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Natural pigment betacyanin as tracking dye for gel electrophoresis

Archana Deka, Bhushan Subhash Chougule, Assma Parveen, Jyoti Prasad Lahan,
Madhumita Barooah and Robin Chandra Boro*

Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat-785013, Assam, India

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Several dyes including bromophenol blue are used as tracking dye in gel electrophoresis. The stability of betacyanin extracted from spinach vine fruit (*Basella rubra* L.) was studied in relation to degradative factors such as pH ranging from 2 to 12 and at 100ºC. Betacyanin pigment was found to be stable at pH range 3-8 while the stability of the pigment at high temperature was found to be moderate. The effectiveness of betacyanin as a tracking dye was checked along with the bromophenol blue for agarose gel electrophoresis. The present study revealed that betacyanin from spinach fruit can be a potential alternative to conventional dye like bromophenol blue used in the loading dye preparation for agarose gel electrophoresis.

Keywords: Betacyanin, *Basella rubra*, Gel electrophoresis, Natural Dye, Fruit.

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Introduction

Natural pigments derived from plant sources including chlorophylls, carotenoids, flavonoids, anthocyanins, quinones, betacyanins, etc. are extensively used in food and cosmetic industries[1]. Food and Drug Administration (FDA) assigned “exempt colour additives” label to specify that colourings are from natural sources[2]. The pigments are soluble in water and often used as a potential source of natural dye. Betalains are water-soluble nitrogen-containing pigments found in two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins[3]. A red pigment, extracted from the fruit of spinach vine (*Basella rubra* L.), is bright purple at pH 3 to 7[(Ref.4)]. Spinach vine belongs to the *Basellaceae* family and is commonly used as vegetable in many Asian countries such as India, China, Thailand, Japan, Vietnam, Philippines, etc. The pigment isolated from Spinach vine contains betanidin monoglucoside as the major betacyanin. In contrast to anthocyanins, betacyanin maintain their colour over a wide range of pH varying from 3 to 7. This property makes betacyanin ideal for colouring low-acid foodstuff such as dairy products[5]. Betacyanins have a high molar absorbency index and are equivalent to synthetic colourants[3]. Cell-culture techniques have been used to produce betalains as it is easier to control quality and availability of pigments independently of environmental changes[6].

Synthetic dyes are extensively used in many fields, such as textile industry, leather tanning industry, paper production, food technology, agricultural research, photochemical cells and molecular biology experiments[7-12]. Extensive application of synthetic dyes can pose serious health risk and can cause considerable environmental pollution. As such research on alternative natural dye have gained considerable attention during the recent years[13-15].

Loading dyes are important in molecular biological experiments as they give colour and density to the sample and allow monitoring the progress of the sample on the gel. The most commonly used dyes are bromophenol blue (3', 3'', 5', 5'' tetrabromophenol sulfonphthalein) and xylene cyanol. Since these are of chemical origin, dyes from biological sources such as anthocyanin and betacyanin can play a vital role. Earlier studies reported the use of leaf extracts of *Lawsonia inermis* L. for protein staining in polyacrylamide gel[16]. In this paper, we report the stability of betacyanin pigments present in spinach vine fruit (*Basella rubra* L) at different pH and temperature and demonstrated the effectiveness of betacyanin as a tracking dye in agarose gel electrophoresis.

*Correspondent author:
E-mail: robinboro@gmail.com
Mob.-+91- 09435704178

Materials and Methods

Plant sample and pigment extraction

Fresh, ripe fruit samples of spinach vine collected from Assam Agricultural University Campus, Jorhat (N = 26°43′110″, E = 94°11′ 812″, Altitude = 86 m) were used for betacyanin extraction. Compared to polar anthocyanins, betacyanins are more hydrophilic. They are more soluble in water as compared to non-polar solvents and thus can be easily extracted and separated[3]. 10 g of fruit samples were crushed using mortar and pestle to get thick puree which was centrifuged later at 10,000 rpm for 5 min to get a clear extract of betacyanin and stored at 4°C. 10g of spinach vine fruit yields 4.5 mL of pigment extract.

Quantification of betacyanin content

Betacyanin content was measured by diluting the sample 500 fold in deionised water to obtain the absorption values at wavelength 540 nm[17]. After 10 min of equilibration, the quantification of betacyanin was carried out by applying the equation: $BC=A\times DF\times MW\times 1000/\varepsilon\times L$, where BC is the betacyanin equivalent concentration in mL/lt; A is the absorption value at the absorption maximum (λ max = 535 nm); DF is the dilution factor, MW is the molecular weight of betanin (550 g/mol), ε is the molar extinction coefficient of betanin (ε = 60,000 L/ mol/ cm in H_2O) and L is the path length of the cuvette.

Study of betacyanin stability at different pH and temperature levels

Effect of pH on stability of betacyanin was measured in a pH range of 2 to 12 by adding either HCl or NaOH. Betacyanin (10 µL) was added to 2990 µL distilled water of different pH. Absorbance of the solution was measured from 400 to 600 nm wavelength. Heat stability of betacyanin pigment was studied at 25°C and 100°C for 5 min and absorbance was recorded. Absorbance of the solutions was measured from 400 to 600 nm wavelength in spectrophotometer (CECIL-CE7250, Bio Aquarius, Germany).

Agarose Gel Electrophoresis

The use of betacyanin as a tracking dye in agarose gel electrophoresis was evaluated by using different concentrations of betacyanin. Conventional dye consisting of 0.25% bromophenol blue (Himedia, India), 0.25 % xylene cyanol (Himedia, India), 4 % sucrose and dH_2O (Lane 1) were used as a control. Different concentrations of betacyanin were used to replace bromophenol blue. Lane 2 consisted of 0.25 % xylene cyanol, 500 µL (0.70 mg) betacyanin, 4 % sucrose and 1X TBE buffer; Lane l and 3 consisted of 0.25 % Xylene Cyanol, 250 µL (0.35 mg) Betacyanin, 4 % sucrose and 1X TBE buffer and lane 4 consisted of 500 µL (0.70 mg) Betacyanin, 4% sucrose and 1X TBE buffer.

Results and Discussion

Betacyanin content

The betacyanin content in spinach vine was calculated by diluting the sample in distilled water and recording the absorbance. Concentration of betacyanin was found to be 1397.91 mg/L of extract. In a recent study[18] reported comparatively higher amount of betacyanin content in dragon fruit [*Hylocereus polyrhizus* (Weber) Britt. & Rose] when extracted with and without ethanol.

Stability of betacyanin at different pH range and temperature

The stability of betacyanin at different pH range (pH 2 to 12) was investigated. Betacyanin was stable within a pH range of 3 to 8. Stability is decreased at pH 2 and above pH 9 (Fig. 1). Maximum absorbance

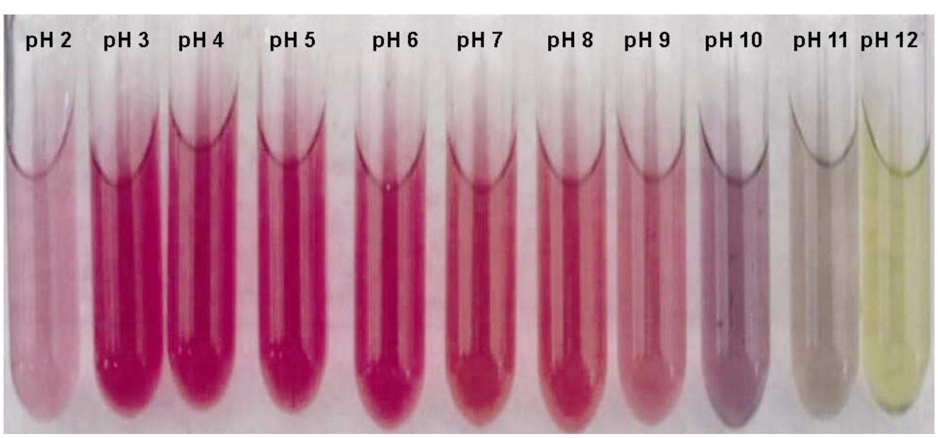

Fig. 1—Stability of betacyanin at different pH range

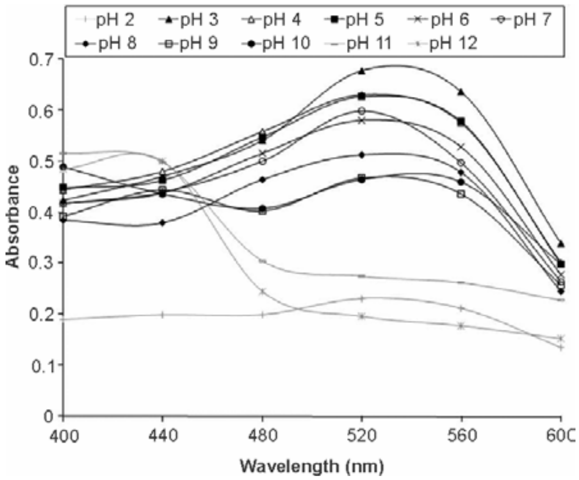

Fig. 2—Spectral analysis of betacyanin

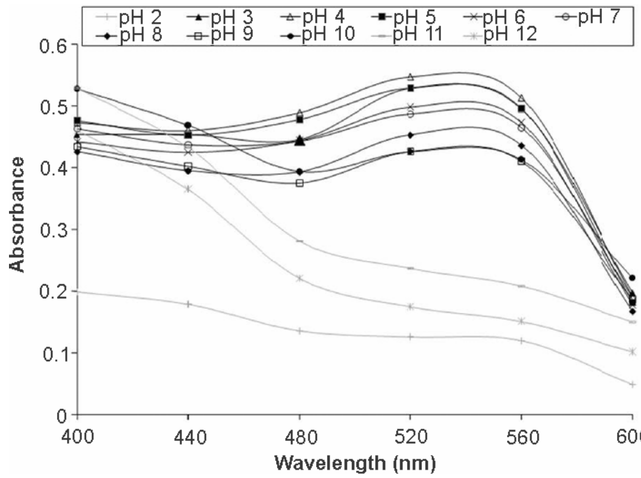

Fig. 3—Spectral analysis of betacyanin after heating for 5 minutes

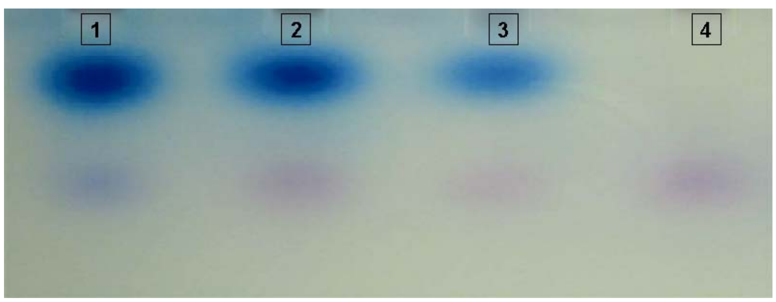

Fig. 4—Agarose gel electrophoresis. Lane 1: Conventional dye (0.25 % bromophenol blue, 0.25% xylene cyanol), Lane 2: 0.25 % xylene cyanol, 500 µL (0.70 mg) betacyanin, Lane 3: 0.25 % xylene cyanol, 250 µL (0.35 mg) betacyanin, Lane 4: 500 µL (0.70 mg) betacyanin

was recorded between 520 to 540 nm at pH 3 (Fig. 2). In earlier reports using betacyanin pigment extracted from Djulis (*Chenopodium formosanum* Koidz.) was shown to be stable between pH 2 to 9 with a maximum absorbance was recorded between pH 2 to 5[Ref.19]. Tang and Norziah[20] reported that the betacyanin pigment extracted from red purple pitaya fruit (*Hylocereus polyrhizus*) was most stable at pH range between 5 and 6. In the present study betacyanin was found to be moderately stable even after heating at 100°C (Fig. 3) for 5 min. Previous reports of betacyanin pigment extracted from different sources indicated instability at higher temperature. The rate of the betacyanin pigment degradation increased with increase in temperature[19].

Agarose Gel Electrophoresis

Betacyanin was used as an alternative dye to bromophenol blue in agarose gel electrophoresis. Migration of betacyanin was found to be parallel to that of bromophenol blue as shown in Fig. 4. The gel was examined after running the gel for 1h at 60 Volt.

Conclusion

To the best of our knowledge, our study is first of its kind to investigate betacyanin as an alternative to chemical dye in molecular biological experiment. The study revealed that the stability of the betacyanin pigment extracted from fruits of spinach vine was stable over a wide range of pH but moderately stable at high temperature. The betacyanin pigment was stable showing pink colour between the pH range of 3 to 8. The results clearly suggest that the betacyanin pigment from spinach vine can be used as a novel tracking dye instead of bromophenol blue in agarose gel electrophoresis due to its stability at a broad pH range.

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