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A rapid thin-layer chromatographic method for the anthocyanins in grape varieties

In the course of our studies on the distribution of anthocyanins in various grape varieties, it was necessary to obtain a quick method of separation for the various pigments involved. Although paper chromatography seems to be preferred by most workers in this field, it is a cumbersome and time-consuming method. In view of the success achieved recently by BIRKOFER *et al.*[1], HESS AND MEYER[2], TANNER *et al.*[3], NYBOM[4], ASEN[5] and MORTON[6] whereby anthocyanins were separated using thin-layer chromatography (TLC), we decided to use this technique.

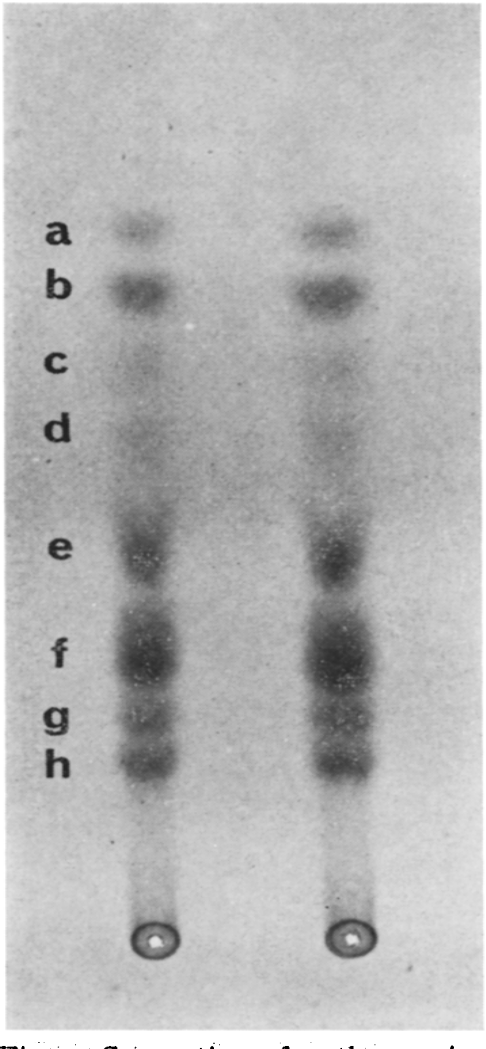

Fig. 1. Separation of anthocyanins from skins of *Vitis vinifera* cultivar Barlinka on TLC plates coated with a 0.3 mm layer of silica gel (Camag D-5). Developing solvent: 1-butanol–ethyl acetate–benzene–formic acid (1:1:1:1, v/v) saturated with solid paraformaldehyde. Concentrations: 1 μl (left) and 2 μl (right) of methanolic anthocyanin extract. The spots are identified as: (a) = Mixture of peonidine-3-monoglucoside acylated with *p*-coumaric acid (pca) and caffeic acid (ca); (b) = mixture of malvidine-3-monoglucoside acylated with pca and ca; (c) = mixture of petunidine-3-monoglucoside acylated with pca and ca); (d) = mixture of delfinidine-3-monoglucoside acylated with pca and ca; (e) peonidine-3-monoglucoside; (f) = malvidine-3-monoglucoside; (g) petunidine-3-monoglucoside; (h) = delfinidine-3-monoglucoside.

TLC was carried out on 10 × 20 cm chromatoplates with 0.3 mm thickness layers of silica gel. To prepare eight chromatoplates, 20 g of silica gel (Camag D-5 for partition chromatography) and 0.5 g of sodium acetate was mixed with 50 ml of distilled water and shaken vigorously for about 2 min. The required thickness layer was obtained with a Camag applicator. Smoothness and uniform thickness of the layer was found to be of importance for satisfactory and reproducible separations. The chromatoplates were dried in the air at room temperature for at least eight hours (preferably overnight). Prior to use the plates were heated for 15 min at 120°. Application of the sample and development followed immediately after cooling of the plates. Chromatograms were developed in a mixture of 1-butanol–ethyl acetate–benzene–formic acid (1:1:1:1, v/v) saturated with solid paraformaldehyde. Development time for a 15 cm shift of the solvent front was approximately 2 h. Fading of the colour of the separated anthocyanins occurred if the development time was more than 2 h. This was probably due to alkaline hydrolysis of the anthocyanins by the sodium acetate present in the thin layer. Since the solvent mixture separated into two phases after standing for a period of 3–4 h it was necessary to use a freshly made up solvent for individual runs. The water content of the chromatoplates was found to be the most critical factor in the successful separation of the anthocyanins in question. Any deviations in the above mentioned procedure resulted in unsatisfactory separations.

Anthocyanins were extracted with 5 % HCl in methanol. In Fig. 1 a separation of anthocyanins obtained from *Vitis vinifera* cultivar Barlinka is depicted. Eight distinct spots which can be divided into two subgroups are visible. Spots were identified by co-chromatography with known standards. The first four spots with the lower R_F-values are the 3-monoglucosides of delfinidine, petunidine, malvidine and peonidine whereas the other four spots are the same anthocyanins acylated with *p*-coumaric and caffeic acids. The success of this method led us to investigate other plant material containing anthocyanins. Various flowers and fruits were investigated and in all cases a satisfactory separation of the above anthocyanins was obtained.

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