, see caption to Fig. 1.

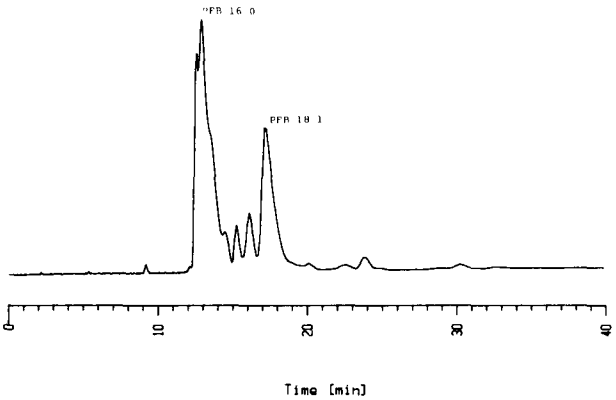

FIG. 3. PFB esters from butter. Contains PFB 18:0, PFB 16:0, (.197 AU), probably PFB 14:0 to PFB 8:0, PFB 18:1 plus PFB 6:0 (?), PFB 18:2 (contaminated). For details, see caption to Fig. 1.

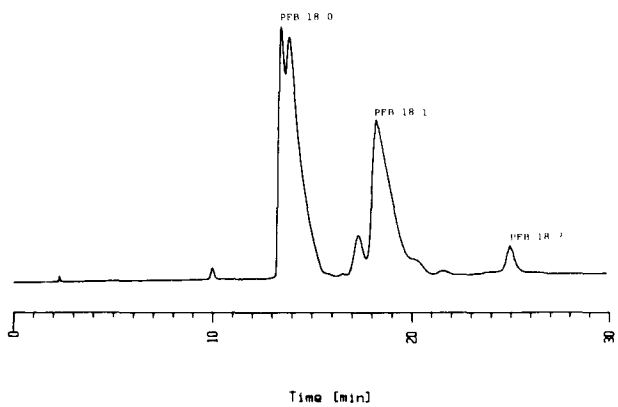

FIG. 4. PFB esters from suet. Contains PFB 18:0 (0.194 AU), PFB 16:0, unknown PFB ester, PFB 18:1 and PFB 18:2 (contaminated). For details, see caption to Fig. 1.

Figures 4 and 5 show chromatograms for the PFB esters derived from suet and lard. As expected, the major peaks in each were PFB 18:0 and PFB 16:0, PFB 18:1 and PFB 18:2 with similar proportions in each fat. However, the suet gave rise to an unidentified PFB ester eluting at 17.3 min.

Figure 6 is the chromatogram obtained for the PFB esters from olive oil. This shows the expected dominance of PFB 18:1. In fact, the column is probably slightly overloaded here because the PFB 18:1 elutes a fraction

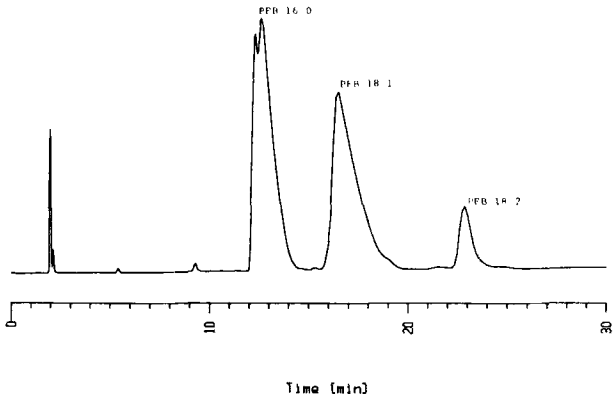

FIG. 5. PFB esters from lard. Contains PFB 18:0, PFB 16:0 (0.261 AU), PFB 18:1, PFB 18:2. For details, see caption to Fig. 1.

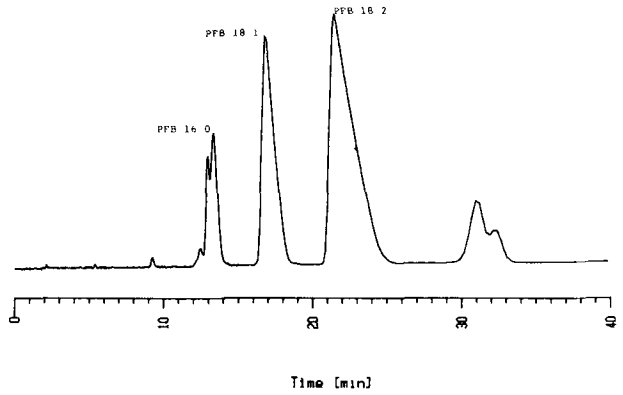

FIG. 7. PFB esters from sunflower oil. Contains PFB 18:0, PFB 16:0, PFB 18:1 PFB 18:2 (0.141 AU), two unknowns. For details, see caption to Fig. 1.

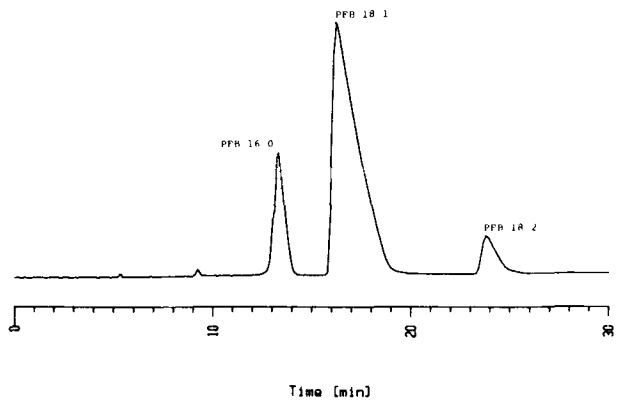

FIG. 6. PFB esters from olive oil. Contains PFB 18:0, PFB 16:0, PFB 18:1 (0.234 AU), PFB 18:2. For details, see caption to Fig. 1.

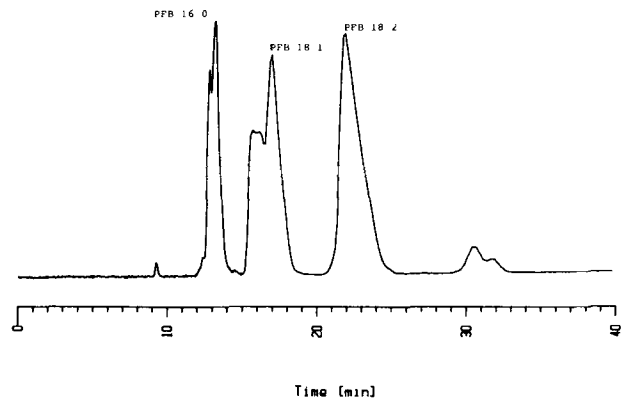

FIG. 8. PFB esters from polyunsaturated margarine. Contains PFB 18:0, PFB 16:0 (0.116 AU), unknown PFB ester, PFB 18:1, PFB 18:2, two unknowns. For details, see caption to Fig. 1.

earlier than would be predicted from the log retention data. Other esters present are PRB 18:0 (leading shoulder at 13.0 min), PFB 16:0 and PFB 18:2. Finally, Figures 7 and 8 show the chromatograms for sunflower oil and polyunsaturated margarine, respectively. The major peaks in each case are PFB 20:0, PFB 18:0 and PFB 16:0, PFB 18:1 and PFB 18:2. The double peaks eluting between 30 and 33 min are not fatty acid PFB esters judging from their UV spectra. Margarine gives rise to a peak (at 10 min), perhaps double, that gives spectra indicating it contains only PFB esters. The chromatographic properties of these esters suggest they contain one double bond and are longer in chain length than oleic acid, but they have not been investigated further.

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