Thin-layer chromatography of anthocyanins from blackcurrant juice

In recent years a number of workers have studied the separation of anthocyanins by thin-layer chromatography (TLC) using a variety of adsorbents. Thus NYBOM[1] used cellulose powder, BIRKHOFER *et al.*[2] polyacrilonitrile–polyamide, and TANNER *et al.*[3] silica gel. ASEN described the use of layers composed of cellulose and silica gel mixtures[4], and more recently BIRKHOFER *et al.* have used alumina[5].

In the present communication, a simple method is described for the rapid resolution and characterisation of the anthocyanins of blackcurrant juice. Kieselgel G (E. Merck, Darmstadt) was found to be a suitable adsorbent, and separations of the glycosides of cyanidin and delphinidin were obtained within one hour. The aglycone cyanidin was also resolved satisfactorily.

Experimental

Kieselgel G (110 g) was agitated with 500 ml acidified methanol (CH_3OH–0.5 *N* HCl, 80:20, v/v) to remove metallic ions, filtered, washed with 400 ml distilled water and dried in a shallow dish for 8 h at 80°. The material was sieved to pass 100 mesh and stored in a glass container. The quantity of Kieselgel G produced was sufficient to coat twenty 20 × 20-cm TLC plates. Five plates (20 × 20 cm) were coated with a 250 μ layer prepared from a slurry of 25 g adsorbent and 56 ml distilled water. The plates were dried for 25 min at 110° and cooled in a desiccator. Samples were always applied to freshly prepared plates. The solvent system ethyl acetate–ethyl methyl ketone–formic acid–water (6:3:1:1, v/v) was used, and the development tank was allowed to equilibrate overnight before use. Several variations[6] of this solvent system were investigated, but were found to be less satisfactory. Plates were run three times in the same direction with intermediate drying. The solvent was allowed to ascend for 5 cm on the first development and for 10 cm on the last two runs. The shortened first development prevented diffusion of the spots.

Extracts of blackcurrant juice in *n*-butanol were separated on cellulose columns by the procedure of CHANDLER AND HARPER[7]. Several bands were eluted and concentrated by rotary evaporation *in vacuo*. The fractions containing the glycosides of cyanidin and delphinidin were subjected to preparative TLC. Concentrated fractions containing 240 μg were applied in 30 μl of solvent as narrow bands to the base line of the plate. After development and subsequent evaporation of the solvents, the separated bands were scraped off into filter funnels packed with prewashed pledgets of glass wool, and the glycosides were then eluted with acidified methanol. The eluates were clarified by centrifugation, the absorbances read at 525 mμ, and the concentration of the anthocyanins determined by the technique of SWAIN AND HILLIS[8]. The recovery of anthocyanins was calculated by reference to standard curves prepared from pure cyanidin and delphinidin glycosides.

Results and discussion

Recoveries of 98 ± 2 % of cyanidin glycosides, and 94 ± 4 % of delphinidin glycosides were obtained in four determinations of each glycoside. Up to 600 μg glycosides could be separated when applied to the plate as a thin streak, and not more than 80–90 μg could be resolved satisfactorily when applied as a single spot. The separation achieved with a range of concentrations of cyanidin-3-rhamnoglucoside

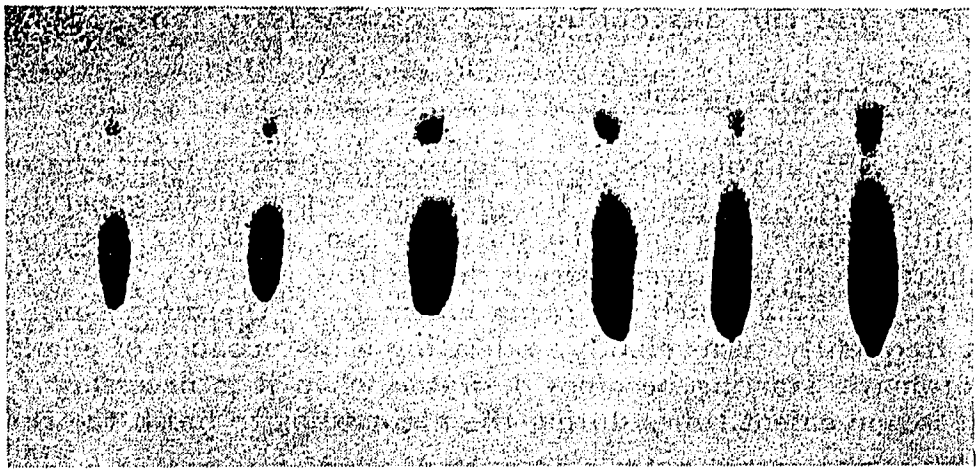

Fig. 1. Separation of cyanidin-3-rhamnoglucoside (lower) from cyanidin-3-glucoside (upper) on TLC plates coated with a 250 μ layer of Kieselgel G. Developing solvent: ethyl acetate–ethyl methyl ketone–formic acid–water (6:3:1:1, v/v). First development for 5 cm, and redeveloped twice for 10 cm. Concentrations (left to right) were 5, 10, 30, 50, 90 μg mixed glycosides.

contaminated with cyanidin-3-glucoside from a fraction of blackcurrant juice is shown in Fig. 1. The cyanidin glycosides migrated in front of the delphinidin glycosides, and in each case the glucosides had higher R_F values than the corresponding rhamnoglucosides. No hydrolysis was observed when the eluted glycosides were centrifuged and concentrated under a gentle stream of nitrogen, and the technique could be readily used to prepare small amounts of pure anthocyanins. TLC of partially hydrolysed cyanidin glycosides[7] showed that the aglycone cyanidin was well resolved from the unhydrolysed material, and moved close to the solvent front. The aglycone faded about 5 min after evaporation of the solvents, but with rapid handling the aglycone could be recovered by elution with a suitable acidic solvent[9]. By contrast delphinidin was not well resolved from the glycosides, and it faded too rapidly for satisfactory recovery.

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Department of Food Science, University of Strathclyde,
1, Horselethill Road, Glasgow, W.2 (Great Britain) A. D. MORTON

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