Korean J. Crop Sci. 45(4): 257~260(2000)

Antioxidative Activity and Varietal Difference of Cyanidin 3-glucoside and Peonidin 3-glucoside Contents in Pigmented Rice

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ABSTRACT: The content of anthocyanin, cyanidin 3-glucoside (C3G) and the peonidin 3-glucoside (P3G) from 591 rice cultivars and the antioxidative activities of MeOH extract from 8 rice cultivars were evaluated. Among them, C3G content of pigmented rice were ranged from 0 to 451.9 mg in 100 g brown rice, while the P3G contents were in the range from 0 to 42.7 mg in 100 g brown rice. There was no correlation between C3G and P3G content. Total anthocyanin content ranged from 0 to 475.1 mg in 100 g brown rice. The antioxidative activity, the scavenging activity on DPPH (2,2-diphenyl-picryl-hydrazyl) radical of MeOH extracts from rice grain, were different according to cultivars. The activity of blockish purple pericarp rice cultivars was twice stronger than that of white pericarp cultivar. Especially, the antioxidative activity of Heugjinjubyeo was four times stronger than that of white pericarp cultivar. The scavenging effect on DPPH radical in rice extracts was related to the total anthocyanin contents of the extracts.

Keywords : pigmented rice, C3G, P3G, HPLC, antioxidative activity, DPPH.

In living system, oxygen species such as hydroxy radicals, superoxide anion radicals, and singlet oxygens are thought to be agents that attack polyunsaturated fatty acids in cell membranes and give rise to lipid peroxidation. Lipid peroxidation is strongly associated with aging and carcinogenesis (Yagi 1987, Harman 1982, Cutlar 1984).

Tsuda *et al.* (1994) reported that the anthocyanin pigments, cyanidin-3-glucoside (C3G) and cyanidin (Cy), had antioxidative activity and they showed stronger activity than *a*-tocopherol in the rat liver microsomal system. Among these anthocyanin, Kuromanin (cyanidin 3-glucoside) had the highest oxygen radical absorbing capacity (ORAC) activity, which was 3.5 times stronger than Trolox (Vitamin E analogue), while pelargonin had the lowest antioxidant activity but was still as potent as Trolox (Wang *et al.* 1997).

Antocyanins are natural pigments belonging to the flavonoid family. They are widely distributed among flowers, fruits and vegetables and are responsible for the bright colors such as orange, red and blue (Wang *et al.* 1997). They play a definite role in attracting animals in pollination and seed dispersal. They may also have a role in the mechanism of plant resistance to insect attack (Strack and Wray 1993).

Antocyanin has recently begun to be regarded as a biologically active substance, as well as a colorant. For example, anti-inflammatory activity, redox potential and antioxidative activity have been studied (Vlaskovska *et al.* 1990, Gabor 1988, Drenska *et al.* 1989, Contantion *et al.* 1992, Meunier *et al.* 1989, Igarashi *et al.* 1989).

Black rice, having dark purple pigmented pericarp, is well known for its rich nutrients and medicinals. Pigments of rice cultivars vary greatly in distribution and intensity, and thus provide a fascinating array of topic for taxonomic and genetic studies. Various pigmented rice can be characterized by anthocyanin and nonanthocyanin pigments, which are found in the bran or hull.

Ryu *et al.* (1998) reported ten rice varieties containing two major anthocyanins, cyanidin 3-glucoside (C3G) and peonidin 3-glucoside (P3G). Total anthocyanin contents varied greatly in the range of 0~493 mg/100 g grain among varieties. Also, Kim *et al.* (2000) reported that the pigment contents of 100 segregating lines in colored rice were in the range of 0~12,877 ppm.

The objective of the present study was to find varietal differences of anthocyanin (C3G and P3G) content and antioxidative activity

MATERIALS AND METHODS

Material and reagent

Anthocyanin pigments in 591 rice cultivars including 186 white rice, 90 pigmented rice, 245 collected weedy red rice in domestic and 70 pigmented rice accessions from gene bank of Rural Development Administration were quantified by high performance liquid chromatography (HPLC).

The rice seeds were cleaned and stored at 4°C until use.

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<Received August 21, 2000>

The rice seeds were polished in sieve to obtain uniform seed fractions. Trifluoroacetic acid (TFA) were obtained from Sigma Chemical Co. (St. Louis Mo, USA).

Quantification of anthocyanin pigment level

HPLC analysis were performed with waters 501 pump, millipore gradient controller, waters 480 UV-Vis spectrophotometer. About 2 g of sample was accurately weighed and shaken with 20 ml of 0.1% TFA-95% EtOH for 3 hours in triplicate at room temperature. The crude extract was filtered through filter paper (Whatman #2, Whatman International Ltd., England) in vacuo. The fliterate was filtered again through 0.45 μm PVDF syringe filter (Whatman Co. Ltd., USA), and then injected on HPLC. HPLC was performed by using a Develosil ODS-5 column (4.6 × 250 mm, Nomura chemical, Japan), Spectrophotometric detector with UV 530 nm and 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.

The standard solutions in various concentration of cyanidin 3-glucoside and peonidin 3-glucoside was injected on the HPLC. Standatd calibration curve was made by average value of peak area for the triplicate determinations of cyanidin 3-glucoside and peonidin 3-glucoside.

Antioxidative activity

Seven pigmented rice cultivars (Heugjinjubyeo, Yongjung 4, Iksan 440, Suwon 425, Sanghaehyanghyeolla, Heugnambyeo, Milyang 175) and one common white cultivar (Hwasungbyeo) were tested to examine antioxidative activity.

From each rice cultivar, 5 g of polished grain was dissolved in 50 ml MeOH and shaken for 24 hours in triplicate to extract antioxidants. The extract was filtered through Whatman No. 2 filter paper. The filterate was dried with a rotary evaporator in vacuo at room temperature, and immediately frozen at -70°C and then freeze-dried.

Each extract was dissolved in MeOH and diluted to various concentration. One ml of methanolic solution containing DPPH (1,1-diphenyl-2-picrylhydrazyl radical, 1.5 × 10^{-4}M) added to 4ml sample solution at various concentration.

The reaction mixture was shaken vigorously and then kept at room temperature for 30 min in air. The absorbance of the remaining DPPH was measured with a UV-Vis spectrophotometer at 517 nm. The radical scavenging activity of each compound was expressed as a 50% decrease (IC_{50}) in the absorbance of DPPH relative to the absorbance of DPPH solution in the absence of the sample, and the concentrations of the test compounds were based on experiments in which the compounds were tested at 5~7 different concentrations, in triplicate.

RESULT AND DISCUSSION

Varietal difference of C3G and P3G content

When extracts from Heugjinjubyeo were injected onto Develosil ODS-5 column (4.6 × 250 mm) and eluted with linear gradient from 0.1% TFA-H_2O to 0.1% TFA-CH_3CN at 1 ml/min, two peaks were observed at different retention time (Fig. 1). The peak (*R*t=12.33 min) corresponded to standard C3G, and the peak (*R*t=13.045 min) corresponded to standard P3G. It was reported that most widely distributed anthocyanins in blackish purple rice grain were cyanidin 3-glucoside, cyanidin 3-rhamnoside, malvidin 3-galactoside, peonidin 3-glucoside (Nagai *et al.* 1960). However, only two kinds of anthocyanin, C3G and P3G were detected in this study.

No anthocyanins are detected in 74 common white rices and 124 collected weedy red rices in domestic (data not shown).

Among 126 pigmented rice varieties accessions from gene bank of Rural Development Administration, three kinds of variety (Cheng Chang : 321.4, PI 160979-2 : 223.5, Hong-

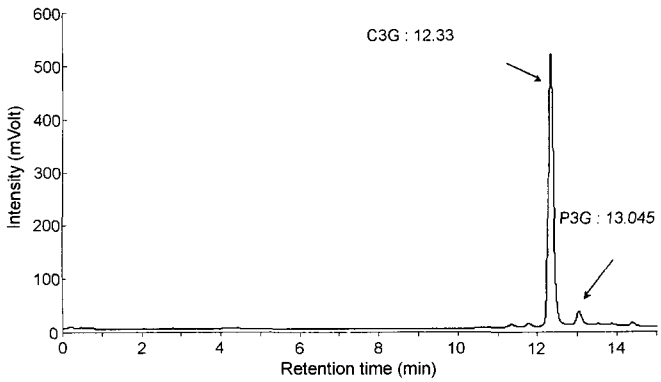

Fig. 1. HPLC chromatogram of Heugjinjubyeo extract. The Develosil ODS-5 column (4.6 × 250 mm, Nomura chemical co.,) was eluted with linear gradient from 0.1% TFA-H_2O to 0.1% TFA-CH_3CN at 1 ml/min.

Table 1. Frequency distribution of anthocyanin content in world collection pigmented rices.

Anthocyanin content (mg/100 g brown rice)		No. of cultivars
C3G	Below 50	113
	50-100	2
	100-200	5
	200 over	6
P3G	Below 5	119
	5-20	2
	20 over	5

Table 2. Content of anthocyanin, cyanidin 3-glucoside (C3G) and peonidin 3-glucoside (P3G) in high pigmented rice cultivars investigated.

Cultivar	Pericarp color	Anthocyanin content (mg/100 g brown rice)			Origin
		C3G	P3G	Total	
Heugjinjubyeo	blackish purple	451.9	23.2	475.1	Korea
Cheng-Chang	〃	321.4	42.7	364.1	China
Kilimheugmi	〃	278.3	8.6	286.9	〃
Yongjung 4	〃	276.8	10.5	287.3	〃
PI 160979-2	〃	223.5	31.4	254.9	〃
Hong-Shei-Lo	〃	220.5	31.9	252.4	〃
Suwon 425	〃	191.5	5.6	197.1	Korea
PI 160979-1	〃	185.9	26.7	212.6	China
Mitak	〃	185.6	trace	185.6	India
Iksan 440	〃	138.0	3.7	141.7	Korea
Sanghaehyanghyeolla	〃	115.7	3.5	119.2	China
Milyang 175	〃	87.1	trace	87.1	Korea
Heugnambyeo	〃	84.2	〃	84.2	〃
Hongmi	redish brown	trace	〃	trace	〃
Jagwangbyeo	〃	〃	0.0	〃	〃
Hwasungbyeo	white	0.0	0.0	0.0	〃

Shei-Lo : 220.5) were above 200 mg/100 g brown rice in C3G content (Table 1). But in most of pigmented rices C3G content was below 50 mg/100 g brown rice. P3G content was also investigated. P3G content was over 20 mg/100 g brown rice in 4 varieties, while most of tested varieties had less than 5 mg/100 g brown rice.

Table 2. shows the anthocyanin content of pigmented rices which was evaluated as high pigmented rice cultivars. C3G and P3G were only detected in blackish purple pigmented rices, while no anthocyanins were detected in redish brown or white pericarp cultivars.

C3G content showed variation from 0 to 451.9 mg/100 g grain. Also, P3G content was 0 to 42.7 mg/100 g grain.

C3G, an agent having important oxygen radical absorbing capacity (Wang *et al.* 1997) was most abundant in Heugjinjubyeo, while P3G content was Cheng-Chang variety. As for the distribution ratio of individual anthocyanin, Cheng-Chang was characterized by relatively high percentage of P3G (42.7 mg/100 g grain), while Kilimheugmi and Suwon 425 were characterized by relatively lower amount of P3G.

As presented, the C3G content was not correlation with the P3G content in blackish purple pericarp rice. However, peonidin was synthesised through cyanidin methylation by methyltransferase, which was controlled by a separate gene (Mazza G. and E. Miniati 1993).

Antioxidative activity of pigmented rice

For the determination of the scavenging activity of pigmented rice cultivars, the bleaching of DPPH by MeOH extracts was measured. Table 3 shows the DPPH radical scavenging activity of the extracts of tested rice cultivars. The result showed that all of pigmented rice cultivars have the scavenging activity (IC_{50} : 57.4~106.9 ppm) compared with common white rice cultivar Hwasungbyeo (IC_{50} : 284.8 ppm). Especially, the extract of Heugjinjubyeo showed strong scavenging activity (IC_{50} : 57.4), which was almost half concentration to the other pigmented rice cultivars (IC_{50} : 90.8~106.9).

Table 3. DPPH radical scavenging activity of MeOH extracts of rice grain.

Cultivars	Anthocyanin content	Radical scavenging activity (IC_{50})
Heugjinjubyeo	475.1	57.4
Yongjung 4	287.3	90.8
Suwon 425	197.1	92.9
Heugnambyeo	84.2	96.1
Sanghaehyanghyeolla	119.2	101.1
Milyang 175	87.1	106.3
Iksan 440	141.7	106.9
Hwasungbyeo	–	284.8

The scavenging effects on DPPH radical of rice MeOH extracts were negatively related with the total anthocyanin contents of the extracts (Fig. 2).

The radical scavenging effects of antioxidants on the DPPH radical are thought to have been due to their hydro-

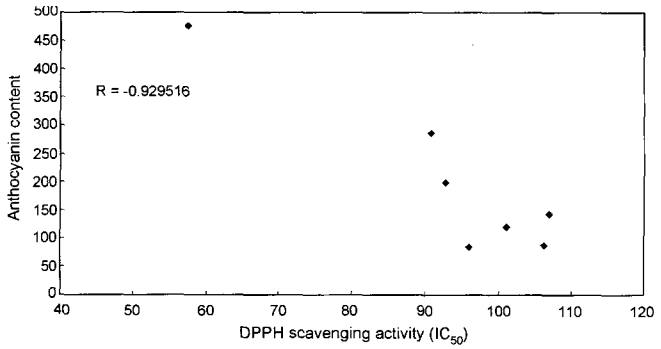

Fig. 2. Relationship between anthocyanin content and DPPH radical scavenging activity (IC_{50}).

gen donating ability. The test compounds contained anthocyanins, and anthocyanin had phenolic hydroxyl groups in the structure, and phenolic antioxidants have been recognized to function as electron or hydrogen donors (Husain S.R. *et al.* 1987). Thus, the DPPH radical scavenging activity of these compounds may be mostly related to not only their phenolic hydroxyl group but their anthocyanin content.

Thus, rice cultivars Heugjinjubyeo may be also related to high antioxidative activity.

REFERENCES

Costantion, L., A. Albasini, G. Rastell, and S. Benvenuti. 1992. Activity of polyphenolic crude extacts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med.*, 58 : 342-344.

Cutlar, R. G. 1984. Antioxidants, aging, and longevity. In Free Radicals in Biology; Pryor, W. A., Ed.; Academic Press; Orlando, FL, Vol. 6. Chapter 11.

Drenska, D., I. Bantutova and R. Ovcharov. 1989. Anticonvulsant effect of anthocyanins and antioxidants. *Farmatsiya*(Sofia) 39, 33.

Gabar, E. 1988. Possible biological role of some anthocyanins in food. *Bull Liaison-Group Polyphenols.* 14 : 130-133.

Harman, D. 1982. The free-radical theory of aging. InFree Radicals in Biology; Pryor, W. A., Ed.; Academic Press: Orlando, FL, Vol. 5. Chapter 8.

Husain S. R., J. Cillard, and P. Cillard. 1987. Hydroxyl radical scavenging activity of flavonoids. *Phytochemisty.* 26 : 2489-2491.

Igarashi, K., K. Takanashi, M. Makinl, and T. Yasui. 1989. Antioxidative activity of mahor anthocyanin isolated from wild grapes (Vitis coignetiae), *Nippon Shokuhin Kogyo Gakkaishi.* 36 : 852-856.

Kim, S. L., J. J. Hwang, J. Song, J. C. Song, and K. H. Jung. 2000. Extraction, purification, and quantification of anthocyanins in colored rice, black soybean, and black waxy corn. *Korean J. Breed.* 32(2) : 146-152.

Mazza, G. and E. Miniati. 1993. Anthocyanins in Fruits, Vegetables, and Grains. *CRC Press* : 20-23.

Meunier, M. T., E. Duroux, and P. Bastide. 1989. Antioxidant activity of procyanidol oligomers and anthocyanins with regard to superoxide anion and lipid peroxidation. Plant Med. *Phytother.* 23 : 267-273.

Nagai, I., G. Suzushino, and Y. Tsuboki. 1960. Anthoxanthins and anthocyanins in Oryzaceae. *I. Jap. J. Breed.* 10(4) : 47-56.

Park, S. Z., J. H. Lee, S. J. Han. H. Y. Kim, and S. N. Ryu. 1998. Quantitative analysis and varietal difference of cyanidin 3-glucoside in pigmented rice. *Korean J. Crop Sci.* 43(3) : 179-184.

Strack, D. and V. Wray. 1993. The anthocyanins, The Flavonoids : Advances in research since. 1986 : Harborne, J. B., Ed., Chapman Hall. London. 1-22

Tsuda, T., M. Watanabe, K. Ohshima, S. Norinobu, S. W. Choi, S. Kawakishi, and T. Osawa. 1994. Antioxidative activity of the anthocyanin pigments cyanidin 3-O-β-D-glucoside and cyanidin. *J. Agric. Food Chem.* 42 : 2407-2410.

Vlaskovska, M., D. Drenska, and R. Ovcharov. 1990. Effect of antioxidants, alone and in combination, on the inflammatory process, *Probl. Vutr Med.* 18 : 13-19.

Wang, H., G. Cao, and R. L. Prior. 1997. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* 45 : 304-309.

Yagi, K. 1987. Lipid peroxides and human disease. *Chem. Phys. Lipids.* 45 : 337-341.