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Research Article

Optimization and Kinetics of Anthocyanin extraction from *Musa paradisiaca* Bracts

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Abstract

Anthocyanin is a natural pigment that gives red to purple colour present in banana bracts. It was extracted in this present study. Five different pretreatment methods were used to increase the efficiency of extraction among which ultrasonication was found to increase the yield. Extraction was carried out by five different methods using ten different solvent systems. Optimization of extraction parameters was performed. The maximum anthocyanin yield of 261.33 mg/100 g was achieved at 12.45% moisture content, 0.063 mm particle size, 400 rpm mixing intensity, with 60°C for 120 min and solvent to solid ratio of 30:1. The anthocyanin extracted was characterized using High Performance Liquid Chromatography (HPLC) analysis. Kinetic studies were performed and the activation energy (Ea) was calculated as 90.822 KJ/mol. Hence, the anthocyanin extracted from banana bracts was found to be valuable natural pigment for the various environmental applications.

Keywords: Banana bracts; Anthocyanin; Ultrasonication; pH differential method; Optimization; Extraction kinetics.

Introduction

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2 – phenylbenzopyrylium (flavylium) salt [1]. Anthocyanins encompasses a varied group of colored pigments responsible for the attractive, pleasing red, purple and blue color of many fruits, vegetables, flowers, leaves, roots and other plant storage organs [2]. These pigments are soluble in water which facilitates their easy inclusion into various aqueous food systems [3].

Color is an essential component of food because it is one of the first characteristics perceived by the senses and is used by consumers for the rapid identification and eventual acceptance of food [2]. The safety of synthetic colorants has been questioned in the past years, and provided to a decrease in the number of permitted colorants. Anthocyanins have recently received increasing attention as natural colorants in food systems [3]. Investigations concerning the development of anthocyanin containing food colorants have led to the development of anthocyanin molecules with complex patterns of glycosylation and acylation that show noteworthy stability to pH changes, heat treatment and light exposure. The enhanced stabilization has been attributed to intramolecular and intermolecular co-pigmentation, self-association, meta complexing and presence of organic salts [2].

Above and beyond the color attributes, importance in anthocyanins has intensified because of their promising health benefits. Health benefits linked with anthocyanin extracts embrace improvement of vision acuteness, antioxidant capacity, treatment of different blood circulation disorders due to capillary weakness, vaso-protective and anti-inflammatory properties, inhibition of platelet aggregation, protection of normal vascular permeability, controlling diabetes, anti-neoplastic, chemo

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protective agents and radiation protective agents [2].

Special consideration is focused on anthocyanin extraction from inexpensive or residual sources from agricultural industries. Banana is a tropical, tree like herb of the genus Musa belonging to the family musaceae. India has first position in the world in banana production. Species of the plant are native to Southeast Asia but are now grown extensively in all tropical countries for their fruit, fiber and foliage. Most bananas' have red, purple or violet bracts and seem to be a potential source of anthocyanin [4,5]. In the present investigation banana bracts were used to extract anthocyanin and the parameters influencing extraction were optimized.

Materials and Methods

Materials

Bracts of banana were collected from local markets in Chennai, India. The bracts present in the outer layers were used for extraction. Organic solvents of analytical grade were purchased and they were used for extraction.

Preparation of Sample for extraction

The bracts were washed with distilled water to remove impurities. The fresh bracts were then ground to powder form. It was then dried under shade for 2 h and was then dried in hot air oven for 3 h at 50±5°C.

Extraction of anthocyanin

Extraction of anthocyanin from the dried banana bracts in powder form was carried out using five different methods as described below: In the first method, extraction procedure proposed by Suganya Devi et al [6] has been used. The prepared sample was added to the solvent (1% hydrochloric acid (HCl) in methanol) in the ratio of 1:10. Then it was stirred for 2 h at low speed and it was then stored at -20^{o}C overnight in the dark to allow for maximum diffusion of phenolics from the cellular matrix. It was then equilibrated to room temperature and centrifuged at 7000 rpm for 10 min. The residue was rinsed with 10 ml volumes of solvent for twice with shaking and it was centrifuged and analysed using pH differential method.

In the second method, extraction procedure proposed by Chen et al [7] has been used. The prepared sample was mixed with solvent (acidified ethanol) in the ratio 1:10 in a 500ml round bottom flask fitted with a cooling system. Extraction was established at 71°C for 60 min. The extract was cooled to room temperature and filtered through Whatman No.1 filter paper. The residue was taken and extracted again by maintaining the same conditions. Both the extracts were mixed and analyzed using pH differential method.

In the third method, extraction procedure proposed by Longo et al [3] has been used. Extraction was carried out with the prepared sample with 0.1% HCl in methanol for 20 h at room temperature in dark. The mixture was filtered using Whatman No. 1 filter paper. The remaining solids were washed with 0.1% HCl in methanol until a clear solution was obtained. The combined filtrate was concentrated by evaporation. The concentrate was dissolved in 0.01% HCl in distilled water and analysed using pH differential method.

In the fourth method extraction procedure proposed by Garcia-Viguera et al [8] has been used and this procedure was slightly modified and used in this study. The prepared sample was extracted at room temperature with extracting solvent acetone in the ratio of 1:10, using a magnetic stirrer at 200 rpm. The extraction was performed four times with the solvent. Each extract was filtered through filter paper, concentrated (30°C with acetone) and the residue was redissolved in 5 ml acidified water (3% formic acid) and analyzed using pH differential method.

In the fifth method, extraction procedure proposed by Abdel-Aal et al [9] has been used and a little modification was made. Extraction was done by mixing the sample with the solvent methanol acidified with 1 N HCl (85:15) and it was stirred at 1800 rpm for 30 min. The apparent pH of the mixture was adjusted to 1.0 before shaking and was again adjusted if necessary after 15 and 30 min of shaking. The crude extract was centrifuged at 7000 rpm at 5 °C for 20 min and then refrigerated for 2 days to precipitate large molecules. The extract was centrifuged at 7000 rpm at 5 °C for 20 min. The partially purified extracts were concentrated. The precipitated pellets were separated by centrifugation as described above. The concentrated extract was vigorously mixed and filtered through filter

paper for further analysis using pH differential method

Quantification of anthocyanin

The total anthocyanin content was calculated using pH differential method [10]. The method can be used for the quantification of total anthocyanin content, based on the structural alteration of the anthocyanin chromophore between pH 1.0 and pH 4.5. Thus the difference in absorbance at the $\lambda_{vis\text{-}max}$ of the pigment is proportional to the concentration of pigment. 1 cm path length cuvettes were used for spectral measurements at 515 nm. Pigment content was calculated as cyanidin-3-glucoside, using an extinction coefficient of 29600 L $cm^{-1}mg^{-1}$ and molecular weight of 449.2 g/mol.

Effect of different solvent system in anthocyanin extraction

After selecting a suitable method the anthocyanin extraction was carried out using different solvent system. The solid to solvent ratio was maintained as 1:10. The different solvent selected were 80% methanol, 0.15% HCl in 60% methanol, 0.15% HCl in 80% methanol, 99.9% methanol, 3% formic acid in methanol, methanol-acetic acid-distilled water, citric acid, HCl-citric acid, 3% formic acid in water and acetone.

Sample pretreatment

The dried and grounded banana bracts were pretreated using different techniques to improve the extraction of anthocyanin. The pretreated samples were then extracted using the suitable solvent using the optimal method and the total anthocyanin content was determined using pH differential method. The different pretreatment methods employed are stated below: (i) Ultrasonication - The sample was exposed to ultra sonication using ultra sonic probe at 24 kHz with constant temperature (50 ± 1°C) for 15 min and anthocyanin was extracted from the sample and analysed [11]. (ii) Deep freezing - The dried sample was placed under freezing conditions at -20^{o}C. (iii) Microwave pretreatment - Microwave pre-treatment was conducted in the microwave oven for 10 min time duration at 60^{o}C, 500W and 2455 MHz [12]. (iv) Lyophilization - Lyophilization was carried out at 4^{o}C under vacuum pressure (14 Pa) using lyophilizer. (v) Bead beater method - Pre-treatment was performed with 1 mm glass beads at high speed of 1500 rpm. After the pretreatment of the sample, it was then extracted using the selected solvent by the selected method and the anthocyanin content was determined.

Optimization of extraction parameters

The parameters influencing the extraction of anthocyanin such as moisture content (5.26% - 17.43%.), particle size (0.246 mm - 0.044 mm), mixing intensity (150 – 500 rpm), temperature (35°C –70°C), extraction time (20-160 min), and solvent-to-solid ratio (5:1 - 40:1) were optimized to increase the yield of anthocyanin extraction.

High Performance Liquid Chromatography Analysis

High Performance Liquid Chromatography (HPLC) was performed on C18 column using diode array detector. The mobile phase consisted of water/formic acid/ acetonitrile (87:10:3, v/v/v; eluent A) and water/formic acid/acetonitrile (40:10:50, v/v/v; eluent B). The flow rate was maintained at 1.0 ml min^{-1}.

Results and Discussion

Effect of extraction methods

Extraction was carried out using five different methods and the anthocyanin content was determined and it was found to be higher in the method proposed by Devi et al [6]. A maximum extraction yield of about 96.23 mg/100 g (Figure. 1) was achieved. It can be seen that the levels of total anthocyanins were significantly higher in the extracts corresponding to method I than in the extracts obtained with other methods. This can be due to well mixing of solvent and the biomass followed by extraction overnight which allows maximum diffusion of phenolic into the solvent.

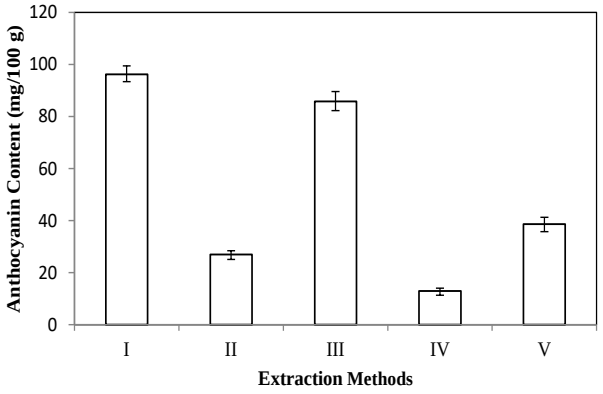

Figure 1. Effect of extraction Methods

Effect of solvent system

The results of the present study showed that the solvent used for extraction had a significant effect on the amount of anthocyanin extracted. The solvent 0.15% HCl in 80% methanol yielded the maximum amount of anthocyanin about 102.25 mg/100 g (Table 1). Most of the studies done so far used ethanol and methanol as solvents for extraction. For example, Puzmino-Duran, et al [13] extracted anthocyanin from banana bracts using acidified methanol. The outcomes of this experiment was consistent with that of Ju et al [14], who found that acidified methanol was more effective than acidified ethanol in extracting anthocyanin from dry red grape skin.

Table 1. Effect of solvent system on extraction

Sl. No.	Solvent	Ratio/ Percentage	Anthocyanin Yield (mg/100 g)
1	Methanol	80%	27.69±2.3
2	Acidified Methanol	60%	90.65±2.3
3	Acidified Methanol	80%	102.25±3.2
	Methanol	99.9%	21.89±2.1
5	Formic acid in Methanol	3%	80.38±3.1
6	Methanol : Acetic Acid : Distilled Water	25:1:2	30.32±2.4
7	Citric acid	-	53.43±2.5
8	HCl – Citric acid	-	89.33±2.7
9	Formic acid in Water	3%	86.58±2.5
10	Acetone	-	43.51±3.5

Effect of pretreatment methods

Cell rupture significantly enhances the bioavailability and the assimilation of pigments from the cells. The banana bracts used for anthocyanin extraction was pretreated using five different methods among which ultrasonication improved the yield of anthocyanin. An increased yield of about 151.94 mg/100 g of anthocyanin was got using ultrasonication. This is mainly due to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave. As a result the solute rapidly diffuses from the solid phase to the solvent [11].

Scanning Electron Microscope Analysis

The microstructure of banana bracts before and after ultrasonication was investigated by Scanning Electron Microscope (SEM). The significant differences were revealed in Figure 2a and 2b. The structure of banana bract after ultrasonication was found to be looser than that the sample before ultrasonication. It seems that ultrasonication results in an explosive disruption of the physical structure of bracts, leading to a direct migration of the desired anthocyanin components into the surrounding solvent. It was also observed that the cell wall breakage caused by the ultrasonic cavitations energy and porous surface was found to be more on the surface of the biomass after making the pre-treatment when compared to the biomass without pre-treatment.

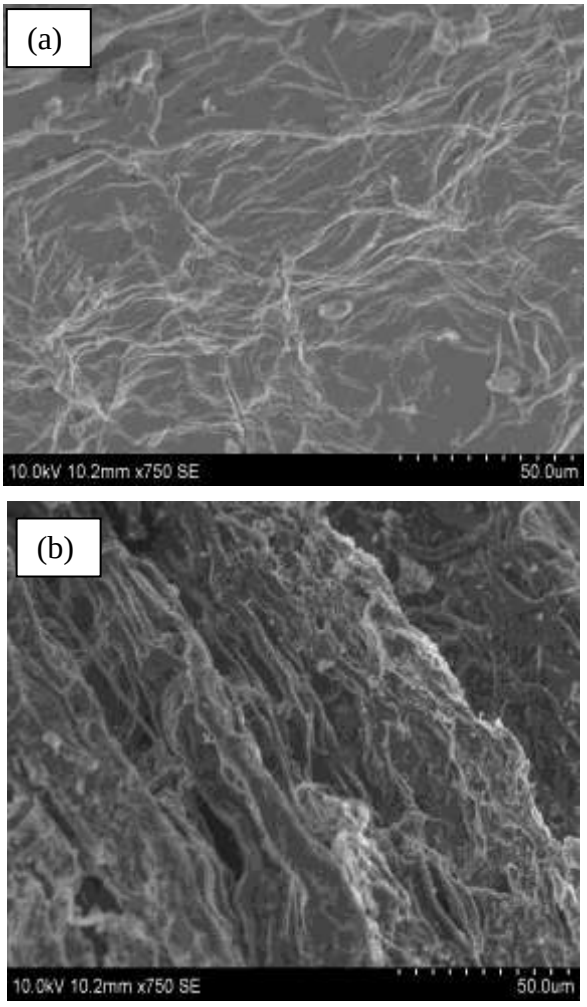

Figure 2. SEM analysis of *Musa paradisiaca* bracts biomass **(**a) Before Ultrasonication pretreatment (b) After Ultrasonication pretreatment

Optimization of parameters influencing extraction

In anthocyanin extraction, moisture content is one of the most important factors to be considered [15]. Figure 3a shows the effect of

moisture content on anthocyanin extraction. Moisture content was varied between the range 5.26 % to 17.43 % for anthocyanin extraction. The effect of moisture content on anthocyanin extraction from banana bracts was studied by maintaining the other parameters constant (particle size - 0.114 mm, stirrer speed - 300 rpm, extraction temperature - 50°C, extraction time – 60 min, solid to solvent ratio – 1:20). From the Figure 3a it was inferred that anthocyanin extraction was found to be increased with increase in moisture content from 5.26 % to 12.45% and then gradually decreased as the moisture content increased further. Maximum extraction of anthocyanin with 138.81 mg/100 g was achieved at 12.45% moisture content.

Particle size is a vital factor which influences the extraction of anthocyanin from banana bracts. Lesser the size of biomass leads to greater the interfacial area between the solid and liquid. Therefore the increase in interfacial area increases the anthocyanin extraction yield. The maximum extraction of 160.31 mg/100 g was achieved at 0.063 mm diameter of particle size with 12.45% optimum moisture level at 50°C temperatures with 300 rpm and solid to solvent ratio of 1:20 for 60 min. From the Figure 3b, it was observed that the anthocyanin extraction yield was found to be gradually increased from 125.38 mg/100 g to 160.31 mg/100 g with decrease in particle size from 0.246 mm to 0.063 mm diameter. This shows that the maximum anthocyanin extraction of 160.31 mg/100 g was obtained with the sample particle size of 0.063 mm diameter. However, further decrease in the particle size did not show much improvement in the anthocyanin extraction. Gao et al [16] showed that size of the ground hulls also influenced the extraction of anthocyanin from sunflower. More finely ground hulls gave a higher extraction yield than the coarse hulls.

The effect of stirrer speed on anthocyanin extraction is illustrated in Figure. 3c. Mixing intensity plays a major role in anthocyanin extraction. The effect of stirrer speed on anthocyanin extraction in the range of 150–500 rpm was evaluated with other parameters as constant. The anthocyanin extraction was found to be increased from 146.93 mg/100 g to 169.11 mg/100 g with an increase in the stirrer speed from 150 rpm to 400 rpm. The maximum anthocyanin extraction was obtained as 169.11 mg/100 g at 400 rpm. However, for stirrer speed more than 400 rpm, there was no significant increase in the anthocyanin extraction.

The effect of temperature on anthocyanin extraction from banana bracts was examined over the range of 35–70°C (Figure 3d). The extraction was found to be enhanced with the rise in the temperature. This is due to the increase in the dissolution capacity of the solvent system. The rise in the temperature from 35 to 60°C leads to increase in the yield from 142.88 mg/100 g to 189.25 mg/100 g. At 60°C, highest anthocyanin extraction of 189.25 mg/100 g was obtained at optimum condition of 12.45% moisture content, 0.063 mm particle size and 400 rpm stirrer speed for 60 min. Solid to solvent ratio of 1:20 was maintained. Ku and Mun [17] investigated the effect of extraction temperature on anthocyanin extraction from Bokbunja. Maximum yield was obtained at 60°C.

In the present study, the effect of time on the anthocyanin extraction was investigated with different time intervals varying from 20 to 160 min (Figure 3e). The results showed that anthocyanin extraction was found to be increased with increase in time. The extraction was established with the optimum condition of 12.45% moisture content, 0.063 mm diameter of particle size, stirrer speed of 400 rpm and temperature at 60°C. After 120 min of extraction, the anthocyanin extraction yield was obtained as 240.22 mg/100 g. Although the time was extended up to 160 min, the increase in extraction time above 120 min did not show any further significant improvement in the extraction. Hence, 120 min was found to be an optimum extraction time.

The effect of solvent-to-solid ratio on the oil extraction is shown in Figure. 3f. The influence of solvent-to-solid ratio from 5:1 to 40:1 on anthocyanin extraction was studied by maintaining all other parameters at optimum conditions. As the solvent-to-solid ratio increased from 5:1 to 30:1, the anthocyanin yield was found to be increased from 213.48 mg/100 g to 261.33 mg/100 g. Further increase in solvent-to-solid ratio above 30:1 did not show much improvement in the anthocyanin extraction. Therefore the solvent-to-solid ratio of 30:1 was found to be an optimum ratio. Zou et al [11] obtained 23.8: 1 as optimum solvent to solid

ratio for ultrasound assisted extraction of anthocyanin from mulberry using response surface methodology.

Extraction kinetics

Extraction of anthocyanin from banana bracts can be defined by the rate equation (1).

$$dY/dt = kY^n \qquad (1)$$

where Y is the anthocyanin yield (mg/100g), t is the extraction time (min), k is the extraction rate constant (min^{-1}) and n is the order of the reaction. Extraction was carried out at optimum conditions at constant temperature ranging from 35 to 70°C and the yield was determine at frequent intervals of extraction time between 20 min to 120 min. Using the obtained yields, plot of ln Y versus ln (dY/dt) was made at different temperatures and the graph got was found to be linear according to the equation above (Table 2).

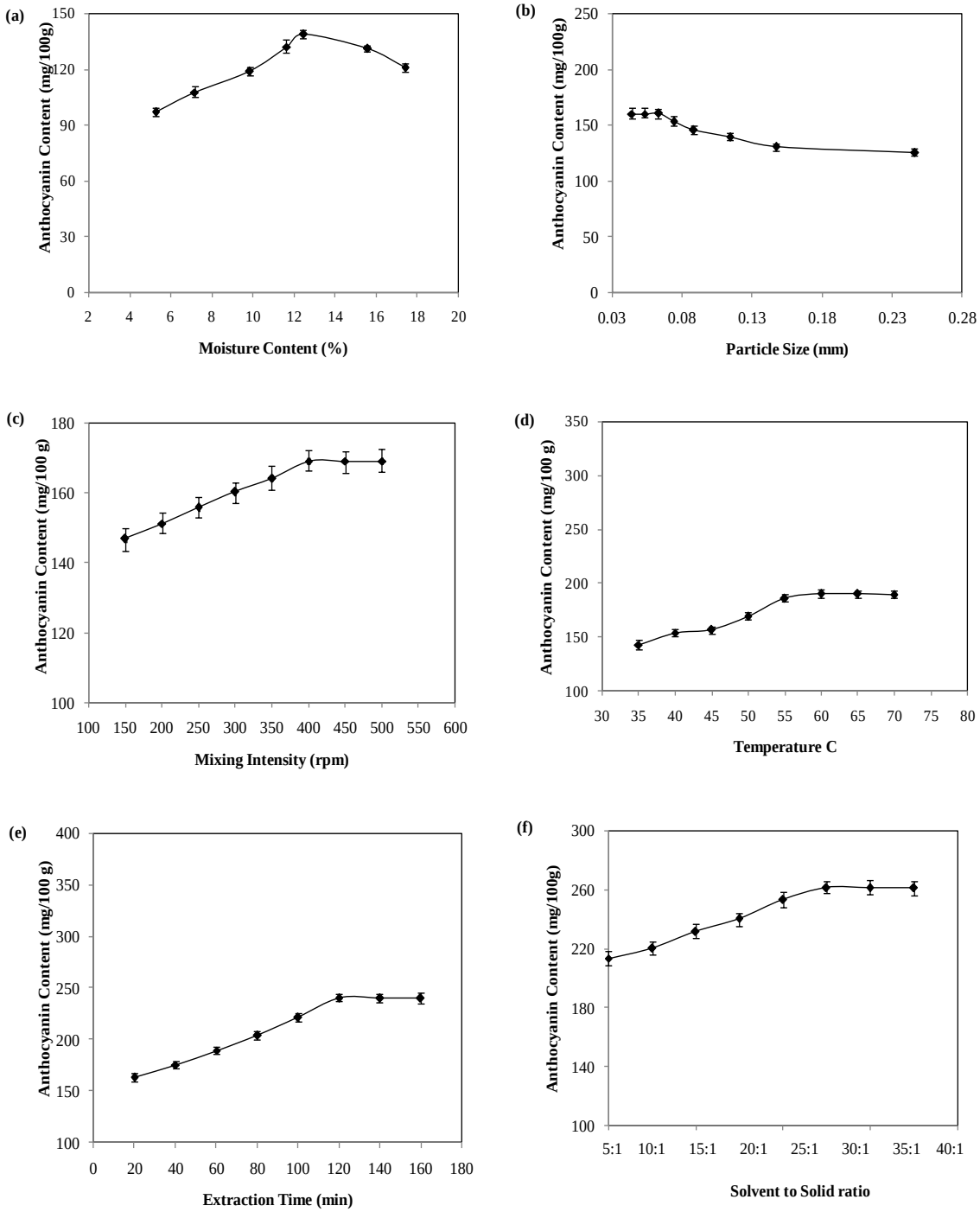

Figure. 3**.** Optimization study for parameters influencing extraction (a) Effect of Moisture content (b) Effect of Particle size (c) Effect of mixing intensity (d) Effect of Temperature (e) Effect of Extraction time (f) Effect of Solvent to Solid ratio

Table 2. First order kinetic model for extraction of anthocyanin from banana bracts with regression coefficient at different temperature and time

Temperature °C	Liner equation from plot	R^2 value	Reaction rate constant, k (min^{-1})
35	ln(dY/dt) = 1.941 lnY – 10.39	$R^2 = 0.966$	3.07×10^{-5}
40	ln(dY/dt) = 1.897 lnY – 10.24	$R^2 = 0.939$	3.57×10^{-5}
45	ln(dY/dt) = 1.785 lnY – 9.701	$R^2 = 0.952$	6.12×10^{-5}
50	ln(dY/dt) = 1.761 lnY – 9.599	$R^2 = 0.980$	6.77×10^{-5}
55	ln(dY/dt) = 1.631 lnY – 8.977	$R^2 = 0.964$	12.62×10^{-5}
60	ln(dY/dt) = 1.396 lnY – 7.703	$R^2 = 0.995$	45.14×10^{-5}
65	ln(dY/dt) = 1.326 lnY – 7.329	$R^2 = 0.900$	65.62×10^{-5}
70	ln(dY/dt) = 1.303 lnY – 7.188	$R^2 = 0.928$	75.5×10^{-5}

A first order kinetic model was well fitted with mean regression coefficient of 0.958. From the analysis of the data the reaction rate constant were found to increase with increase in temperature. The rate constant and the extraction temperature can be related using the Arrhenius equation (2).

$$k = Ae^{-Ea/RT} \quad (2)$$

Where k is the rate constant (min^{-1}), A is the Arrhenius constant (s^{-1}), Ea is the activation energy (kJ/mol), T is the absolute temperature (K) and R is the gas constant (J/mol K). The activation energy was calculated using the plot of ln k versus 1/T. Activation energy (Ea) was found to be 90.822 kJ/mol.

HPLC analysis of anthocyanin

The anthocyanin extract was separated and purified using HPLC [18]. Analysis of anthocyanin glucosides was performed using HPLC. Peaks were detected at 520 nm and identified by comparing with standards. Individual anthocyanin monoglucosides were quantified using authentic standards of anthocyanin. 13 peaks were got at 13 different retention time which confirms the presence of 13 different kinds of anthocyanin compounds (Figure 4 and table 2).

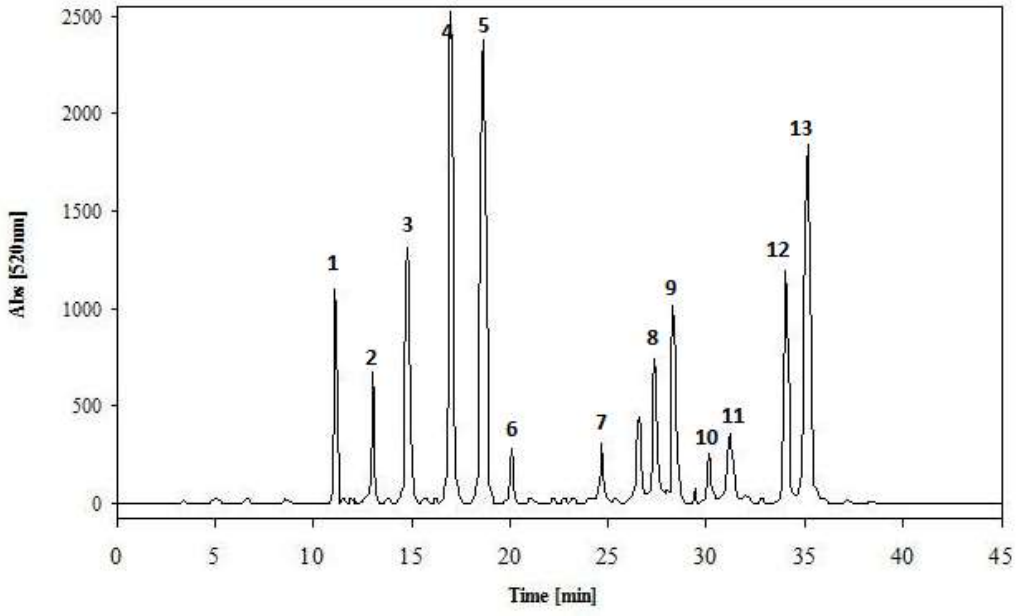

Figure. 4. HPLC analysis of anthocyanin extract

Table 2. Composition of anthocyanin extract by HPLC analysis

Peak	Retention time (min)	Compound
1	11.6	Delphinidin-3-glucoside
2	13.4	Cyanidin-3-glucoside
3	14.9	Petunidin-3-glucoside
4	17.9	Peonidin-3-glucoside
5	19.2	Malvidin-3-glucoside
6	20.3	Delphinidin-3-acetylglucoside
7	24.9	Petunidin-3-acetylglucoside
8	28.6	Peonidin-3-acetylglucoside
9	29.4	Malvidin-3-acetylglucoside
10	30.2	Cyanidin-3-p-coumaroylglucoside
11	31.4	Petunidin-3- p-coumaroylglucoside
12	34.2	Peonidin-3- p-coumaroylglucoside
13	35.4	Malvidin-3- p-coumaroylglucoside

Conclusion

From this research work, it can be concluded that extraction of anthocyanin from banana bracts using ultrasound pretreatment method showed better results when compared with other methods studied. Acidified 80% methanol solvent achieved high anthocyanin yield. The maximum anthocyanin of 261.33 mg/100 g was obtained with optimum condition of 12.45% moisture content, 0.063 mm of particle size, 400 rpm mixing intensity, 60°C extraction temperature, 120 min extraction time and solvent to solid ratio as 30:1. Kinetic studies confirmed that this extraction follows first order. The anthocyanin extracted from banana bracts serves as a natural pigment for environmental applications.

Conflicts of interest

Authors declare no conflict of interest.

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