

Thin-Layer Chromatographic Separation of Beet Pigments

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ABSTRACT

A new simple method for the thin-layer chromatographic (TLC) separation of betalaines has been developed and is described in this paper. The individual components were separated by preparative TLC on cellulose-coated plates and analyzed by a multiple development TLC technique with solvent mixtures consisting of ethanol, isopropanol, water, and acetic acid. Separated components of various hues were clearly visible after development without application of an indicator. Spectral analyses of fractions also were conducted. By this TLC procedure, analytical data were obtained more rapidly and conveniently than could be obtained by other methods such as electrophoresis.

INTRODUCTION

AMONG VARIOUS PLANTS that produce natural colorants, the red table beet (*Beta vulgaris*) is a potential source of valuable water-soluble pigments, the betalaines, which are comprised of two main groups: betacyanines (red) and betaxanthines (yellow) pigments. However, efficient and economical methods for isolation and identification of these components still present a problem. Commonly, the separation of individual pigments is carried out by column chromatography (von Elbe et al., 1972) or paper electrophoresis (Powrie and Fennema, 1963). Since these methods are costly and time consuming, multiple development TLC was investigated as a rapid and more convenient method of separating and quantitating betalaines.

Preparative TLC

The multiple development technique of TLC is used when one solvent is incapable of separating some of the substances (Stahl and Kaltenbach, 1961). It is often possible to separate the components first with one solvent mixture and then with another, each component being developed to a different length on the plate. Separation of betalaine pigments was accomplished by the preparative TLC procedure with the sequential use of two distinct solvent mixtures consisting of various proportions of isopropanol, ethanol, and water containing 5% acetic acid. 500 μ m thick cellulose-coated TLC plates provided by Brinkmann Instruments, Inc., Westbury, NY 11590 (Cat. No. 66-14-100-4), were used. Each sample was applied on preparative TLC plates by means of a stainer made by Applied Science Laboratories Inc., State College, PA 16801 (Cat. No. 17700). The solvent mixtures, containing acetic acid, denoted by Roman Numerals I, and II, listed in Table 1 were used for development.

Two hundred mg of beet juice powder, obtained by the procedure previously described (Bilyk, 1979), were dissolved in 4 ml of distilled water and applied on the cellulose-coated plate. The sample was developed first with the more polar System II which separates the pigments from insoluble substances. After the solvent moved 10 cm, the plate was dried under a nitrogen atmosphere, and two successive developments were made with the less polar System

Table 1—Solvents used for TLC separation of betalaine components

Solvents	Solvent mixtures, ml	
	I	II
Isopropanol	55	30
Ethanol	20	35
Distilled water	20	30
Acetic acid	5	5

cessive developments were made with the less polar System I to a height of 15 cm each time.

RESULTS & DISCUSSION

BETALAINES, being compounds of ionic nature, are difficult to separate. Previously, the most reliable methods for separating, isolating, and quantifying the betacyanines (Piatelli and Minale, 1964) and betaxanthines (Piatelli et al., 1965) in plant material were column chromatography and paper electrophoresis (Powrie and Fennema, 1963; Nilsson, 1970). Thin-layer chromatography with unacidified solvents gives poor results because the migration of unprotonated betalaine is irregular and separation of individual components incomplete. However, when acid is incorporated in the developing solvent, the movement of betalaine on the TLC plate is facilitated, due to the protonation of the carboxyl group of the betacyanine molecule. The acid anion provides an electrically neutral system with the quaternary nitrogen. The same phenomenon occurs with betaxanthine. Such a protonated pigment acquires a chromatographic mobility which facilitates the preparative TLC development.

No indicator is needed for visualization of the separated pigments; they appear clearly on the TLC plate in their natural colors. During the development procedure, the TLC tanks should be covered with aluminum foil to shield the plate from light, which may cause pigment degradation.

The success of TLC separation is greatly influenced by the proper application of water-soluble pigment. For preparative TLC a special sample stainer is recommended. The streak applied on the plate should be narrow, and solvent (water) should be evaporated immediately at room temperature under a nitrogen stream. In order to avoid band irregularities during the development, the plate must be dried thoroughly before it is placed in new developing mixture. Material extracted from the TLC bands should be stored at 5°C after solvent removal to prevent degradation.

The separation of individual components on the TLC plate is shown in Figure 1. The band denoted by B represents the red betacyanines; band C, the yellow betaxanthines, which consist of Vulgaxanthine-I and Vulgaxanthine-II. The top band D was a very light-yellow pigment which had no measurable absorption maximum in the 375-650 nm range (color picture can be obtained from the author).

Figure 2 illustrates the spectrophotometric evaluation of the individual fractions obtained by preparative TLC with the multiple development technique. Absorbance

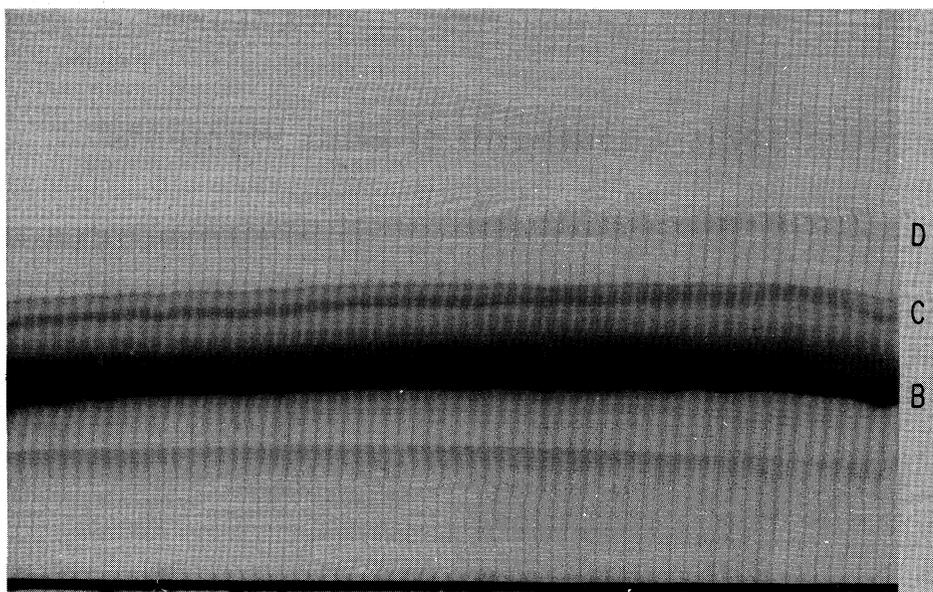


Fig. 1—Preparative thin-layer chromatography: (B) betacyanine fraction (red); (C) betaxanthine fraction (dark yellow); (D) light yellow fraction, no peak in the visible spectrum.

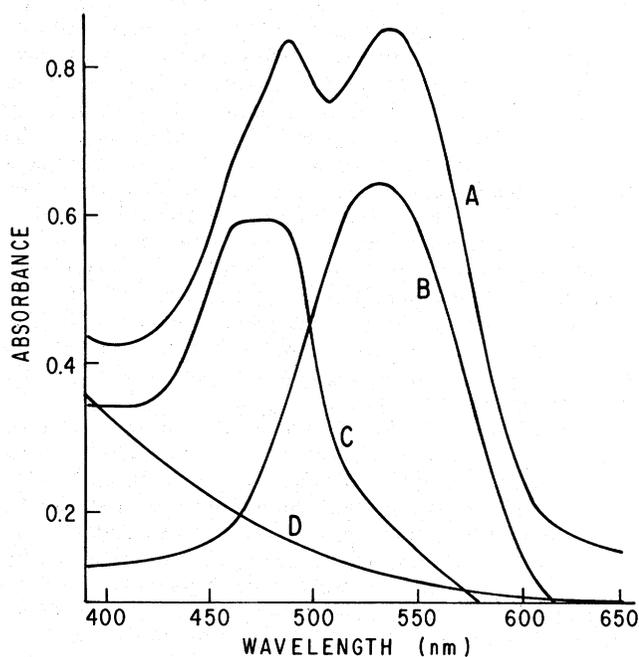


Fig. 2—Visible spectra of betacyanines and betaxanthines obtained from the preparative thin-layer chromatography: (A) beet juice pigments; (B) betacyanine fraction; (C) betaxanthine fraction; (D) top layer light yellow fraction, no peak in the visible spectrum.

measurements were made between 375 and 650 nm with a Bausch and Lomb spectronic 505 recording spectrophotometer. The absorption spectrum of crude beet juice powder, denoted by A, shows two absorption peaks, one at 537 nm, which is typical of the red betacyanines, and the other around 480 nm, characteristic of the yellow betaxanthines. The fraction of red pigment, denoted as spectrum B, exhibited a single peak at 537 nm. The yellow pigment fraction, spectrum C, gave a single peak at 480 nm. Spectrum D represents the topmost light yellow TLC band; no peak was evident for this pigment in the 375-650 nm range spectrum.

This method provides a rapid and satisfactory separation of the red and yellow pigments of beet, in quantities suitable for studies of their chemical and physical properties.

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