Determination of Anthocyanins Content in Different Parts of *Indosasa hispida* McClure cv. 'Rainbow'

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Abstract. Three types of anthocyanins present in different parts of a new variety of *Indosasa hispida* were determined and quantified simultaneously by reverse-phase high-performance liquid chromatography (RP-HPLC) for the first time. The HPLC analysis in a single run was performed on a C18 column (particle size 5 μm, i.d. 4.6 mm, length 150 mm) with a diode array detector (DAD), and all calibration curves showed good linearity. The concentration of tested anthocyanins increases during the entire growing period, and the highest amounts were all found in red mature stem. These applicability results could be used as a good reference for a wide variety of colored bamboos.

Keywords: *Indosasa hispida* MeClure cv. 'Rainbow', Anthocyanins, RP-HPLC, Determination.

1. Introduction

Indosasa hispida MeClure cv. 'Rainbow' (Y. M. Yang et J. Wang) with high ornamental value, a new variety of *Indosasa hispida*, exhibits different degree of purplish red at different stages of its growth, and the purplish red material was ascertained as anthocyanins after isolation and analysis in our previously research [1-3]. Anthocyanins existing widely in the plant world has been studied extensively [4-7], nevertheless, the question about composition and content of anthocyanins in bamboo species is rarely known.

Recently, three types of anthocyanidins (cyanidin-3-*O*-glucoside chloride, pelargonin chloride and delphinidin chloride) of this new bamboo variety were simultaneously determined by reverse-phase high-performance liquid chromatography (RP-HPLC) for the first time in our group. The aim of the study reported herein was to establish an HPLC analytical method to determine and quantify some of most important anthocyanidins in culm tissues along the mature process of *I. hispida* MeClure cv. 'Rainbow'.

2. Experimental

2.1. Regents and standards.

Acetonitrile and methanol were of HPLC grade, and hydrochloric acid (HCl) was of reagent grade. Deionized water was prepared in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Three types of anthocyanin standards, cyanidin-3-*O*-glucoside chloride, pelargonin chloride and delphinidin chloride, were purchased from Sigma (Shanghai, China). The purity of all standards was higher than 90.0%. Stock standard solutions of three mixed standards were prepared at concentration of 1 mg ml^{-1} by using methanol containing 1% HCl. The solutions were stored in amber glass at 4℃ for HPLC analysis.

2.2. Test sample.

I. hispida MeClure cv. 'Rainbow' was obtained from Puer region of Yunnan province, China, which was divided into five parts after harvest and stored in a freezer at 4℃ until extraction. The five parts were designated as shoot, red shoot, intermediate part, mature part and red mature part. (Fig. 1)

Fig. 1 Sample of *I. hispida* MeClure cv. 'Rainbow' and site classification (1.shoot; 2. red shoot; 3. intermediate part; 4. mature part; 5. read mature part)

2.3. Sample preparation.

Each bamboo sample of 10.0 g was ultrasonic extracted with methanol containing 1% HCl for 20 minutes at room temperature followed by the filtration, and then the filtrate was transferred into a round-bottom flask. After repeating the extraction step once more, the combined filtrate was evaporated to approximately 10 mL at 35℃ under reduced pressure. The concentrated solutions were transferred to a 20 mL volumetric flask and reconstituted to 20 mL using 1.0% HCl. At the end, the solutions of each sample were filtered through a 0.25 μm syringe filter into a small glass vial for HPLC analysis.

2.4. HPLC analysis of anthocyanins.

The analyses were performed on an Agilent 1200 series HPLC coupled to a diode array detector using a Zobax Eclipse XDB-C18 column of 150 mm ×4.6 mm diameter, 5 μm particle size (Agilent Technologies, USA) thermostated at 25 ℃. The mobile phase consisted of a mixture of a solvent A (2% formic acid in Milli-Q water) and B (2% formic acid in acetonitrile). Elution was performed at a flow rate of 0.8 mL·min^{-1} using a linear gradient from 5% A and 95% B to 35% A and 65% B over a period of 30 min and the injection volume was 10 μL. Detection was carried out at 280 nm. Peaks were assigned by comparing their retention times with those of pure standards and by spiking sample with standards. All data was recorded and processed by data analysis software from Agilent.

3. Results and Discussion

3.1. Linearity and method validation.

The linearity was determined by five different concentrations of the mixed standard solutions, and each solution was injected 3 times. The standard curves were obtained by plotting peak area (Y) against concentration of standard solutions (X). All calibration curves showed good linearity within the tested ranges, as shown in Table 1.

The sample stability was assessed using measurements from a single sample solution stored at room temperature for 0, 2, 4, 6 and 8 h. The relative standard deviations (RSDs) of peak area of three standards were 0.8%, 1.2% and 0.5%, respectively. The average recovery rates of three standards were 96.7%, 100.5% and 98.2% with RSD 0.8%, 1.3%, 2.3%, respectively.

Table 1 Regression equation and correlation coefficient of standards

Compounds	Regression equation	Correlation coefficient (r)	Linear range (mg·mL^{-1})
Cyanidin-3-*O*-glucoside chloride	Y = 178.07X – 12.46	0.9998	2 – 20
Pelargonin chloride	Y = 523.42X – 158.87	0.9973	2 – 20
Delphinidin chloride	Y = 125.13X + 18.83	0.9996	2 – 20

3.2. Amount of anthocyandins in the analyzed sample.

The content of anthocyanins varied from 0.13 mg/10 g to 1.03 mg/10g in different parts of *I. hispida* MeClure cv. 'Rainbow', and the pelargonin chloride was the highest among the three tested anthocyanins. The highest values for the sum of three anthocyanins were found in red mature stem and the lowest values were found in shoot with sheath enclosed. The experimental results manifested anthocyanins gradually accumulated along with the bamboo maturity and the increase of light exposure.

Table 2 Average contents (mg/10g) of anthocyanins from different parts of sample (n=3)

No.	Different parts of the sample	Cyanidin-3-*O*-glucoside chloride	Pelargonin chloride	Delphinidin chloride
1	shoot	0.13	0.23	0.12
2	red shoot	0.26	0.58	0.35
3	intermediate stem	0.32	0.65	0.44
4	mature stem without color	0.54	0.98	0.51
5	red mature stem	0.87	1.03	0.81

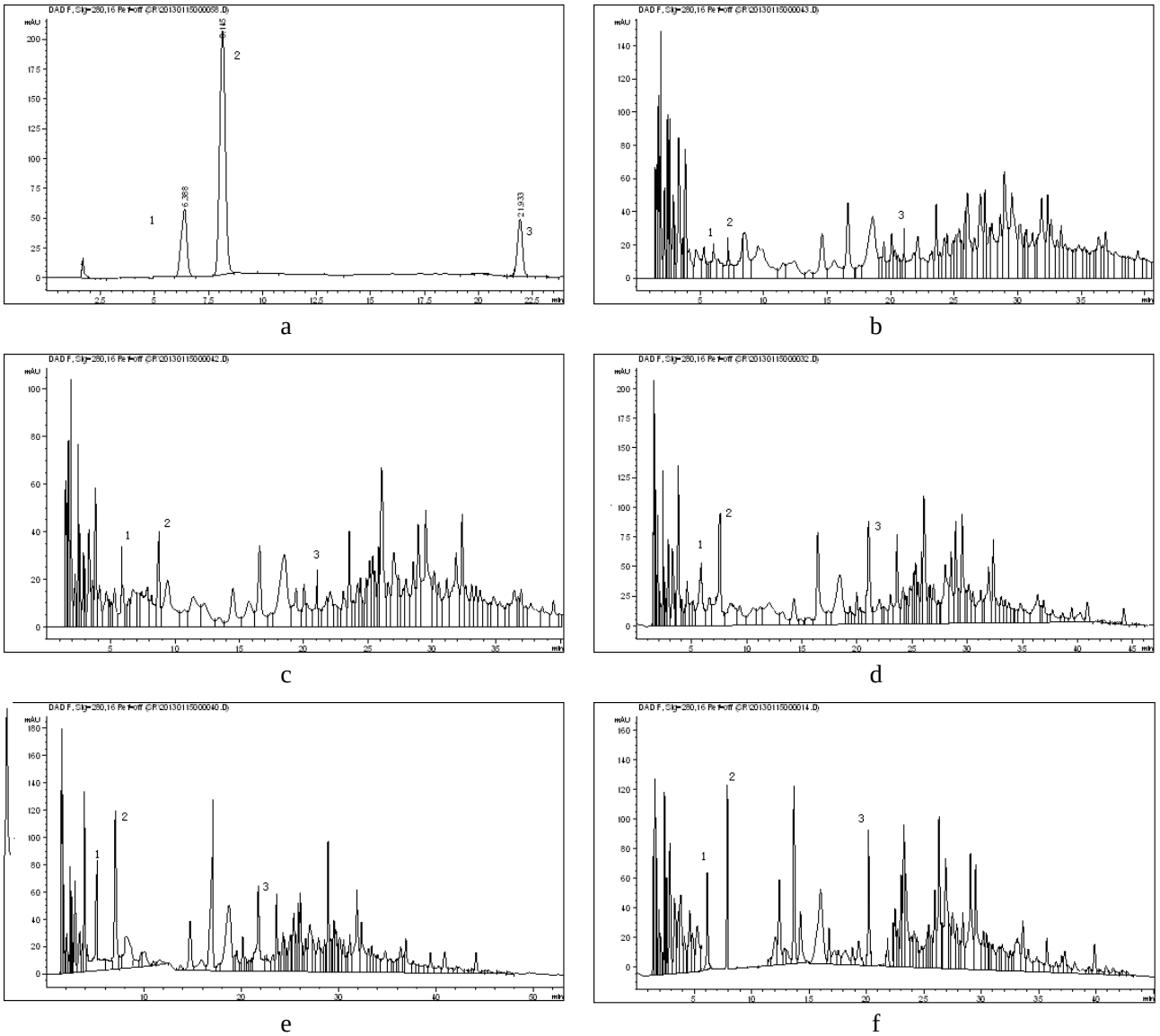

Fig. 2 HPLC chromatography of mixed standard (a) and different parts of sample (b. shoot; c. red shoot; d. intermediate stem; e. mature stem without color; f. red mature stem)
Peak 1. Cyanidin-3-*O*-glucoside chloride; 2. Pelargonin chloride; 3. Delphinidin chloride

4. Conclusions

The proposed extraction and RP-HPLC methods were successfully applied for measuring the content of three types of anthocyanins in *I. hispida* MeClure cv. 'Rainbow' simultaneously. The concentration of tested anthocyanins increases during the entire growing period, and the highest amounts were all found in red mature stem. These applicability results could be used as a good reference for a wide variety of colored bamboos.

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