

## EXTRACTIVE FRACTIONATION OF BETALAINES

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## ABSTRACT

A fractionation procedure, based on extraction with acidified ethanol, was developed for the separation of betalaine pigments in beet juice powder. Extraction of lyophilized fresh beet juice with boiling 95% ethanol and subsequent chilling of the extract to  $-25^{\circ}\text{C}$  yielded two fractions: an orange precipitate containing betaxanthines and a yellow pigment obtained from the filtrate. Three additional extractions of the beet juice powder residue were carried out at room temperature with 95% ethanol containing 1.0, 0.6 and 0.4% HCl, respectively, and the resulting fractions were combined. This extract contained mainly the betacyanines. This procedure achieved rapid and satisfactory separation of the principal constituents of betalaine, comparable to other more complex methods. Fractions were analyzed by thin-layer chromatography (TLC) on cellulose-coated plates developed sequentially with solvent mixtures consisting of ethanol, isopropanol, water and HCl.

## INTRODUCTION

RECENT REGULATORY restrictions and consumer concern over the safety of food additives based on new evidence that artificial colorants may be hazardous to human health have evoked increased interest in plant pigments that may eventually be used as food colorants (von Elbe et al., 1974a; Pasch et al., 1975). Among various sources of natural colorants, the red table beet (*Beta vulgaris*) is a potential source of valuable water-soluble pigments, so-called betalaines, which are comprised of two main groups: the red betacyanines and the yellow betaxanthines. Studies conducted at the Eastern Regional Research Center have indicated that the betacyanines are about ten times more stable than the betaxanthines (Sapers and Hornstein, 1978). This makes it desirable to separate the yellow pigments from beet juice in order to prevent differential pigment losses which would cause the color to change during storage. However, efficient and economical methods for the isolation and purification of beet pigments still present a problem. Commonly, the pigments are purified by ion-exchange resin chromatography. Separation of individual pigments is carried out by paper electrophoresis (Piattelli and Minale, 1964; Piattelli et al., 1965). Both of these methods are costly, time-consuming, and impracticable for commercial use. Having this in mind, we sought a more convenient procedure for the separation of the main betalaine components by extractive fractionation of beet juice powder.

## EXPERIMENTAL

## Material

Fresh red beet roots (cultivar "Slowbolt R-2289"), grown by the W. Atlee Burpee Company, at their Doylestown, PA, experiment station, were washed and diced. The beet juice was extracted from 2.2 kg of beet dice with an Acme Supreme Juicerator Model 6001 (Acme Juicer Manufacturing Co., Lemoyne, PA) lined with 6.3 x 56.6 cm strip of Whatman No. 1 filter paper; 1600 ml of beet juice were collected, filtered through cheese-cloth, and placed in four lyophilization bottles (1200 ml). Water was removed with a Virtis Lyophilizer (Research Equipment, Gardiner New York, NY); 137.7g

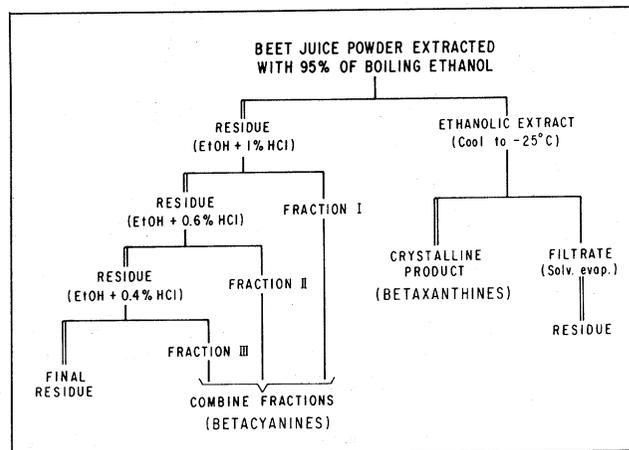


Fig. 1—Schematic diagram of extractive fractionation of betalaines.

of dry beet juice powder were obtained and stored under a nitrogen atmosphere in the freezer.

## Extractive fractionation

The extractive fractionation procedure is shown schematically on Figure 1. Twenty grams of beet juice powder were extracted with 500 ml ( $10 \times 50$  ml) of boiling 95% ethanol in a 150 ml beaker with stirring. The combined ethanolic extract was filtered through a folded Whatman 2V filter paper and held overnight at  $-25^{\circ}\text{C}$ . An orange precipitate was formed which was separated by filtration through Whatman 2V paper, dried under vacuum at room temperature, and stored at  $5^{\circ}\text{C}$ . After solvent was removed by distillation in reduced pressure at  $30^{\circ}\text{C}$ , the residue from the filtrate consisted of light-yellowish material.

Further extraction of the remaining beet juice powder residue, free of yellow pigments, was carried out at room temperature with acidified ethanol. First, the residue was extracted with  $2 \times 50$  ml of 95% ethanol containing 1.0% HCl. The second extraction was performed with  $3 \times 50$  ml of 95% ethanol including 0.6% HCl. The third extraction was carried out with  $4 \times 50$  ml of 95% ethanol containing 0.4% HCl. In all cases the ethanolic extract was neutralized with dilute NaOH and then solvent was removed under vacuum at  $30^{\circ}\text{C}$ . The fractions were combined after spectrophotometric and TLC analyses showed that they were similar. The remaining gum-like residue, after all ethanolic extractions, consisted of substances which were soluble in concentrated HCl. Another extraction similar to that described above for separation of betacyanines was performed, with lower concentrations of HCl in ethanol, namely, 0.6, 0.4 and 0.2%, respectively. However, the purity of the product obtained from this extraction was lower, as shown in Figure 2.

## RESULTS &amp; DISCUSSION

THE MAIN OBJECTIVE of this investigation was to separate the yellow and red pigments from beet juice powder. This was achieved by a simple procedure of extractive fractionation with 95% ethanol. In the first stage of extraction with boiling ethanol, yellow pigments were separated. Under cooling, the betaxanthines, which consist of Vulgaxanthine-I and Vulgaxanthine-II, were isolated from the ethanolic extract in the form of a precipitate. The yield of the crystalline fraction was 3.9g, which represented 19.5% of the original sample (20g) of crude beet juice powder. The residue obtained from the filtrate consisted of 4.4g of pro-

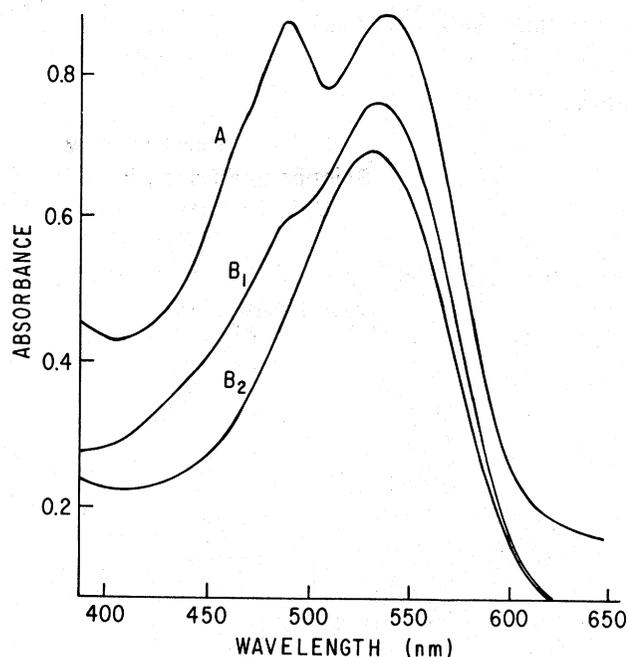


Fig. 2—Visible spectra of betacyanine fractions extracted with ethanol and various concentrations of HCl: (A) beet juice pigments; (B<sub>1</sub>) combined betacyanine fractions, extracted with 0.2–0.6% ethanolic HCl; (B<sub>2</sub>) combined betacyanine fractions, extracted with 0.4–1.0% ethanolic HCl.

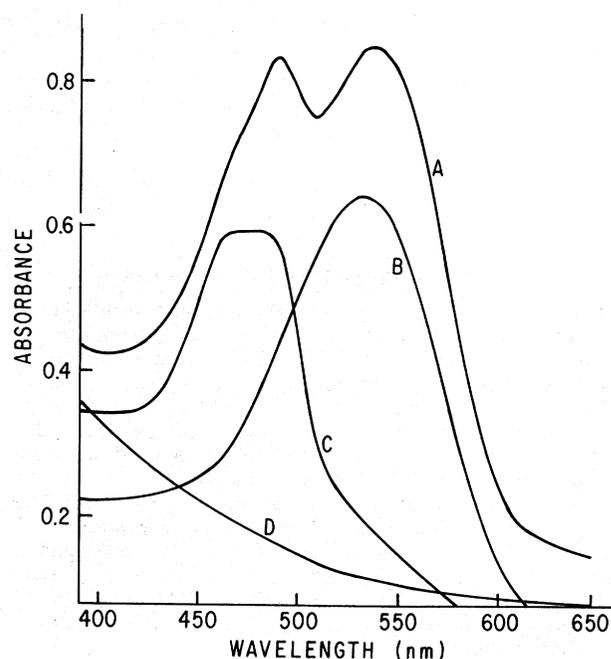


Fig. 3—Visible spectra of betacyanines and betaxanthines obtained from the extractive fractionation: (A) beet juice pigments; (B) combined betacyanine fractions, extracted with 0.4–1.0% ethanolic HCl; (C) betaxanthines crystallized from ethanol; (D) dried filtrate.

duct, which was equivalent to 22% of the total sample. Overall yellow pigments extracted from the sample of beet juice powder represented 41.5% (Table 1). Fraction I, obtained from extraction with 95% ethanol containing 1.0% HCl, yielded 6.1g of product and represented 30.5% of the original weight of beet juice powder. The second fraction (II) extracted with 95% ethanol containing 0.6% HCl gave 1.8g of product (9.0% of beet juice powder). The third fraction (III), obtained from the extraction with 95% ethanol containing 0.4% HCl, yielded 1.1g (5.5%) of material. The combined pigment from fractions I, II and III amounted to 9.0g, which represents 45% of the original sample of beet juice powder, and contained largely betacyanines. The final residue contained 2.7g (13.5%) of a gum-like material. Yields of fractions are listed in Table 2.

Extraction of yellow pigment was carried out with ethanol alone to avoid any degradation of betaxanthines (Piatelli et al., 1965). The separation of betacyanines was performed sequentially with acidified ethanol. Then the extract was neutralized and solvent was removed under reduced pressure at low temperature in order to prevent the alteration of betacyanines (von Elbe et al., 1974b). Beside the pigments, ethanolic extracts contain considerable amounts of sugars. These can be separated by column chromatography or partially reduced during an anaerobic fermentation by *Candida utilis* (Adams et al., 1976).

Spectral analyses of fractions are shown in Figure 3. Absorbance measurements were made between 375 and 650 nm with Bausch and Lomb Spectronic 505 Recording Spectrophotometer. The crude beet juice powder (A) gave two absorption peaks, one at 537 nm, which is typical of betacyanines (Wyler and Dreiding, 1957), and the other around 480 nm, characteristic of betaxanthines (Piatelli et al., 1965). The combined fractions extracted with acidified ethanol (B) exhibit a single peak at 537 nm, and the crystalline fraction from the yellow ethanolic extract (C) shows an absorption maximum at 480 nm. The residue from filtrate

(D) has no peak in the visible spectrum. Absorbance values in Figure 2 reflect the purity of betacyanines extracted by ethanol containing various concentrations of HCl. As seen on the spectral band (B<sub>1</sub>), the product extracted with acidified ethanol containing 0.6, 0.4 and 0.2% HCl has impurities in the region of betaxanthines (480 nm), as compared to the single band (B<sub>2</sub>), which represents the product extracted with ethanol containing 1.0, 0.6 and 0.4% HCl.

These fractions were analyzed by a thin-layer chromatography procedure developed in our laboratory (Bilyk, 1979), which achieved rapid and satisfactory separation of betalaine components comparable to results obtained with more complex methods entailing electrophoresis. The separ-

Table 1—Extraction of beet juice powder with boiling ethanol

Fractions	Yield, % of total beet juice powder	Total, %
Crystalline product	19.5	
Filtrate (solvent evaporated)	22.0	41.5
Residue		58.5

Table 2—Residue free of yellow pigments extracted with acidified ethanol

Fractions	Yield, % of total beet juice powder	Total, %
Fraction I (1.0% HCl)	30.5	
Fraction II (0.6% HCl)	9.0	45.0
Fraction III (0.4% HCl)	5.5	
Final Residue		13.5

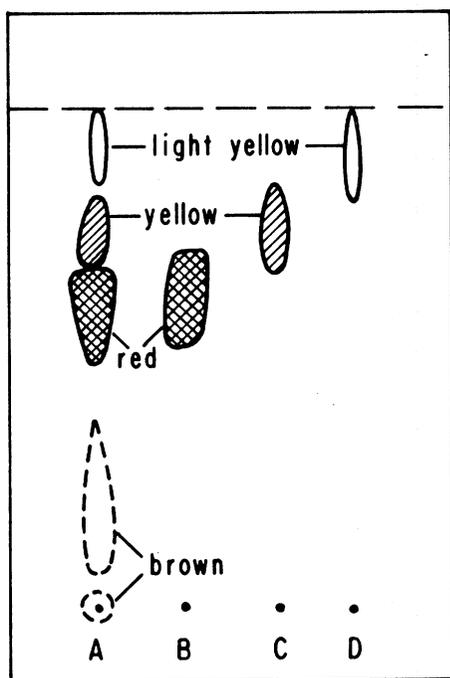


Fig. 4—TLC analysis of betalaines: (A) beet juice pigments; (B) betacyanine fraction; (C) betaxanthine fraction; (D) dried filtrate.

ations, shown in Figure 4, include: (A) crude beet juice powder, containing red and yellow components and other matters of low polarity, mainly sugars; (B) betacyanines

(single spot) extracted with acidified ethanol; (C) betaxanthines, less polar than betacyanines; and (D) residue from the filtrate, of still lower polarity (top spot).

Pigments purified by extractive fractionation will be tested as colorants for food and beverages. Subsequently, their stability and other functional properties will be evaluated.

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- Ms received 11/25/78; revised 2/3/79; accepted 2/10/79.

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