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ORIGINAL RESEARCH

The Impact of pH and Solvent on the Extraction of Anthocyanin Pigments

Lalitkumar D. Katre[1], Manoj R. Patle[2], Rajkumar M. Patle[2] and Chandrakant Bisen[1]
[1]Department of Chemistry, M.B. Patel College of Arts, Commerce and Science, Gondia, Maharashtra, India
[2]Department of Chemistry, Dhote Bandhu Science College, Gondia, Maharashtra, India

ABSTRACT

As photosensitizers, anthocyanins offer the primary advantages of being readily accessible, completely biodegradable, and generally environmentally benign. To improve the energy conversion efficiency of natural photosensitizers, the analyst employed a range of strategies, including acidification, co-pigmentation, blending, and other methods, which yielded satisfactory results. This article discusses the pH dependency of dyes and the effects of various solvents on the economical extraction of anthocyanin colors. Anthocyanin dyes are also naturally used as photosensitizers in dye-sensitized solar cells (DSSCs), which ultimately balance out their intrinsic usage. At low pH levels, anthocyanins are stable and more soluble in CH_3OH and C_2H_5OH solvents.

KEY WORDS: Dye-sensitized solar cell, Photo sensitizer, Anthocyanin dyes, Stability of dye

INTRODUCTION

Developing organic pigments derived from various naturally existing materials that exhibit high light-acquiring efficiency, stability, easy fabricability, high molar absorptivity, and biodegradability is a continual research endeavor. Plants are the source of naturally occurring pigments extracted and used as photosensitizing agents for DSSC (Meng *et al*., 2008; Mahajan *et al*., 2024). The majority of vividly colored flowers, fruits, and foliage contain anthocyanins, which are large-scale photosensitizing agents (Mehmood *et al*., 2014; Aziz *et al*., 2020). The brilliant color of plant extract is a sign of anthocyanin pigments, primarily cyanidin and its byproducts. Natural colorants are the primary use for anthocyanins, which are pigments that belong to the flavonoid class (Pazmiño-Durán *et al*., 2001; Ghann *et al*., 2017). Environmental and genetic variables determine the kind and quantity of anthocyanin pigments present in fruits and vegetables (Yu *et al*., 2023). This pigment gives different sections of plants their hues, which include blue, pink, violet, orange, and red. Greek terms anthos and kyanose denote flowers and blue, respectively (Miraje *et al*., 2015). When illuminated, the dye molecule adsorbed on TiO_2 sheets absorbs photons of wavelength corresponding to the energy difference between its highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) (Amogne *et al*., 2020). Anthocyanidins are the fundamental anthocyanin structures, factors affecting their stability and degradation. According to the current study, anthocyanins offer major antioxidant, anticancer, anti-inflammatory, and antibacterial qualities that can be utilized to prevent illnesses such as diabetes, obesity, and even cancer. However, anthocyanins have a significant disadvantage: their limited stability. As a result, a variety of variables impact their stability, including pH, light, temperature, co-pigmentation, sulphites, ascorbic acid, oxygen, and enzymes (Enaru *et al*., 2021; Mahajan *et al*., 2024). An aromatic ring is joined to a heterocyclic ring that contains oxygen, and this ring is joined to another aromatic ring by a carbon-carbon bond to form anthocyanidins (Khooa *et al*., 2017). Glycoside-derived anthocyanidins are anthocyanins. Titrimetric analysis detects endpoints by observing color changes from one medium to another (e.g., acidic or basic) when chemicals are added referred to as indications. Synthetic indicators can pollute the environment and increase costs. Many synthetic indicators are hazardous to humans. Finding alternative indicators from natural

*Corresponding author email: lalitkumarkatre@gmail.com

sources is crucial for cost-effectiveness and reducing environmental toxicity and pollution (Enaru *et al*., 2021; Nag *et al*., 2023). Anthocyanin pigments deteriorate rapidly during food preparation and storage, which can have a significant influence on color quality and nutritional characteristics. Simple spectrophotometric techniques may quickly assess the total anthocyanin pigment concentration as well as indexes for polymeric color and browning. The intensity of color is caused by fleavylium ions due to their resonance structure.

The effect of the aging period on the amount of anthocyanin pigments, trans-resveratrol, and colorimetric characteristics (color intensity, color density, and tonality) of Fondillón and other Monastrell wines (young, crianza, and reserva) with different aging periods, and to propose a new traceability method for determining Fondillón originality (Uysal *et al*., 2023). The number of sugars bound to the structure of anthocyanin pigments, the number of hydroxyl groups and their location in the molecule, and the presence of aliphatic or aromatic acids bonded to sugar are the factors that differentiate them (Kong *et al*., 2003). The anti-oxidant and anti-inflammatory effects of anthocyanins from various dietary sources and Anthocyanins' anti-ultraviolet property and their physiological activity in treating osteoporosis (Li *et al*., 2022). Anthocyanins play a role in illness prevention and health enhancement, particularly by counteracting oxidative stress, inflammation, and microbial infections (Chikane *et al*., 2022). Based on many reviews, about 23 anthocyanidins and over 500 distinct anthocyanins have been reported (Andersen & Jordheim, 2006; Gencdag *et al*., 2022). Glycoside derivatives of cyanidin, delphinidin, and pelargonidin are the most prevalent anthocyanins found in nature. Polyphenols are among the most coveted phytochemicals because of their antioxidant properties. These components, known as secondary plant metabolites, have antibacterial, antiviral, and anti-inflammatory effects, as well as a high antioxidant capacity (Ignat *et al*., 2011).

As naturally occurring pigments that dissolve in water, anthocyanins are known to be unstable in a variety of environmental settings (Laleh *et al*., 2006). Several factors may influence the stability of anthocyanins. Through structural modifications with hydroxyl, methoxy, glycosyl, and acyl groups, or environmental factors like anthocyanin structure, pH, temperature, light, and oxygen, several factors may influence and several other factors, these factors may affect anthocyanin stability chemically or physically, resulting in color change and degradation (Roobha *et al*., 2011; Nag *et al*., 2023). The anthocyanins that have been isolated are extremely brittle and prone to breaking down (Giusti & Wrolstad, 2003; Kang *et al*., 2021).

The most widely utilized solvents in extractions include aqueous mixtures of ethanol, methanol, acetone, and water, as well as their water combinations with or without acid, because anthocyanins are polar molecules (Rababah *et al*., 2010). Phenol solubility is enhanced by the polarity of the solvent (Shahidi *et al*., 2021). Anthocyanins cannot be effectively extracted by high-polarity solvents like water or low-polarity solvents like hexane (Silva *et al*., 2017).

Depending on the pH of the solution, anthocyanins can exist in many chemical forms (Kennedy & Waterhouse, 2000; Enaru *et al*., 2021). The positive charge of oxygen is carried by anthocyanins, which are primarily found as red flavylium cations at pH 3 (Jackman *et al*., 1987). They have sparked significant scientific and industry attention as possible natural food colorings. However, individual anthocyanins are intrinsically reactive compounds with electrophilic, nucleophilic, and electron-donating characteristics (Dangles, 2024). The stability of anthocyanin pigments is also affected by light. They have sparked significant scientific and industry attention as possible natural food colorings. However, individual anthocyanins are intrinsically reactive compounds with electrophilic, nucleophilic, and electron-donating characteristics (Lopez *et al*., 2014; Sunarya *et al*., 2024). Scholars have examined the effects of light on the stability of anthocyanins in grape juice and found that exposure to light speeds up the breakdown of anthocyanin pigments (Pervaiz *et al*., 2017). By preventing the hydration of chromophores in the pigments, co-pigmentation interactions between anthocyanin pigments and other substances, such as metallic ions, improve and stabilize the color of anthocyanins (Cortez *et al*., 2017).

Table 1: Absorbance of sweet potato, eggplant, and pomegranate in different solvents

Sample	Solvent	λ(nm)	Absorbance
Sweet Potato	D.W.	334.00	4.30
	Ethanol	356.00	4.80
	Methanol	345.50	4.80
	Acetone	324.5	3.41
Eggplant	D.W.	237.50	4.35
	Ethanol	395.00	4.80
	Methanol	242.50	4.36
	Acetone	311.00	4.80
Pomegranate	D.W.	330.00	4.32
	Ethanol	230.00	4.50
	Methanol	272.00	4.36
	Acetone	361.00	4.80

Temperature has an impact on the molecular structure of anthocyanins because they are thermally vulnerable substances. Anthocyanin content from black soybean skins was found to be 1.992 mg/L in distilled water + 30% citric acid and 1.936 mg/L in 70% ethanol + 1% HCl. The color stability temperature of Black soybean skin extract was tested at temperatures of 40°C, 50°C, 60°C, 70°C, 80°C, and 90°C, yielding absorbance values of 0.274, 0.247, 0.237, 0.227, and 0.214. At increasing temperatures, anthocyanin content diminishes (Charmongkolpradit *et al*., 2021).

The pH test conducted having pH 5, 6, 7, 8, and 9, yielding absorbance values of 1.831, 1,759, 1,229, 1,118, and 1,066. The test revealed that as the pH increased, the anthocyanin level decreased (Sunarya *et al*., 2024). Studies on the relationship between temperature and anthocyanin stability have demonstrated that higher storage temperatures dramatically hasten the degradation of pigment (Yusoff *et al*., 2014).

MATERIALS AND METHODS

Locally sourced sweet potatoes, eggplants, and pomegranates were sun-dried after being purchased from the Gondia market. Before being used, the samples were crushed in a blender and stored at room temperature in plastic bags (Fig. 1).

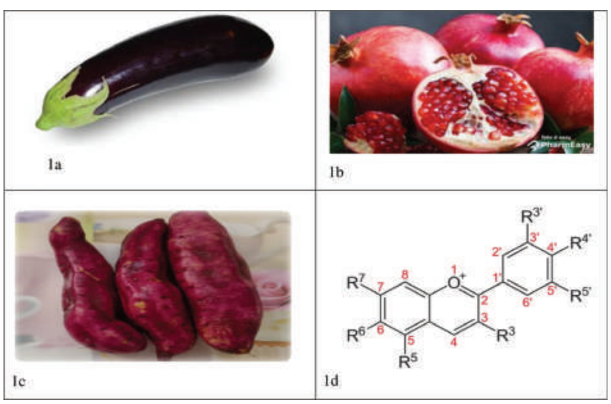

Fig. 1: a) Eggplant, b) pomegranate, and c) sweet potatoes, d) Structure of anthocyanin

Technique for Extracting Anthocyanins

Soak 8 grams of each plant powder in 50 milliliters of methanol with 1% HCL for five to ten minutes in a 100 mL beaker. Then, filter the mixture through Whatman No. 1 filter paper. The filtrate is put in a rotary evaporator to get the five original sizes, Fig. 2 (a). The solution is then allowed to dry at room temperature in the shade on a glass plate (petri dish) until the sticky material becomes amorphous shown in Fig. 2 (b).

Fig. 2(a): Rotatory evaporator

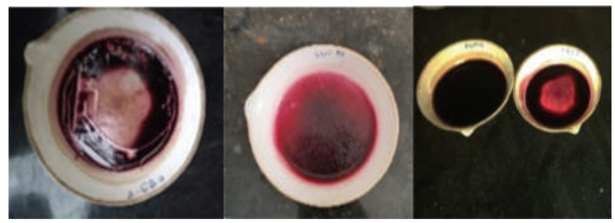

Fig.2(b): Extracted pigments

Examining the effects of solvents

Several solvents were used to demonstrate the solvent effect on extracted anthocyanin pigments. 0.03 gm of pigment was dissolved in 5 ml of different solvents that differed in nature, polarity, and dielectric constant, such as distilled water (H_2O), methanol (CH_3OH), ethanol (C_2H_5OH), and acetone (CH_3COCH_3) in series of test tubes for different pigments obtained from sweet potato, pomegranate, and eggplant. Absorbance for different pigments was determined in various solvents using a UV-visible spectrophotometer to examine the λmax value shown in Fig. 3, 4, and 5 and Table 1.

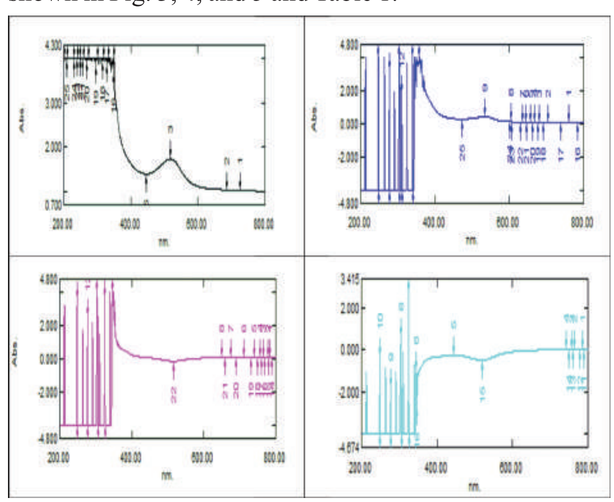

Fig. 3: Sweet potato in distilled water, ethanol, methanol, and acetone

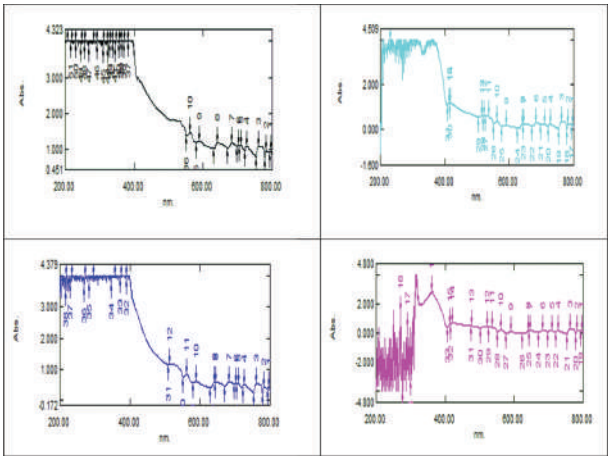

Fig. 4: Pomegranate in distilled water, ethanol, methanol, and acetone

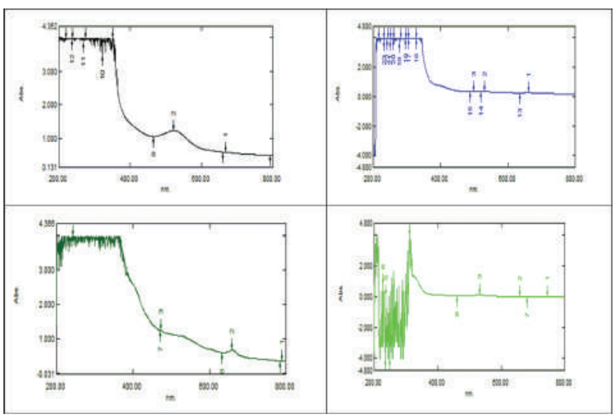

Fig. 5: Eggplant in distilled water, ethanol, methanol, and acetone

Examination of pH's Effects

Following preparation using (1N) hydrochloric acid, distilled water, and (1N) sodium hydroxide, three solutions for extracted pigments were created and adjusted for acidic, Neutral and alkaline pH: the first solution was pH = 1, the second was pH = 7, and the third was pH = 10.5. Color changes were noted for each solution immediately as well as after 24, 48, and 72 hours. At the same intervals, the absorbance at λ_{max} was determined.

The effect of the acidic function at pH = 1, pH = 7, and pH = 10.5 on eggplant, pomegranate, and sweet potato was depicted graphically. The solution was examined immediately, as well as 24, 48, and 72 hours after manufacture, shown in Fig. 6, Fig. 7, Fig. 8, and Table 2.

Table 2: Absorbance of sweet potato, eggplant, and pomegranate at different pH values and time intervals

Sample	Solvent	pH	Time (hr.)	Wavelength (nm)	Absorbance
Sweet potato	HCl	< 7	0	319.00	0.09
			24	312.50	4.00
			48	313.00	3.46
			72	347.50	4.00
	NaOH	> 7	0	347.00	4.00
			24	384.00	4.00
			48	244.00	4.00
			72	408.50	4.00
	H2O	= 7	0	347.00	4.00
			24	384.50	4.00
			48	244.00	4.00
			72	408.00	4.00
Egg Plant	HCl	< 7	0	361.00	4.00
			24	324.00	4.00
			48	352.00	4.00
			72	305.00	4.00
	NaOH	> 7	0	212.00	4.00
			24	302.00	4.00
			48	239.00	4.00
			72	331.00	4.00
	H2O	= 7	0	237.00	4.00
			24	254.00	4.00
			48	212.00	4.00
			72	201.00	4.00
Pome-granate	HCl	< 7	0	267.00	4.0
			24	311.00	4.0
			48	249.00	4.0
			72	263.00	4.0
	NaOH	> 7	0	285.00	4.0
			24	329.00	4.0
			48	385.00	4.0
			72	346.5	4.0
	H2O	= 7	0	254.00	4.0
			24	257.00	4.0
			48	214.00	4.0
			72	222.50	4.0

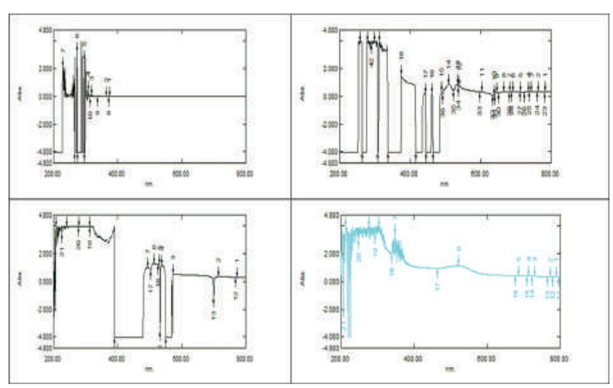

Fig. 6: Effect of pH on eggplant (HCl, NaOH, D.W.) at different time intervals (0, 24 h, 48 h,72 h).

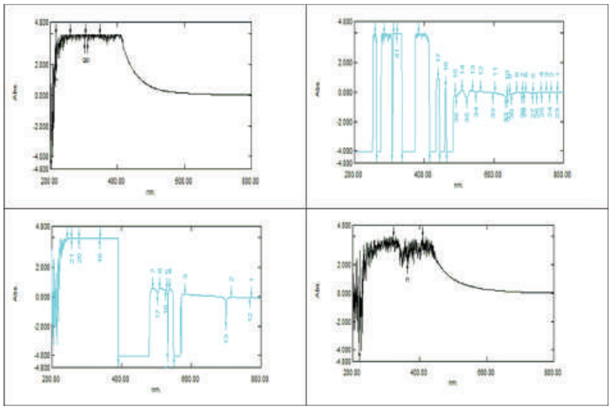

Fig. 7: Effect of pH on pomegranate (HCl, NaOH, D.W.) at different time intervals (0,24,48,72hours)

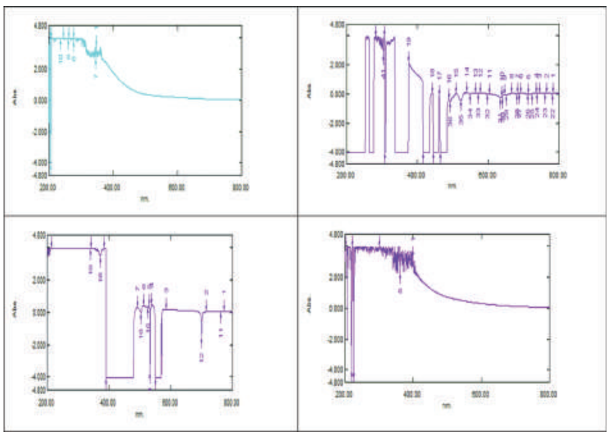

Fig. 8: Effect of pH on sweet potato (HCl, NaOH, D.W.) at different time intervals (0, 24 h, 48h, 72 h).

RESULTS AND DISCUSSION

The present investigation isolated anthocyanin pigments from three distinct sources: sweet potatoes, pomegranates, and eggplant. Water, methanol, ethanol, and acetone are among the solvents in which the extracted anthocyanin pigments dissolve have also been reported earlier (Rababah *et al*., 2010; Shahidi *et al*., 2021). The present investigation suggests that, the maximum λ value for sweet potatoes appears in ethanol at 356.5 nm (theoretical λ_{max} = 475 nm). Pomegranate peel max-λ values are found in ethanol at 230.5 nm (theoretical λ_{max} = 250 nm). The eggplant's max-λ values indicate that the theoretical λ_{max} is 476 nm, or 395.0 nm, in ethanol. These findings clearly show that anthocyanin pigments are insoluble in acetone and only slightly soluble in distilled water. It demonstrates good solubility in methanol and ethanol. Earlier it was reported that, one band appears in the visible area of the UV-Vis spectra in all extracts; this band is caused by the n-π* transition (Amogne *et. al*., 2020).

The stability of the anthocyanin pigment is significantly impacted by pH (Nag *et al.,* 2023). Sunarya *et al*. (2024) suggested that Anthocyanins can withstand low pH levels. The hue transitions from pale yellow to faint dull at pH 7. As a result, they keep their structure (Roobha *et al.,* 2011; Enaru *et al.,* 2021). In the present study it was observed that at an acidic pH, the pomegranate pigment transforms from a light brown to a dark yellow tint. The color transitions from reddish brown to light yellow to light orange to yellow at basic pH.

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