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Determination of C3G Content in Blackish Purple Rice using HPLC and UV-Vis Spectrophotometer

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ABSTRACT: Cyanidin 3-glucoside (C3G) content contained in the grains of blackish purple rice varieties, Heugjinjubyeo, Kilimheugmi, Heugnambyeo, Sanghaehyanghyeolla, and the progenies derived from their crosses was evaluated by HPLC and UV-Vis spectroscopy. C3G content was higher in the range of 10~30% by using UV-Vis method compared to HPLC method. A significant linear relationship was, however, observed between two analytical methods. The correlation coefficient was 0.98. Thus, this results suggested that it would be able to use UV-Vis spectroscopy to determine C3G content which does not demanded precise value like selection.

Keywords: Blackish purple rice, anthocyanins, C3G, HPLC

Recently, consumers are increasing the demand for new natural, functional and healthful food. These demands have encouraged a better understanding of the biochemical, chemical and nutritional composition of plant products.

Blackish purple rice composition is of great interest because of the possible beneficial health effects associated with their anthocyanins content (Gabar, 1988; Vlaskovska *et al.*, 1990; Costantion *et al.*, 1992). The known anthocyanins in blackish purple rice are cyanidin, peonidin, malvidin, pelagonidin, delphinidin (Nagai *et al.*, 1960; Reddy *et al.*, 1994; Tsuda *et al.*, 1994; Cho *et al.*, 1996; Choi & Oh, 1996; Ryu *et al.*, 1998).

The anthocyanins are part of the group of flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium of flavylium salts (Fig. 1). Anthocyanins are individualized by the differences of the number of hydroxyl groups, the degree of methylation of these hydroxyl groups, the nature and number of sugars attached to the molecule and the position of the attachment (Mazza & Miniati, 1993).

The analysis of anthocyanins is complicated because of their ability to undergo structural transformations and complexation reactions and they are difficult to measure independently of other flavonoids because they have similar structural and reactivity characteristics. Thus, so far, the most satisfactory method for mixture analysis is the multistep method by HPLC.

Preliminary treatment for HPLC, however, is very difficult and time consuming work for breeder just to select the high pigmented blackish purple rice varieties. This is the reason why it is practically impossible to breed a high anthocyanins rice cultivar. In order to alternate HPLC for quantitative analysis to relatively convenient UV-Vis spectroscopy, we compare the C3G content according to the analytical instruments, UV-Vis and HPLC.

MATERIALS AND METHODS

Plant Materials

Five blackish purple rice cultivars (Heugjinjubyeo, Kilimheugmi, Heugnambyeo, Sanghaehyanghyeolla, and Suwon 425), and their hybrid progenies (F_1, F_2, F_4, and F_5) were cultivated and harvested in an experimental field at the National Crop Experiment Station at Suwon, Korea in 2000.

Extraction Method

Each hulled rice was ground to pass 30-40 mesh screen and 2 g of pulverized rice was extracted three times with 25 m*l* of 0.1% trifluoroacetic acid (TFA) -95% ethanol in a 100 flask for 4 h under vigorous agitation and reflux conditions. The extract was pre-filtered through Whatman No. 2 filter paper and concentrated to a final volume of 5 in a

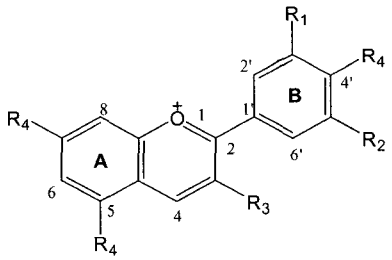

Fig. 1. The flavylium cation. R_1 and R_2 are H, OH or OCH_3; R_3 is a glycosyl or H; and R_4 is OH or a glycosyl.

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rotary evaporator at 30°C. The aqueous extract was placed in a volumetric flask and made up to 25 m*l* with extraction solvent and then replaced at a refrigerator (4°C) until analysis.

Pigment Quantification

Anthocyanins content in blackish purple rice were determined by two different analytical methods, HPLC and UV-Vis spectrophotometer. The aqueous solution of the anthocyanins (4°C) was passed through a Whatman 0.45 μm-PVDF syringe filter. The anthocyanins of the filtrate was evaluated by UV-Vis spectrophotometer at 530 nm and then injected to HPLC system.

The standard solutions in various concentration of cyanidin 3-glucoside and peonidin 3-glucoside was injected on the HPLC. C3G standard was isolated from Heugjinjubyeo (Park *et al.*, 1998). Peonidin 3-glucoside was purchased from Extrasynthese (Genay, France).

Individual anthocyanins were analyzed by reversed-phase HPLC equipped with two model 510 pumps; Millipore gradient controller; Waters 480 UV-Vis detector, 530 nm fixed wavelength; ODS-5 column (4.6 mm×250 mm, Nomura Chemical, Japan); linear gradient from 0.1% TFA-H_2O to 0.1% TFA-CH_3CN for 30 min as elution solvent at a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

It is theoretically impossible to determine anthocyanins content in acidic alcohol extract by UV-Vis spectroscopy. Because several kinds of anthocyanins(cyanidin 3-glucoside, cyanidin 3-rhamnoside, malvidin 3-galactoside, peonidin 3-glucoside, etc.) exist in blackish purple rice bran(Nagai *et al.*, 1960). Also, Ryu *et al.* (1998) reported that major anthocyanins of blackish purple rice cultivated in korea were C3G and peonidin 3-glucoside (P3G). However, content of C3G was about twenty times higher than that of P3G, and other anthocyanins was not detected in mostly cultivated blackish purple rice in domestic (Ryu *et al.*, 2000). Among anthocyanins which could be contained in blackish purple rice, C3G is dominant. Thus, to select a greater pigment content rice variety would be selection higher C3G content rice variety.

Anthocyanins from blackish purple rice varieties were separated by HPLC. The HPLC chromatogram pattern of anthocyanins and its UV-Vis absorption spectra are shown in Fig. 2 and 3, respectively. The blackish purple rice varieties used in this study showed the simplest anthocyanins pattern

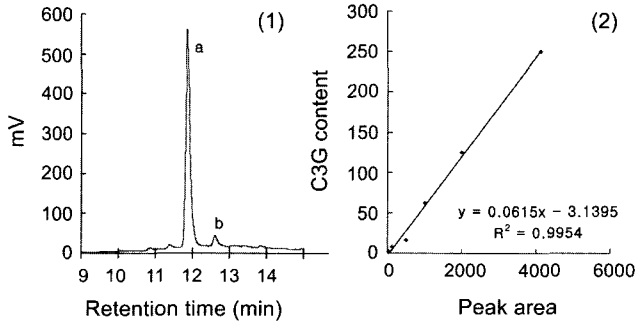

Fig. 2. HPLC chromatogram of anthocyanin extracts of Heugjinjubyeo(1) and calibration curve prepared by plotting different concentration of standard C3G versus area measurements in HPLC(2). Peak identification as follow: (a) cyanidin 3-glucoside (*Rt*=11.83); (b) peonidin 3-glucoside (*Rt*=12.60).

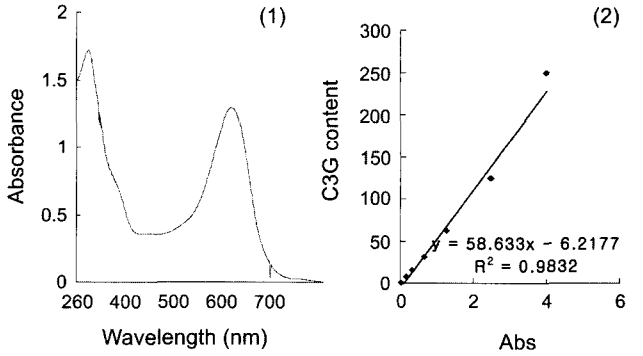

Fig. 3. UV-Vis absorption spectra of anthocyanin extracts of Heugjinjubyeo(1), and calibration curve prepared by plotting different concentration of standard C3G versus absorbance intensity measurements at 530 nm(2).

Table 1. The difference of C3G content depending on the analytical instrument, UV-Vis spectrophotometer and HPLC.

Cultivar	Anthocyanin content (mg/100g brown rice)			I- II
	UV-Vis (I)	HPLC (II)		
		C3G	P3G	
Heugjinjubyeo	592.0	417.2	5.9	169.1
Killimheugmi	591.4	407.4	6.8	177.2
Suwon425	192.3	153.2	4.4	34.7
Heugnambyeo	193.6	147.7	3.7	42.2
Sanghaehyanghyeolla	57.1	41.1	2.9	13.1

with only two peaks, cyanidin 3-glucoside and peonidin 3-glucoside(P3G).

Quantitative results for the tested varieties are shown in Table 1. Heugjinjubyeo showed the greatest amounts of C3G and P3G (417.2 and 5.9 mg/100 g brown rice, respectively), Kilimheugmi showed its C3G and P3G as 407.4 and 6.8 mg/100 g brown rice, respectively. Suwon 425, Heugnambyeoo and Sanghaehyanghyeolla showed their C3G content as 153.2, 147.7, and 41.1, respectively, and their P3G content was as low as 5 mg/100 g brown rice.

According to Ryu *et al.* (2000) a great part of anthocyanins of blackish purple rice cultivated in korea was C3G. Therefore we assuemed that the value at 530 nm measured by UV-Vis spectrophotometer was nearly C3G. Hence, C3G content was more highly determined as using UV-Vis spectroscopy compared to HPLC, and its difference depended on C3G content. C3G content determined by UV-Vis spectrophotometer showed Heugjinjubyeo as 592.0 mg/100 g brown rice, Kilimheugmi 591.4 mg/100 g brown rice. The difference of C3G content between two analytical methods, HPLC and UV-Vis spectroscopy, was about 170 mg/100g brown rice. On Suwon 425, Heugnambyeo, and Sanghaehyanghyeolla the C3G content difference were in the range of 13.142.2 mg/100g brown rice. C3G content by UV-Vis was overall high in the range 1030% compared to HPLC in 253 plants of progenies of the crosses between blackish purple rice varieties(data not shown). There appeared to be the relationship between C3G content determined by HPLC and that by UV-Vis spectroscopy. C3G content of 253 plants of which pedigree were different were investigated both by HPLC and UV-Vis. The relationship between C3G content determined by HPLC and UV-Vis spectroscopy in all tested varieties is presented Fig. 4. A significant linear relationship was observed between HPLC and UV-Vis spectroscopy. The correlation coefficient was much higher (r=0.98). This results suggested that it would be able to use UV-Vis spectroscopy to determine C3G content which does not demanded precise value like selection, for instance.

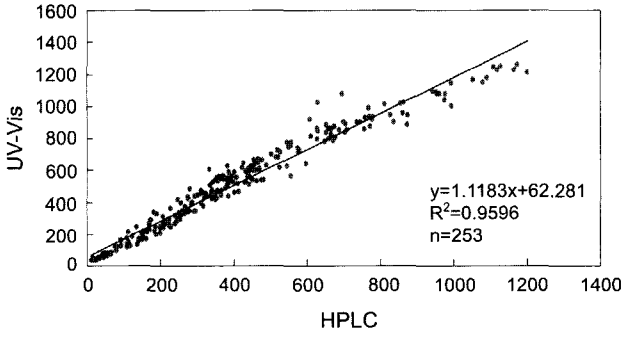

Fig. 4. Relationship between C3G content (mg/100g brown rice) determined by different analytical methods, HPLC and UV-Vis spectroscopy.

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