

Antioxidant Activity of Blackish Purple Rice

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ABSTRACT : Anthocyanin in blackish purple rice is composed of cyanidin, peonidin, malvidin, pelargonidin and delphinidin flavylum ion and their glucosides, which shows antioxidant activity similar to α -tocopherol. It has been demonstrated that cyanidin-3-glucoside(C3G) of anthocyanin has a high-potency antioxidant activity, and is a prominent in anticancer and antithrombotic activity. In analysis of content of anthocyanin from the genetic source of blackish purple rice, the level of C3G was approximately 80% of total contents of anthocyanins, and showed the highest content in Heugjinjubyeo, of which contains an amount of 500 mg by weight of 100g brown rice in comparison that most of varieties has less than 50mg by weight of 100g brown rice. We found that C3G pigment in blackish purple rice was considerably affected by cultural season, cultivation area, ripening temperature, and weather condition, etc. as well as genetic properties. In cross combination between Heugjinjubyeo and Suwon425, intermediate parent F₃ generation which was three times higher or more in C3G content than that of Heugjinjubyeo was bred, of which it contained an amount of 1678 mg by weight of 100g brown rice, and we called it "C3GHi" Rice. And, we found that the C3GHi rice was more excellent than that of the existing Heugjinjubyeo in anticancer and antithrombotic activity. Methanol extract from Heugjinjubyeo was fractionated by organic solvents in order of n-hexane, CH₂Cl₂ and n-BuOH, and then oryzafuran, quercetin, vanillic acid and protocatechuic acid and their structures from the n-BuOH fraction were ascertained. Oryzafuran of these compounds was a natural compound found firstly in nature. Quercetin and protocatechuic acid besides new compound oryzafuran showed excellent antioxidant ability to vitamin C. These results suggest that blackish purple rice has very high value as a source of various functional food as well as staple food.

Keywords: blackish purple rice, anthocyanin, cyanidin-3-glucoside(C3G), antioxidant activity, anticancer, antithrombotic activity, C3GHi, Heugjinjubyeo, oryzafuran, quercetin, protocatechuic acid

The amount of annual production of rice in Korea has been maintained an average of about 4,632 thousand tons for the recent 10 years, but the per capita consumption of rice is reduced 24.2 kg in 104.9 kg(1996) to 80.7 kg(2004). This reflected the fact that a national dietary life pattern come to be various.

On the other hand, demand for functional agriculture-products was increased because of latest well-being syndrome, and which also served as a momentum for needing various blend agricultural products. Soft rice and powdered rice including low-amylose rice, saccharinity enhanced saccharide rice, flavor rice enhanced with a fragrance ingredient are the example of functional rice(Choi *et al.*, 1996). Moreover, rice containing functional ingredient has been developed to be efficient at the prevention and treatment of an every kind disease(Ryu *et al.*, 2005).

This report relates to the recent research result about anthocyanin in blackish purple rice, we hereby expect to bring into relief a functionality of rice and to promote an industrial usage, and thus to contribute to increasing the income of farmers and a national health improvement, further to prepare a opportunity of maintaining farmland area which is dwindling.

Biosynthesis of anthocyanin in blackish purple rice and its contents

Anthocyanin identified in blackish purple rice is composed of cyanidin, peonidin, malvidin, pelargonidin, and delphinidin flavylum ion and their glucosides (Choi *et al.*, 1996; Choi and Oh, 1996; Nagai *et al.*, 1960; Reddy *et al.*, 1994; Ryu *et al.*, 1998; Tsuda *et al.*, 1994), which shows antioxidant activity similar to α -tocopherol (Kahkonen & Heinonen, 2003).

Anthocyanin in rice is accumulated at the pericarp and makes rice into blackish purple color. In analysis of anthocyanin content from the genetic sources of blackish purple rice (280 pieces) kept in Korean Collection for Type Cultures, the level of cyanidin-3-glucoside (C3G) was approximately 80% of total anthocyanin content, peonidin-3-glucoside(P3G) was distributed about 5%, and malvidin, pelargonidin, and delphinidin etc. were presented very low level (Ryu *et al.*, 1998; 2000).

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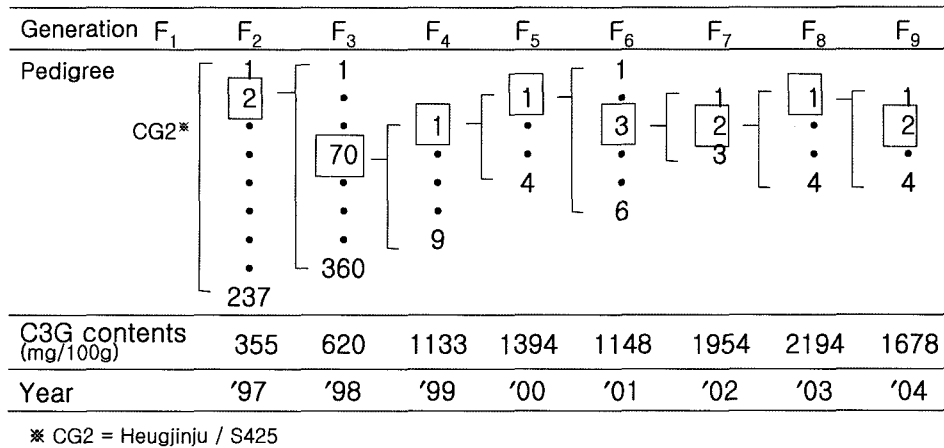


Fig. 1. Pedigree of high C3G selection.

The most breeds of blackish purple rice contained C3G an amount of less than 50 mg by weight of 100 g brown rice, only 6 breeds contained above 200 mg. In the most breeds of blackish purple rice, P3G was contained less than 50 mg per 100 g unpolished rice, only 5 breeds contained above 200 mg of C3G. Among these, Heugjinjubyee contained C3G 500 mg per 100 g unpolished rice has highest content.

However, In cross combination between Heugjinjubyee and Suwon425, lines (F₉, 1,678mg/100g brown rice) which was 3.4 times higher than that of Heugjinjubyee in C3G content was bred (Fig. 1). This line containing high C3G is expected to be used as intermediate parent.

A synthesis of anthocyanin in a plant is achieved through synthesis pathway of phenylpropanoid and synthesis pathway of flavonoid using phenylalanine as precursor (Fig. 2). The enzyme to play important role in anthocyanin synthesis is phenylalanine lyase (PAL), which undertakes the first step in synthesis pathway of phenylpropanoid to turn phenylalanine into cinnamate. Further, chalcone synthase(CHS) associated with flavonoid synthesis as former step of anthocyanin synthesis, dihydroflavonol 4-reductase (DFR) acting to connecting-ring of anthocyanin, and anthocyanidine synthase (ANS) directly dominating anthocyanin synthesis, are known to play important role.

As comparing blackish purple rice of high anthocyanin content (1,500 mg per 100 g of brown rice) with that of low anthocyanin content (500 mg per 100g of brown rice), it was revealed that quantity of PAL did not have the difference, but CHS was increased to about 70 times, DFR was increased to more than 100 times, and ANS was increased to near 2,000 times (unpublished). Accordingly, it can be perceived a relationship between anthocyanin content and expression of CHS, DFR and ANS-dase genes in biosynthesis pathway of anthocyanin in blackish purple rice

Antioxidant activity of blackish purple rice

1) Antioxidant material in plant

Antioxidant activity in human body is very important. Active oxygen varieties such as superoxide anion (O₂⁻) or hydroxyl radical (OH⁻), can be essentially functioned as defense material in one respect, but it can be also functioned as a strong oxidant to cause a disease such as tissue damage, inflammation, cancer, senility, hardening of the arteries, hyperpiesia and diabetes (Madavi *et al.*, 1995). In this way, as antioxidant material coped with oxidation pathway, superoxide dismutase (SOD) or catalase (CAT) *et al.*, have enzymatic antioxidant activity, and vitamin C, α-tocopherol, β-carotene, glutathione, flavonoid *et al.*, have unenzymatic antioxidant activity(Madavi *et al.*, 1995).

In the field of food processing, peroxides of lipid are main factor to decrease a quality of food, thus synthetic oxidation inhibitors have been used to prevent such factor and BHA, BHT, PG and TBHQ are widely used (MAAdavi *et al.*, 1995). But, as most of synthetic inhibitors can induce the cancer, the effort to find alternative natural antioxidant materials which are harmless to human beings has been widely processed.

As worthy of fulfilling such ability, alternate antioxidant material have been distributed widely in Plants, which is phenolic compound such as anthocyanin, catechin and flavonoid *et al.*, Green tea contains such phenolic compound in the level of 30% (Lin *et al.*, 1998). Especially, anthocyanin widely distributed in Plants, is known as a strong natural antioxidant material, and extracted from grape and strawberry etc. and used as food additives (Satue-Gracia *et al.*, 1997, Wang *et al.*, 1997). In recent, antioxidant activity of Anthocyanin in Blackish purple rice and catechin in reddish brown rice has been continually reported (Choi *et al.*, 1996, Ryu *et al.*, 2005).

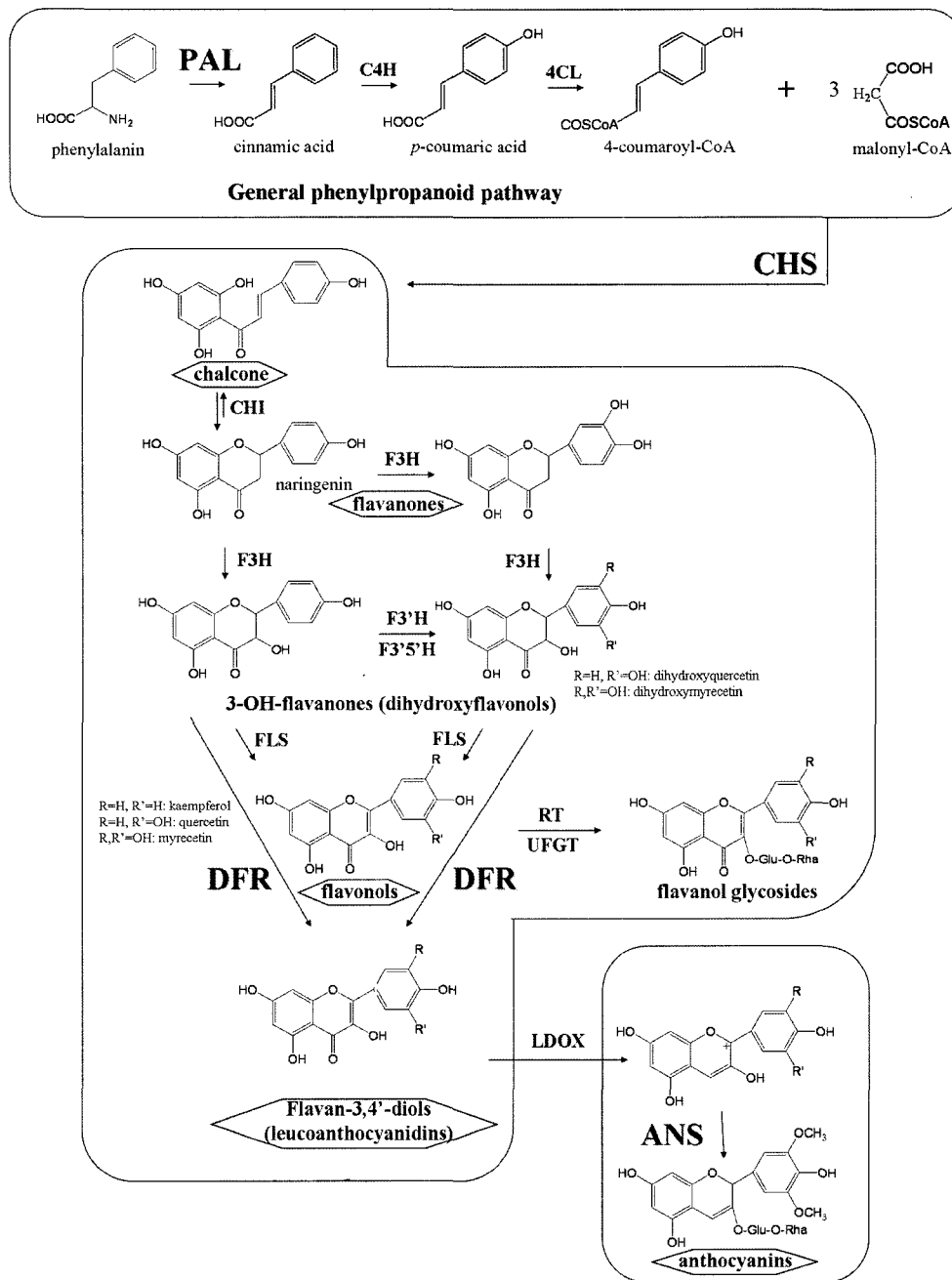


Fig. 2. Biosynthesis pathway of flavonoid and anthocyanin.

2) Antioxidant material in blackish purple rice and its activity

Brown rice contains plenty of essential natural nutrients such as vitamin E, for example α , β , γ , δ , tocopherol and tocotrienol etc., carotinoid(vitamin A) and vegetable sterol. Also, it contains various kinds of phenolic compounds including anthocyanin (Choi *et al.*, 1996).

Tocopherol was known for its anti-aging effect (Harman, 1980; Blackett & Hall, 1981), immunopotentiating and anti-

cancerous activity (Machlin, 1991; Moon & Micozzi, 1999; Nikiforva *et al.*, 1995) etc., after it had been found for the first time in 1922, and was also known for its skin-moisturizing effect (Eberlein-Konig *et al.*, 1998) and affirmative function on the therapy of dementia (Fahn, 1992). The γ -oryzanol which extracted from rice bran oil and reported firstly in 1954, is compound group in which ferulate bonded to vegetable sterol, and it have a basic structure of C27-C30 that OH group attaches to C-3 and one double bond exists in

5/6, 7/8, 8/9 or 22/23 etc.. The γ -oryzanol which is similar to cholesterol in its structure so that it is absorbed to the small intestine competitively with cholesterol to reduce cholesterol content in the blood (Rukmini & Raghuram, 1991). Furthermore, these ingredients in brown rice are compounds having strong antioxidant activity. By measuring FTC, reducing power and DPPH radical scavenging activity of brown rice to valuate antioxidant activity, it is recognized that polished rice such as Ilpumbyeo and Chujeongbyeo, and colored rice such as Heugjinjubyeo, Heughyangchalbyeo and Jeogjinjubyeo have good antioxidant activity similar to pure vitamin E (Fig. 3). Especially, reducing power and DPPH radical scavenging activity of colored rice extracts are superior to those of polished rice extracts (Fig. 4).

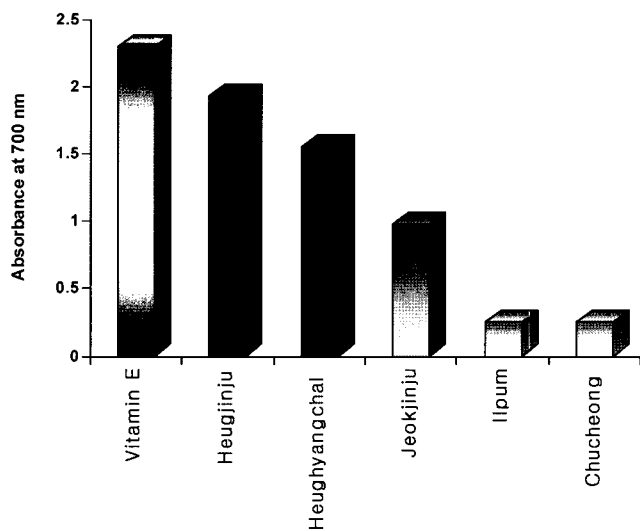


Fig. 3. Reducing power of 80% MeOH extract.

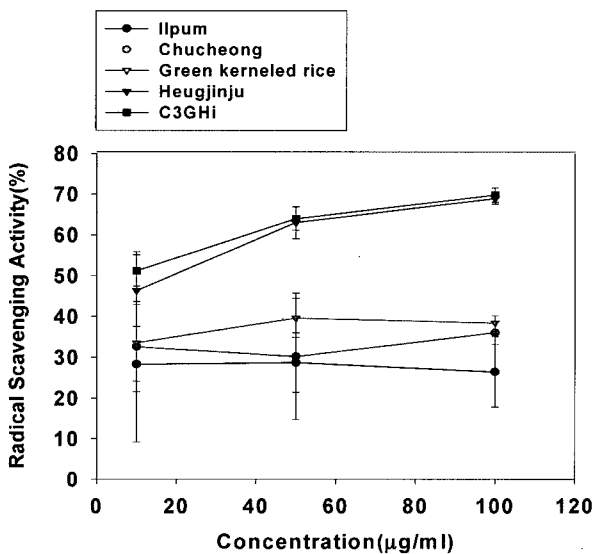


Fig. 4. Effects of rice extracts on radical scavenging activity.

Among colored rice, antioxidant activity of blackish purple rice (Heugjinjubyeo) is found superior to that of reddish brown rice (Jeogjinjubyeo), this can be determined to the general reason as blackish purple rice contains anthocyanin.

In order to measure a food stability of rice containing C3G highly, pigment partition of Heugjinjubyeo was orally administrated to ICR mice for 14 days, and then acute toxicity was estimated, such that the safety was proved from all target mice (Ryu *et al.*, 2000)

Also, rice bran containing C3G was fed to 15 pigs(average weigh 19.91 ± 1.80 kg) to evaluate a physiological availability(not published). Growth Performance of treating zone, to which 2% of general rice bran and 4% of rice bran containing C3G are added respectively, did not show significant difference. However, there are observed that cholesterol and neutral fat contents of the pig which fed rice bran containing C3G was low than that of the pig which fed general rice bran, and especially, cholesterol and neutral fat contents of the treating group which fed 4% of rice bran containing C3G was lowest. Also, GOP(glutamate oxaloacetate transaminase), GPT(glutamate pyruvate, oxaloacetate transaminase) level, which can be called as an index of antioxidant activity, was low in the treating group which fed rice bran containing C3G, but it is not significant statistically.

3) Environmental variation of the anthocyanin content in blackish purple rice

We have been found that the anthocyanin content contained in blackish purple rice was considerably affected by cultural season, cultivation area, ripening temperature, weather condition, etc. as well as genetic properties(Kang, 2002).

In investigation for the effect of cultural season which influences on anthocyanin content in Heugjinjubyeo, C3G content was higher in case of lately seeding (May 30) than that of early seeding (April 1) or ordinary seeding (April 20). Also, in the experiment to change a temperature condition in the day and night during grain filling, C3G content was increased when a temperature variation between the day and night (day:27, night:18) was high and temperature variation in the night (18) was low. Additionally in cultivation area, C3G content was higher at a plain region than a mountain region.

Further, ethephon[(2-chloroethyl)phosphonic acid][Faragher and Brohier, 1984) solution known as closely connected with coloring of anthocyanin, was sprayed on an ear of Heugjinjubyeo at interval of 5 days after earing time, and consequently C3G content was increased to 2~3 times in the treatment on the 18th day after earing time.

Methanol extract from Heugjinjubyeo rice bran was fractionated by n-hexane, CH_2Cl_2 and n-BuOH in sequence, and

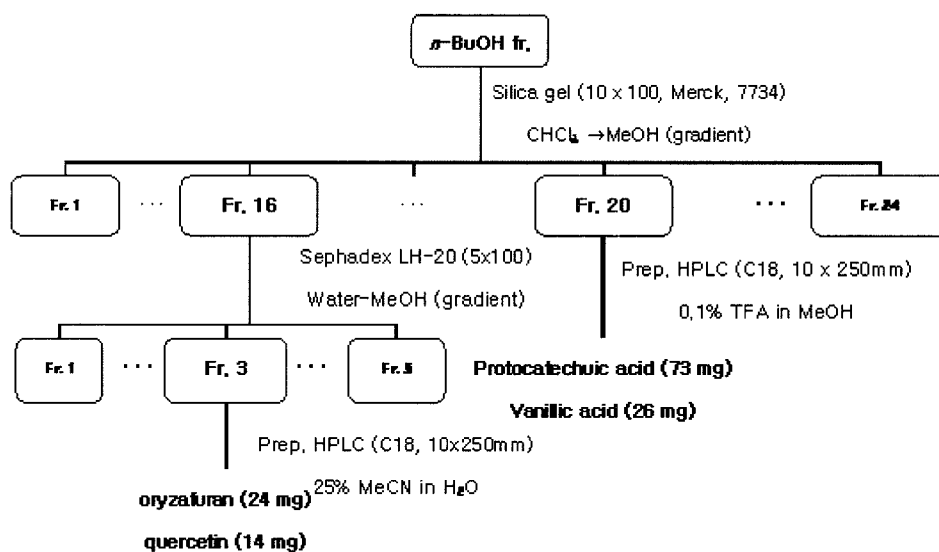


Fig. 5. Isolation scheme of compounds from the BuOH fraction of Heugjinjubyeo.

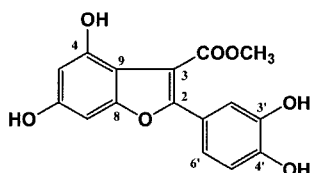


Fig. 6. Chemical structure of oryzafuran.

Table 1. DPPH free radical scavenging activity of compounds isolated from BuOH fraction of Heugjinjubyeo.

Compounds	EC50 ($\mu\text{g}/\text{ml}$)
Oryzafuran	1.58 \pm 0.001
Quercetin	2.73 \pm 0.004
Protocatechuic acid	2.33 \pm 0.007
Vanillic acid	>20.00
Ascorbic acid	3.35 \pm 0.006

then oryzafuran, quercetin, vanillic acid and protocatechuic acid was obtained by separation of the *n*-BuOH fraction to determine their structures (Fig. 5). Among these compounds, oryzafuran was a natural compound found firstly in nature, and has a structure showed in Fig. 6 (Han *et al.*, 2004).

In the result of the examination about antioxidant activity of phenolic compounds isolated from *n*-BuOH fraction, three remainder compounds except vanillic acid showed excellent ability to vitamin C.

Summary

Anthocyanin in blackish purple rice is composed of cyani-

din, peonidin, malvidin, pelargonidin, and delphinidin flavylium ion and their glucosides, which showed antioxidant activity similar to α -tocopherol. It has been known that Cyanidin-3-glucoside (C3G) among anthocyanin showed excellent antioxidant, anticancer and antithrombosis ability.

In analysis of content of anthocyanin from the genetic source of blackish purple rice, the level of C3G was approximately 80% of total contents of anthocyanin, and showed the highest content in Heugjinjubyeo of which it contains an amount of 500 mg by weight of 100 g brown rice (most of varieties has less than 50 mg by weight of 100 g brown rice). We found that C3G in blackish purple rice was considerably affected by cultural season, cultivation area, ripening temperature, weather condition, etc. as well as genetic properties.

In cross combination between Heugjinjubyeo and Suwon-425, intermediate parent (F9, 1,678 mg/100 g brown rice) generation which was the three times higher or more in C3G than that of Heugjinjubyeo was bred, and we called it "C3GHi" rice. We found that C3GHi rice was more excellent than that of the existing Heugjinjubyeo in anticancer and antithrombotic activity.

Oryzafuran, quercetin, vanillic acid and protocatechuic acid were separated from *n*-BuOH fraction which is Methanol (MeOH) extracts of Heugjinjubyeo, and Oryzafuran of these compounds was firstly found in nature as a nature compound.

Quercetin and protocatechuic acid besides new compound oryzafuran showed excellent antioxidant activity to vitamin C.

These results suggest that blackish purple rice has very high value as a source of various functional food as well as staple food.

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