Ind. J. Nutr. Dietet., 2014, **51**, 348

Total Phenol, Flavonoid and Antioxidant Activity of Black and White Rice in Raw and Cooked Form

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(Received 4th July, 2014)

Abstract

This study was carried out to evaluate the antioxidant activities of ethanolic and aqueous extracts of black and white rice in raw and cooked form in various in vitro systems. Antioxidant and radical scavenging activities of black and white rice were assessed by using DPPH radical scavenging activity and reducing power assays. The antioxidant activity of samples was increased in a concentration or dose dependent manner. In DPPH radical scavenging activity, black rice raw ethanolic extract showed a higher inhibition activity of 88.24%. The reducing ability of ethanolic extract of raw sample of black rice was found to be 1.3 at OD_{700}, high among all the rice samples in different processing and in different extracts. Natural antioxidants like total phenols and total flavonoids were found to be higher in the raw sample of black rice in ethanolic extract i.e. 569.67±1.98 and 144.67±0.89 mg/100 g.

Keywords: *Black rice, white rice, total phenol, flavonoid, antioxidant, raw, cooked*

Introduction

Phytochemicals are bioactive non nutritive plant secondary metabolites belonging to the category of polyphenols, alkaloids, terpenoids, carotenoids and organosulfur compounds which are potent antioxidants. Whole grains, fruits, vegetables, spices and tea are natural sources of antioxidants[1]. Free radicals are unpaired electrons that can damage the cells and tissues of our body which lead to the degenerative diseases. Enzymatic and non-enzymatic antioxidants which neutralize the pro-oxidants, scavenge free radicals and suppress the reactive oxygen species which occur in tissues[2]. The role of antioxidants, either exogenous or endogenous, whether synthetic or natural, are scavenging the free radicals or preventing the free radical formation[3]. The secondary metabolite of polyphenolic compounds exhibits antioxidant, antimutagenic,

anticarcinogenic and antiglycemic properties[4]. The mechanisms of action of these compounds are by scavenging or chelating process[5]. Plant sources are good sources of antioxidants since they contain high amount of phenolic and flavonoid compounds[6].

Black rice commonly known as forbidden rice, is one of the whole grain rice varieties largely cultivated in Indonesia, China, Japan and Korea. It contains high amount of fibre and minerals and also supplies important essential amino acids and phytonutrients. It is purple and dark purple in raw and cooked form[7]. Many ongoing studies are seeking new uses for black rice and its seed-coats in nutraceutical and functional food formulations[8]. The color of black rice is due to the presence of anthocyanidins, mainly cyanidin-3-glucoside, delphidin -3-glucoside and peonidin-3-glucoside[9-15]. However, data on the effect of cooking on antioxidant activities of black and white rice was found to be meager. Hence, the present study was carried out to evaluate the *in vitro* antioxidant activities of ethanol and water extracts of black and white rice.

Materials and Methods

Black rice (Kavun rice) and white rice (Ponni rice) in raw form were collected from the village of Keelapoongudi, Karaikudi district, Tamil Nadu, India. The rice samples were stored in air tight plastic pouches and transported to the laboratory. Since white rice is commonly consumed, it was chosen as control for the study.

Preparation of rice samples

Raw rice powder

The raw samples of black rice and white rice were powdered separately by a heavy duty mixer grinder for two minutes and stored in air tight plastic pouches.

Pressure cooked rice powder

One hundred grams of white rice was weighed and washed with deionized water to remove impurities. After decanting excess water completely, the rice was mixed with deionized water in the ratio of 1:2 (w/v) and cooked for 20 min at 15 psi in a pressure cooker. The pressure cooked white rice sample was dried in a hot air oven at 30°C and powdered finely. Similarly, one hundred grams of black rice was weighed and washed with deionized water to remove impurities. After removing excess water, the rice was cooked in 1:3 ratio of water for about 35 min in the pressure cooker at 15 psi. The pressure cooked black rice sample was dried in a hot air oven at 30°C and powdered finely. The powders were stored in the air tight plastic pouches separately. Deionized water was used for washing and cooking the rice samples so as not to affect the mineral content in the samples.

Conventional cooked rice powder

One hundred grams of white rice was weighed and washed with deionized water to remove impurities. After decanting thoroughly, water in the ratio of 1:5 (w/v) was added to the rice and cooked by conventional method for 38 min. The excess water was drained out and was dried in a hot air oven at 30°C and powdered finely. In a similar way, 100g of black rice was weighed and washed with deionized water to remove impurities. After pouring out the excess water, the rice was cooked in 1:7 ratio of water for about 55 min by conventional cooking method. The conventionally cooked black rice sample was dried in a hot air oven at 30°C and powdered finely. The powders were stored in air tight plastic pouches separately.

Preparation of extracts

Rice samples were weighed (0.5g) and ground in a mortar and pestle in 5 ml of ethanol or water individually to prepare ethanolic and aqueous extracts of rice samples. Extracts were centrifuged at 2000 rpm for 10 min and the supernatant was collected in 50 ml volumetric flask. Then the residue was evaporated which is dissolved in known volume of water and used for the estimation of total phenolic and flavanoid content.

Estimation of total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method[16]. To 0.5 ml of sample solution, 0.5 ml of 2% $AlCl_3$ in ethanol solution was added and incubated for one hour at room temperature. The developed yellow color was measured at 420 nm in a UV visible spectrophotometer. Using the quercetin, a standard graph was prepared and the total flavonoid content was expressed as quercetin equivalent (mg/g).

Estimation of total phenol content

Folin-Ciocalteu method was used for phenolic estimation[17]. The sample extract of 0.1 ml was added to 3 ml of distilled water and then Folin-Ciocalteu reagent of 0.5 ml was added. After 3 min 2 ml of 20 % sodium carbonate was added and mixed thoroughly. The tubes were kept in a boiling water bath for exactly one minute. The absorbance was measured at 650 nm in a spectrophotometer against the reagent blank after the tubes were cooled. The standard graphs were plotted using the different concentrations of gallic acid (0.01-0.1 mM) and the total phenolic content were expressed as mg of Gallic Acid Equivalents (GAEs) per g of extract.

In vitro antioxidant activity

The antioxidant activity of rice extracts were determined by *in vitro* methods such as DPPH free radical scavenging assay and reducing power assays. Fifty grams each of powdered rice samples of raw, pressure cooked

and conventionally cooked black rice and white rice were homogenized with ethanol and water (250 ml) and the homogenate was kept in a shaker at 27°C for 24 hrs and then filtered using Whatman No. 1 paper. The residue was then extracted with two additional 250 ml portions of corresponding solvents as described above and the supernatants of each extract were combined. The dried extract was stored at 4°C until further used. Fifty mg of the crude extract was made up to 50 ml with ethanol or water and these aqueous and ethanol extracts of rice samples were analyzed for antioxidant scavenging assays. All the *in vitro* assays were carried out in triplicate and average values were considered.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by the Blois method[18]. Various concentrations of rice extracts (4.0 ml) were added with 1.0 ml of methanolic solution containing DPPH radicals (the concentration of DPPH is 0.1 mM). The tubes were shaken vigorously and allowed to stand for 30 min and measured at 517 nm BHT was used as control. The percentage of DPPH decolorization of the sample was calculated using the formula.

$$\text{\% Radical scavenging activity} = \frac{\text{Control Optical Density} - \text{Sample Optical Density}}{\text{Control Optical Density}} \times 100$$

Concentration of 50% Inhibition value was also calculated.

Reducing power assay

Oyaizu method was used for the reducing power assay[19]. The total mixture contained 2.5 ml of various concentrations of rice extracts, 2.5 ml of 1 % potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control had all the reagents except the sample. The tubes were incubated at 50°C for 20 min and then 2.5 ml of 10 % (w/v) of trichloroacetic acid was added. The tubes were then centrifuged at 3000 rpm for 10 min. Five ml of the supernatant was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1 % ferric chloride. The absorbance was read at 700 nm. The blank sample contained distilled water and phosphate buffer. Increased absorbance directly indicates the increased reducing power of the sample.

Results and Discussion

Total phenols and flavonoids

Table I gives the total phenols and flavonoids in black and white rice raw and cooked samples.

The presence of phytochemical has been directly linked to the antioxidant activity against free radicals. Many phytochemicals containing phenolic compounds exhibit antioxidant activity[20]. Table I reveals that the ethanolic extract of raw black rice exhibited 569.67±1.98 mg/g of gallic acid equivalent of phenols followed

TABLE I

Total Phenols and Flavonoids in Black Rice and White Rice Samples

Parameters (mg/100 g)	Raw		Pressure cooked		Conventionally cooked	
	Water	Ethanol	Water	Ethanol	Water	Ethanol
Black rice						
Total flavonoids	123.45±0.98	144.67±0.89	117.33±0.23	131.23±0.12	98.67±0.33	116.43±0.12
Total phenols	441.67±1.21	569.67±1.98	423.56±0.52	531.78±0.33	417.22±0.56	520.34±0.33
White rice						
Total flavonoids	17.32±0.41	28.69±0.66	11.23±0.22	9.11±0.23	6.20±0.54	10.43±0.12
Total phenols	23.45±0.32	42.50±0.78	10.32±0.40	19.55±0.78	8.44±0.56	9.44±0.56

by pressure cooked and conventionally cooked black rice (531.78±0.33 mg/100 g and 520.34±0.33 mg/100 g respectively). Jeong and Lee stated that the methanolic extracts prepared from red sorghum and black rice showed higher polyphenolic content than other grains[21]. Though there is a considerable loss in phenolic content of the cooked samples, the loss in phenolic content is higher in conventionally cooked rice varieties.

This is in agreement with the reports of Barroga *et al.* who found that boiling and cooking reduced the amount of phenolics in legumes by 75%[22]. Hiemori *et al.* revealed that cooking methods caused reduction of antioxidant content in the black rice and it was shown that pressure cooking resulted in greater loss (79.8%) followed by the rice cooked in rice cooker (74.2%) which indicates that cooking black rice results in the thermal degradation[23].

The white rice exhibited very low phenolic content in ethanolic extract and water extract and was 42.50±0.78 mg/100 g and 23.45± 0.32 mg/100 g when compared to black rice varieties (Table I). It was noted that there was a loss of phenolic content in all pressure cooked rice varieties and further loss was observed in conventionally cooked rice varieties in ethanolic and water extracts. Finocchiaro *et al.* reported that there was a loss of antioxidants in cooking but it was limited when there was a full uptake of cooking water by the grains[24].

With regard to the flavonoid content of the black and white rice samples, the ethanolic extract of black rice raw established higher content of 144.67±0.89 mg/100 g than the pressure cooked samples of black rice 131.23±0.12 mg/100 g as seen in Table I. The considerable losses were found in the water extract than the methanolic extract of all the rice samples and further losses were seen in the cooked rice varieties. White rice exhibited very low flavonoid content in

ethanolic extract and water extract to the extent of 28.69±0.66 mg/100 g and 23.45±0.32 mg/100 g than black rice samples. Shen *et al.* reported that total phenolics and flavonoids displayed an increasing order in the white rice, red rice and black rice[25]. Naveena and Bhaskharachary reported that soaking and germination processes significantly reduced the total and individual polyphenolic contents in the commonly consumed millets and legumes[26].

DPPH Radical Scavenging activity

DPPH is a stable free radical which is reduced by Blois method[18]. This method is used widely to evaluate the antioxidant activity of plant extracts and foods[27,28]. Per cent DPPH radical scavenging activities of all the extracts were dose dependent. The dose dependent DPPH radical scavenging activities of raw and processed samples of *O. sativa* black and white rice varieties are shown in Table II.

The values of the 50% inhibition concentration (IC_{50}) of BHT and black rice raw ethanolic extract were 42.3 and 18.9 µg/ml respectively. A significant difference was seen between the raw and cooked forms of black rice. The IC_{50} of raw, pressure cooked and

TABLE II

DPPH Radical Scavenging Activity of Black and White Rice in Raw and Cooked Form

Samples	Concentration (µg/ml)					IC_{50} Value µg/ml
	200	400	600	800	1000	
BHT (Control)	29.67	48.37	65.33	86.37	88.37	42.3
Black rice						
REE	54.0268	61.9127	73.0872	81.2617	88.2483	18.9
RWE	30.7578	41.8220	52.1169	62.3080	71.3805	56.7
PCEE	24.7117	36.2438	48.7644	55.4036	61.6639	66.2
PCWE	19.4876	30.4118	42.6029	49.1927	58.5832	82.1
CCEE	17.4629	21.6425	27.7265	32.7182	39.7446	130.8
CCWE	12.2405	18.2322	22.7759	27.3311	35.9670	134.4
White rice						
REE	21.0873	27.0181	36.1285	42.7182	49.1927	101.9
RWE	15.7001	19.6425	27.2322	33.7067	40.2965	128.5
PCEE	14.0527	21.3014	27.2322	36.2932	44.2009	117.6
PCWE	12.4052	16.2438	25.7644	31.2240	39.6952	125.4
CCEE	10.7578	17.2899	21.3509	28.0593	34.2009	148.6
CCWE	9.06425	15.2553	19.3506	23.9061	26.3426	242.7

BHT–Butylated Hydroxy Toluene; REE– Raw Ethanolic Extract; RWE– Raw Water Extract; PCEE–Pressure Cooked Ethanolic Extract; PCWE–Pressure Cooked Water Extract; CCEE–Conventionally Cooked Ethanolic Extract; CCWE–Conventionally Cooked Water Extract

conventionally cooked samples of black rice in ethanolic and water extract were found to be 18.9, 66.2, 130.8, 56.7, 82.1 and 134.4 µg/ml respectively. This radical scavenging activity of raw and cooked rice samples is due to the presence of phenolics by the action of electron transfer / hydrogen donating ability[29]. According to Tsai and She[30] change in phenolic compounds after heating might be contributed to decrease of DPPH scavenging ability. However, the IC_{50} of raw, pressure cooked and conventionally cooked samples of white rice in ethanolic and water extracts were found to be 101.9, 117.6, 148.6, 128.5, 125.4 and 242.7 µg/ml respectively. Htwe *et al*[31] observed that DPPH of red rice showed a higher scavenging activity of 88.95% than the black rice 81.15%. In the present study, the high activity in black rice may be due to the high polyphenolic content of anthocyanin in the outer fraction than the red rice.

Reducing power assay

Table III gives the reducing power activity of black and white rice in raw and cooked forms.

TABLE III

Reducing Power Activity of Black and White Rice in Raw and Cooked Form

Samples	Concentration (µg/ml)				
	200	400	600	800	1000
BHT (Control)	0.19	0.36	0.53	0.71	0.89
Black rice					
REE	0.87	0.91	1.02	1.27	1.34
RWE	0.71	0.89	1.04	1.09	1.21
PCEE	0.59	0.61	0.71	0.94	1.14
PCWE	0.51	0.59	0.62	0.74	0.86
CCEE	0.41	0.49	0.51	0.59	0.63
CCWE	0.29	0.38	0.41	0.52	0.58
White rice					
REE	0.47	0.58	0.69	0.74	0.79
RWE	0.38	0.48	0.52	0.63	0.67
PCEE	0.31	0.39	0.46	0.51	0.58
PCWE	0.21	0.30	0.39	0.41	0.44
CCEE	0.20	0.25	0.28	0.31	0.33
CCWE	0.14	0.17	0.19	0.21	0.25

BHT–Butylated Hydroxy Toluene; REE–Raw Ethanolic Extract; RWE–Raw Water Extract; PCEE–Pressure Cooked Ethanolic Extract; PCWE–Pressure Cooked Water Extract; CCEE–Conventionally Cooked Ethanolic Extract; CCWE–Conventionally Cooked Water Extract

Antioxidants are also known as reductants, which can inactivate oxidants. They are involved in redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of the antioxidant (reductant). The test solution which has the various shades from green to blue depends upon the reducing power of the sample. The presence of reductants that is antioxidants in the rice extracts reduces the Fe^{3+}/ Ferric cyanide complex to ferrous form. Therefore the Fe^{2+} complex can be measured by the formation of Perl's Prussian blue at 700 nm[32]. Table III shows the reducing power of raw and processed rice samples of black and white varieties. In general the increasing absorbance value was directly proportional to the concentration of extracts. Therefore the increasing OD value is directly proportional to the increasing trend of reducing power.

From Table III, it is evident that raw samples of black rice ethanolic extract had a higher ferric reducing ability. The reducing ability of ethanolic and water extract of raw, pressure cooked and conventionally cooked samples of black rice were found to be 1.34, 1.14, 0.63, 1.21, 0.86 and 0.53 respectively at OD_{700}, high among all the rice samples in different processing and in different extracts. Tsai and She[30] concluded that there was change in the phenolic compounds after heating and contributed to the increase in reducing power. Similarly, the presence of anthocyanins in black rice might be attributed to the reducing power of the black rice sample extracts.

The raw, pressure cooked and conventionally cooked samples of white rice had reducing ability in ethanolic and water extracts of 0.79, 0.58, 0.33, 0.67, 0.44 and 0.25 respectively at OD_{700} least value compared to black rice samples. The decrease in reducing power of pressure cooked samples correlates with the low level of phenolic contents, because during cooking, part of phenolics diffused from the grain coat to cooking water[33]. The results revealed that the rice sample extracts can act as an electron donor and react with free radicals and convert them to stable products, thus terminating the radical chain reaction.

Conclusion

The findings of this study showed the high antioxidant activity in black rice may be due to the high polyphenolic content of anthocyanin in the outer fraction. The black rice sample extracts showed higher radical scavenging activity with the lower concentration and indicated the contribution of anthocyanidins mainly like cyanidin-3-glucoside and peonidin-3-glucoside contributes to the antioxidant activity of black rice through its strong radical scavenging activity.

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