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**Note**

# Separation of low-polarity lipid oxidation products by a combination of gel permeation chromatography and liquid column chromatography

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Lipid oxidation products can be separated by various chromatographic techniques, column liquid chromatography[1,2] being the most suitable method in many instances. Analytical methods are usually based on separations according to different polarities of fractions. If fats and oils are oxidized at high temperatures and with limited access of oxygen, *e.g.*, under conditions of deep fat frying, relatively non-polar oxidation and polymerization products predominate[3,4]. Peaks of various monomers then overlap in the chromatogram with those of polymers, so that a satisfactory separation is not achieved.

In contrast, oxidized lipids are separated by gel chromatography into fractions of similar molecular sizes[5,6] (*e.g.*, into monomeric, dimeric and higher oligomeric fractions) and an inadequate resolution into subfractions of different polarities is obtained.

Therefore, a combination of silica gel column chromatography and gel permeation chromatography was tested for the separation of mixtures containing low-polarity lipid oxidation products.

## EXPERIMENTAL

### *Materials*

Standards were prepared according to the literature[7–10]. Model reaction mixtures were prepared by heating 90% of component A (butyl palmitate, butyl oleate, palmitic acid, oleic acid or butyl 9,10-dihydroxystearate) with 10% of component B (butyl 9,10-epoxystearate or butyl hydroperoxyoleate) in a sealed test-tube under nitrogen at 150° (with butyl hydroperoxyoleate) or 180° (with butyl 9,10-epoxystearate) for 2 h.

TABLE I

CONDITIONS FOR SEPARATION OF A HEATED MIXTURE OF LIPIDS ON SILICA GEL COLUMNS

Conditions: silica gel for column chromatography (Merck), 70–325 mesh ASTM (particle size 0.05–0.20 mm); infrared detection (gravimetric for hydrocarbons)

| *Condition* | *After pre-fractionation* | |
|---|---|---|
| | *On Sephadex LH-20 with chloroform* | *On silica gel with n-heptane–diethyl ether (95:5)* |
| Column | 300 × 10 mm | 300 × 30 mm |
| Sample size | 450 mg | 2 g |
| Flow-rate | 0.5 ml/min | 2 ml/min |
| Elution system: | | |
| (a) *n*-Heptane–diethyl ether (95:5) | 150 ml | 250 ml |
| (b) *n*-Heptane–diethyl ether (50:50) | 110 ml | 250 ml |
| (c) Diethyl ether | 60 ml | 200 ml |
| (d) Methanol | 100 ml | 200 ml |

*Procedures*

Pre-fractionation of the heated mixtures was carried out on a 300 × 30 mm column of Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Samples of 1–3 g were separated with chloroform (flow-rate 2 ml/min), 3-ml fractions were collected and the amount of eluted esters was determined by infrared spectrometry of the effluent at the maximum absorbance of the carbonyl peak.

Column liquid chromatography was carried out with silica gel for column chromatography (E. Merck, Darmstadt, G.F.R.), 70–325 mesh ASTM, containing 5% of water, using infrared detection at the absorption maximum of the carbonyl peak (with *n*-heptane and diethyl ether as solvent) or weighing (with methanol as solvent or with the first, hydrocarbon fraction). The elution conditions are given in Table I.

The gel permeation chromatographic separation was carried out under essentially the same conditions as reported previously[6]. Elution volumes of some monomeric model substances are given in Table II.

TABLE II

RELATIVE ELUTION VOLUMES OF MODEL SUBSTANCES ON GEL PERMEATION CHROMATOGRAPHY

| *Compound* | *Relative elution volume* | |
|---|---|---|
| | *S-GEL-832 (conditions as in Fig. 3)* | *Sephadex LH-20 (conditions as in Fig. 1)* |
| Butyl palmitate | 1.000 | 1.000 |
| Butyl oleate | 0.990 | 0.981 |
| Palmitic acid | 0.982 | 1.691 |
| Oleic acid | 0.971 | 1.430 |
| Butyl hydroperoxyoleate | 0.985 | — |
| Butyl 9,10-epoxystearate | 1.004 | — |
| Butyl 9,10-dihydroxystearate | 0.929 | 1.168 |
| Butyl 9,10-diacetoxystearate | 0.933 | — |

Thin-layer chromatography was used to test the purity of fractions; only pure fractions were combined for subfractionation. Pre-coated Silufol UV-254 plates (Glass Works Kavalier, Votice, Czechoslovakia), 150 × 150 mm, activated at 105° for 30 min, were used. The running distance was 120 mm, the solvent system was *n*-heptane–diethyl ether (60:40) and detection was effected with a 5% methanolic solution of molybdophosphoric acid.

## RESULTS AND DISCUSSION

The separation consisted of the following steps: (a) pre-fractionation by gel permeation chromatography on Sephadex LH-20 in order to remove the bulk of unreacted substances, if only the oligomeric fraction was to be studied, or pre-fractionation on silica gel if all substances more polar than the original lipids were to be investigated; (b) fractionation on a column of silica gel according to polarity, followed by a check of purity by thin-layer chromatography (only pure fractions being combined for further subfractionation); (c) subfractionation on an S-GEL-832* (cross-linked polystyrene–divinylbenzene gel) column according to molecular size.

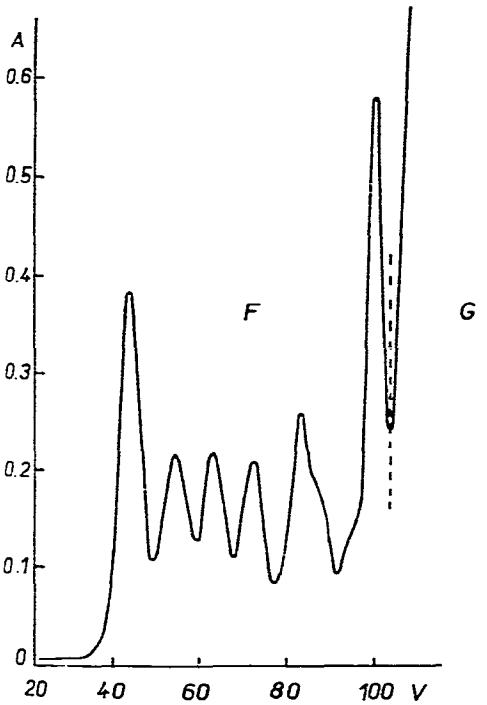


Fig. 1. Pre-fractionation of the reaction mixture of butyl palmitate and butyl 9,10-epoxystearate on Sephadex LH-20. Conditions: column, 300 × 30 mm; solvent, chloroform; flow-rate, 2 ml/min; infrared detection at the absorption maximum of the carbonyl peak; 3-ml fractions. A = Absorbance (in 0.5-mm cells); V = elution volume (ml); F = oligomeric fractions; G = monomeric and low-molecular-weight fractions.

* Developed at the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia.

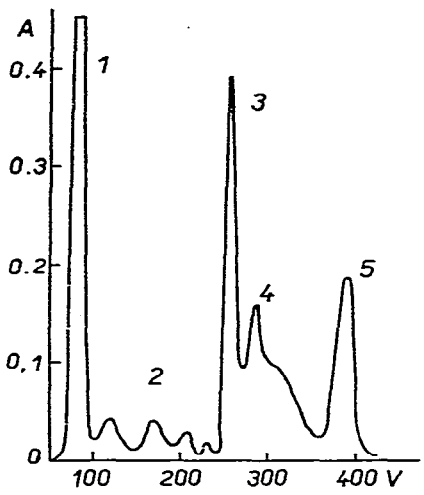


Fig. 2. Separation of oxidized lipids on silica gel column. Conditions as in Table I; sample, combined fractions F (see Fig. 1); sample size, 450 mg. A = Absorbance at the absorption maximum of the carbonyl peak (0.5-mm cells); V = elution volume (ml).

Chromatographically pure oligomeric compounds were obtained in the above way from all heated mixtures.

The separation of products prepared by heating a mixture of butyl palmitate and butyl 9,10-epoxystearate (90:10, w/w) at 180° for 2 h is shown as an example (Fig. 1). Most unreacted butyl palmitate was removed by gel chromatographic prefractionation on the Sephadex LH-20 column; all small peaks preceding the main peak were collected as one broad fraction.

The broad fraction was fractionated on the silica gel column and five fractions were collected (Fig. 2). These fractions consisted mainly of one component, as shown by thin-layer chromatography.

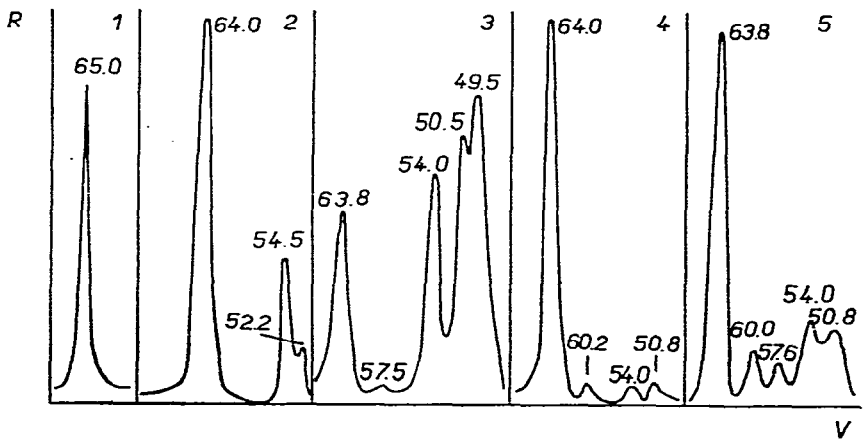


Fig. 3. Subfractionation of oxidized lipids by gel permeation chromatography. Conditions: column, 6 m × 8 mm; gel, S-GEL-832; solvent, tetrahydrofuran; flow-rate, 35 ml/h; Waters R-4 differential refractometer. R = Detector response; V = elution volume (counts, indicated in the peaks); sample, fractions from silica gel chromatography (see Fig. 2, fractions Nos. 1–5).

Each fraction obtained by silica gel column chromatography was subfractionated on an S-GEL-832 column (Fig. 3); one to five subfractions were found in the fractions.

A total of 18 chromatographically pure subfraction was obtained by using a combination of silica gel liquid and gel permeation chromatography.

A combination of chromatographic techniques, consisting of pre-fractionation by column chromatography on Sephadex LH-20 or silica gel, followed by fractionation on silica gel and subfractionation on cross-linked polystyrene–divinylbenzene S-GEL-832, was therefore useful for separating low-polarity lipid oxidation products such as frying fats. The amounts of the fractions obtained are sufficient for further characterization by IR spectroscopy or other methods. Acylglycerols are converted into butyl esters before the fractionation.

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