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Analysis of inhibitors in edible fats and oils by gel chromatography

The determination of inhibitors present in edible oils and fats consists, as a rule, of a selective extraction of the sample with aqueous alcohol, fractionation of the extracted mixture of compounds into their components, followed by the determination of the content of the individual compounds. Chromatographic procedures are most frequently used for the fractionation of the mixture. Formerly, paper chromatography was the method most in use; it is still recommended[1], mainly for the detection of inhibitors. At present, the commonest method is thin-layer chromatography (TLC) on silica gel[3–5], impregnated silica gel[6–7], polyamide[8–10] or acetylated cellulose[11]. Gas chromatography (GC)[12,13] or GC combined with TLC[14] is employed for the determination of inhibitors to a lesser extent. Fractionation on a column of aluminium oxide has also been described[15].

In our previous work, gel chromatography was used for fractionation and qualitative and quantitative analysis of the stabilizing systems for polyolefins[16]. This method can also be applied advantageously in the analysis of inhibitors in edible oils and fats.

Experimental

Materials. The antioxidants were purified by column adsorption chromatography. Their purity was checked by TLC on silica gel[2]. No impurities were revealed, even if gel chromatography was used. The stabilized substrates were beef tallow, pork fat obtained by the Titan method and freshly refined sunflower seed oil.

Extraction of inhibitors. A 25% solution of a stabilized substrate in heptane was extracted three times, each time with the same volume of 80% ethyl alcohol. The solvents and water were evaporated from the extract *in vacuo* at a bath temperature not exceeding 40°. If necessary, free fatty acids were removed from the extract with a solution of sodium carbonate.

Gel chromatography. The non-volatile extract residue, after evaporation of the solvent, was diluted with tetrahydrofuran, which was used as an elution agent for gel permeation chromatography (GPC), to yield an approximately 2% solution. A 0.5 ml quantity of this solution was injected into the gel chromatograph (Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences) with an accuracy of $\pm$0.05 ml. The analysis was performed in five columns connected in series (8 $\times$ 1200 mm) on the S-832 gel (Institute of Macromolecular Chemistry) at room temperature. The flow-rate of the elution agent was 35 ml/h. Detection was performed by means of a series-connected Waters Model R-4 differential flow refractometer and a flow UV analyzer (Development Works, Czechoslovak Academy of Sciences, Prague) with monochromatic light, wavelength 254 nm. One count corresponded to an eluate volume of 2.7 ml.

Results and discussion

Gel chromatography is a suitable method for an efficient fractionation and determination of the common antioxidants (Table I).

TABLE I

ELUTION CHARACTERISTICS OF THE ANTIOXIDANTS

V^{+}_{0} = 47.0 counts.

Antioxidant	V_e	*Relative velocity of the zone* $\frac{V^{+}_{0}}{V_e}$
D- + L- Ascorbyl palmitate	58.9	0.798
Dodecyl gallate	61.2	0.768
Nordihydroguaiaretic acid (NDGA)	65.1	0.722
Propyl gallate	69.3	0.678
2,6-Di-*tert.*-butyl-4-methylphenol (BHT)	74.2	0.633
2-*tert.*-Butyl-4-methoxyphenol (BHA)	76.7	0.613

After quantitative calibration of the differential refractometer and the UV detector with pure inhibitors, the content of the individual components can be determined directly. It follows from the chromatograms of the extracts of unstabilized fats and oils (Fig. 1) that only two fractions of the natural fat components, extractable with ethanol, can interfere in the determination of antioxidants. The elution volume of monoglycerides and tocopherols is close to the elution volume of dodecyl galate,

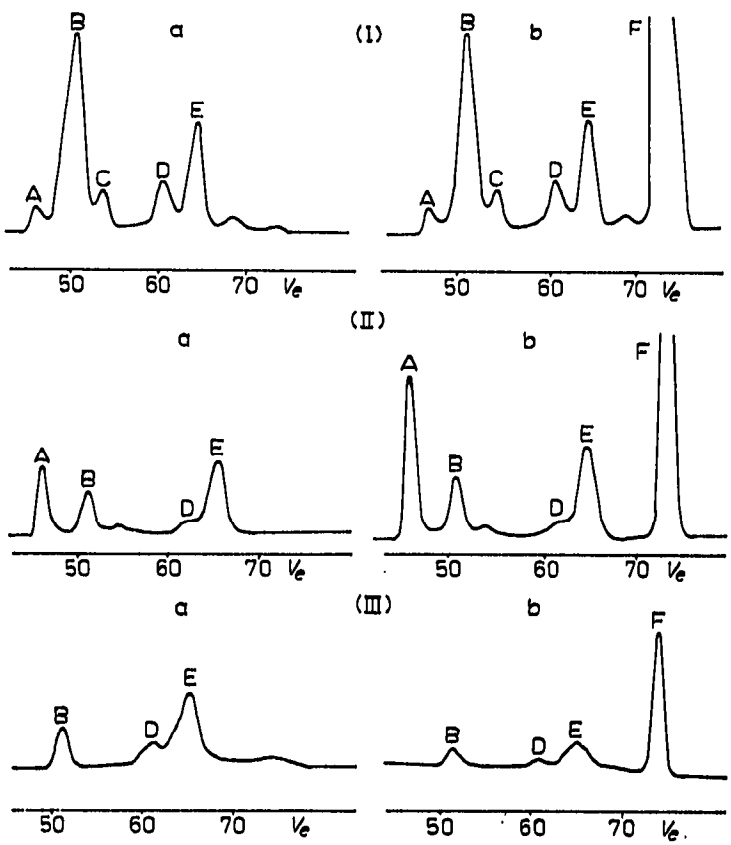

Fig. 1. Gel chromatograms of aqueous ethanol extracts of oils and fats. (I) Sunflower seed oil: (a) unstabilized and (b) stabilized with BHT. (II) Pork fat: (a) unstabilized and (b) stabilized with BHT. (III) Beef tallow: (a) unstabilized and (b) stabilized with BHT. Meaning of chromatographic peaks: A, unidentified high-molecular-weight compounds; B, triglycerides; C, unidentified compound; D, tocopherols + monoglycerides; E, fatty acids + sterols; F, antioxidant, BHT.

so that their signals on the chromatogram interefere with the differential refractometric detection. Monoglycerides do not interfere if the UV detector is used, since they do not absorb in the region above 220 nm. If, however, tocopherols are present, the presence of dodecyl gallate has to be confirmed by some of the selective colour reactions, *e.g.* with ferric tartrate[17] or with alkalies[18,19]. These reactions can also be used for a quantitative determination of the above antioxidant. The elution volume of the nordihydroguaiaretic acid is close to the elution volume of higher fatty acids and sterols extracted from the sample. Free fatty acids can be removed from the extract by treating with sodium or potassium carbonate. The fact that the UV absorption of free fatty acids is negligible compared to the absorption of the nordihydroguaiaretic acid can also be made use of. We followed the response of the UV flow detector to the

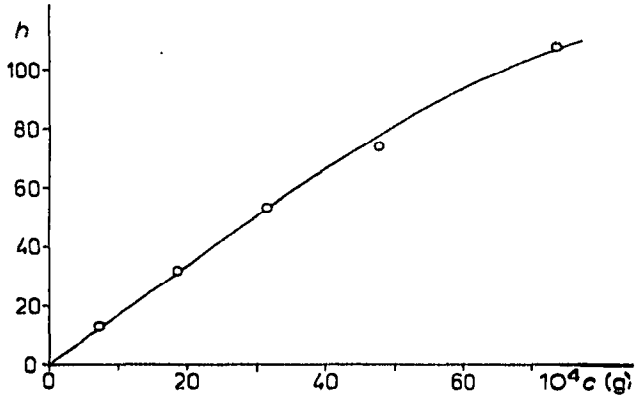

Fig. 2. Calibration dependence of the response of the UV detector on the injected amount of NDGA in the presence of lauric acid. *h*, peak height in mm.

amount of NDGA in a mixture with lauric acid, which was used as a model fatty acid whose elution volume was close to that of the antioxidant under investigation. The presence of the acid did not interfere in this case and the calibration curve (Fig. 2) can therefore be used to read off the NDGA content also in a mixture with free fatty acids. A similar procedure allows antioxidant to be determined in the presence of sterols.

Compared with the labour-consuming methods based on the fractionation of the individual components of the mixture followed by their determination, the gel chromatography of inhibitors in edible oils and fats is a method which is both fast and relatively exact.

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