



Antioxidant Potential of Brown Rice

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ABSTRACT

A tastier food which attracts most of the population is rice. Taking antioxidants artificially will be troublesome for people depending upon their age group. But, taking antioxidant rich foods through diet will be a natural remedy as it helps in aiding our immune system. Since, brown rice is edible, it was planned to study the secondary metabolites and antioxidant activity of aqueous extract containing 25, 50, 75, 100mg of brown rice by adopting standard protocol. According to our results, brown rice was found to contain phenolics and flavonoids in higher concentration. Presence of these metabolites ensures higher antioxidant activity. Among the various antioxidants studied, nitric oxide scavenging, reducing power activity was higher on comparison with total antioxidant and metal chelating activity. Results showed that it will be a good antioxidant.

Keywords: Antioxidant, Brown rice, Medicinal value, Plant metabolites, Staple food.

INTRODUCTION

Rice is a staple cereal for over half of the world's population.¹ Brown rice is a whole grain and contains more fiber than white rice. Coloured rice's are reported as a potent source of antioxidants and encouragements as viable sources of antioxidants for functional foods were made.² Brown rice contains Thiamine-0.34, Riboflavin-0.05, Niacin-4.7, Iron-1.9, Magnesium-187.0mg/100g, Whereas, polished rice contain Thiamine- 0.07, Riboflavin- 0.03, Niacin-1.6, Iron-0.5, Magnesium- 13.0mg/100g, showing its richness in nutrients compared to white rice.³ Brown rice is a nutritionally valuable food source. The bran layers of the brown rice kernel are rich in dietary fiber, minerals, oil and vitamins, particularly the B vitamins. The complete milling and polishing that converts brown rice into white rice destroys 67% of vitamin B3, 80% of vitamin B1, 90% of vitamin B6, half of the manganese, phosphorus, 60% of iron and dietary fiber, essential fatty acids. Fully milled and polished white rice is required to be "enriched" with vitamins B1, B3 and iron. Brown rice is important for health because its bran components of pericarp, seed coat, nucleus, and aleurone layer contain a number of functional components, such as vitamin E and γ -oryzanol.⁴

Studies report that the bran layers have cholesterol reducing properties.^{5,6} An alternative to white rice would be to consume brown rice, since it lowers insulin and glycemic indices and may confer other health benefits.⁷ Since, rice is our staple food, it is essential to have an alternate which is able to give satiety as well as overall health benefits. Hence, the present study was aimed to assess the secondary metabolites and antioxidant activities of brown rice.

MATERIALS AND METHODS

Sample collection

The brown rice was purchased from super market located at Chennai, Tamil Nadu, India during February, 2014. The purchased rice were cleaned thoroughly and ground to powder using blender for further use.

Aqueous extract preparation

Aqueous extract was prepared by taking 25 to 100mg of brown rice powder. Each concentration was dissolved in 10ml water mixing with a magnetic stirrer at 4°C for 4h. The mixture was filtered through nylon cloth and centrifuged at 20,000g for 30min. 0.1ml of supernatant was used for the analysis. Each experiment was performed three times and 0.1ml was used for each experiments. Each experiment was repeated thrice.

Determination of secondary metabolites

The phenol and flavonoid content of aqueous extract was analysed.

Determination of total phenol content

Total phenolic content were determined by Folin-ciocalteau method. The extract (0.1ml) was mixed with folinciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and added aqueous NaCO₃ (4ml, 1M). The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. The standard curve was prepared. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.^{8,9}

Estimation of flavonoids

The aluminium chloride method was used for the determination of the total flavonoid content. Extract



solution were taken and to this 0.1ml of 1M potassium acetate, 0.1ml of AlCl_3 (10%), 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415nm was recorded after 30min of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.¹⁰

Determination of antioxidant activities

Nitric oxide scavenging assay, Reducing power assay, Total antioxidant assay and Metal chelating activities were performed.

Nitric oxide scavenging activity

This procedure is based on the principle that, sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate buffered saline was mixed with extract and incubated at room temperature for 150min. After the incubation period, 0.5ml of griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Ascorbic acid was used as a positive control.¹¹

Reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20min. 1.0 ml of Trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and FeCl_3 (0.1ml, 0.1%) after mixing, the contents were incubated for 10min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control.¹²

Total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of green phosphate/Mo (V) complex at acidic pH by adding 4ml reagent solution containing 0.6M Sulphuric acid,

28mM Sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to RT, the absorbance of mixture was measured at 695nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid.¹³

Metal chelating activity

Add extract (0.1ml) to a solution of 2mM FeCl_2 (0.05ml). The reaction was initiated by the addition of 5mM Ferrozine (160 μl), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6ml) instead of sample solution was used as a control. Distilled water (160 μl) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution.¹⁴

For all estimations, readings were taken using UV- Visible spectrophotometer- Shimadzu, Japan make. Model UV 1800. Standard graph were plotted for all experiments using their respective standards and samples were plotted against standard by taking concentration in X axis and OD in Y axis.

Statistical tool

Each experiment was carried out in triplicate and the results are given as the mean \pm standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(x-M)^2}}{n-1}$

RESULTS AND DISCUSSION

Secondary metabolites and antioxidant activities

Table 1 shows the results of Secondary metabolites and Antioxidant activities. Among the secondary metabolites assessed, total flavonoids were higher at all concentrations studied compared to phenolics. Higher values obtained with flavonoid signify that flavonoids are the major class of phenolic compounds in plants. Likewise, nitric oxide scavenging as well as reducing power activity was found to be higher. But, Total antioxidant and metal chelating activity was found to be lower.

Table 1: Secondary metabolites and Antioxidant activities

Brown rice (mg)	Total Phenolics (mg/g)	Total Flavonoids (mg/g)	Reducing power activity (mg/g)	Total antioxidant assay (mg/g)	Nitric oxide scavenging activity (mg/g)	Metal chelating activity (mg/g)
25	10.66 \pm 1.15	12.66 \pm 2.08	31.0 \pm 1.0	11.33 \pm 1.15	34.33 \pm 2.51	5.83 \pm 0.57
50	15.53 \pm 3.32	38.2 \pm 2.76	67.96 \pm 1.35	15.53 \pm 2.04	50.63 \pm 1.35	17.06 \pm 1.66
75	30.66 \pm 1.15	60.66 \pm 1.15	118.66 \pm 1.15	34.66 \pm 1.15	72.66 \pm 3.05	23.33 \pm 6.35
100	128 \pm 6.92	126.66 \pm 4.61	154.66 \pm 6.11	68.0 \pm 4.0	204.0 \pm 6.92	58.0 \pm 4.0

Values are Mean \pm SD for three experiments



Rice possess phenolic compounds with different antioxidant activity, but discrepancies observed in the concentration of these compounds in the grains are primarily because of variation in genotype, pericarp colour, processing of grain and environmental factors.¹⁵ Phenolic compounds exert their antioxidant activity in different ways by directly scavenging some reactive species, including hydroxyl, peroxy and superoxide radicals, acting as chain breaking antioxidants and also act by suppressing lipid peroxidation recycling other antioxidants, such as tocopherol. Some phenolic compounds may bind pro-oxidant metals, such as iron and copper, preventing the formation of free radicals from these pro-oxidants while simultaneously maintaining their capacity to scavenge free radicals.^{15,16,17} Besides, the effects of some phenolics are related to the increase in the activity of antioxidant enzymes¹⁸ and induction of the synthesis of antioxidant proteins.¹⁹ Brown rice, too, has plenty of phenol, which is said to be effective in fighting cancer²⁰ and in reducing the risk of diabetes. The main phenolics in rice grains with light brown pericarp color are the phenolic acids, mainly ferulic and coumaric acids.^{21,22} The concentration of total phenolics in the rice grains has been positively correlated with the antioxidant activity.^{23,24,25}

CONCLUSION

From our results, it is concluded, that brown rice will be a good antioxidant as it contains phenolics and flavonoids. These secondary metabolites are able to induce an antioxidant activity which is an essential factor in scavenging free radicals. Eventhough, brown rice is not consumed by everyone like white rice, we must be aware of its nutrient content and health benefits in order to increase the consumption among the population.

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