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Research Article

THE EFFECT OF LIGHT, TEMPERATURE, PH ON STABILITY OF BETACYANIN PIGMENTS IN *BASELLA ALBA* FRUIT

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ABSTRACT

The fruit of *Basella alba* is often considered as waste so the study was aimed at exploring the feasibility of using the fruit as natural colorant using acidified methanol extraction. The extracted betacyanin pigments then were exposed to number of environmental conditions, which could destabilize the betacyanin molecules. These environmental conditions were included fourteen different pHs, various temperatures and presence or absence of light. The temperature stability of the betacyanin extract was calculated by reaction velocity constants (k) as well as the half-life time (t1/2). The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the betacyanin molecule. Copigmentation of betacyanin resulting in increase in both hypochromic effects and bathochromic shifts. *Basella alba* betacyanin extract was more stable at pH 4.1 and 6.0, temperature at 10° C, 20° C and 30° C both in the presence and absence of light. The results shows that betacyanin obtained from *Basella alba* fruit has a high potential to be used as a natural food colorant.

Keywords: *Basella alba,* betacyanin, color stability, pH, temperature, light

INTRODUCTION

Natural colourants from plant sources are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes [1, 2]. Natural dyes are colarant obtained from biological matter through mechanical retention, covalent chemical bond formation or complexes with salt or metal formation, physical absorbtion or by solution [3]. Nature produces a variety of compounds adequate for food colouring, such as the water-soluble anthocyanins, betalains, and carminic acid, as well as the oil soluble carotenoids and chlorophylls [4]. However, replacing synthetic dyes with natural colorants offers a challenge because the colour and stability of plant pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity, presence of, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products [5]. Identifying stable aqueous colorant extracts (e.g. fruit and vegetable juices) is attractive because their GRAS (Generally Recognized As Safe) status makes them easily commercialized. Betacyanins are the group of reddish to violet betalain pigments they are common in many flowers and fruits. Betacyanin can be classified into four kinds: betanin, amaranthin, gomphrenin and bouginvillein [6]. They are water-soluble betalain pigments derived by glycosylation of betanidin, which can be considered as a condensation product of cyclodopa with betalamic acid [7, 8]. They have antioxidant, anti-inflammatory and anticancerous property. That it can be better studied as the natural source of food colorant.

However, the lower stability of natural plant pigments against environmental factors could pose restriction to their utilization as food colorant in industry [9]. Therefore, the present study characterize the stability of *Basella alba* fruit betacyanin extracts. Our approach involved determining: (1) the pH effect on chromaticity (including the alkaline region) and (2) the effects of acylation, pH, temperature, and light on betacyanin stability through time. Hence, we can determine the optimal condition for extracting the dye as natural colorant.

MATERIALS AND METHODS

Sample collection

Basella alba fruit were collected from Coimbatore, Tamilnadu, India and stored in sealed polyethylene bags at -20°C until extraction.

Extraction

0.5 gm of *Basella alba* fruit were treated with 10 ml acidified methanol. And the mixture was centrifuged at 10,000rpm for 10 min and supernatant was taken for analysis [10].

Analytical study

Stability studies on temperature

The effect of temperature on colorant stability was done with samples inside capped glass vials covered with aluminium foil sealed with parafilm and kept at different temperatures ranging from 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, and 70°C. The UV/Vis spectra were recorded for freshly made samples (‘‘0’’), after 1 h, 1 day, and then subsequently after 2, 3, 4 and 5 days at 540nm.

Stability studies on Light

Light effect on colorant stability was performed with samples inside capped glass vials sealed with parafilm and kept at different temperatures ranging from 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, and 70°C. The UV/Vis spectra were recorded for freshly made samples (‘‘0’’), after 1 h, 1 day, and then after 2, 3, 4, and 5 days at 520 nm. The colorant half-life (t1/2) was determined for samples exposed to light and temperature only.

Stability studies on pH

a) Buffer solutions and betacyanin solubility

0.05M (2μg/ml) of *Basella alba* were dissolved in acidified methanol. Each pigment solution was then divided into 14 equal portions, dried, and dissolved in appropriate volumes of buffers. The betacyanin solutions were similarly prepared. All solutions were sealed, and kept at 10 °C during storage. The solvents used for preparations of the buffer-solutions were 0.2M KCl (A), 0.2M HCl (B), 0.1M $KHC_8O_4H_4$ (C), 0.1M HCl (D), 0.1M NaOH (E), 0.1M KH_2PO_4 (F), 0.025M borax (G), 0.05M Na_2HPO_4 (H), and Table 1 shows the solvent proportions. The accurate pH-values were measured with a pH-meter. The pH values of the various samples did not change during storage.

b) Colour measurements

UV/Vis absorption spectra were recorded at 540nm for betacyanin solutions at fourteen different pH-values (see Table 1) on a UV–Vis Spectrophotometer. As references, the respective buffer solutions were used. The UV/Vis spectra were recorded for freshly made samples (‘‘0’’), after 1 h, 1 day, and then after 2, 3, 4, 5, 7, 9, 11, 19 and 27 days. The samples were kept in a refrigerator (25ºC) between the measurements.

Table 1: Colour stability solvent proportions (v/v) used in the buffer solutions

S.No	pH	A	B	C	D	E	F	G	H
1	1.1	27.17	72.83						
2	3.0			69.16	30.84				
3	4.1			99.80	2.0				
4	5.1			68.87		31.13			
5	6.0					10.07	89.93		
6	6.6					24.70	75.30		
7	6.8					30.94	69.06		
8	6.9					36.79	63.21		
9	7.2					40.97	59.03		
10	7.3					43.88			
11	8.0				29.08		56.12		
12	8.9				8.42				
13	9.9					26.79		73.21	
14	10.5					7.58			92.42

A-0.2M KCl, B-0.2M HCl, C-0.1M $KHC_8O_4H_4$, D-0.1M HCL, E-0.1M NaOH, F-0.1M KH_2PO_4, G-0.025M Borax, H-0.05M Na_2HPO_4

RESULTS AND DISCUSSION

Stability of betacyanin from *Basella alba* at various parameters

In our experiments, the betacyanin stability was optimized using various temperature, pH and light. The effects of light, temperature, and pH on the stability of betacyanins were studied by several authors, and relationships between these effects and the decomposition of the betacyanin pigments has always been observed [3, 9, 11].

Spectrophotometric measurements of color intensity

Absorption spectra of *Basella alba beta*cyanin solutions were recorded using a UV-visible spectrophotometer. The change in the maximum absorbance (Amax) at varying wavelengths (λmax) presented the change in the color intensity, and revealed a possible hyperchromic effect (ΔAmax) and bathochromic shift (Δλmax), resulting from a copigmentation reaction.

The effect of temperature on the destruction of betacyanin under light

Temperature is the most important factor on betalain stability during food processing and storage. Some studies reported increasing betalain degradation rates resulting from increasing temperatures [12, 13, 14]. In this research it has demonstrated that increasing time and temperature resulted in changes in betacyanin and the copigmentation complex which resulted an increase in the visible spectrum (hyperchromic effect) and an increase in max (bathochromic shift) in the main peaks [15]. These changes were shown in Fig. 1.

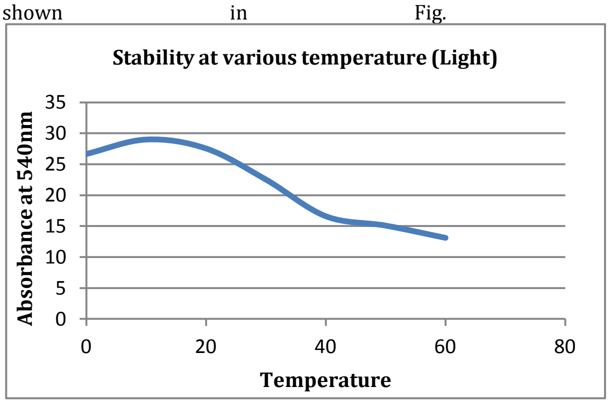

Figure 1 :Stability of betacyanins in various temperature (light)

The effect of seven different temperatures 0,10,20,30,40,50 and 60 °C on level of betacyanin extracted from *Basella alba* during 5 days were measured at 540 nm. The results show that decrease in the copigmentation of betacyanin at 40, 50 and 60°C. There was an increase in absorbance of *Basella alba* betacyanin which is stable at temperature 0, 10, 20°C. It was previously reported increased temperature can accelerate betanin [16], the main structure of betacyanin, to be regenerated into two products, betalamic acid and cyclodopa-5-Oglycoside, in unstable form. During heat processing, betanin may be degraded by isomerisation, decarboxylation or cleavage, resulting in a gradual reduction of red colour, and eventually the appearance of a light brown colour [17, 18]. Dehydrogenation of betanin leads to neobetanin formation, bringing about a yellow shift. Cleavage of betanin and isobetanin, which can also be induced by bases [19, 20], generates the bright yellow betalamic acid and the colourless cyclo-Dopa-5-O-glycoside.

The effect of temperature on the destruction of betacyanin under dark

The effect of seven different temperatures 0, 10, 20, 30, 40, 50 and 60°C on level of betacyanin extracted from *Basella alba* during 5days were measured in separate instances. The results show that decrease in copigmentation of betacyanin in 40°C, 50°C and 60°C. There was an increase in absorbance of *Basella alba* betacyanin which is stable at temperature 10°C, 20°C and 30°C (Fig. 2). It was reported that destruction of betacyanin in dark condition will be less when compared to the exposure of betacyanin under light because the light affects the electron of double bond in betacyanin molecule to be in excited stage, resulting in higher destruction of betacyanin [21]. Earlier studies also reported that betacyanin are light-sensitive pigments and tend to degrade due to light absorption in the visible light and the ultra-violet range of betalain molecule [6, 22].

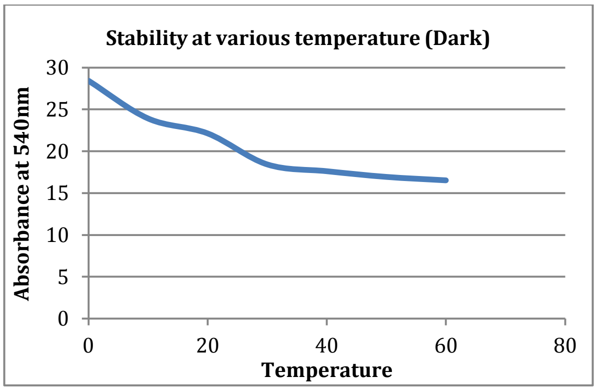

Figure 2 :Stability of betacyanins in various temperature (dark)

The effect of pH on the stability of betacyanin

Although altering their charge upon pH changes, betalains are not as susceptible to hydrolytic cleavage as the anthocyanins. Our results showed that increasing pH cause greater destruction of betacyanin in samples. Betacyanin solutions are stable and in red color under neutral and weekly acidic condition, while unstable under alkaline conditions. When the pH of betacyanin solution is higher than 7.5, the absorbance decreases slowly and the color of solution become yellow from red-violet, which is the typical alkaline-acidic reaction feature of betacyanins. The reason for the color change is that the betacyanins are changed to betaxanthins (yellow) under alkaline condition [24].

Colours of betacyanins in freshly made samples at pH 1.1 to 10.5

All the experiments were performed in a fixed temperature of 20ºC in an 20 days period. The colour differences between the *Basella alba* betacyanin vary with pH**.** These pigments had pinkish at the lowest pH values. By stepwise pH increase at 8.9, 9.9 and 10.5 the colour gradually changed toward yellow tones. Bathochromic shifts were observed for colourants at pH 1.1, 6, 6.6, 7.2, 8 and highest bathochromic shifts were observed at 4.1. Hypochromic shifts were observed for all colourants at pH 3, 6.9, 7.3, 10.5. At higher pH, destruction of betacyanin occur (Fig. 3). It have been alrealy reported that in warm agricultural area, high pH of the grapes at the time of harvest could cause problem for the juice making industry. Higher pH in grapes can cause fading the colour and decrease in stability of the products [24].

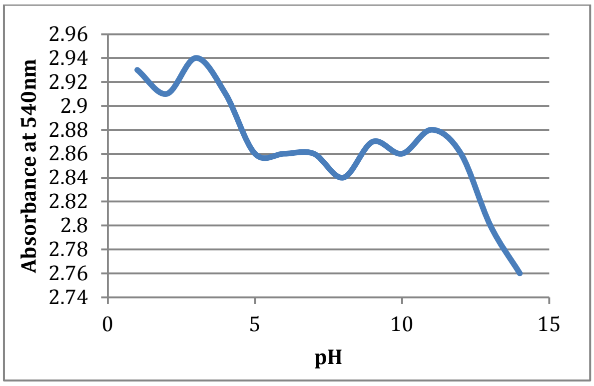

Figure 3: Stability of 0.05M crude betacyanins samples taken initially at various pH

Colour variation of anthocyanins in the pH range 1.1–10.5 after 1 hour dissolution

All the experiments were performed in a fixed temperature at 20ºC and after 1 hour incubation, the absorbance was recorded at 540nm. The same colour differences occurs between the *Basella alba* betacyanin like colours of betacyanins in freshly made samples. The visible absorption maxima for *Basella alba* suffered a bathochromic shift at pH 1.1, 4.1, 6.6, 7.3. Hypochromic shifts were observed at higher pH 3, 6, 6.8, 6.9, 10.5. Red sweet potato extracts, at higher pH,showed hypochromic shifts with time related to chemical changes in the molecule[25] due to interactions with the buffer used (Fig. 4).

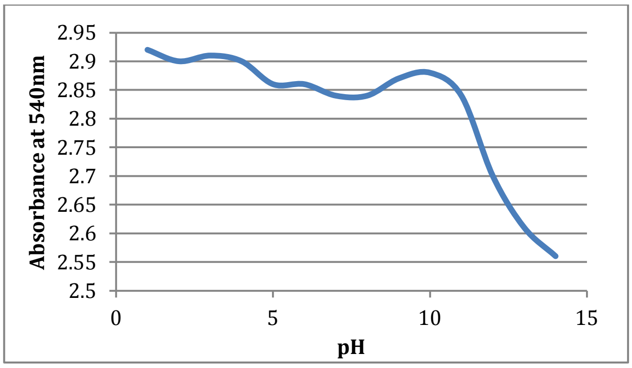

Figure 4: Stability of betacyanins samples taken after 1 hour at various pH

Colour variation of betacyanins in the pH range 1.1–10.5 during storage

The experiments were performed in a fixed temperature at 20ºC and absorbance was recorded at 540nm after storage. The colour differences between the *Basella alba* betacyanin vary with pH. These pigments had light pinkish and brown colour at lowest pH values and light yellow colour at higher pH 10.5. Bathochromic shifts were observed at pH 1.1, 4.1, 6, 6.8, 7.2. Hypochromic shift were observed at pH 3, 5.1, 6.6, 6.9 and at higher pH from 8 to 10.5. Under alkaline condition, most of betacyanins (red-violet) change to betaxanthins (yellow) [23]. Alkaline conditions cause aldimine bond hydrolysis, while acidification induces recondensation of betalamic acid with the amine group of the addition residue [19]. At low pH values, C_{15} isomerisation [26] and dehydrogenation of betanin [27] were observed (Fig. 5).

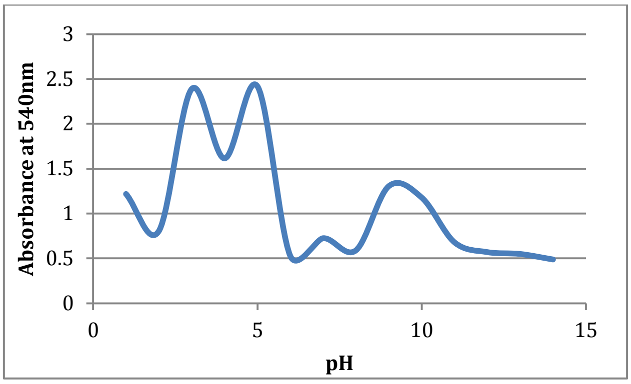

Figure 5 :Stability of Anthocyanins samples taken after 20 days storage at various pH

CONCLUSION

From the results it can be concluded that betacyanin extracts of *Basella alba* fruit were highly or moderately resistant to the pH, temperature and light factors tested. *Basella alba* betacyanin extract was more stable at pH 4.1 and 6.0, temperature at 10° C , 20° C and 30° C both in the presence and absence of light. Increase in environmental factors like pH, temperature and light accelerates destruction of betacyanins. This studies verify our results.

There is a need for replacement of the artificial dyes used in the food industry with natural dyes because of the general toxicity presented by artificial dyes, making them undesirable for human consumption. Thus these results suggest that betacyanin extract from *Basella alba* fruit represent inexpensive crops with high pigment yield that could be sources of betacyanins for the food colorant market.

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