Chapter 4
Nutrition Profiles of Black Rice

Black rice is truly remarkable in its abilities which are loaded with antioxidants, vitamin E, fiber, and valuable anti-inflammatory properties. Black rice anthocyanins are extracted from the aleurone layer of rice seed which is a major cereal crop existing since ancient times in China and other Eastern Asian countries (Ling et al. 2002). Little is known about the phytochemical profiles and antioxidant activities of different black rice varieties. Research shows that there are significant differences in phytochemical content and antioxidant activity among the different black rice varieties (Zhang et al. 2010). One half cup serving of cooked or about one-fourth cup uncooked black rice contains approximately (in daily recommended values) (www.blackrice.com).

- 160 cal (669 kJ) carbohydrates,
- 1.5 g of fat,
- 34 g of carbohydrates,
- 2 g of fiber, and
- 7.5 g of protein.

Black rice has a number of nutritional advantages over common white rice such as higher protein content, higher vitamins and minerals, although the letter varies with cultivar, production location and production technique (Suzuk et al. 2004). One serving of black rice contains only around 160 cal, but offers a high amount of flavonoid phyto nutrients, a good source of important fiber, substantial mineral content including iron and copper, and even a good source of plant based protein. This rice has the highest levels of anthocyanin, an antioxidants found in any food. Black rice is a healthy source of minerals especially iron. It contains vitamin E and is lower in sugar than berries that have similar phytochemical qualities. Black rice is fiber rich and nutritionally similar but not identical to whole grain brown rice. It contains 18 amino acids, minerals and vitamins like copper, iron, zinc, and carotene.

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It is estimated that 50 g of black rice provides about 35 % of the recommended dietary allowance of Se, Cu, Zn, and Mn per day.

> "one spoonful of black rice bran contains more anthocyanin antioxidants than a spoonful of blueberries and better yet, black rice offers more fiber and vitamin E antioxidants, but less sugar." 240th National Meeting of the American Chemical Society (ACS).

4.1 Nutrition of Different Rice Types

Different rice types are available today and the most common one is the white rice. Out of all the types, black rice is the one that contains the highest amount of nutrition. A comparison of different rice types that differ in terms of nutrient content when compared with 100 g serving of each kind (Table 4.1) (www.naturalnews.com).

The antioxidant activity of all rice bran extracts indicates high antioxidant efficiency in the following order: red>black>white color rice brans (Muntana and Prasong 2010). The Fe, Zn, Cu, and Mn concentration of the colored rice is higher than that of the white rice, and the four mineral concentrations in brown rice is Zn>Fe>Cu>Mn. In addition, Fe and Zn concentration of colored rice are affected by pigment content in pericarp of it, Cu and Mn of color rice are not influenced by pigment content in pericarp of it. Fe concentration of the colored rice is black rice>green rice>brown rice>red rice>yellow rice; Zn concentration of the colored rice is green rice>red rice>black rice>brown rice>yellow rice; Cu concentration of the colored rice is black rice>brown rice>red rice>yellow rice>green rice and Mn concentration of the colored rice is brown rice>black rice>red rice>yellow rice>green rice. There are some differences among Fe, Zn, Cu, and Mn average concentrations in brown rice. Of the different colored rice, black rice and brown rice are rich on Fe, Zn, Cu, and Mn; green rice are rich on Fe and Zn and red rice is rich on Zn and Cu. Similarly, Fe, Zn, Cu, and Mn concentrations of yellow rice are lower than that of the other colored rice (Guo 2011) (Table 4.2).

Table 4.1 Nutritional composition of different rice types

Polished white rice	6.8 proteins, 1.2 irons, 0.5 zinc, and 0.6 fibers
Brown rice	7.9 protein, 2.2 iron, 0.5 zinc, and 2.8 fiber
Purple rice	8.3 proteins, 3.9 irons, 2.2 zinc, and 1.4 fibers
Red rice	7.0 proteins, 5.5 irons, 3.3 zinc, and 2.0 fibers
Black rice	8.5 proteins, 3.5 irons, 0.0 zinc, and 4.9 fibers

Table 4.2 Total Phenolic Content (TPC), Total Anthocyanin Content (TAC), Anti-DPPH Radical Activity and R-Glucosidase Inhibitory of red, purple, and black rice, purple corn, black barley, black soybean, and black soybean coata (Yao et al. 2009)

Crop types	TPC	TAC	DPPH	IC50
Red rice	0.10 ± 0.01[d]	0.05 ± 0.01[f]	1.63 ± 0.15[c]	>1000
Purple rice	4.62 ± 0.18[b]	1.22 ± 0.08[c]	30.92 ± 1.58[b]	475.14 ± 25.46
Black rice	8.58 ± 0.56[a]	3.83 ± 0.04[a]	73.47 ± 4.63[a]	13.56 ± 1.2
Purple corn	1.11 ± 0.09[c]	0.31 ± 0.01[d]	1.68 ± 0.19[c]	833.33 ± 56.31
Black barley	0.46 ± 0.04[c,d]	0.27 ± 0.05[d,e]	2.21 ± 0.37[c]	>1000
Black soyabean	0.75 ± 0.06[c,d]	0.19 ± 0.02[e]	4.59 ± 0.27[c]	>1000
Black soyabean coat	5.26 ± 0.42[b]	1.63 ± 0.03[b]	13.94 ± 4.86[b]	111.11 ± 21.24

[a]Data are expressed as mean (SD of triplicate samples. TPC is expressed as grams of GAE/100 g. TAC is expressed as milligrams of anthocyanin/gram. The anti-DPPH capacity is expressed as micromolar TE/gram. Values in the same column sharing different letters are expressed as significantly different ($p < 0.05$). a IC50 was expressed as mg/mL

4.2 Nutritional Facts of Black Rice

Black rice provides the richest nutritional value, a higher level of vitamins, minerals, and fiber as well as a comprehensive range of amino acids, proteins, vegetable fats, and essential trace elements compared to other rices needed by the body. Finocchiaro et al. (2010) reported that on average, the pigmented rices had a TAC four times higher than the white ones.

A typical nutritional data of black rice is reported here (www.blackrice.com, www.chinese-black-rice.com) (Table 4.3).

4.3 Factors Affecting Rice Nutrition

A detailed analysis of nutrient content of rice suggests that the nutrition value of rice varies based on a number of factors such as sunlight, water, soil nutrient content, organic matter content, variety, and method of rice milling etc. It also depends on the strain of rice that is between white, brown, black, red, and purple varieties of rice prevalent in different parts of the world. It also depends on nutrient quality of the soil where rice is grown, whether and how the rice is polished or processed, the manner it is enriched, and how it is prepared before consumption. Comparative nutrition studies on red, black, and white varieties of rice suggest that pigments in red and black rice varieties may offer nutritional benefits over white rice. Red or black rice consumption is found to reduce or retard the progression of atherosclerotic plaque development, induced by dietary cholesterol in mammals. White rice consumption offers no similar benefits (Table 4.4).

Table 4.3 Nutritional data of black rice per 100 g

Nutrient	Amount
Alanine	0.437 g
Arginine	0.569 g
Ash	1.27 g
Aspartic acid	0.702 g
Calcium, Ca	33 mg
Carbohydrate, by difference	76.17 g
Copper, Cu	0.277 mg
Cystine	0.091 g
Energy	1515 kJ
Energy	362 kcal
Fatty acids, total monounsaturated	0.971 g
Fatty acids, total polyunsaturated	0.959 g
Fatty acids, total saturated	0.536 g
Fiber, total dietary	3.4 g
Folate, DFE	20 mcg_DFE
Folate, food	20 mcg
Folate, total	20 mcg
Glutamic acid	1.528 g
Glycine	0.369 g
Histidine	0.190 g
Iron, Fe	1.80 mg
Isoleucine	0.318 g
Leucine	0.620 g
Lysine	0.286 g
Magnesium, Mg	143 mg
Manganese, Mn	3.743 mg
Methionine	0.169 g
Niacin	4.308 mg
Pantothenic acid	1.493 mg
Phenylalanine	0.387 g
Phosphorus, P	264 mg
Potassium, K	268 mg
Proline	0.352 g
Protein	7.50 g
Riboflavin	0.043 mg
Serine	0.388 g
Sodium, Na	4 mg
Thiamin	0.413 mg
Threonine	0.275 g
Total lipid (fat)	2.68 g
Tryptophan	0.096 g

(continued)

Table 4.3 (continued)

Nutrient	Amount
Tyrosine	0.281 g
Valine	0.440 g
Vitamin B-6	0.509 mg
Water	12.37 g
Zinc, Zn	2.02 mg

Table 4.4 Test results of quality raw materials of sticky black rice (by the volume of dry substance) (Minh 2014)

Parameter	Unit	Result
Moisture	%	11.7
Crude protein	%	8.9
Lipid	%	3.3
Glucid	%	75.5
Dietary fiber	%	13.18
Anthocyanin	%	0.49
The absolute mass of the particle	(g/1000 grains)	25.1
Percentage of impurities	%	2.12
Decay	%	0
Sprout	%	95.4
Color of the rice husk		Specific colored rice
Colors likely when peeled husks		Black purple
Aroma		Characteristic of rice, no strange smells, does not smell musty

4.4 Chemicals Present in Black Rice

Black rice is an excellent source of minerals such as iron, zinc, manganese, selenium, and magnesium. It contains substantial amounts of protein, dietary fiber, vitamin E, B vitamins, unsaturated fats, γ-oryzanol, γ-amino butyric acid (GABA), antioxidants, phenolic compounds, and other phytochemicals (Roohinejad et al. 2009). The color of black glutinous rice is caused by anthocyanins which are a group of reddish purple water-soluble flavonoids (Shen et al. 2009) located on pericarp, seed coat, and aleurone layer (Sompong et al. 2011). Anthocyanins are the main antioxidants in black glutinous rice and are composed of anthocyanidin and sugar. Six anthocyanin pigments are identified and the predominant anthocyanins are cyanidin-3-glucoside (572.47 µg/g, 91.13 % of total) and peonidin-3-glucoside (29.78 µg/g; 4.74 % of total). Minor constituents include three cyanidin-dihexoside isomers and one cyanidin hexoside (Hiemori et al. 2009). Several research show that only six types of anthocyanins, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin, cyanidin, pelargonidin, and malvidin are present in raw black rice bran (BRB) and black rice bran colorant powder (BCP). Zhang et al. (2006) reported four main anthocyanidins in black rice are malvidin, pelargonidin-3,5 glucoside, cyaniding-3-gluconside, and cyaniding-3,5-diglucoside. Cyaniding-3-glucoside and pelargonidin-3-glucoside show aldose reductase inhibitory activities, and therefore, they would be beneficial for the prevention of diabetes (Yodmanee et al. 2011). Cyanidin 3-glucoside (Cy-3-G) was found in the highest amounts in unprocessed and extruded rice bran (Ti et al. 2015). Cyanidin-3-O-glycoside has been shown to have antioxidant activity 3.5 times more potent than Trolox, a vitamin E analog (Zuo et al. 2012). Notably, a black rice variety that contains the highest amount of anthocyanins possesses the lowest antioxidant activity and total phenolic content. This indicates that the overall phenolic components rather than the anthocyanin pigments contribute to the antioxidative capacity of black rice brans (Pitija et al. 2013). Eleven flavonoids are detected and six of them are found for the first time in black rice bran are taxifolin-7-O-glucoside, myricetin-7-O-glucoside, isorhamnetin-3-O-acetylglucoside, isorhamnetin-7-O-rutinoside, 5,6,3′,4′,5′-pentahydroxyflavone-7-O-glucoside, and 5,3′,4′,5′-tetrahydroxyflavanone-7-O-glucoside. The quantitative results reveal that different rice varieties possess flavonoids in different concentrations. The most abundant glycoside derivative of flavonoids that widely distributed among the rice varieties is monoglucoside (quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside and myricetin-7-O-glucoside) (Sriseadka et al. 2012). The black rice bran contains most of the antioxidant compounds including phytic acid, γ-oryzanol, anthocyanins, and vitamin E homologues (Kong and Lee 2010). The thermal degradation of both the visual color and the anthocyanin content in the BCP follow a first-order kinetic reaction model. The temperature-dependent degradation is adequately fit to the Arrhenius equation (Loypimai et al. 2015).

The phenolic constituents of rice are mainly distributed in rice pericarp which accounts for 2–3 % of rice caryopsis, and can be separated in three different groups: phenolic acids, flavonoids, and proanthocyanidins. The simple phenolic acids and flavonoids are the most common phenolic compounds and they generally occur as soluble conjugated (glycosides) and insoluble forms (bound) (Nardini and Ghiselli 2004). Free and soluble conjugated phenolic forms are absorbed in the stomach and small intestine, the dietary intake of bound phenolics presents a chemopreventive activity against colon cancer (Acosta et al. 2014). Anthocyanins (cyanidin-3-O-β-glucoside and peonidin-3-O-β-glucoside) and tocopherols are identified in black rice which prove that they have aldose reductase inhibitory activity (Yawadio et al. 2007). Polar extractions of rice bran are high in antioxidative compounds and activities than nonpolar extractions (Pengkumsri et al. 2015). Interestingly, the phenolics, flavonoids, and anthocyanins of black rice bran are mainly present in free form. Nonfermented black rice bran extract (NFBE) show greater antioxidant activities than fermented black rice bran extract (FBE). This result suggests that the cytotoxic activity of black rice bran improve through fermentation while antioxidant activity gets reduced (Yoon et al. 2014).

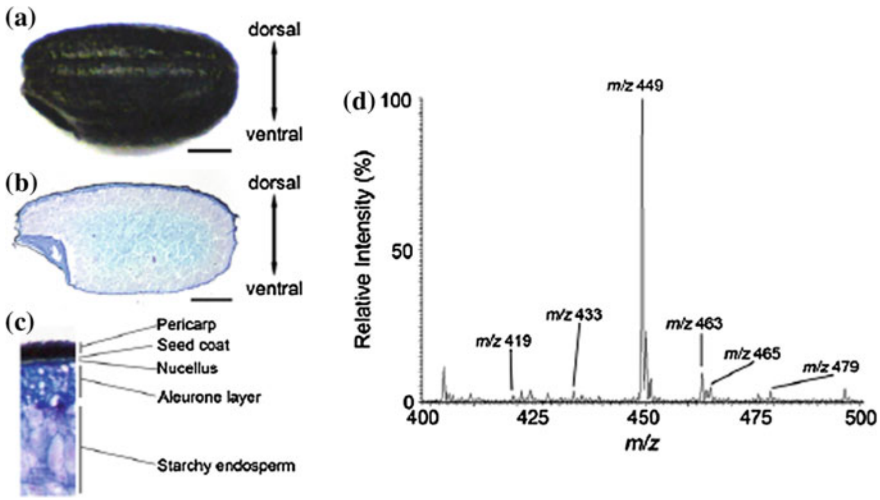


Fig. 4.1 Identification of anthocyanin species in black rice crude extract. **a** Appearance of black rice. **b** Black rice section stained with toluidine blue O. Scale bar: 1.0 mm. **c** Enlarged image of the dorsal region of the longitudinal black rice section. **d** Mass spectrum of the black rice crude extracts (Yoshimura et al. 2012)

Currently, 250 anthocyanins have been identified; however, only six of them pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are commonly found in fruits and cereal grains (Escribano et al. 2004). These compounds have been recognized as health enhancing substances, owing to their antioxidant activity, anti inflammatory properties, and hypoglycaemic effects (Nam et al. 2006; Philpott et al. 2004). They also show other biological effects including antimutagenic and anticarcinogenic activities (Hyun and Chung 2004) (Fig. 4.1).

4.4.1 Anthocyanin Profile of Black Rice

Anthocyanins are naturally occurring plant pigments belong to the flavonoid family. They are widely distributed in nature and are responsible for the attractive red (rasp berries), purple (grasses and purple cabbage), and blue (blue berries) colors of many flowers, fruits, and vegetables (Jackman et al. 1987), and when ingested they protect cells against oxidative damage from free radicals and are widely used for their antioxidant and pharmacological properties (Guo 2008). The accumulation of anthocyanins in leaves, shoots, and roots is stimulated by various environmental cues such as temperature, light, and nutrients. The six common anthocyanidins are delphinidin, cyanidin, peonidin, pelargonidin, petunidin, and malvidin (Table 4.5).

Anthocyanins have been shown to be beneficial to health due to their high antioxidant capacity. Scientists says that one reason of fruits and vegetables are so

Table 4.5 Common anthocyanidins present in nature

Anthocyanidins	Substitution pattern	Molecular weight
Delphinidin	3,5,7,3′,4′,5′-OH	303
Cyanidin	3,5,7,3′,4′-OH	287
Pelagonidin	3,5,7,4′-OH	271
Petunidin	3,5,7,3,4,5′-OH; 3′-OMe	317
Peonidin	3,5,7,3,4′-OH; 3′-OMe	301
Malvidin	3,5,7,4′-OH; 3,5-OMe	333

healthy is that they are rich in the vitamins and phytochemicals that help to defend against this damage. Though the best known antioxidants are vitamin C, vitamin E, selenium, zinc, and beta-carotene; hundreds or thousands of other similar healthy compounds (flavonoids, anthocyanins, polyphenols, and catechins) are found in foods like nuts, berries, beans, and tea. Antioxidants have been shown to have an important role in fighting a number of diseases including heart disease and cancer. Anthocyanins have many biological properties such as prevention of inflammation, reduction of DNA cleavage, antimutagenicity in bacterial model, and gastric protective effects. Black rice containing cyanidin and peonidin suppresses oxidative modification of human low-density lipoprotein and reduces the formation of nitric oxide (Hu et al. 2003). Hyun and Chung (2004) reported that cyanidin and malvidin mediate cytotoxicity through the arrest of the G2/M phase of the cell cycle and by induction of apoptosis. Furthermore, Ling et al. (2001) showed that reduction of atherosclerotic plaque formation is due to anthocyanins present in red and black rice. The antioxidants in black rice may help fight heart disease and reduce blood levels of “bad” cholesterol which is low-density lipoprotein or LDL. Anthocyanin antioxidants deliver multiple powerful health benefits such as

1. Eliminating harmful free radical molecules
2. Protecting arteries from plaque buildup
3. Protecting against DNA damage that causes cancer.

Anthocyanins are the pigments responsible for the bran color of black rice (Escribano et al. 2004) which has been proven to have 96 times more anthocyanin content than pigmented (blue, pink, purple and red) corn, wheat, and barley with the values reaching as high as 3276 μg/g (Abdel et al. 2006). Meanwhile, proanthocyanidins are the pigments responsible for red rice color (Oki et al. 2002), although according to several studies (Abdel et al. 2006; Yoshinaga et al. 1986; Morimitsu et al. 2002), anthocyanins can also be found in red rice in lower concentration up to 35 times less than that of black rice. Black rice has high anthocyanin content located in the pericarp layers and is a good source of fiber, minerals, and several important amino acids. Black glutinous rice contains many nutritious substances such as essential amino acids, vitamins, minerals, and especially effective antioxidants (Minh 2014). Black waxy rice is one of the most potential plant sources of dark purple color of anthocyanin pigment. The bran layer of the rice kernel contains high level of bioactive compounds such as oryzanol, anthocyanins, and phenolic compounds. In some varieties of black rice, anthocyanins are present in the stem and leaves as well as the kernels in others only the grains are pigmented.

Only little is known about the phytochemical profiles and antioxidant activities of different black rice varieties. Total anthocyanin content in the black rice extract is 43.2 %; other components of the extract are carbohydrate, 21.6 %; protein, 4.9 %; flavonoids, 16.6 %; water, 5.5 %; and others, 8.2 % (Xia et al. 2006). The spectroscopy analysis indicated that the four active components of the antioxidative extract of black rice are four anthocyanin compounds of malvidin, pelargonidin-3,5-diglucoside, cyaniding-3-glucoside, and cyaniding-3,5-diglucoside. Thus the anthocyanin compounds are the most important substantial foundations for antioxidation (Zhang et al. 2006). Several works were done on rice to either determine the anthocyanin profile (Ichikawa et al. 2001; Yawadio et al. 2007) the antioxidant activity of rice (Choi et al. 2007) or more recently with selected fractions there of, e.g., rice bran (Chotimarkorn et al. 2008; Lai et al. 2009) (Table 4.6).

An experiment on the phytochemical profiles and antioxidant activity of rice bran samples from 12 diverse varieties of black rice were determined. The average values of total phenolic contents of black rice bran were six times higher than those of white rice bran respectively ($p < 0.05$). The percentage contribution of free anthocyanins to the total ranged from 99.5 to 99.9 %. Cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside were detected in black rice bran samples and ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively. The percentage contribution of free antioxidant activity to the total ranged from 88.7 to 96.0 %. The average values of total antioxidant activity of black rice bran were six times higher than those of white rice bran respectively ($p < 0.05$). These results indicate that there are significant differences in phytochemical content and antioxidant activity among different black rice varieties. Interestingly, the phenolics, flavonoids, and anthocyanins of black

Table 4.6 Anthocyanin content and visual color of raw black rice bran (BRB) and black rice bran colorant powder (BCP) (Loypimai et al. 2015)

Characteristics	BRB	BCP
Anthocyanins (μg/g)	1042.4 ± 10.5	7235.5 ± 18.3
Cyanidin-3-O-glucoside	27.3 ± 3.19	93.25 ± 10.2
Cyanidin-3-O-rutinoside	451.3 ± 7.33	723.8 ± 19.7
Delphinidin	184.3 ± 8.35	638.4 ± 23.3
Cyanidin	334.9 ± 10.2	1654.2 ± 54.2
Pelargonidin	51.6 ± 3.98	101.8 ± 11.2
Malvidin	2947.3 ± 22.5	12,540.8 ± 85.9
Total contents		
Color value		
L^a	37.2 ± 0.84	39.4 ± 0.95
C^a	15.2 ± 0.38	17.2 ± 0.48
$h°$	310.9 ± 6.61	348.8 ± 6.74

[a]Values are means ± SD of triplicate samples ($n = 3$) (on a wet weight basis)

rice bran are mainly present in free form. The total antioxidant activity of black rice bran was correlated to the content of total phenolics, total flavonoids, and total anthocyanins and also was significantly correlated to the contents of cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside. Knowing the phytochemical profile and antioxidant activity of black rice bran gives insights to its potential application to promote health (Zhang et al. 2010) (Fig. 4 2).

Yoshimura et al. (2012) identified seven species of anthocyanin monoglycosides and two species of anthocyanin diglycosides in crude extracts from black rice by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis. They also analyzed black rice sections by MALDI-IMS and found two additional species of anthocyanin pentosides and revealed different localization patterns of anthocyanin species composed of different sugar moieties. Anthocyanin species composed of a pentose moiety (cyanidin-3-O-pentoside and petunidin-3-O-pentoside) were localized in the entire pericarp, whereas anthocyanin species composed of a hexose moiety (cyanidin-3-O-hexoside and peonidin-3-O-hexoside) were focally localized in the dorsal pericarp. These results indicate that anthocyanin species composed of different sugar moieties exhibit different localization patterns in the pericarp of black rice.

Anthocyanin types and total content vary among different rice cultivars. The antioxidant capacity of colored rice is mainly found in the seed capsule. Colored rice cultivars show stronger antioxidant activities than white rice. Antioxidant

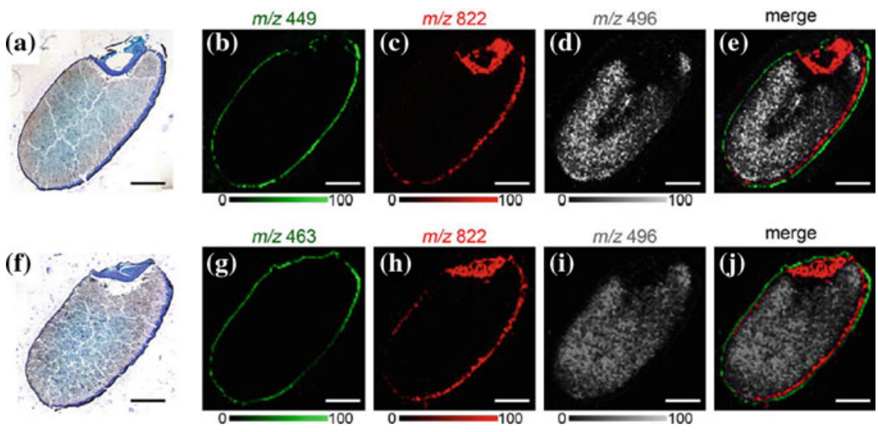


Fig. 4.2 MALDI-IMS analysis of anthocyanins in black rice sections. **a** Black rice section stained with toluidine blue O after MALDI-IMS analysis of the ions at m/z 449. **b** Localization pattern of the ions at m/z 449. **c** Localization pattern of the ions at m/z 822 corresponding to PC (diacyl C36:3), which marks the nucellus epidermis/aleurone layer. **d** Localization pattern of the ions at m/z 496 corresponding to LPC (C16:0), which marks the endosperm. **e** Merged ion image of m/z 449 (*red*), m/z 822 (*green*) and m/z 496 (*white*). **f** *Black* rice section stained with toluidine blue O after MALDI-IMS analysis of the ions at m/z 463. **g** Localization pattern of the ions at m/z 463. **h** Localization pattern of the ions at m/z 822 corresponding to PC (diacyl C36:3). **i** Localization pattern of the ions at m/z 496 corresponding to LPC (C16:0). **j** Merged ion image of m/z 463 (*red*), m/z 822 (*green*) and m/z 496 (*white*). Scale bar: 1.0 mm (Yoshimura et al. 2012)

capacity is mainly due to the other phenolic compound instead of anthocyanin content. Colored rice particularly black rice has been shown to possess bioactive properties and rice bran contains high levels of several antioxidant compounds. The black rice bran contains most of the antioxidant compounds including phytic acid, γ-oryzanol, anthocyanins, and vitamin E homologues (Kong and Lee 2010). The spectroscopy analysis indicates that four active components of the antioxidative extract of black rice are cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-rutinoside (Hou et al. 2013). The predominant anthocyanins are cyanidin-3-glucoside (572.47 μg/g, 91.13 % of total) and peonidin-3-glucoside (29.78 μg/g, 4.74 % of total). Minor constituents include three cyanidin-dihexoside isomers and one cyanidin hexoside. It is concluded that the anthocyanin compounds are the most important substantial foundations for antioxidation (Zhang et al. 2006). Zhang et al. (2015) identified the breeding black rice line YF53 has the highest total phenolic content (23.3 mg ferulic acid equiv./g), total anthocyanin content (2.07 mg cyanidin-3-glu equiv./g), and antioxidant activities; total anthocyanin content and antioxidant activity of black rice is significantly higher in germinated rice (Sutharut and Sudarat 2012). Phonsakhan and Ngern (2014) reported that the accumulation of anthocyanins in black glutinous rice varieties range from 0.262 to 2.539 mg/g.

Colored rice has red or purple pigments in its bran. The anthocyanins contain approximately 95 % of cyanidin-3-O-glucoside and 5 % of peonidin-3-O-glucoside. The 100 g/ml concentration of the anthocyanin extract exhibits 88.83 % inhibition on the peroxidation of linoleic acid, 55.20 % DPPH free radical scavenging activity, 54.96 % superoxide anion radical scavenging activity, and 72.67 % hydrogen peroxide scavenging activity, and it also shows high ferrous ion reducing capability. These data suggest that the anthocyanin extracted from black rice may be utilized as a possible antioxidant agent against ROS (J. Microbiol. Biotechnol, 01/2008). The total anthocyanin content varies greatly among black rice cultivars (79.5–473.7 mg/100 g), but is lower in red rice (7.9–34.4 mg/100 g). Total phenolic content is similar between red (460.32–725.69 mg/100 g) and black (417.11–687.24 mg/100 g) rice. The oxygen radical absorbing capacity is ranked as follows: red (69.91–130.32 μmol Trolox/g)>black (55.49–64.85 μmol Trolox/g)>green (35.32 μmol Trolox/g)>white (21.81 μmol Trolox/g) rice. The antioxidant capacity results mainly from the seed capsule, not the endosperm. The anthocyanin pigments contributed little to the total antioxidant capacity of red (0.03–0.1 %) and black (0.5–2.5 %) rice cultivars. Hence, the antioxidant capacity is derived mainly from other phenolic compounds (Chen et al. 2012). Anthocyanin can be a key factor in functional property of black rice and "Heugjinjubyeo" a black rice variety may be very important source concerning nutritional value (Lee 2010). A new 2-aryl benzofuran, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester, oryzafuran (1), has been isolated from the black colored rice bran of *Oryza sativa* cv. Heugjinjubyeo. This compound showed strong antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (Han et al. 2004).

Different methods of anthocyanin extraction from black rice are available nowadays. Studies show that high-performance countercurrent chromatography (HPCCC) coupled with reversed-phase medium pressure liquid chromatography RP-MPLC method is more rapid and efficient than multi-step conventional column chromatography for the separation of anthocyanins (Jeon et al. 2015). Similarly, the VIS/NIRS method would be applicable only for rapid determination of cyanidin-3-glucose content in black rice flour samples (Sig et al. 2012) (Fig. 4.3).

Blue characters indicate biosynthetic enzymes of each steps of the pathway. Enzyme names are abbreviated as follows: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, Flavanone 3-hydroxylase; F3′H, Flavanone 3′-hydroxylase; F3′5′H, Flavanone 3′5′-hydroxylase; FLS, Flavonol synthase; DFR, Dihydroflavonol reductase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; ANR, anthocyanidin reductase; UGTs, UDP-glucosyl transferases. This figure was redrawn from data described by Petroni and Tonelli (2011).

Antioxidant activity was studied for anthocyanins extracted from purple black rice (PBR) by a 3 % aqueous trifluoroacetic acid solution (TFA), as well as for anthocyanins extracted from blueberry (Bluetta, high bush type). Capillary zone electrophoresis revealed that the PBR extract contained almost exclusively a single anthocyanin, which was identified as cyanidin 3-O-β-D-glucoside (Cy 3-Glc) after purification by polyvinyl pyrrolidone column chromatography. In contrast, 11 anthocyanins were identified in the blueberry extract. PBR extract showed slightly weaker superoxide scavenging and crocin bleaching activities than blueberry

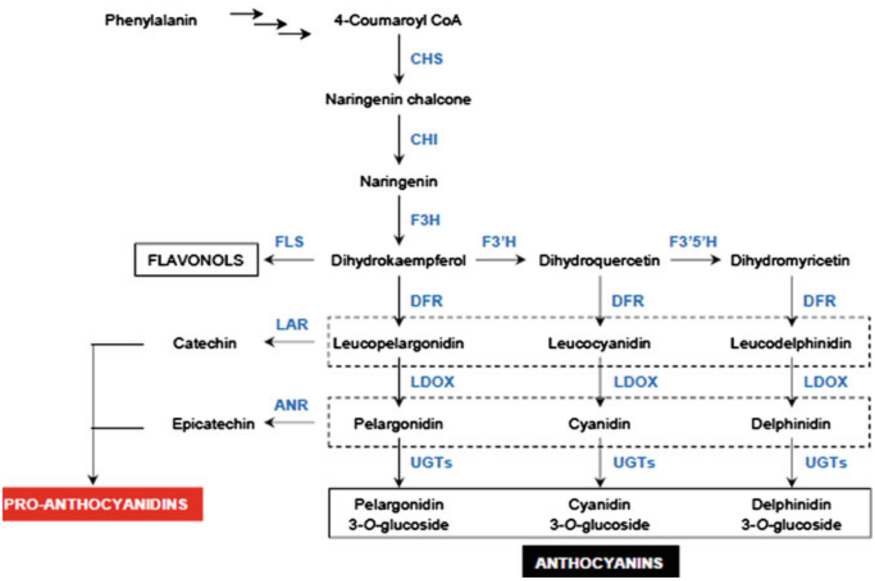


Fig. 4.3 Schematic diagram of the anthocyanin and proanthocyanidin biosynthesis pathway in plants

extract did. Both PBR and blueberry extracts, however, showed 10–25 times stronger activity than the same concentration of Trolox used as a reference antioxidant. It was further noted that the purified Cy 3-Glc from PBR extract retained approximately 74 % of the antioxidant activity (both crocin bleaching and superoxide scavenging) observed in the original TFA extract. The hydroxyl radical scavenging activity of both extracts was several times weaker than that of the same concentration of Trolox, although the PBR extract showed approximately two times stronger activity than blueberry extract did. The hydroxyl radical scavenging activity of the purified Cy 3-Glc from PBR, however, decreased to approximately 20 % of that of the original PBR extract. These results indicate that the anthocyanin Cy 3-Glc contributes to the antioxidant activity of PBR through its strong superoxide radical but not hydroxyl radical scavenging activity (Ichikawa et al. 2001). A new compound of 2-aryl benzofuran, 2-(3,4-dihydroxy phenyl)-4,6-dihydroxy benzofuran-3-carboxylic acid methyl ester, oryzafuran (1), was isolated which showed strong antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (Han et al. 2004).

4.4.2 Cyanidin-3-Glucoside (C3G)

The most abundant anthocyanins are cyanidin 3-glucoside in black and red rices and in blue, purple, and red corns, pelargonidin 3-glucoside in pink corn, and delphinidin 3-glucoside in blue wheat (Abdel et al. 2006). Cyanidin 3-glucoside and peonoidin 3-glucoside are confirmed as the dominant anthocyanins in black rice varieties with contents ranging from 19.4 to 140.8 mg/100 g DM and 11.1–12.8 mg/100 g DM, respectively (Muntana and Prasong 2010). Park et al. (2008) reported the anthocyanins in black rice include cyanidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside, pelagonidin 3-O-glucoside, and delphinidin 3-O-glucoside. Similarly, four anthocyanins are detected in black rice are identified as cyanidin-3-glucoside (91.01 %), peonidin-3-glucoside (7.13 %), cyanidin-3,5-diglucoside (0.92 %), and cyanidin-3-rutinoside (0.94 %). The main anthocyanins are cyanidin-3-glucoside and peonidin-3-glucoside (Hou et al. 2013) (Table 4.7).

Cyanidin-3-O-β-d-glucoside (C3G), the best known and most investigated anthocyanin, has many pharmacological properties and black rice bran is an outstanding source of C3G due to its high C3G content and a simple anthocyanin

Table 4.7 Anthocyanin profile in black rice varieties (Sompong et al. 2011)

	Thailand		China
	Niaw Dam Pleuak Khao (PK)	Niaw Dam Pleuak Dam (PD)	China black Rice (CNB)
Anthocyanins			
Cyanidin 3-glucoside	137.41 ± 16.66	19.39 ± 0.09	140.83 ± 2.0
Peonidin 3-glucoside	11.07 ± 0.97	12.75 ± 0.51	11.1 ± 0.16

composition. 1.43 g of pure C3G (purity 94.38 %) was obtained from 3.0 g of anthocyanin enriched extract by reversed-phase C_{18} column chromatography (Li et al. 2012). Two novel oxidized products are isolated from radical oxidation of cyanidin 3-O-glucoside and the products obtain from the anthocyanin oxidation are dependent on the nature of the solvent (Kamiya et al. 2014). Cyanidin 3-glucoside, peonidin 3-glucoside, and cyanidin occurs in black and some light purple rice samples. The average anthocyanins content in black rice bran is over 3.5 mg/g and vitamin E content is between 0.01 and 0.05 %, oryzanol is 0.1–0.3 % and the fiber content is 7–11 %. A large-scale isolation of C3G from the crude black rice bran extract is obtained by combining high-speed countercurrent chromatography (HSCCC) and reversed-phase C18 column chromatography. The method is time-saving, low risk of sample denaturation, high sample recovery, and so is suitable for large-scale preparation of C3G for further studies of bioactivities (Du et al. 2012) (Fig. 4.4).

Black purple rice is becoming popular among health-conscious food consumers. A study was conducted where the secondary metabolites in dehulled black purple rice cv. Asamurasaki were analyzed using HPLC–PDA–MS^2. The results showed that seeds contains a high concentration of seven anthocyanins (1400 μg/g fresh weight) with cyanidin-3-O-glucoside and peonidin-3-O-glucoside predominating. Five flavonol glycosides, principally quercetin-3-O-glucoside and quercetin-3-O-rutinoside and

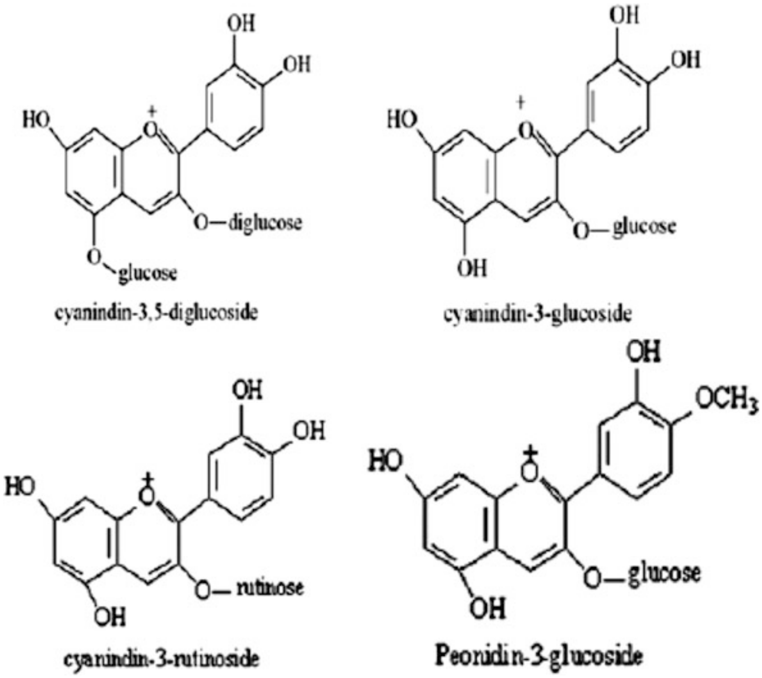


Fig. 4.4 Chemical structure of different anthocyanin compounds

flavones. The seeds also contained 3.9 μg/g of carotenoids consisting of lutein, zeaxanthin, lycopene, and β-carotene. γ-Oryzanol (279 μg/g) was also present as a mixture of 24-methylenecycloartenol ferulate, campesterol ferulate, cycloartenol ferulate, and β-sitosterol ferulate (Caro et al. 2013).

4.5 Rice Preparation for Consumption and Its Effects on Nutrition

4.5.1 Washing or Rinsing Rice Before Cooking

This is a common practice in places where rice packaging and storage methods leave rice exposed to dust and other contaminants. Rinsing rice, sometimes multiple times results in the loss of water-soluble nutrients, including starch, protein, vitamins, minerals, and fats. Use of clean packaged rice reduces or removes the need for washing and thus prevents the loss of nutrients (FAO). Similar nutrient loss would also occur, however, if rice is presoaked and then drained before cooking, or if rice is cooked in excess water that is drained away before consumption (Ricepedia). Washing of milled rice prior to cooking is a common practice in Asia to remove bran, dust, and dirt from the food, since rice is often retained in open bins and thus exposed to contamination. During washing, some water-soluble nutrients are leached out and removed.

Boiling in excess water results in leaching out of water-soluble nutrients including starch and their loss when the cooking liquor is discarded. For example, 0.8 % of the starch is removed on two washings of three milled rices, but 14.3 % of the starch by weight is in the rice gruel after cooking for about 20 min in 10 weights of water (Perez et al. 1987) Protein removal is 0.4 % during washing and 0.5 % during cooking (Table 4.8).

4.5.2 Cooking

Cooking milled (polished/white) rice by boiling in water (without washing) results in the loss of up to 7 % of protein, 36–58 % of crude fat, 16–25 % of crude ash, 21 % of calcium, 47–52 % of thiamine, 35–43 % of riboflavin, and 45–55 % of niacin (Ricepedia).

4.5.3 Wet Milling

Water-soluble nutrients are also lost during wet milling of rice flour (a process used for making rice noodles, egg roll wrappers, and some rice flourcakes etc.), in the

Table 4.8 Percent nutrient losses during washing and cooking in excess water (Hayakawa and Igaue 1979; Perez et al. 1987)

Nutrient	Washing[a]			Washing and cooking[b]	Cooking without washing[c]		
	Raw milled rice	Brown rice	Parboiled milled rice	Milled rice	Milled rice	Brown rice	Parboiled milled rice
Weight	1–3	0.3–0.4		5–9	2–6	1–2	3
Protein	2–7	0–1		2	0–7	4–6	0
Crude tat	25–65			50	36–58	2–10	27–51
Crude fiber	30						
Crude ash	49				16–25	19	29–38
Free sugars	60			40			
Total polysaccharides	1–2			10			
Free amino acids	15			15			
Calcium	18–26	4–5		1–25	21		
Total phosphorus	20–47	4		5			
Phytin phosphos	44						
Iron	18–47	1–10		23			
Zinc	11	1					
Magnesim	7–70	1	1				
Potassium	20–41	5	15				
Thiamine	22–59	1–21	7–15	11	47–52		
Riboflavin	11–26	2–8	12–15	10	35–43		
Niacin	20–60	3–13	10–13	13	45–55		

filtration step. This includes vitamins, minerals, free sugars and amino acids, water-soluble polysaccharides, protein (albumin), fat and starch (Ricepedia).

4.5.4 Parboiling

Parboiling rough rice before milling, as is common in India and Bangladesh, allows a portion of the vitamins and minerals in the bran to permeate the endosperm and be retained in the polished rice. This treatment also lowers protein loss during milling and increases whole grain recovery (Ricepedia). Parboiling reduced the TSPCs concentration in the grains due to the loss of part of them in the processing water, thermal decomposition and, possibly, interaction with other components.

This reduction is related to the lower AOA in these grains. In a similar way, cooking also reduced the concentration of TSPCs, especially in brown and polished grains (Walter et al. 2013).

4.5.5 Fermentation

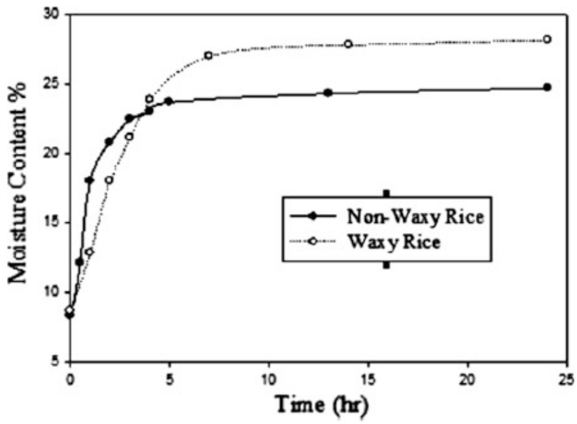
Fermentation of rice is another popular preparation method. Reportedly, protein content of fermented rice decreases from 1.54 % after one day of fermentation to 1.14 % after 3 days at 70 % moisture (Ricepedia).

4.6 Rice Cooking Methods and Anthocyanin Content

All cooking methods cause significant ($P < 0.001$) decreases in the anthocyanins. Pressure cooking results in the greatest loss of cyanidin-3-glucoside (79.8 %) followed by the rice cooker (74.2 %) and gas range (65.4 %). Conversely, levels of protocatechuic acid increase from 2.7 to 3.4 times in response to all cooking methods. These findings indicate that cooking black rice results in the thermal degradation of cyanidin-3-glucoside and concomitant production of protocatechuic acid (Hiemori et al. 2009). Proximate analysis and light micrographs revealed higher migration of red rice proteins than black rice proteins to the endosperm as a result of parboiling. Parboiling reduces the ash content of red rice while no difference is determined in black rice. Polishing removes more than 90 % of free phenolics while parboiling allow the partial preservation of free phenolics content in polished rice. Parboiling induces an increase in the cooking time of red rice, but a decrease in the cooking time of black rice (Paiva et al. 2015). Roasting result in the greatest decrease (94 %) in anthocyanins, followed by steaming (88 %), pan-frying (86 %), and boiling (77 %). The contents of phenolic compounds decrease drastically after cooking, with significantly lower retention in the black rice cultivar that had higher amylose content. DPPH radical scavenging activity of black rice decrease after cooking compared to the raw ones. In contrast, metal chelating activities significantly increase after cooking. The contents of anthocyanins shows a high positive correlation with total phenolic compounds whereas a significant negative correlation with metal chelating activity. These results indicate that cooking degrades anthocyanins and other phenolic compounds, but concomitant increase in phenolics from possible degradation of the anthocyanins which results in the enhancement of metal chelating activity (Surh and Koh 2014) (Fig. 4.5).

Antioxidants in black rice bran tend to leach out into the water during cooking as rice is usually cooked in excess water which is discarded after cooking. The percentages of antioxidant extractability from pigmented rice into the cooking water are 88.42 and 103.26 %, respectively, for red rice and black rice. However, red rice drink possess significantly ($p < 0.05$) higher antioxidant activity than black rice

Fig. 4.5 Water absorption curves of waxy and nonwaxy black rice (Tang et al. 2015)



drink, except for its total monomeric anthocyanin content. Black rice and red rice cooking water have the potential of being new antioxidant drinks (Handayani et al. 2014). All processed black rice exhibit significantly ($p < 0.05$) lower TPC, TFC, CTC, MAC, Cy3glc, Pn3glc, and antioxidants as compared to the raw rice. Different processing methods significantly degrade the content and activities of antioxidants of both waxy and nonwaxy black rice. Under the same cooking time, black rice porridge retain more active substances than that of cooked rice by rice cooker. Therefore, to maintain bioavailability of active components, black rice porridge may gain more health promoting effects (Tang et al. 2015). The risotto cooking allows a complete absorption of water, could be considered a recommended cooking method to retain phenolic compounds and total antioxidant capacity and, in the case of the black rice. Consumption of whole meal rice particularly the black rice should be recommended as a good source of phenolic compounds in a diet, especially when it is cooked with the complete absorption of water (Zaupa et al. 2015) (Table 4.9).

Table 4.9 Cooking properties of rice varieties ($n = 3 \pm$ s.d.) (Thomas et al. 2013)

Rice samples	Min cooking time (min)	Elongation	Cooked l/b (mm)	Water uptake ratio	Cruel soild loss (%)
White local	10.00 ± 0.0^{a}	1.43 ± 0.0^{b}	3.27 ± 0.1^{b}	2.44 ± 0.3^{a}	6.43 ± 0.0^{f}
Brown	31.67 ± 0.6^{d}	1.68 ± 0.3^{d}	2.25 ± 0.4^{a}	3.95 ± 0.3^{d}	3.37 ± 0.6^{b}
Bario	12.67 ± 0.6^{b}	1.37 ± 0.2^{a}	3.07 ± 0.2^{b}	2.75 ± 0.2^{b}	5.46 ± 0.3^{d}
Black (imported)	22.66 ± 0.6^{c}	1.57 ± 0.1^{c}	3.45 ± 0.3^{c}	2.73 ± 0.1^{b}	4.22 ± 0.4^{c}
Glutinous	22.67 ± 0.0^{c}	1.41 ± 0.1^{b}	3.17 ± 0.4^{b}	2.33 ± 0.8^{a}	5.76 ± 0.2^{e}
Basmati	22.00 ± 0.6^{c}	1.77 ± 0.0^{d}	4.18 ± 0.1^{d}	3.77 ± 0.6^{c}	3.17 ± 0.2^{a}

Same letter in the same column are not significantly different from each other at $p < 0.05$

Table 4.10 Anthocyanin content of raw and cooked black rice and percentage of losses by cooking (Zaupa et al. 2015)

	Raw	Risotto	Boiled
Cyanidin 3-O-glucoside	255.7 ± 4.2[a]	184.6 ± 13.7[b] (−27.8 %)	96.2 ± 0.6[c] (−62.4 %)
Peonidin 3-O-glucoside	50.8 ± 0.7[a]	28.2 ± 0.6[b] (−44.4 %)	15.5 ± 0.9[c] (−69.5 %)
Cyanidin 3-O-rutinoside	7.5 ± 0.1[a]	6.5 ± 1.0[a] (−13.0 %)	1.9 ± 0.4[b] (−74.7 %)
Peonidin 3-O-rutinoside	1.3 ± 0.1[a]	0.8 ± 0.2[b] (−38.5 %)	0.5 ± 0.0[c] (−61.5 %)
Cyanidin-O-diglucoside	18.6 ± 0.3[a]	14.4 ± 0.7[b] (−22.6 %)	5.4 ± 0.3[c] (−71.0 %)
Total	333.9 ± 4.4[a]	234.5 ± 14.1[b] (−29.8 %)	119.5 ± 1.2[c] (−64.2 %)

[a]Peonidin-3-O-glucoside is quantified as cyanidin 3-O-glucoside equivalents. Peonidin 3-O-rutinoside and cyanidin O-diglucoside are quantified as cyanidin 3-O-rutinoside equivalents
[b]Values are presented as mean ± SD, $n = 3$ and expressed as lg/g dry weight. Different letters in the same row correspond to significantly ($p < 0.05$) different samples. Values in parentheses represent the percentage of loss with cooking with respect to raw rice
[c]Cyanidin O-diglucoside content is the sum of the three peaks (r.t. 4.22, 4.61 and 4.86 min)

Hou et al. (2013) presented a study and provided detail information regarding the changes in kinetic stability of the four anthocyanins in black rice at temperature (80 °C, 90 °C, and 100 °C) at different pH levels (1.0–6.0). The data showed that degradation of anthocyanins followed first-order reaction kinetics. The four anthocyanins degraded more quickly with increasing heating temperature and pH values, especially, as heating temperature increasing to 100 °C and pH value to 5.0. Thus higher stability of anthocyanins is achieved by using lower temperature, lower pH values, and short time. This confirms the stability of anthocyanins depend on temperature and pH (Table 4.10).

Cooking and physiochemical properties of rice are strongly dependent on amylose content. Black rice variety has the higher protein content (8.16 %) with lower fat content (0.07 %) (Thomas et al. 2013). Variations in composition and cooking quality of rice mainly depend on the genetic as well as surrounding

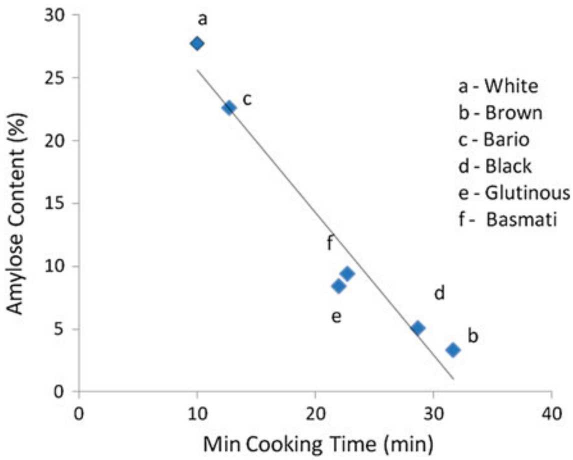


Fig. 4.6 Relationship between amylose content and cooking time of various rice varieties (Thomas et al. 2013)

environmental factors where they are grown (Giri and Vijaya 2000; Singh et al. 2005). Rice grain quality is influenced by various physicochemical characteristics that determine the cooking behavior as well as the cooked rice texture (Bocevska et al. 2009). Physicochemical properties and cooking characteristics of rice depend on amylose content (Saikia et al. 2012) (Fig. 4.6).

4.7 Nutrient Content and Degree of Milling

Black rice shows a thicker bran layer than red rice. Around 80 and 65 % of the ash content of red and black rice is distributed in the bran layer, respectively. 4 % degree of milling (DOM) reduce 47 % of the fat content in red rice, while in order to reduce similiar fat content in black rice, a 7 % DOM is necessary. The total free phenolics are around 6 and 7 fold higher than bound phenolics for black and red rice respectively. Although the nonmilled black rice presents higher free and bound phenolics contents than nonmilled red rice, the red rice shows higher DPPH and ABTS + antioxidant activities (Paiva et al. 2014). Milling for 20 s is sufficient to remove most of the bran layer of the black rice sample, but 10 s of milling retains higher contents of nutritional components and rice antioxidants (Laokuldilok et al. 2013). Thus the relationship between milling time and DOM show a nonlinear curve. After milling, the amount of bran left on milled rice kernels is measured and is termed as Degree of Milling (DOM) (Fig. 4.7).

Glutinous black rice flour appears to have significantly higher gelatinization temperature and pasting viscosities including peak, trough, breakdown, and final

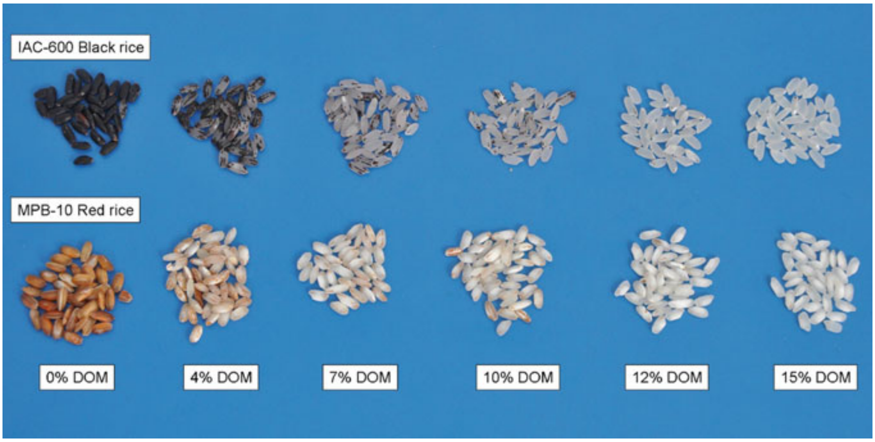


Fig. 4.7 Dehulled black rice and red rice samples subjected to different degrees of milling (Paiva et al. 2014)

viscosities. Compared to dry milled black rice flour, wet milled black rice flour show lower peak viscosity and higher final viscosity resulting in increase setback value (Lee 2013).

4.8 Aromatic Chemistry of Black Rice

Black rice (*Oryza sativa* L.) is a special aromatic rice popular in Asia. Characteristic flavor of black rice varieties plays an important role in the rice's reputation and its acceptance by customers. It has a unique flavor, but the volatile chemistry has not been reported in detail yet. Thirty five volatile compounds have been identified in black rice by gas chromatography mass spectrometry. Aldehydes and aromatics have been found quantitatively in the greatest abundance accounting for 80.1 % of total relative concentration of volatiles. The concentration of 2-acetyl-1-pyrroline (2-AP) is high, exceeded only by hexanal, nonanal, and 2-pentylfuran. But 2-AP, guaiacol, indole, and *p*-xylene largely influence the difference between the aroma in cooked black and white rice. 2-AP and guaiacol are major contributors to the unique character of black rice based on odor thresholds, relative concentrations, and olfactometry (Yang et al. 2008). Similarly, 21 and 23 odorants have been detected using GC-O for cooked samples of premium quality, waxy, and black pigmented rice cultivars, respectively. Hexanal is the main odorant in premium quality and waxy cultivars; however, waxy cultivars have 16 times higher hexanal odor activity values (OAVs) than premium quality cultivars indicating that the premium quality rice has a less-pronounced overall aroma. 2-Acetyl-1-pyrroline was found to be the main contributor to overall aroma in black pigmented rice followed by guaiacol. Six odor-active compounds (2-acetyl-1-pyrroline, guaiacol, hexanal, (E)-2-nonenal, octanal, and heptanal) contributed the most in discriminating the three types of specialty rice (Yang et al. 2010). Ajarayasiri and Chaiseri (2008) reported that benzaldehyde was found in the highest concentration (1,203.7 ng/kg) but 4-vinyl-2-methoxyphenol (sweet, spicy and smoky odor) had the highest odor active value (OAV = 133) in black glutinous rice. However, the results from gas chromatography-olfactometry (GCO) indicated that there were four aroma active compounds in black glutinous rice. The most prominent odorants were (E,E)-nona-2,4-dienal (sweet, fatty) and 2-acetyl-1-pyrroline (pandan) that had a flavor dilution (FD) factor of 3. Two other compounds that had an FD factor of 1 were 2,3-butanediol (sweet, balsamic) and 1-octen-3-ol that had straw and earthy-aroma characteristics.

A research on red and black rice oil chemistry was conducted. The red rice bran essential oils yield was 0.031 %, and its major constituents were (E)-β-ocimene (3.12 %), nonanal (11.32 %), (2E, 4E)-decadienal (2.54 %), myristic acid (41.32 %), geranyactone (2.41 %), and methyl oleate (2.46 %). The black rice bran essential oils yield was 0.053 %, and its major constituents were nonanal (8.31 %), acrylic acid (3.21 %), 2-hydroxy-6-methylbenzaldehyde (2.81 %), pelargonic acid (4.21 %), and myrisitc acid (28.07 %) (Chung et al. 2011). Trans-β-carotene, quercetin, and isorhamnetin were present in the bran of all black rice cultivars within the range of 33.60–41, 1.08–2.85, and 0.05–0.83 µg/g, respectively (Nakornriab et al. 2008). Similarly, Sukhonthara et al. (2009) reported one hundred twenty nine (129) volatile compounds in red and black rice bran. He said

> Myristic acid, nonanal, (E)-beta-ocimene and 6,10,14-trimethyl-2-pentadecanone are main compounds in red rice bran, whereas myristic acid, nonanal, caproic acid, pentadecanal, and pelargonic acid are main compounds in black rice bran.

Guaiacol, presented at 0.81 mg/100 g in black rice bran is responsible for the characteristic component in black rice (Sukhonthara et al. 2009). The main constituents of the volatile compounds are nonanal, butylated hydroxytoluene, 1-hexanol, naphthalene, and dodecamethyl-cyclohexasiloxane. The changes of variety and content of volatile compounds from raw and processed materials could be attributed to the soaking and heating during the processing which might induce some chemical reactions and promote the extraction of some compounds. Ninety four of volatile compounds have been identified in black rice. And processing can significantly change the volatile compounds of black rice (Wu et al. 2013). Names of 94 volatile compounds are listed here:

1. Hexamethyl-cyclotrisiloxane
2. Methyl-pyrazine
3. Ethylbenzene
4. *p*-Xylene
5. 1-Hexanol
6. Styrene
7. 1,3-Dimethyl-benzene
8. 5-(1-Methylet hylidene)-1,3-cyclopentadiene
9. 1,3-Dimethyl-benzene
10. 2-Chloro-pentane
11. 2-Amino-5-methylbenzoic acid
12. 2,5-Dimethyl-pyrazine
13. Ethyl-pyrazine
14. Morpholine
15. 6-Propyltetrahydro-2H-thiopyran-2-one
16. (Z)-2-Heptenal
17. Benzaldehyde
18. Benzoyl bromide
19. N-Methyl-3-nitro-N-(2-phenylethyl)-benzeneethanamine
20. 1,2,4-Trimethyl-benzene
21. N-methyl-3-nitro-N-(2-phenylethyl)-benzeneethanamine
22. 1-Butanol
23. 1-Octen-3-ol
24. 6-Methyl-5hepten-2-one
25. 1-Butyl-cyclohexene
26. 2-Pentyl-furan

27. 2-Ethyl-6-methyl-pyrazine
28. Hexanoic acid, ethyl ester
29. 1-Methyl-2-pyrrolidinone
30. Octamethyl-cyclotetrasiloxane
31. 1,4-Dichloro-benzene
32. 2-Ethenyl-6-methyl-pyrazine
33. 2-Ethyl-1-hexanol
34. Indane
35. Benzeneacetaldehyde
36. (E)-2-Octenal
37. Acetophenone
38. 1-Heptanol
39. 3-Ethyl-2,5-dimethyl-pyrazine
40. 2-(Cyclopropylmethyl)-4,5-dimethoxy-benzenamine
41. Hexamethyl-cyclotrisiloxane
42. 1-Ethyl-2,4-dimethyl-benzene
43. 2-(1-Methylvinyl)thiophene
44. 1-Methyl-azetidine
45. Nonanal
46. 1-Ethyl-2,3-dimethyl
47. 4-Hydroxy-benzonitrile
48. 1,2,3,5-Tetramethyl-benzene
49. 1,7,7-Trimethyl-(1R)-Bicyclo[2.2.1]heptan-2-one
50. 1,2,3,4,5-Tetramethyl-benzene
51. 1-Ethyl-2,3-dimethyl-benzene
52. (E)-2-Nonenal
53. Octyl-oxirane
54. 3,4-Dimethylcyclohexanol
55. Naphthalene
56. Decanal
57. 2,3-Dihydro-benzofuran
58. Benzaldehyde,4-methyl-oxime,(Z)-
59. Isoquinnoline
60. 6-Methyl-tridecane
61. Ether,hexyl pentyl
62. 2-Methyl-naphthalene
63. Hexadecane
64. Oxalic acid, isobutyl pentyl ester
65. 1-Methyl-naphthalene
66. 2-Methoxy-4-vinylphenol
67. Docosane
68. Hexadecane
69. 1-Iodo-nonane
70. (Aminomethyl) cyclopropane
71. Dodecamethyl-cyclohexasiloxane

72. 5-Butyl-5-ethyl-1,3-bis(trimethylsilyl)-2,4,6- (1H,3H,5H)-pyrimidinetrione
73. Heptacosane
74. 2,3,6-Trimethyl-decane
75. Heptadecane, 2,6,10,14-tetramethyl
76. Propanoic acid,2-methyl-,hexyl ester
77. Biphenyl
78. Tetradecane
79. 2,2-Dimethyl-butane
80. 1-Phenyl-3-methylpenta-1,2,4-trien
81. 1,3-Dimethyl-naphthalene
82. 5,9-Undecadien-2-one,6,10-dimethyl-,(Z)-
83. Heptadecane
84. Pentadecane
85. Tetradecamethyl-cycloheptasiloxane
86. Butylated Hydroxytoluene
87. Fluorene
88. 2,3,4-Trimethyl-hexane
89. Phenanthrene
90. Carbon dioxide
91. Phthalic acid, isobutyl octadecyl ester
92. Hexadecanoic acid, methyl ester
93. 4,5-Nonadiene
94. Cyclohexanemethanol.

Fragrance is the most important trait among the domesticated characteristics of rice (*O. sativa* L.). It is highly valued trait and known to be primarily associated with 2-acetyl-1-pyrroline. It has been previously determined that the fragrance gene is located on chromosome 8 that controls the level of aromatic compound 2-acetyl-1pyroline (Bradbury et al. 2005). The recessive fragrance gene on chromosome 8 is associated with rice fragrance. The structure of fragrance gene (fgr) comprises 15 exons interrupted by 14 introns. Fragrance is a recessive trait, the alleles from fragrance varieties show the presence of mutations portion (i.e., the 8 bp deletion in exon 7), resulting in the loss of function of the fragrance gene product. This allele is responsible for rice fragrance. Interestingly, the concentration of 2-acetyl-1-pyroline (2-AP) is high in cooked black rice (Yang et al. 2008; Prathepha 2008).

Black nonglutinous rice contains the greater number of monoterpenoids. An herbaceous odorant, myrcene, occurs in the bran of all black rice varieties but not in the bran of white rice. This monoterpene could, therefore, be considered as a characteristic odorant of black rice (Chumpolsri et al. 2015). Terpenoids found in black glutinous rice (Adams 2001; Mottaram 2004) are as follows:

Compounds name—Odor description

1. α-Pinene-Green, pine-like
2. Camphene-Herbal, woody

3. Sabinene-Fresh, citrus note
4. β-Pinene-Sweet, green
5. Myrcene-Herbaceous, metallic
6. 1,4-Cineol-Eucalyptol-like
7. β-Cymene-Citrus, woody
8. Limonene-Green, citrus-like
9. 1,8-Cineol-Spicy, camphor-like
10. Trans-β- ocimene-Tropical, green
11. γ-Terpinene-Herbaceous, fruity
12. Trans-linalool oxide-Sweet, floral
13. Cis-linalool oxide-Sweet, floral
14. Linalool-Fruity, green
15. Camphor-Camphor-like
16. Terpinen-4-ol-Sweet, herbaceous
17. Fenchyl acetate-Coniferous, herbaceous
18. 10-(Acetylmethyl)-3-carene
19. Carveol-Citrus-like, fruity
20. α-Copaene-Woody, honey
21. α-Ylangene
22. α-Elemene-Herbal, fresh
23. α-Gurjunene-Wood, balsam
24. Trans-caryophyllene-Spicy, dry
25. α-Neo-clovene
26. Trans-cadina-1(6),4-diene
27. β-Bisabolene-Balsamic, woody
28. 7-Epi-α-selinene-Amber.

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