Rapid detection of gallic acid esters by centrifugal chromatography

Gallic acid esters are used in the food industry as antioxidants for oils and fats. Some of them are even among the antioxidants most often used (*e.g.* propyl gallate, being intermediate between the natural antioxidants (α-tocopherol, flavonoids, etc.) and the synthetic ones, not occurring in nature (BHA, BHT*). The amount of antioxidant that is permitted as additive to fats is very low, and usually does not exceed 0.01–0.05 % of fat weight; hence accurate identification and determination of these substances is often difficult, especially where various mixtures of antioxidants have been used for fat stabilisation.

In all the methods so far described, antioxidants are isolated from fats by extraction, usually with alcohol. Their detection is effected either by chromatographic separation on paper[1–4] (acetylated paper, or with a stationary less polar phase), or by separation on chromatoplates[5–8] (silica gel or polyamide powder). The latter method is preferable, mainly on account of its greater rapidity.

Centrifugal chromatography has recently been found increasingly useful in the detection of various groups of substances; it has the advantage of shortening the time taken for chromatographic separation. Centrifugal chromatography can be carried out in the simple apparatus described by DEYL, PAVLÍČEK AND ROSMUS[9]. In the present work the method was used for rapid separation and detection of gallic acid esters.

Experimental

Apparatus. Centrifugal chromatography was carried out in a simplified version of the rotary chromatographic apparatus described by PAVLÍČEK AND DEYL (number of revolutions 700/min). Whatman No. 3 paper was used for the chromatographic separation; the diameter of the chromatographic paper disc was 18 cm.

Solvent systems. Methanol–benzene in various ratios; methanol–carbon tetrachloride in various ratios; water–ethyl acetate (97.5:2.5); ethyl acetate; ethyl acetate saturated with water; methyl acetate; butyl acetate; and the classical mixture: *n*-butanol–acetic acid–water (4:1:5).

Procedure. About 0.05 ml of methanolic solution of the respective gallate is spotted on the start at a distance of about 1.5 cm from the centre of rotation. After drying, the chromatographic paper is stretched on and fixed in the rotary chromatographic apparatus, which after having been perfectly sealed is put into operation. The inflow of the solvent introduced through a special capillary tube, is adjusted in such a way as to prevent any possible irregularity of the flow during the chromatographic process. When the solvent system has advanced about 14 cm, the operation is interrupted, the chromatogram removed, dried and the substances detected with an alcoholic 1 % ammoniacal 1 % silver nitrate solution.

Results

Various mixtures of gallates were separated by centrifugal chromatography, using the above-mentioned solvent systems. The results are given in Table I. The most suitable solvent system proved to be a mixture of carbon tetrachloride and methanol in the

* BHA = 2(and 3)-*tert.*-butyl-4-methoxyphenol; BHT = 2,6-di-*tert.*-butyl-*p*-cresol.

TABLE I

R_F VALUES OF GALLIC ACID AND ITS ESTERS

Solvent system	R_F					
	GA	MG	EG	PG	OG	LG
Ethyl acetate	0.53	0.81	0.88	0.94	0.97	0.98
Ethyl acetate satd. with water	0.50	0.89	0.92	0.93	0.94	0.95
Methanol–benzene (1:9)	0.0	0.30	0.38	0.58	0.88	0.98
Methanol–benzene (1:4)	0.06	0.35	0.46	0.58	0.95	0.98
n-Butanol–acetic acid–water (4:1:5)	0.78	0.88	0.91	0.93	0.97	0.97
Carbon tetrachloride–methanol (4:1)	0.08	0.33	0.45	0.50	0.86	0.95
Methanol–benzene (1:19)	0.00	0.07	0.12	0.29	0.62	0.78
Methanol–benzene–amyl alcohol–water (2:1:1:1)	0.67	0.92	0.97	0.97	0.98	0.98

GA = gallic acid; MG = methyl gallate; EG = ethyl gallate; PG = propyl gallate; OG = octyl gallate; LG = lauryl gallate.

ratio 4:1. The advantage of centrifugal chromatography lies especially in its rapidity; in addition, the separation of some substances with close R_F values is better than with the usual arrangement. Thus, a good separation of gallic acid esters in the solvent system carbon tetrachloride–methanol (4:1) takes 5–6 hours without centrifugal force; in the rotary arrangement the same substances are separated within 30–45 minutes. Whatman No. 3 chromatographic paper proved best for the purpose; the capacity of the method is also fairly high.

We were successful in separating even highly complex mixtures of gallic acid esters. The method was verified in the separation of gallic acid esters isolated from stabilized fats. For this purpose about 100 g of fat was weighed out and mixed with 100 ml petroleum ether. This solution was then extracted 3 times with 50 ml 60% ethanol each time; the combined alcoholic extracts were evaporated on a water bath, and the dry residue dissolved in 1 ml methanol. This solution was then spotted on the chromatographic paper. We have observed that the R_F values of substances isolated from fats are lower than those of the corresponding pure gallates, owing to the presence of residues of fat, fatty acids and other lipoidic substances. Their presence, however, has no substantial influence on the quality and accuracy of the separation.

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