Saudi Journal of Biological Sciences 28 (2021) 7472–7480

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Application of Cyanidin-3-Glucosides as a functional food ingredient in rice-based bakery products

Habibul Bari Shozib [a,*], Md. Mariful Islam [a], Sultan Abu Saleh Mahmud [a], Md. Nazmul Bari [b], Nasima Akter [c], Saima Jahan [d], Shakir Hosen [a], Mohammad Nazir Hossain [e], A.H.M. Nurun Nabi [f], Muhammad Ali Siddiquee [a], Md. Manjurul Haque [g]

[a] *Grain Quality and Nutrition Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh*
[b] *Entomology Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh*
[c] *Agronomy Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh*
[d] *Mathmetical and Physical Sciences, East West University, Aftab Nagar, Dhaka 1212, Bangladesh*
[e] *Genetic Engineering and Biotechnology, Bangabandu Sheikh Mujibur Rahman Maritime University, Mirpur, Dhaka 1216, Bangladesh*
[f] *Biochemistry and Molecular Biology, Dhaka University, Dhaka 1205, Bangladesh*
[g] *Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh*

ARTICLE INFO

Article history:
Received 21 April 2021
Revised 11 August 2021
Accepted 15 August 2021
Available online 23 August 2021

Keywords:
Black pericarp rice
Bangladesh Rice Research Institute (BRRI)
Grain Quality and Nutrition (GQN)

ABSTRACT

Background: Black pericarp rice has recently become popular among rice consumers for its diverse health benefits specially anti-cancer effect. Cyanidin-3-Glucosides (C3G), an prominant bioactive component of anthocyanins which is abundantly present in black pericarp rice.
Objectives: We investigated, how effectively it can be used to fortify Cyanidin-3-Glucosides (C3G) content in red and white pericarp polished rice or rice based bakery products for more nutritional value.
Method: In the present study, we have characterized several black pericarp rice cultivars along with some red pericarp and white pericarp rice cultivars by physicochemical including mineral profiling, and quantified the C3G by UFLC and LCMS.
Results: C3G content was significantly reduced from raw rice to cooked rice condition. All the black pericarp rice cultivars synthesized C3G, while this content was not detected in red and white pericarp rice cultivars. However, when 25% of black pericarp rice were mixed with 75% red or white pericarp polished rice, C3G content was significantly retained in cooked rice conditions. Formulation of rice-based bakery food product using black pericarp rice powder was also remarkably retained the C3G content as compared to that of cooking. Black rice is harder in texture, difficult to digest and needs higher energy for cooking. Therefore, we tried to circumvent these challenges by fortifying 25% of black pericarp rice with white or red pericarp rice.
Conclusion: Fortification of C3G enriched black rice (25%) with red or white pericarp rice (75%) might bring a better nutritional quality in both cooking and baking condition. This may lead a way to the effective management of the non-communicable disease such as cancer for common rice consuming population.

1. Introduction

Rice is a major cereal crop that has a wide genetic diversity, along with thousands of varieties grown worldwide. Among the pigmented rice varieties, black rice has received growing consideration due to its high nutritious value (Ito, et al., 2019; Kushwaha, 2016). Based on characterization features such as physicochemical, extraction, quantification, and identification of phytochemicals with antioxidant potential several studies have been reported (Pang et al., 2018; Zhang et al., 2010; Shao et al., 2018). Rice has

* Corresponding author at: Grain Quality, and Nutrition (GQN) Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh.
E-mail address: habibul.gqn@brri.gov.bd (H.B. Shozib).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.08.042
1319-562X/

versatile functional nutraceutical properties and possesses an important role in the relation between diet and health. Several antioxidant activity compounds such as phenolic compounds, tocopherols, tocotrienols, and γ-oryzanol (Iqbal et al., 2005) are identified in rice. Red and black pericarp pigmented rice contain higher amounts of polyphenols (Tian et al., 2004; ZHOU, 2004). (Itani et al., 2002) (Itani et al., 2002) positively correlated the antioxidant activity with the concentration of total phenolics in the grain. Therefore, it reduces oxidative stress (Ling, et al., 2001) cancer (Hudson, 2000; Hu, 2003) blood lipids and related diseases, and hence prevents cardiovascular problems (Ling, et al., 2001) and diabetes complications (Morimitsu, et al., 2002; Yawadio, 2007). Food intolerance became an important public health concern, and the identification of effective strategies for prevention is obligatory. Gluten causes coeliac related to allergic disease. This intolerance can be at any age, from early childhood to the elderly. Since rice does not have gluten protein naturally, it has advantages to prepare rice-based bakery products over wheat-based products**.**

Black rice is an anthocyanin enriched pigmented rice with high market value. In current days people are very health conscious and intend to consume high-value food products which tend to be enriched with nutraceutical properties such as pigmented rice. Black rice is low in calories, higher in protein, and lower in carbohydrates compared to white and brown rice. Since black rice is usually consumed as unpolised or brown rice condition but due its high fiber content, sometimes it is difficult to get it gelatinated by cooking. Therefore, it is not recommended to consume brown rice alone but better to cook with a portion with polished white or red rice which might be beneficinal for human beings.The senior citizen might not be comfortable with brown rice only rather they will prefer if nutrition comes through a mixture of polished and brown rice together. It will improve the cooking condition as well as will be more tasty than brown-black rice alone. Considering the above-mentioned scenario, we tried to evaluate the loss of anthocyanins specially C3G while cooking and sorted out a way to retain a significant amount of C3G by mixing with red or white pericarp rice. So, present studies focused on the characterization of available black rice cultivars by physicochemical and estimation of C3G as a potential antioxidant. We further quantified the amount of C3G in cooked rice and baked rice with a combination of both white and red pericarp rice to evaluate the performance of black rice. Since this is an expensive value added product therefore we would like to further evaluate whether combination with other ordinary red and white pericarp rice could retain C3G significantly through cooking and baking fortification procedure.

2. Material and methods

2.1. Chemicals and standards used in this study

Cyanidin-3-glucoside was purchased from Sigma Aldrich (44689, Lot BCCC0843). HPLC grade Acetonitrile, Formic Acid, and Ethanol were used for the preparation of mobile phase and sample elution, respectively. 18.2 MΩ deionized distilled water was collected from Barnstead (Model no D4642-33).

2.2. Collection of rice germplasms

Fifteen (15) rice cultivars comprised of 11 black pericarp (such as BK1, BK2, BK3, BK4, BK5, BK6, BK7, BK8, BK9, BK10 and BK11), two red pericarp (such as Laxmideega, and BRRI dhan84) and two white pericarp (such as BRRI dhan80 and Gabura) rice were collected and used in this study. We collected black pericarp rice cultivars from hilly regions of Sylhet, Bandarban and Khagrachori districts of Bangladesh and rest of the white red pericarp rice and white pericarp rice were collected from GQN Division. In order to increase the seeds, all these germplasms were grown at area of 5 × 5 square meter for each in BRRI westbyed farm located in Gazipur, Bangladesh (BRRI Latitude: 23.99, BRRI Longitude: 90.40) during Aman and Boro 2020–21 seassion (i.e., Aman season July to November 2020 and Boro season November 2020 to April 2021). Standard agronomic practices were followed to ensure high quality seeds. After maturation, crop was hervested, and seeds were collected. The collected seeds were dried and stored at −20 °C for further use at GQN Division, BRRI.

2.3. Milling and physical characteristics

Each sample was milled unparboiled and analyzed for physicochemical properties. Milled rice outturn was determined by dehulling 200 g rough rice in Satake Rice Mill, followed by 45 s polishing in a Satake Grain Testing Mill TM-5 (Japan). Slide calipers were used for the measurement of grain length and breadth. Based on length, milled rice was classified into three groups including long (>6.0 mm in length), medium (5–6 mm in length), and short (<5.0 mm in length). They were again classified into three classes according to the length/breadth (L/B) ratio; slender (ratio > 3.0); bold (ratio 2.0–3.0) bold, round (ratio < 2.0) to determine size and shape.

2.4. Determination of amylose and protein content

Amylose content was determined by the method described in Juliano (Juliano, 1971). Alkali spreading value was determined according to the method of Little et al. (Little, 1958). Protein contents were calculated from nitrogen, and were determined by the method of Micro Kjeldahl.

2.5. Estimation of iron, zinc, and calcium and phytic acid (PA)

Each sample was digested, and estimated by the method of the Association of Official Agricultural Chemists, AOAC (Little, 1958). Rice powder (0.5 g) was taken into a 25 mL conical flask. Then for extraction of minerals, a 5 mL mixture of nitric acid: perchloric acid (5:2) was added to the flask. The sample was heated at 350 °C for digestion until the color became clear. Then the digested sample was cooled and filtered through a Whatman filter paper No. 1 and the volume was made up to 25 mL with de-ionized distilled water. Iron (Fe), zinc (Zn), and calcium (Ca) were determined by the atomic absorption spectrometry (Shimadzu Atomic Absorption Spectrophotometer AA-7000) using a different standard curve for each. For the preparation of standard curve for iron, absorbance of 0.0, 1.0, 2.0, 4.0, 8.0 μg mL^{-1} iron solutions at 348.3 nm, for zinc 0.05, 0.10 0.20, 0.40, 0.80, 1.00 μg mL^{-1} at 213.8 nm and for calcium 1.0, 2.0, 4.0, 8.0, 12.0 μg mL^{-1} at 422.7 nm were taken respectively. Phytic acid present in rice samples were determined colorimetrically by Wheeler and Ferral method (1971). About 200 mg rice powder was weighed and transferred into a 15 mL centrifuge tube. Hen 7.5 mL TCA (5%) solution was added and vortexed the mixture. The mixture was incubated at 60 °C for 10 min and then centrifuged at 5000 rpm for 10 min. He supernatant was transferred into a 25 mL volumetric flask. The extraction was repeated for 2 more times and transferred the supernatant into the volumetric flask and the volume was made up to 25 mL. Twenty mL extracted sample was taken into a 75 mL Technicon tube and then 5 mL ferric chloride solution was added. He tubes were then heated in a block digestor at 95 °C for 45 min. After cooling the tube, it was made up to 75 mL with deionized water and filtered through Whatman filter paper No. 42. Pipetted out 2.5 mL filtrates and then 2 mL potassium thiocyanate (29%) and 5 mL de-ionized water was added in each of the tube for making total volume 9 mL. Hen after mixing, the absorbance of the mixture was measured at 485 nm against water as blank. Pipetted out 5 mL of ferric chloride solution into a tube, and then 20 mL de-ionized water and 2 mL TCA (5%) was added. He flask was heated in a block digestor at 95 °C for 45 min. $fter cooling the tube, it was made up to 75 m L with de-ionized water and filtered through Whatman filter paper No. 42. Hen filtrates were pipetted from 0.5 to 2.5 mL in different tubes. He volume was made up to 7.5 mL with de-ionized water and 2 mL of potassium thiocynate (29%) was added in each tube. After mixing, the absorbance of the solution was measured at 485 nm. A standard graph of ferric ion was plotted. From the graph, the slope was determined and then values for phytic acid was calculated on the

assumption that four ferric ion combine with one molecule of phytic acid ($Fe_4P_6C_6H_6O_{24}$). Calculation was done on the basis of dry weight of the sample.

2.6. Cooking

Only selected germplasms including BK11 (black pericarp rice), BRRI dhan80 (white pericarp rice) and BRRI dhan84 (red pericarp rice) were used in this case. The 5%, 10%, and 25% of BK11 was mixed with both BRRI dhan80 and BRRI dhan84 then cooked at boiling temperature upto respective cooking time (until 90% of gelatinization). These rice samples were cooked using a consistent ratio of 1:1.5 (w/w) using 113 g of deionized distilled water to 75 g of rice. This rice to water ratio resulted in the complete absorption of water by the rice at the end of the cooking time. Rice was cooked using a commercial National view rice cooker (model NV-1 1.8L, 220–240 V, 50/60 Hz). A series of rice samples were presoaked in 113 g of water for one hour before cooking in the rice cooker to compare the effects of presoaking rice on anthocyanidin retention. Rice was cooked for 30 min in the rice cooker. All cooked rice was allowed to steam-cool for 5 min. after the heating stopped. Two aliquots of cooked rice, about 20 g each, were randomly selected from each sample and immediately frozen at −80 °C before freeze-drying. Each cooking test was performed three times on independent samples.

2.7. Formulation of rice-based bakery product

Rice-based bakery product such as rice cakes were prepared at GQN bakery laboratory at BRRI, Gazipur with a minimum of 10 g of protein 100^{-1}g. Ingredients were used as a mixture of rice and sago flours (4:1), vegetable fat or butter, rice bran oil, powder sugar, salt, milk, and egg. In adding the rice powder portion, we used 25% black rice powder with 75% white rice powder (4:1). In the mixing process, all the ingredients were put together for batter formation. The ingredients were fed into the dough mixer, where they were mixed properly for 10–15 min. In the Baking process, cake batter was put into the baking pan at 190 °C for 30 min. After baking cakes, they were passed on to cooling conveyors for natural cooling. We preferred natural cooling as it helps to maintain the texture quality of the cake.

2.8. Extraction of anthocyanin from black rice

Black rice powder and black rice cake were extracted three times with buffer solutions (0.2 M disodium hydrogen phosphate and 0.1 M citric acid, pH 3.2) at a solid–liquid ratio of 1:12 for 120 min. The solutions were first filtered with a 3 kDa membrane at room temperature under an operating pressure of 0.5 MPa, and the filtrate was then loaded on a glass column (20 mm × 1000 mm) of AB-8 resin, and 80% (v:v) ethanol was subsequently used as the eluent at a flow rate of 1.0 $mLmin^{-1}$. The eluate was collected and stored at −20 °C refrigerator for further experiments.

2.9. Identification of C3G from black rice and black rice cake

Shimadzu UFLC system (Japan) was used to identify the anthocyanins from black rice extraction. The diode array detector (SPD-M20A) was set at 520 nm. The anthocyanins were separated by a Prevail C_{18} (4.6 mm × 250 mm) with an average particle size of 5.0 μm at 0.2 $mLmin^{-1}$. at room temperature. The mobile phase consisted of A (water: formic acid = 99:1, v: v) and B (acetonitrile: formic acid = 99:1, v:v), with the following binary gradient elutions:0 ± 10 min, 95% A; 10 ± 15 min, 95 ± 5% A; 15 ± 17 min, 5% A; 17 ± 17.1 min, 5 ± 95% A; and 17.1 ± 22 min, 95% A. Oven temperature was set at 25 °C and the sample injection volume was 10 μL. The data were processed with LabSolutions software (Shimadzu, Japan). UFLC peak of Cyanidin-3-glucoside (Retention Time; 12.99 ± 0.2 min.) in raw black pericarp rice (BK11) and cooked rice were monitored at 520 nm and 280 nm, respectively. C3G concentration of the tested samples was calculated by using standard calibration curve (y = 20784x + 286223, R^2 = 0.9992) which is applied to this present study, and units of C3G were expressed in ppm ($mgKg^{-1}$). UFLC peck of anthocyanins specifically C3G compound in raw rice was monitored at 520 nm and cooked rice was monitored at 280 nm according to Hiemori et al, 2009 (Table 4).

2.10. Preparative HPLC

Before LCMS analysis, the individual anthocyanin C3G from raw black rice and a fraction collected from cooked rice were isolated using a Prevail C_{18} (4.6 mm × 250 mm). The composition of the mobile phase was the same as that described above. The flow rate was 3.0 mL, and the gradient was programmed 0 ± 10 min., 95% A; 10 ± 15 min., 95 ± 5% A; 15 ± 17 min., 5% A; 17 ± 17.1 min., 5 ± 95% A; and 17.1 ± 22 min., 95% A. Fractions collected were applied to a preconditioned PrepSep C_{18} SPE cartridge (Fisher Scientific, Darmstadt, Germany). After flushing buffer salts from the C_{18} SPE cartridge with water, the phenolic compounds were eluted with 85% methanol.

2.11. LCMS identification

Confirmation of the identity of the components isolated by preparative HPLC was carried out using a prominence-I (LC-2030C 3D Plus) and LCMS-2020 (Shimadzu, Japan). Samples (20 μL) were analyzed for scanning mode. The mobile phase was 50% methanol in water, and the flow rate was 0.1 $mLmin^{-1}$. MS interface was operated in positive ion mode using a cone voltage of 20 V, capillary voltage of 3.0 kV, and collision energy of 12 eV. The ion source temperature was maintained at 140 °C and the dissolution gas temperature at 340 °C. The flow rates of nitrogen (Peak Scientific) and cone gases were 460 Lh^{-1} and 67 Lh^{-1} respectively.

2.12. Statistical analysis

Duncan's multiple range test (DMRT) was applied on different parameters such as AAC %, Protein content (%), Minerals such as Zn, Fe, Ca, C3G content, and different combination of black rice mixtures (5%, 10%, and 25%) with red and white pericarp rice at cooking condition, etc. for statistical analysis using SPSS, version 20.0.

3. Results

3.1. Physicochemical chracteristics of local germplasms and HYVs of rice

In this study, we used different pigmented rice cultivars along with high yielding varieties (HYVs) (Fig. 1). Among them, BK1

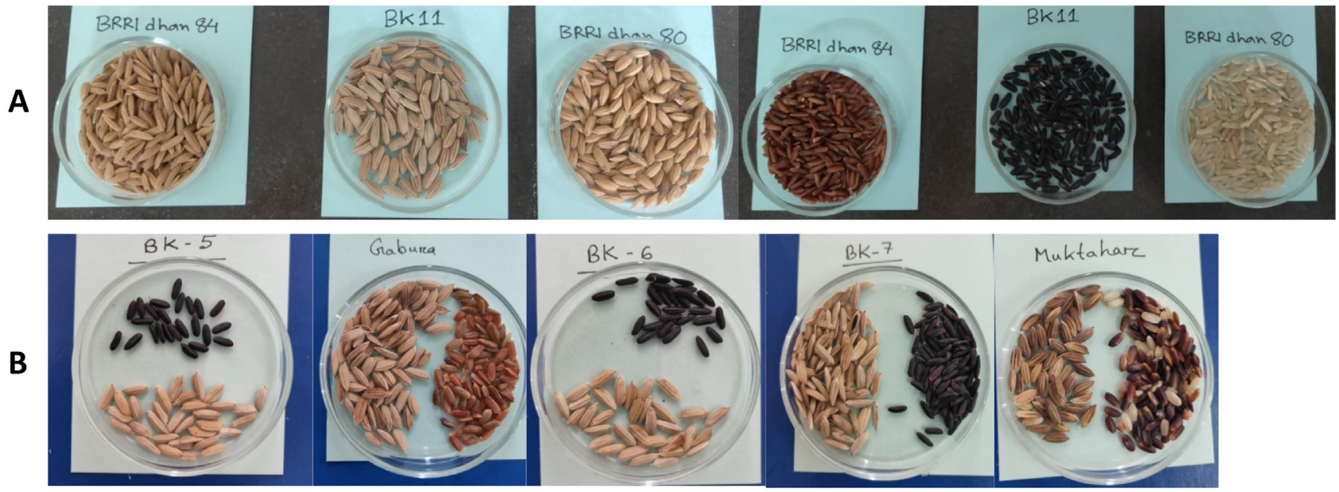

Fig. 1. Different **p**igmented rice cultivars including black, red and white pericarp rice of both local and high yielding varieties (HYVs).

was a mixture of both black and white pericarp rice, while BK2, BK3, BK4, BK5, BK6, BK7, BK8, BK9, BK10 and BK11 were solely black pericarp rice. Laxmidigga was the mixture of black and red pericarp rice, while BRRI dhan84 was exclusively red pericarp rice. On the other hand, BRRI dhan80 and Gabura were white pericarp rice. The decorticated brown rice length ranged from 5.9 to 7.6 mm (Table 1). BK2 and BK11 exhibited the highest length (7.6 mm) of brown pericarp rice, and these cultivars were also extra long slender and extra long bold type grain, respectively. BK3, BK4. BK5. BK6, BK8, BK9, BK10, BRRI dhan84 and Gabura were categorized as long bold type rice grains, while BK1 and Laxmideega were medium bold type rice grain. Among the tested cultivars and HYVs, only BK6 and BK9 have strong scented aroma (Table 1 and Fig. 1).

3.2. Protein (PC) and apparent amylose content (AAC)

The protein content was significantly varied in these cultivars and HYVs (Fig. 2A). This content varied from 8.52 to 12.16% (g 100^{-1}g). Remarkebly the highest protein content was obtained in BK7 (12.16%) followed by BK9 (11.43%) and BK2 (11.30%). Indeed, >10% (g 100^{-1}g) protein content was detected in all the black pericarp rice cultivars except BK6 (9.44%) (Fig. 2A). However, BRRI dhan80 contained the lowest protein content. Like protein content, AAC was also incredibly different in these cultivars which ranges from 2.97% to 25.74% (Fig. 2B). BRRI dhan84 produced the maximum AAC (25.74%) followed by Laxmidigga (24.61%) and BK10 (24.14%). Overall, black pericarp rice cultivars contained very low to intermediate level of AAC. Among the black pericarp rice cultivars, BK10 constructed the highest level of AAC (24.14%) which resembles intermediate AAC. We did not find any high AAC (>25.0%) in black pericarp rice cultivars (Fig. 2B). The minimum AAC was found in BK3 (2.9) which resemble very low or waxy type rice.

3.3. Mineral and phytic acid content of both rice and mixture of rice samples

We quantified different mineral contents including Fe. Zn and Ca in all the rice cultivars. Fe, Zn and Ca content were found to be considerably varied in these cultivars. The content of Fe, Zn and Ca fluctuated from 18.51 to 32.06, 3.65 to 20.15 and 8.63 to 72.71 $mgKg^{-1}$, respectively (Fig. 3). BK5 contained the highest level of Zn (32.06 $mgKg^{-1}$), Fe (20.15 $mgKg^{-1}$), and Ca (72.71 $mgKg^{-1}$) followed by BK11 (28.11, 19.28 and 33.2 $mgKg^{-1}$, Zn Fe, and Ca, respectively mg) among the tested rice samples. BRRI dhan84 exhibited the highest mineral enriched HYV inbred (Zn 27.55 $mgKg^{-1}$, Fe 10.21 $mgKg^{-1}$, and Ca 33.24 $mgKg^{-1}$). Thus, black pericarp rice might contain higher level of Zn, Fe, and Ca as compared to that of inbred HYVs and local germplasms. White rice are consumed as clean or polish rice (Low in Protein and mineral content) but usually black rice is consumed as brown rice (High in Protein

Table 1
Physicochemical parameters of local germplasms and HYVs.

Germplasms	Pericarp color	Length (mm)	Breath (mm)	L/B ratio	Grain size and shape	Aroma
BK1	Black and white	5.9	2.5	2.4	Medium bold	–
BK2	Black	7.6	2.2	3.4	Extra-long slender	–
BK3	Black	6.7	2.5	2.7	Long bold	–
BK4	Black	6.5	2.5	2.6	Long bold	–
BK5	Black	6.5	2.3	2.8	Long bold	–
BK6	Black	6.5	2.6	2.5	Long bold	+
BK7	Black	6.6	2.1	3.1	Long slender	–
BK8	Black	6.2	2.3	2.6	Long bold	–
BK9	Black	6.5	2.1	3.0	Long bold	+
BK10	Black	6.3	2.5	2.5	Long bold	–
BK11	Black	7.6	2.5	3.0	Extra Long bold	–
Laxmideega	Black and red	5.7	2.4	2.3	Medium bold	–
BRRI dhan84	Red	6.5	2.2	3.0	Long bold	–
BRRI dhan80	White	6.8	2.1	3.2	Long slender	–
Gabura	White	6.6	2.7	2.5	Long bold	–

Table 2
Retention of C3G in cooked black rice and mixed with red and white pericarp HYVs.

Samples	C3G (ppm or mg Kg^{-1})	C3G retention (%)
100% BRRI dhan80 (cooked)	Not detected	Not detected
5% BK 11 + 95% BRRI dhan80 (cooked)	25.04 ± 0.99f	3.24
10% BK 11 + 90% BRRI dhan80	38.94 ± 1.51e	4.66
25% BK 11 + 75% BRRI dhan80 (cooked)	109.26 ± 5.24c	12.98
100% BK11 (raw)	806.17 ± 1.06a	Not applied
100% BK11 (cooked)	387.40 ± 1.44b	47.85
100% BRRI dhan84 (cooked)	Not detected	Not detected
5% BK 11 + 95% BRRI dhan84 (cooked)	36.38 ± 5.33e	3.76
10% BK 11 + 90% BRRI dhan84 (cooked)	55.78 ± 2.67d	6.75
25% BK 11 + 75% BRRI dhan84 (cooked)	109.46 ± 6.53c	13.34

Any two-means having common letter (s) are not statistically different at a P < 0.05, as measured by the Duncan Multiple Range Test (DMRT).

and mineral content). So, 25% black variety and 75% white rice mixture will increase higher mineral content than white rice alone. On the top black rice is expensive enough to consume regularly by common people so, it will be economically viable to consume if takes as mixture. We have measured minerals such as Zn, Fe and Ca of white, red and black pericarp rice including mixtures and compared in tabulated form in Table 3 and Fig. 3. We have also measured phytic acid (PA) content of white, red and black pericarp rice including mixtures and compared in tabulated form in Table 3. PA act as anti-nutrient element for bioavailability of minerals specially Zinc and Iron. Since our data reveals there is a significant decrease of PA content in 25% Black variety and 75% red and white rice mixture than black rice alone so, it will increase mineral bioavailability through our daily mixture rice consumption (Table 3).

3.4. Quantification of cynidin 3 glucoside (C3G) from different rice cultivars

The C3G content was considerably varied in the rice cultivares. The C3G content was not detected in both red and white pericarp rice (such as Laxmideega, BRRI dhan84, BRRI dhan80 and Gabura), while all the black pericarp rice synthesized this compound (Fig. 4). Among the tested black rice cultivars, the highest C3G (806.17 $mgKg^{-1}$) was obtained in BK11 which is popularly grown in Khagrachori district, Bangladesh followed by BK10 (608.81 $mgKg^{-1}$) and BK8 (337.89 $mgKg^{-1}$) (Fig. 4).

3.5. Effect of cooking and fortification on Cynidin-3-Glucoside (C3G)

Cooking has a significant effect on C3G content. While cooking, C3G content was significantly reduced from raw rice to cooked rice. In this study, C3G content in BK11 was reduced 48.05% while cooking. In order to retain the C3G while cooking, we mixed 5%, 10%, and 25% BK11 with red pericarp rice BRRI dhan84 and white pericarp rice BRRI dhan80. We found fortification of 25% in both red and white pericarp rice, the C3G content retained 13.34% and 12.98%, respectively (Table 2), while fortification of 5% in both red and white pericarp rice, the C3G content retained only 3.24% and 3.76%, respectively (Table 2). On the other hand, 4.66 and 6.75% of C3G retained in both red and white pericarp rice, respectively when fortified upto 10%.

Only selected germplasms including BK11 (black pericarp rice), BRRI dhan80 (white pericarp rice) and BRRI dhan84 (red pericarp rice) were used in this case. The 5%, 10%, and 25% of BK11 was mixed with both BRRI dhan80 and BRRI dhan84 then cooked at boiling temperature up to respective cooking time (until 90% of gelatinization) of 30 min in the rice cooker. Since our target was to get minimum 90% of gelatinization for all samples but we observed that white rice along with mixture rice samples were 100% gelatinized during same treatment of 30 min as mixture rice definitely needs less time to cook as 75% white rice (Clean or Polished rice, less fiber) with 25% black rice (Cooking time of 25 min and the energy consumed of 0.27 kWh) than black rice alone (Brown rice, high content of fiber, Cooking time 30 mins, highest energy consumed as 0.33 kWh). Red rice and white rice alone need

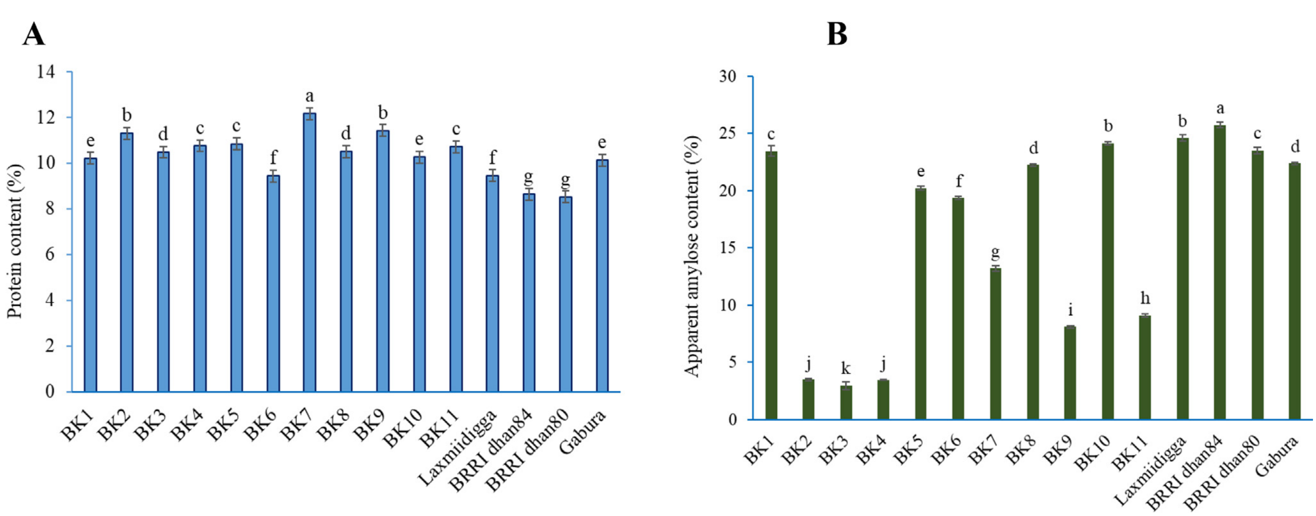

Fig. 2. Chemical properties of different rice samples. Protein content (A) and Apparent amylose content (B). Error bar indicates standard deviation (±).

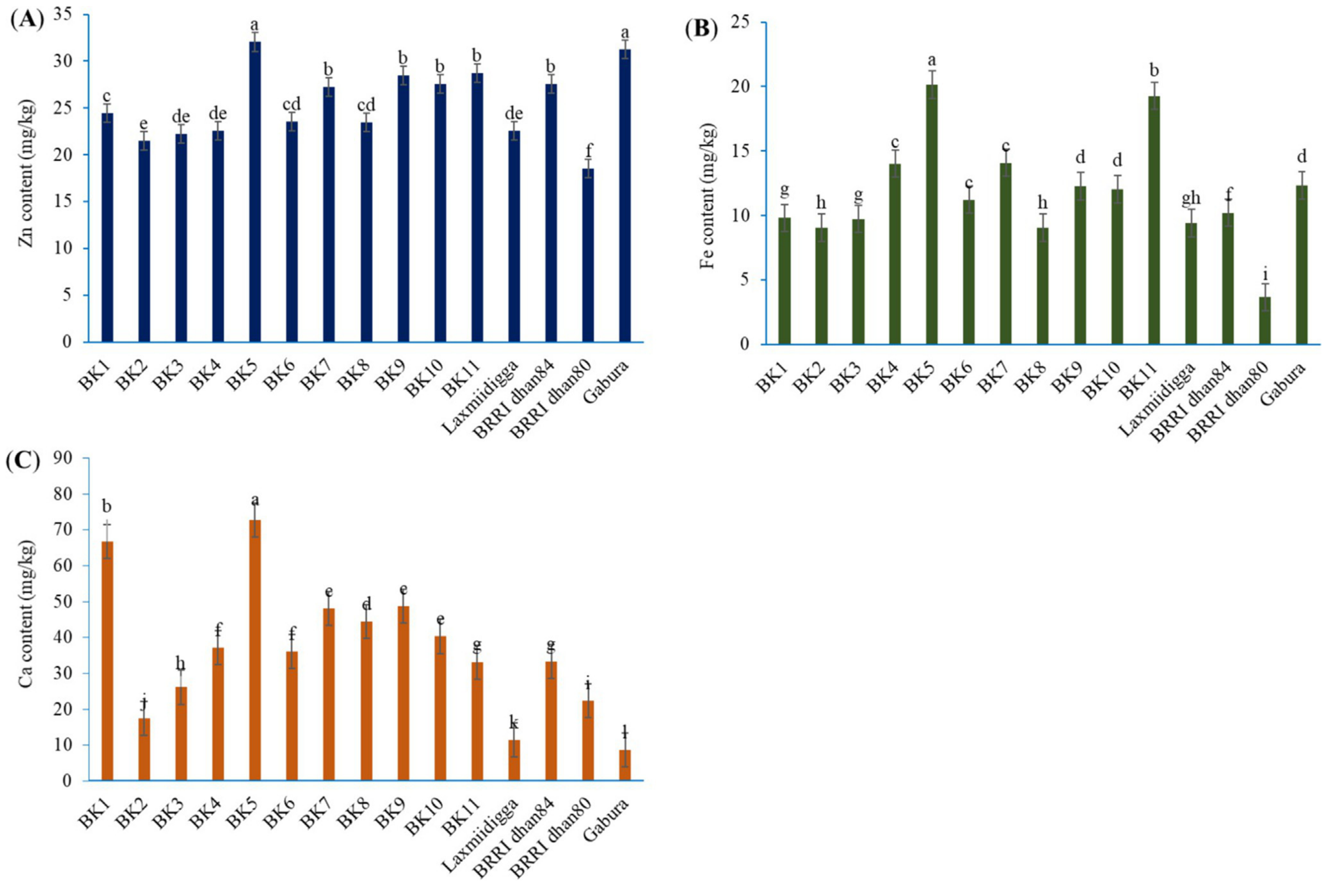

Fig. 3. Mineral profiling in different samples. Zinc (A), Iron (B) and Calcium (C).

Table 3
Mineral and Phytic acid content of both rice and mixture of rice samples

Germplasms	Zinc (Zn, ppm)	Iron (Fe, ppm)	Calcium (Ca, ppm)	Phytic Acid (PA, mgg^{-1})
BK11 (Black Rice)	28.71a	19.28a	33.20a	2.38a
BRRI dhan84 (Red Rice)	27.55b	10.21b	33.24a	1.23b
BRRI dhan80 (White Rice)	18.51d	3.65e	22.42c	0.38d
25% BK11 (Black Rice) + 75% BRRI dhan84 (Red Rice)	28.28a	7.8c	27.21b	1.12bc
25% BK11 (Black Rice) + 75% BRRI dhan80 (White Rice)	25.12c	5.69d	26.45b	0.78 cd

Any two-means having a common letter (s) are not statistically different at a P < 0.05, as measured by the Duncan Multiple Range Test (DMRT).

Table 4
UFLC and LCMS Data of C3G detected in Raw Black Rice.

RT (min)	λmax (nm)	[M]+ (*m*/*z*)	compound
12.99 ± 0.05	280, 520	449	Cyanidin-3-Glucoside

29 and 23 min respectively and both of them consumed energy of 0.32 kWh and 0.25 kWh respectively. So, mixture of rice consumption reveals less cooking time with lesser energy consumption in our study.

3.6. Retention of C3G in the baked cake of black and white pericarp rice.

We compared the retention of C3G in the baked cake of black and white pericarp rice, and cooked rice (Fig. 6). Interestingly, the retention of C3G in the baked cake of black and white pericarp rice was higher than the cooked rice. We also prepared the rice-based cake using 25% BK11 (black rice) with 75% BRRI dhan80 (white rice) as an active ingredient of carbohydrate source. In this case, C3G content was found to be 196.4 ± 1.65 $mgKg^{-1}$in the rice-based cake which resemble higher content than the cooked rice of similar proportion (Table 2 and Fig. 6). We also examined C3G at

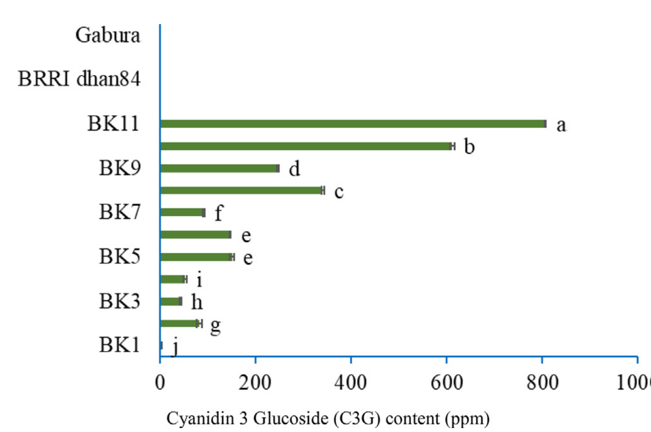

Fig. 4. Cyanidin-3-Glucoside (C3G) content (mg/kg) in different rice samples.

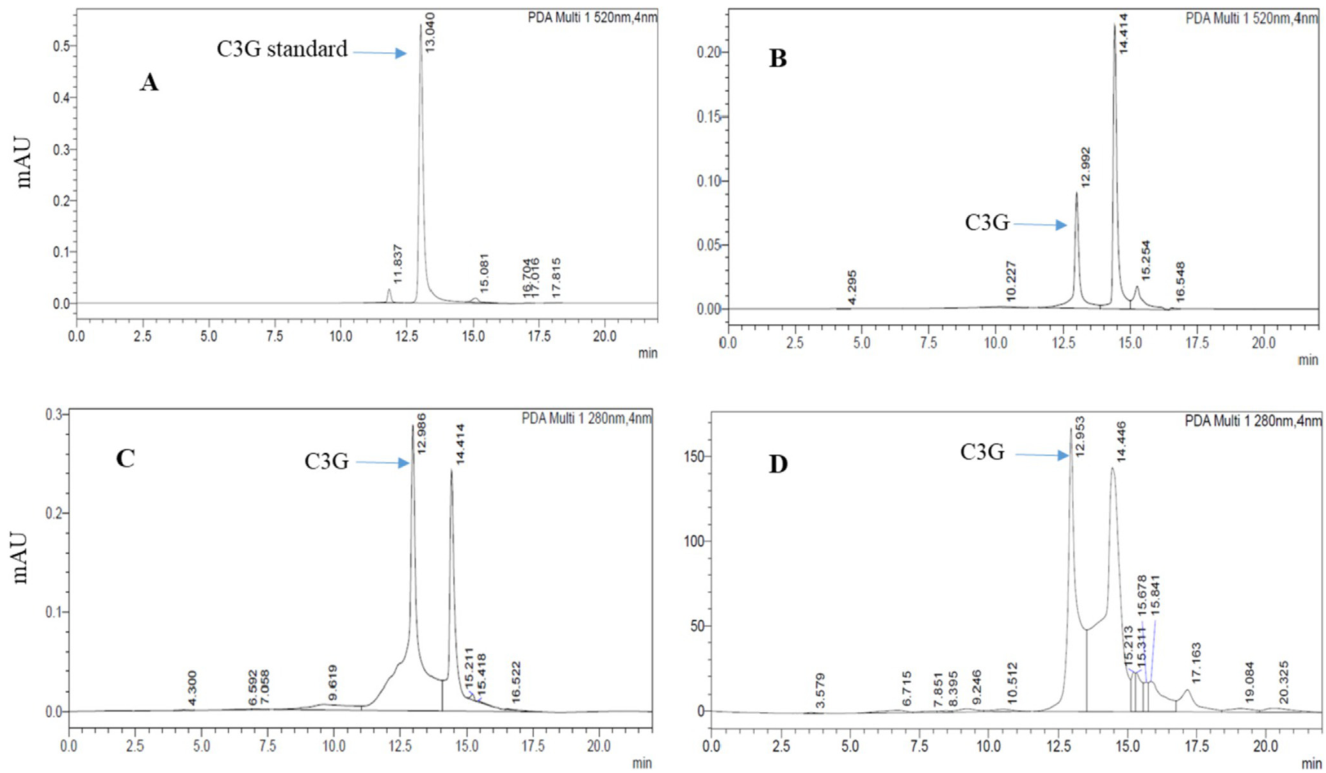

Fig. 5. UFLC peak of Cyanidin-3-glucoside (RT;13.04 mins) in standard solution (A) at 520 nm. UFLC peak of Cyanidin-3-glucoside (RT;12.99 mins) in raw and cooked rice (BK11) was monitored at 520 nm (B) and 280 nm (C) respectively. UFLC peak of C3G in black rice (BK11) based cake (D).

520 nm for raw rice and 280 nm for cooked rice and baked cake in UFLC, and *m*/*z* (499.0) of C3G has validated in LCMS accordingly (Table 4).). We had spiked Cyanidin-3-Glucosides (C3G) standard in rice sample and found retention time 12.99 ± 0.5 min for tested samples which were varies in RT for a range of 0.006 to 0.05 min considering several matrices such as raw rice, cooked rice, rice cake and solvent alone (Fig. 5).

Since anthocyanin specially Cyanidin-3-Glucoside (C3G) was found in all types of black rice except red or white pericarp rice in this study by UFLC method. We have reconfirmed the C3G presence in black rice by LCMS method through mass to ion ratio (*m*/*z*). So, in this consideration we did not run any red and white pericarp rice samples as initially UFLC analysis, we did not find the presence of C3G component in red and white rice.

4. Discussion

Rice is an important source of energy, hypoallergenic, easily digested, providing protein with higher nutritional quality, and has versatile functional nutraceutical properties. Rice has an important role in the relation between diet and health. Black rice is especially rich in anthocyanin pigments, phytochemicals, protein, vitamins, minerals and antioxidant properties. The bran hull of black rice is the outermost layer of the rice grain which contains one of the highest levels of the anthocyanin found in any known food. Anthocyanins are the flavonoid pigments of black rice and are the source of antioxidants that have the ability to inhibit the formation or to reduce the concentrations of reactive cell damaging free radicals. Anthocyanin antioxidants help to prevent cardiovascular disease, protecting against cancer that can be caused by free radical damage, improving brain function, reducing inflammation. Black rice rich in Cyanidin 3-Glucoside (C3G) and has hypolipidaemic effects through regulating hepatic lipogenic enzyme activities (Um, et al., 2013). It also ameliorates diabetic nephropathy via reducing blood glucose, suppressing oxidative stress and inflammation (Zheng, et al., 2020). Chen, P.N., et al. (2006) (Chen et al., 2006) gave molecular evidence associated with the anti-metastatic effects of Peonidin 3-Glucoside and cyanidin 3-glucoside, major anthocyanins extracted from black rice (*Oryza sativa* L. *indica*), by showing a marked inhibition on the invasion and motility of SKHep-1 cells. This effect was associated with a reduced expression of matrix metalloproteinase (MMP)-9 and urokinase-type plasminogen activator (u-PA). C3G is the active component of anthocyanin in black rice. In our study we were aimed to extimate C3G content in our Bangladeshi available black rice cultivars from different parts of Bangladesh. We also brought some red and white pericarp rice for comparitative analysis along with black rice. We characterized our black rice cultivars and found very high level of protein content (≥10%) and low to intermediate level of apparent amylose content (AAC) compare to other red and white rice.

Black rice cultivars posses higher mineral contents such as Zn, Fe and Ca than other rices. Zinc content of Black rice cultivars varied ranges from 21.44 ppm (mgKg^{-1}) to 32.06 ppm (mgKg^{-1}). Our data reveals diverse range of C3G content in black rice cultivars in Bangladesh. C3G content in black rice cultivars varied ranges from 2.58 ppm to 806.17 ppm. BK11 is the highest C3G content black rice cultivars in Bnagladesh followed by BK10, BK8, BK9. But we could not get confirm C3G content in both red and white pericarp rice by UFLC and LCMS in this study. Due to the nutraceutical enriched properties black rice has got immense potential both in domestic as well as overseas market and become expensive to purchase. Since black rice yield is lower than modern HYVs so expenses of cultivating black rice is costier than HYVs.

Thermal effect reduces the concentration of C3G in cooking condition than raw. Considering this into account we were aimed to investigate whether proportionate use of Black rice with other

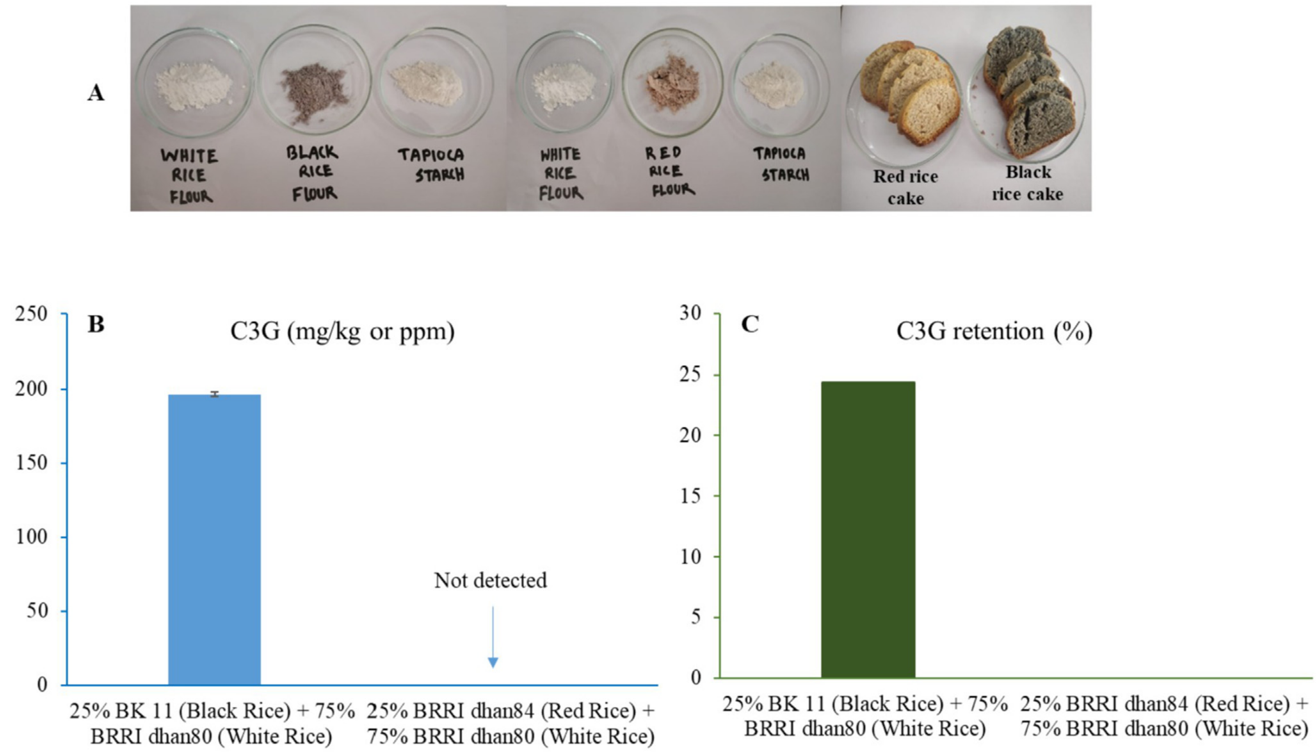

Fig. 6. Pictorial view of brown rice flour powder of Red (BRRI dhan84), Black (BK11), and White (BRRI dhan80) pericarp rice including Tapioca starch powder (A) and Pictorial view of black and red pericarp rice flour in preparation of rice-based cake (A). Graphical representation of C3G content in proportionate mixture of black and white rice (B), and red and white rice (C) at cooking condition.

red and white pericarp rice could bring effectiveness regarding availability of C3G as consumption of red and white rice is not able to provide C3G alone. Our data revealed that the proportionate mixture (1:3) of the highest C3G enriched black rice (BK11) and white (BRRI dhan80) or red (BRRI dhan84) pericarp rice retained 12.98 and 13.34% of C3G, respectively at cooked rice condition. In addition, we investigated whether C3G can effectively be used in baking condition over cooking condition. Our data suggested that black rice would be used as active ingredient of rice flour in baking condition it can retain higher C3G than cooking alone which resumble black rice flour can effectively be utilize in rice based baking industries.

5. Conclusion

Our data concluded that black rice should be consumed with white or red polished rice so that nutraceutical properties of common rice will be fortified which ultimately bring better nutritional value to humans. The utilization of black rice flour can fortify more bioactive compounds such as C3G in rice-based bakery products than rice. Combination of black rice along with red or white pericarp rice will be able to play a significant role in managing non-communicable diseases. Since black rice cultivars have poor yield gain so, BK11 can further be utilized as a superior crossing parent in the molecular breeding of *indica* type black HYV rice research program.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Authors would like to acknowledge all staffs of GQN Division of BRRI for their support. Special thanks to BRRI, Gazipur and PIU_BARC-NATP2, Farmgate, Dhaka authority for providing facilities to conduct the research activities.

References

Chen, P.-N., Kuo, W.-H., Chiang, C.-L., Chiou, H.-L., Hsieh, Y.-S., Chu, S.-C., 2006. Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. Chem. Biol. Interact. 163 (3), 218–229.
Hiemori et al, (2009). Influence of Cooking on Anthocyanins in Black Rice (Oryza sativa L. japonica var. SBR). J. Agric. Food Chem. 57, 1908–1914.
Hu, C. et al., 2003. Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J. Agric. Food Chem. 51, 5271–5277.
Hudson, E.A. et al., 2000. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidem Biomar 9, 1163–1170.
Iqbal, S., Bhanger, M.I., Anwar, F., 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem. 93 (2), 265–272.
Itani, T., Tatemoto, H., Okamoto, M., Fujii, K., Muto, N., 2002. A comparative study on antioxidative activity and polyphenol content of colored kernel rice. J Jpn Soc Food Sci 49 (8), 540–543.
Ito, V.C., et al. (2019). Effects of gamma radiation on the stability and degradation kinetics of phenolic compounds and antioxidant activity during storage of (Oryza sativa L.) black rice flour. Brazilian Archives of Biology and Technology, 62, 01–14.
Juliano, B.O., (1971). A simplified assay for milled rice amylose. Cereal. Sci. Today, 16: 334-338, 340, 360.
Kushwaha, U.K.S., 2016. Black Rice: Research History and Development. Springer International Publishing, 21–31 (Chapter 2).
Ling, W.H., et al (2001). Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. J Nutr, 131, 1421-1426.
Little, R.R. et al., 1958. Differential effects of dilute alkali on 25 varieties of milled white rice. Cereal Chem. 35, 111–126.

Morimitsu, Y. , et al. (2002). Inhibitory effect of anthocyanins and colored rice on diabetic cataract formation in the rat lenses. Int Congr Ser, 1245, 503-508.
Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y., Bao, J., 2018. Bound phenolic compounds and antioxidant properties of whole-grain and bran of white, red, and black rice. Food Chem. 240, 212–221.
Shao, Y., Hu, Z., Yu, Y., Mou, R., Zhu, Z., Beta, T., 2018. Phenolic acids, anthocyanins, proanthocyanidins, antioxidant activity, minerals, and their correlations in non-pigmented, red, and black rice. Food Chem. 239, 733–741.
Tian, S.u., Nakamura, K., Kayahara, H., 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. J. Agric. Food Chem. 52 (15), 4808–4813.
Um, M. Y., et al. (2013). Hypolipidaemic effects of cyanidin 3-glucoside rich extract from black rice through regulating hepatic lipogenic enzyme activities. J Sci Food Agric Sep;93(12):3126-8. doi: 10.1002/jsfa.6070. Epub 2013 Mar 7.
Yawadio, R. et al., 2007. Identification of phenolic compounds isolated from pigmented rice and their aldose reductase inhibitory activities. Food Chem. 101, 1616–1625.
Zhang, M.W., Zhang, R.F., Zhang, F.X., Liu, R.H., 2010. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. J. Agric. Food. Chem. 58 (13), 7580–7587.
Zheng, H., et al. (2020). Cyanidin-3-glucoside from Black Rice Ameliorates Diabetic Nephropathy via Reducing Blood Glucose, Suppressing Oxidative Stress and Inflammation, and Regulating Transforming Growth Factor β1/Smad Expression. J Agric Food Chem. 2020 Apr 15;68(15):4399-4410. doi: 10.1021/acs.jafc.0c00680. Epub 2020 Mar 25.
ZHOU, Z., 2004. The distribution of phenolic acids in rice. Food Chem. 87 (3), 401–406.