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Note

Frontal elution liquid chromatography of a "total artefacts" peak in frying oils

At present, colour reactions and other chemical and physical tests are used to decide when an oil should be discarded[1–5]. None of these tests is very satisfactory, however, as several different mechanisms of oil deterioration may operate at the same time, and normally only one group of products is sensitive to one colour reaction.

Thermal degradation of oils leads to thermal dimeric triglycerides and higher polymers. Oxidative degradation may produce oxidized triglycerides containing polar functional groups and dimeric triglycerides linked by one or more oxygen atoms. Hydrolytic processes will lead to diglycerides and free fatty acids. Depending on fat composition, type of food, frying temperature, agitation of the oil, presence or absence of antioxidants, oil surface area and access of air, cooling periods between usage, and the presence or absence of a layer of water vapor at the oil–air interface, the relative concentration of a particular class of artefacts may vary considerably[3].

A new approach was the introduction of gel permeation chromatography (GPC) for the analysis of used frying oils[6–10]. The importance of this technique lies in the fact that a peak at molecular weight 1800, which is not present in fresh fats, increases considerably during the frying procedure and constitutes a measure of oil deterioration. This peak represents the sum of both non-polar (thermal) and polar (oxidized) dimeric triglycerides.

In a similar approach, a method was developed for the simultaneous elution of polar compounds by liquid chromatography (LC) using an ethanol front[11]. The resolution of oxidized oils into several peaks by LC on silica gel columns has also been described in two earlier papers[10,12].

Both of the two types of bulk frying oil investigation described, GPC[6–9] and frontal elution LC[11], have the shortcoming that they include several, but not all, of the important classes of degradation products. GPC does not determine oxidized triglycerides, and the type of LC described[11] does not include non-polar dimeric triglycerides and products of low polarity in the polar peak P. We wanted to develop a method that would include this class of products in a similar frontal elution peak. This would then constitute a "total artefacts" peak that combines all of the major classes of degradation products.

A composite peak can be quantitated only by a detector that has a similar response for all components of the peak. The Pye detector comes close to this requirement, while UV and refractive index detectors are too specific and may indicate only one or a few components.

Non-polar dimeric triglycerides can be separated from the normal monomeric triglycerides by adsorption chromatography[13] on dry, active silica gel, *e.g.*, in mixtures of light petroleum and diethyl ether[14]. It is difficult to separate the two classes of

compounds if the silica gel is deactivated by small amounts of water or ethanol.

In instrumental LC, polar compounds should be displaced by a polar solvent, and the column must then be reconditioned by a solvent of low polarity. It is well known that deactivated columns may become more active during chromatography with dry solvents[15]. The degree to which adsorption and/or partition takes place will be governed by the nature of the polar displacing solvent and the efficiency of the reconditioning procedure.

By using this effect, it should be possible to produce a "polar materials peak" and a "total artefacts peak" with comparatively simple solvent programs. It should also be possible to achieve both ends with exactly the same solvent program simply by changing the column reconditioning procedure.

Experimental and results

The LC equipment was of the type previously described[11,12,16]. The Ultrograd program is shown in Fig. 1. A program time of 15 min was chosen, and a recorder chart speed of 2.5 mm/min and an attenuation of 5 × 10 were used throughout.

The solvents, which were of reagent grade (E. Merck, G.F.R.) and used without further purification or drying, were mixtures of heptane (H), diisopropyl ether (D), ethanol (E) and water (W). The nitrogen pressure on the solvent reservoirs was 1.0 atm. The columns were made of ordinary glass tubing, 20 cm × 4 mm I.D., and were filled with Merckogel SI 50, 36–75 μm grain size. No water-jacket or cooling was used.

Test samples of dimeric triolein were prepared and purified by gel permeation as described[13]. Monoepoxytriolein was prepared by epoxidation of triolein with H_2O_2–acetic acid[17], followed by preparative adsorption chromatography on silica gel. Fats A–C were used frying oils and D was a refined soyabean oil. Samples were injected directly into the column through a Pye injection head[16].

Column re-equilibration procedures other than those already incorporated in the program sheet of Fig. 1 were as described in the captions to the other figures.

Thin-layer chromatography (TLC) was carried out on ready-made silica gel TLC plates (Fertigplatten Merck; E. Merck, G.F.R.). The plates were heated for

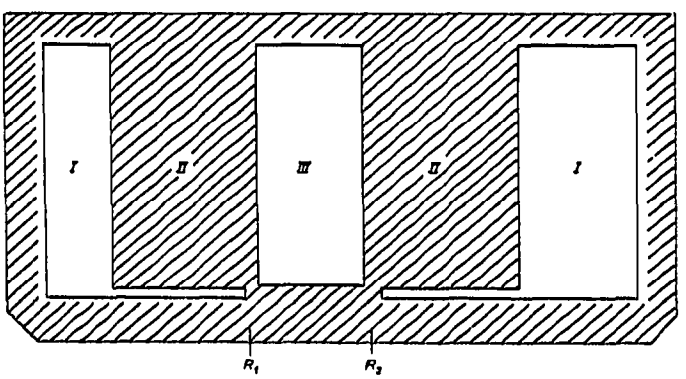

Fig. 1. Ultrograd valve-switching program. Solvents and corresponding column rinsing periods as described in the legends of Figs. 2 and 3.

30 min at 120°. TLC was carried out in light petroleum (boiling range 40–60°)–diethyl ether (80:20). The spots were made visible by spraying with 10% sulfuric acid plus charring. The LC fractions for the TLC investigations were obtained with the aid of automatic LC peak-trapping equipment[16].

Fig. 2 shows that under these conditions only the very polar components are separated from the main peak. Squalane standard (S) appears at 2 mm, normal triglycerides (T) at 8.5 mm and very polar components (P) at 18.5 mm from the point of injection on the chart. Both non-polar dimeric triolein and monoepoxytriolein appear at 8.5 mm, and would run together with the main peak of normal triglycerides in a frying oil. A polar impurity at 18.5 mm was present in the monoepoxytriolein preparation and was found to migrate in the area of dihydroxy compounds on TLC. The frying oils tested contained various amounts of polar peak P at 18.5 mm.

In Fig. 3, the same program as in Fig. 1 was used with a less polar solvent mixture in II. The column reconditioning procedure was also changed. Squalane standard again appears at 2 mm, while normal triglycerides are now recorded at 11 mm and polar materials at 17.5 mm from the injection point. All three peaks are present in the frying fat samples.

The chromatograms show that dimeric triolein now appears at 17.5 mm; thus under the conditions used, non-polar dimeric triglycerides are eluted together with the polar artefacts by the ethanol front. The same is true for monoepoxytriolein, which, together with the polar impurity from which it is not resolved, is also eluted at 17.5 mm with the polar materials peak.

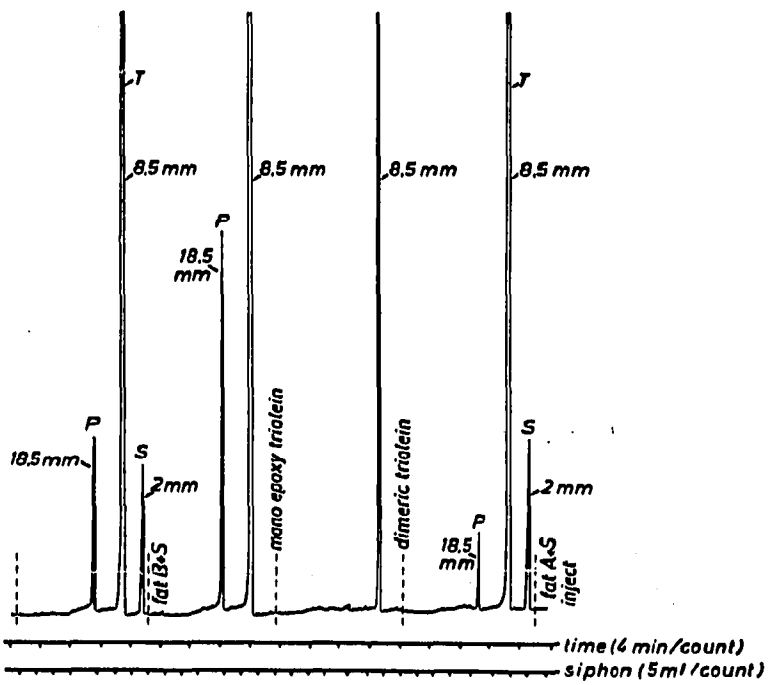

Fig. 2. Frontal elution chromatograms of very polar materials. Program of Fig. 1 with I = H, II = H–D (50:50), III = H–D–E–W (20:30:50:1). Additional column rinsing for 4 min with solvent I. S = squalane standard; T = triglycerides; P = very polar materials.

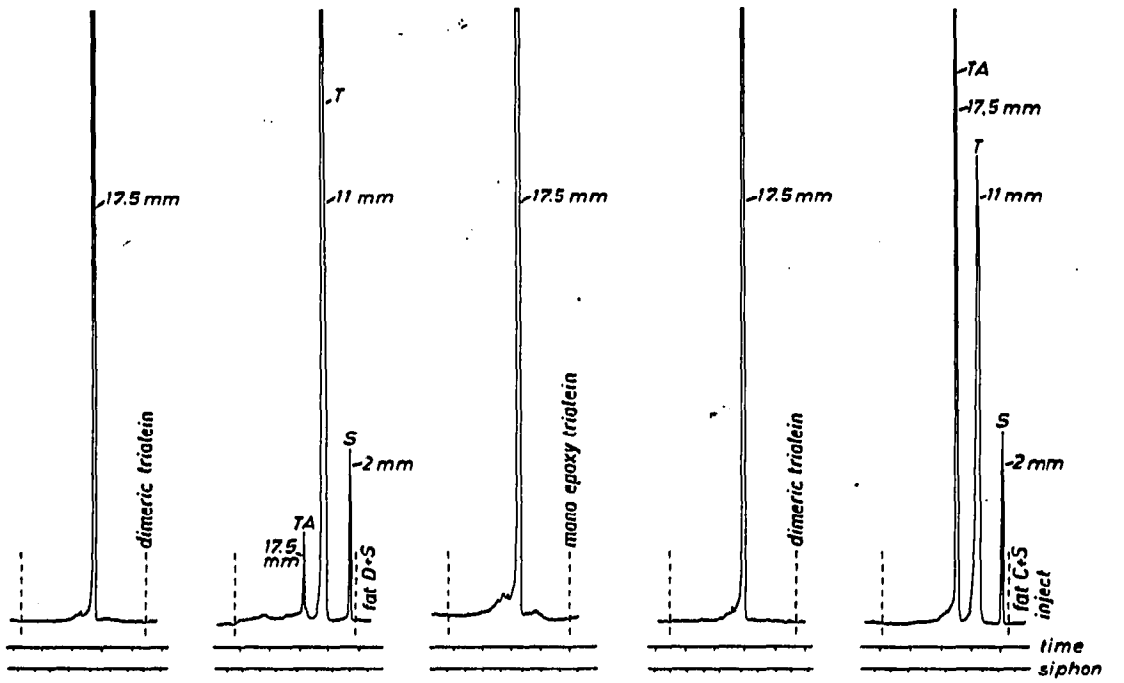

Fig. 3. "Total artefacts" chromatograms of frying fats and test substances. Program of Fig. 1 with I = H, II = H–D (80:20), III = H–D–E–W (30:40:30:1). Column re-equilibration for 12 min with solvent II, then 12 min with solvent I. TA = "total artefacts".

The elution of non-polar, dimeric triolein at 17.5 mm is reproducible. It can be assumed that any non-polar dimeric triglyceride artefacts, if present, have been eluted and recorded together with the polar artefacts of frying fat C in Fig. 3. To check this assumption, and to test the original assumption that both types of frontal elution chromatograms can be produced simply by changing the column rinsing procedure, the peak-trapping experiment of Fig. 4 was carried out. The same frying oil was injected twice using the same Ultrograd program (Fig. 1), the same solvents and the same column, but with different column reconditioning conditions. The solvents used for LC were the same as for Fig. 3. The triglyceride peaks, T_1 and T_2, and the polar materials and total artefacts peaks, P and TA, were trapped automatically by using the equipment described earlier[16] and spotted on thin-layer chromatograms together with the starting material, a fat which had been used for fish frying. After the preceding Ultrograd program, column reconditioning for a period of 12 min with solvent II was carried out before the usual column rinsing for 12 min with solvent I. The sample was injected, and the 15-min program was followed as usual with solvents I, II and III. The reconditioning with solvent II was then omitted and the column was rinsed for 12 min with solvent I only. The sample was injected and the same program carried out again.

Of the recorded peaks, T_1 is smaller than T_2, while TA is larger than P. The thin-layer chromatograms of the trapped peaks show that in the first run, T_1 consisted of pure triglyceride, while the spot corresponding to non-polar dimeric tri-

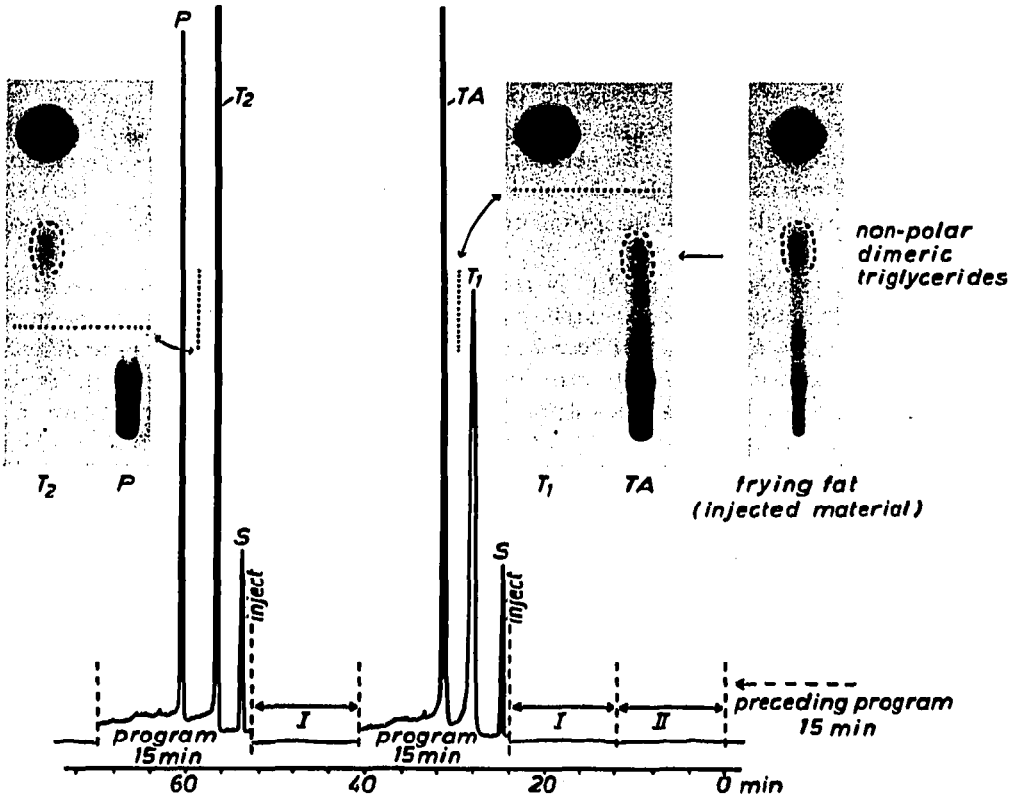

Fig. 4. Peak-trapping experiment. A comparison of "polar materials" and "total artefacts" chromatograms as obtained from the same frying oil, same column, solvents and program. Program of Fig. 1, solvents as for Fig. 3. The only difference between the two chromatograms shown is in the column re-equilibration period. TLC spots above the dotted line were eluted in the peaks T_1 and T_2, while spots below the dotted line fell into the peaks TA and P, respectively. The wire movement of the Pye LC detector was switched off during rinsing periods, so the baseline appears to be slightly lowered.

glyceride was found in TA. In the second run, non-polar dimeric triglyceride was found in T_2, so that this peak is no longer a "pure triglyceride" peak but also contains the first (*i.e.*, the least polar) artefact. Peak P contains only the more polar artefacts, as was the case in the procedures used for the determination of "total more polar products"[11].

Discussion

The production of a "total artefacts peak" from frying oils seems to be feasible according to the chromatograms shown in Fig. 3 and the right-hand side of Fig. 4. This is possible by the appropriate choice of column equilibration procedures, which permit the least polar major class of artefacts, non-polar dimeric triglycerides, to move further away from the normal triglycerides and into the polar peak eluted by the ethanol front. As all the major artefacts are now combined in one peak, this peak should be a still more sensitive indicator of general oil degradation.

While the relative elution positions of peaks S, T and P or TA do not change

very much when the same solvent program is operated with a more active or a less active column, the elution of non-polar dimeric triglycerides does change considerably. The rinsing period with solvent II, *i.e.*, 20% diisopropyl ether in heptane, will remove more efficiently some of the ethanol and water deactivating the column at the end of the preceding solvent program. As a result of this, the column may become more active in the sense that more adsorption and less partition will take place. Without rinsing with solvent II, the separations (Fig. 2 and the left-hand side of Fig. 4) are likely to be governed entirely by liquid–liquid partition chromatography.

One of the main advantages of LC is that columns do not have to be refilled and refitted for every determination, as with conventional silica gel column chromatography by gravity. The same column has been used for over 100 LC separations.

Most publications on LC today emphasize its ability to separate pairs or classes of compounds or isomers that are difficult to separate. For the more academic question of the separation of isomers, universality of detection and gradient elution are not necessary requirements. UV detectors will normally detect both isomers, and both isomers will normally be eluted from a column by the same solvent mixture.

In the author's opinion, however, a major commercial breakthrough of LC will come only when it is possible to inject complex samples, such as drinks, oils or food extracts, and to obtain rapid results on simple separations or group separations without too much pretreatment of the sample. To achieve this aim, it seems necessary to combine a universal detector with gradient elution. Most of the commercial LC instruments available today do not fullfil these requirements.

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