Indian Journal of Biochemistry & Biophysics
Vol. 49, April 2012, pp. 115-123

Carbohydrate content and antioxidative potential of the seed of three edible indica rice (*Oryza sativa* L.) cultivars

Supratim Basu[1], Aryadeep Roychoudhury[2*], Saptadwipa Sanyal[3] and Dibyendu N Sengupta[4]

[1,3,4]Department of Botany, Bose Institute, 93/1, A. P. C. Road, Kolkata 700009, West Bengal, India
[2]Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Park Street, Kolkata 700016, West Bengal, India

Received 12 October 2011; revised 19 February 2012

Rice (*Oryza sativa* L.) grains or seeds are known to lose much of their nutrient and antioxidant contents, following polishing. The current study was undertaken to evaluate and compare the carbohydrate content and antioxidant parameters in the unpolished and polished seeds of three edible indica rice cultivars, namely Swarna (SW), the most popular indica rice cultivar in India and aromatic or scented cultivars Gobindobhog (GB) and Pusa Basmati (PB). While both the sucrose and starch content was the maximum in PB seeds (both unpolished and polished), the amylose content was the highest in SW polished seeds. SW polished seeds were superior as compared to GB and PB cultivars in terms of total antioxidant capacity, DPPH radical scavenging and Fe(II) chelation potential, as well as the highest lipoxygenase (LOX) inhibition or H_2O_2 scavenging potential, probably due to the maximum accumulation of total phenolics and flavonoids, the two important antioxidants. The reducing power ability was, however, identical in both SW and GB polished seeds. The PB polished seeds were more potent in superoxide and hydroxyl scavenging, whereas GB in nitric oxide (NO) scavenging. The common observation noted after polishing of seeds was the reduction in the level of carbohydrates and antioxidant potential, though the extent of reduction varied in the three cultivars. The only exception was GB, where there was no alteration in NO scavenging potential even after polishing. Our study showed the better performance of SW polished seeds with respect to higher amylose content and majority of the tested parameters governing antioxidant capacity and radical scavenging potential, thus highlighting the greater dietary significance of SW over the other two cultivars.

Keywords: Antioxidant activity, Carbohydrate content, Iron chelation, Lipoxygenase, *Oryza sativa*, Polished seeds, Radical scavenging, Reducing power, Rice

The proper ratio of prooxidants and antioxidants maintains the normal biomolecular function[1]. A shift in this ratio towards prooxidants or an imbalance between the two gives rise to oxidative stress[2]. It is induced by the excessive or uncontrolled formation of reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, H_2O_2, singlet oxygen and nitroso compounds. The evidences that they can cause oxidative damage to lipids, proteins, nucleic acids, carbohydrates and enzymes under severe oxidative stress and lead to numerous diseased conditions[3,4] are overwhelming. There is widespread interest in defining the possible role of diet in preventing and reversing ROS-induced chronic diseases. These protective effects are considered, in large part, to be related to the presence of various antioxidants in the dietary foodstuffs.

In recent years, research on antioxidants, especially exploration of potent natural compounds with low cytotoxicity from plants has become an important branch of biomedicine. The content, ability, capacity and function of antioxidative systems in several plant sources or the antioxidative potential of various food items are currently being investigated. The foods rich in antioxidants preserve an adequate function of immune cells against homeostatic disturbances[5] and are hence therapeutically beneficial. The different antioxidant nutrients include ascorbic acid, α-tocopherol, cysteine, reduced glutathione, carotenoids, polyphenolic compounds (flavonoids, anthocyanins, phenolic acids), indole carbinols as well as antioxidative enzymes[6,7].

The content of antioxidant molecules is strongly affected by the specific plant genotype and environmental conditions of the plant[8]. In one of our

*Corresponding author
Ph: 9903538297
E-mail: aryadeep.rc@gmail.com

Abbreviations: DPPH, 1, 1-diphenyl-2-picrylhydrazyl; GB, Gobindobhog; LP, lipid peroxidation; LOX, lipoxygenase; PB, Pusa Basmati; ROS, reactive oxygen species; SW, Swarna; TPC, total phenolic content.

earlier communications[9], we noted increase in antioxidants (flavonoids and phenolics) in rice (*Oryza sativa*) during polyethylene glycol (PEG)-mediated water stress together with the induction in radical scavenging and increased reducing power ability. The rice genotypes differing in their susceptibility to salt or water stress also differ with respect to the extent of induction of antioxidants or scavenging mechanisms.

Rice is the staple food for more than half of the world population. The rice grain consists of the endosperm, bran, embryo, outer grain layers and inedible fibrous hull. Unpolished rice refers to the whole grain (starchy endosperm with 6-10% protein) of rice, where the germ and outer layers enclosing the bran have not been removed[10]. The bran is considered as a waste product traditionally, but it is more diverse in composition and a rich natural source of several functional compounds. The polysaccharides present have strong antioxidant properties. In addition, phytochemicals like ferulic, *p*-coumaric, hydroxy-cinnamic, hydroxybenzoic, protocatechuic, vanillic, syringic, chlorogenic, caffeic and sinapinic acids that affect the radical scavenging activities have been reported[11,12]. The phenolic compounds, lipophilic antioxidant vitamin E (α-tocopherol) together with other vitamins (thiamin, riboflavin and niacin) and minerals (phosphorus, potassium and magnesium) also exert an antioxidative effect in unpolished rice[13].

Rice is, however, chiefly consumed in its polished form as a stable food in most countries. The polishing refers to the mechanical processing of the grain by first removing the hull from the grain to obtain brown or hulled rice and then further processing by additionally removing the bran layer from the endosperm to obtain milled rice. Polishing is done due to the consumers' preferences and also to prevent rancidity of the rice oil in the outer layers of the grain[14]. Rice is thus protected from oxidation and can be stored for up to one year without perishing. The predominant form of rice found in today's markets is, therefore, milled or polished rice. However, polishing also leads to the considerable loss of several nutritionally valuable rice components, which are mostly concentrated in the germ and outer layers than in the starchy endosperm.

There is limited published information available about the carbohydrate content and antioxidant activities of the unpolished and polished rice. Swarna (SW) constitutes the most popular indica rice cultivar in terms of food item. It forms an important selling commodity on wholesale markets of India and is preferably consumed by the people. In addition, the scented or aromatic indica rice cultivars like Gobindobhog (GB) and Pusa Basmati (PB) are essential to many delicacies, preferred as important foodstuffs due to the recognition of their good qualities like pronounced or fragrant odour and perfumed aroma. They are often used in the preparation of several delicious dishes[15].

In this study, we have investigated the carbohydrate content and antioxidant potential of the three indica rice seeds, estimated as sucrose, starch and amylose content, along with total phenolic content (TPC) and flavonoids. The antioxidative potential has been evaluated with respect to total antioxidant capacity, reducing power ability, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, superoxide, hydroxyl and NO scavenging activities, Fe(II) chelating activity, inhibition of conjugated diene formation in linoleic acid emulsion, lipoxygenase (LOX, EC 1.13.11.12) inhibition and H_2O_2 scavenging both in unpolished and polished seeds of the three rice cultivars. The comparative evaluation of such parameters may lead to the discovery of rice cultivars with high antioxidative potential and to the development of possible nutraceutical dietary agents rich in natural antioxidants.

Materials and Methods

Plant material

Seeds of Swarna (SW) were obtained from Chinsurah Rice Research Station (Hooghly, West Bengal), Pusa Basmati (PB) from Indian Agricultural Research Institute (IARI, New Delhi) and Gobindobhog (GB) from State Agricultural Research station (Bethuadahari, Nadia district, West Bengal). The seeds were sun-dried, dehulled manually and divided into two sets – one kept unpolished, while the other was polished on sandpaper with continuous shaking (250 rpm) for 8 h[16].

Estimation of sucrose, starch and amylose content

Sucrose was estimated using anthrone reagent[17]. About 0.4 g of dried seeds were homogenized in 10 ml of 80% (v/v) ethanol. The hexoses were destroyed by placing the reaction tubes on boiling-water bath for 10 min. After cooling, 0.1 ml of 30% (w/v) aqueous KOH was added and kept at 100°C for

10 min. When the tubes were cooled to room temperature (25°C), 3 ml of anthrone reagent (prepared by mixing 76 ml of conc. sulfuric acid, 36 ml of water and 0.15 g of anthrone) was added and incubated at 38°C for 20 min. The absorbance was recorded at 620 nm, where sucrose served as the standard. The amount of sucrose was expressed as mg g^{-1}.

For starch estimation[18], about 0.4 g seeds were homogenized in hot 80% (v/v) ethanol, centrifuged and the residue was retained, dried in hot water-bath and resuspended in 5 ml of water. Subsequently, 6.5 ml of 52% (v/v) perchloric acid was added to the residue and the contents were centrifuged. The supernatant was decanted and collected and the whole procedure was repeated thrice. The supernatants of each step were then poured and the total volume was made up to 100 ml with distilled water. The mixture was then filtered and an aliquot of 1 ml of filtrate was analyzed for starch content. To the 1 ml of the supernatant, 4 ml of anthrone reagent was added and boiled for 8 min and cooled rapidly. The absorbance was measured at 630 nm. The quantity of starch was calculated in terms of glucose equivalent and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed as mg g^{-1}.

For amylose estimation[19], homogenized seeds (about 0.4 g) were incubated with 1 ml of ethanol and 10 ml of 1 N NaOH at 100°C for 10 min. The volume was made up to 25 ml, followed by centrifugation. The extract (2.5 ml) was taken along with 20 ml water and three drops of phenolphthalein. 0.1 N HCl was added drop-wise until the pink color disappeared. The iodine reagent (1 ml) was then added, the volume was made up to 50 ml and the absorbance was read at 590 nm.

Estimation of TPC and flavonoids

For TPC estimation, about 0.5 ml of methanolic extract of seed samples was mixed with 2.5 ml of ten-fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5% (w/v) sodium carbonate[20]. The mixture was allowed to stand for 30 min at room temperature (25°C), and the absorbance was measured at 760 nm. The final results were expressed as tannic acid equivalents.

The flavonoid content of the seed samples was measured using a modified colorimetric method[21]. About 0.5 g of each sample was crushed to fine powder, dissolved in 10 ml methanol and extracted thrice with 10 ml of *n*-butanol. The extracts were pooled and concentrated under vacuum at 60°C. The residue was re-dissolved in 5 ml of 60% (v/v) ethanol and washed twice with 5 ml of 30% (v/v) ethanol. All the three parts were pooled together and filtered. The filtrate was diluted up to 25 ml with 30% (v/v) ethanol. An aliquot of 0.5 ml of the solutions was transferred to a test tube containing 4.5 ml of 30% (v/v) ethanol and mixed with 0.3 ml of 5% (w/v) sodium nitrite for 5 min. Then, 0.3 ml of 10% (w/v) aluminium nitrate was added. After 6 min, the reaction was stopped by adding 2 ml of 1 M NaOH. The mixture was further diluted with 30% (v/v) ethanol up to 10 ml. The absorbance of the mixture was immediately measured at 510 nm. The flavonoid content was calculated and expressed as rutin equivalents.

Determination of total antioxidant capacity

The assay was based on the reduction of Mo(VI) to Mo(V) by the methanolic extracts and subsequent formation of a green phosphate/Mo(V) complex at acidic pH[22]. The tubes containing methanolic extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. After the mixture was cooled to room temperature (25°C), the absorbance of each solution was measured at 695 nm. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

Reducing power determination

About 0.2 ml of methanolic extract of each sample was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Next, 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) was added to the mixture and centrifuged at 8000 rpm for 5 min. The aqueous layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% (w/v) $FeCl_3$ and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power[23].

DPPH radical scavenging activity

The antioxidant activity of the extracts was determined based on the scavenging activity of the stable 1, 1-diphenyl-2-picrhylydrazyl (DPPH) free radical[23]. The aqueous extracts (0.5 ml) of each sample were added to 3 ml of a 0.004% (v/v) methanolic solution of DPPH. Water (0.5 ml), in place of the plant extracts was used as a control. The absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated as $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

Assay of superoxide radical (O_2^-) scavenging activity

The determination of superoxide dismutase (SOD) was carried out in the riboflavin-light-nitrobluetetrazolium (NBT) system[24]. Each 3 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, NBT (75 µM) and 1 ml of methanolic extracts. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp. The inhibition percentage was expressed as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

Assay of hydroxyl radical scavenging capacity

The hydroxyl radical was assayed using the deoxyribose method[25]. About 1 ml of final reaction mixture contained 0.5 ml of methanolic extracts and 0.1 ml of each of 100 µM $FeCl_3$, 100 µM EDTA, 20 mM H_2O_2, 100 µM L-ascorbic acid and 30 mM deoxyribose in 0.2 M phosphate buffer (pH 7.4). The reaction mixture was incubated at 37°C for 1 h, followed by heating in a boiling water bath for 15 min after addition of 1 ml of TCA (2.8%, w/v) and 1 ml of 1% (w/v) solution of 2-thiobarbituric acid. The absorbance of the solution was measured at 532 nm against a phosphate buffer blank. The antioxidant capacity was expressed as µmol hydroxyl radical scavenged g^{-1} of seed samples. The inhibition percentage was expressed as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

Assay of NO radical scavenging activity

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent method[26]. About 2 ml of the methanolic extracts and sodium nitroprusside (5 mM) in phosphate buffered saline (PBS) in a final volume of 3 ml were incubated at 25°C for 150 min. After incubation, 0.5 ml of samples were removed and diluted with 0.5 ml Griess reagent [1% (w/v) sulfanilamide and 0.2% (w/v) N-(1-naphthyl) ethylene diamine dihydrochloride (NEDH)]. After 15 min, the nitrite produced was measured colorimetrically at 540 nm. The inhibition percentage was expressed as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

Measurement of Fe(II) chelating activity

The Fe(II) chelating activity of methanolic extracts of seed samples was measured as reported previously[27]. The reaction was performed in an aqueous medium. The seed extracts (2 ml) were mixed thoroughly with 2 mM $FeCl_2$ (0.2 ml) and 5 mM ferrozine (0.4 ml). The mixtures were left at room temperature (25°C) for 10 min. The absorbance of the resultant solution was read at 562 nm. The percentage of Fe(II) chelating activity was calculated as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. The Fe(II) chelating capacity of samples was expressed as µg EDTA equivalents g^{-1} of defatted material using a standard curve prepared with EDTA.

Inhibition of conjugated diene formation in linoleic acid emulsion

An aliquot of 0.2 ml of methanolic extracts was added to 1 ml of 10 mM linoleic acid emulsion (pH 6.6). The mixture was shaken and incubated at 37°C for 15 h. About 0.1 ml of the solution from either 0 h or 15 h incubation period was separately added to 3.5 ml of 80% (v/v) methanol. The absorbance at 234 nm was then measured[28]. The inhibition percentage was expressed as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

Measurement of LOX inhibition and H_2O_2 scavenging

The enzymatic lipid peroxidation (LP) mediated by LOX was measured spectrophotometrically, following an increase in absorbance of lipid hydroperoxide formation at 234 nm[29]. The 1 ml reaction mixture contained 250 mM linoleic acid substrate solution, 5 nM soybean LOX and 50 mM Tris buffer (pH 9.0). The methanolic extracts of different seed samples were incubated with soybean LOX for 2 min prior to the initiation of the reaction with the linoleic acid substrate. The decrease in hydroperoxide formation was calculated. The control contained all the reagents except the extracts.

For H_2O_2 scavenging activity assay, the methanolic extracts (0.2 ml) were added to 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 ml of 43 mM solution of H_2O_2 (prepared in the same buffer). The absorbance value at 230 nm of the reaction mixture was recorded at 0 min and after 40 min. For each concentration, a separate blank sample (devoid of H_2O_2) was used for background subtraction[30]. The concentration (mM) of H_2O_2 in assay media was determined using a standard curve.

Statistical analyses

All the experimental data (values) were mean of three independent series. The results were presented

as mean ± standard error (SE) based on three replications. The statistical significance was evaluated at $P \leq 0.05$ by two-sided Student's t-test.

Results

Determination of sucrose, starch and amylose content

The unpolished seeds of PB showed the maximum content of sucrose and starch; the sucrose level being 1.8-times of SW and 2.1-times of GB, while starch content was 3.1- times of SW and 1.4-times of GB. However, GB unpolished seeds showed higher amylose content, viz., 1.2-1.3-times that of SW and PB. There was statistically significant decrease ($P \leq 0.05$) in the three carbohydrate content in the polished seeds of all the cultivars. PB showed the maximum loss in sucrose (2.8-times less than unpolished seeds), SW in starch (2.5-times less than unpolished seeds) and GB in amylose (1.7-times less than unpolished seeds). The polished seeds of PB showed the highest starch content, but comparable sucrose content with GB. However, the amylose content was higher in SW polished seeds (1.2-times of GB and PB) (Table 1).

TPC and flavonoid content estimation

The unpolished seeds of SW registered the highest TPC content (1.5-times that of GB and PB), while the flavonoid content was similar in the unpolished seeds of all the cultivars. The polished seeds showed a reduction in the level of both TPC and flavonoids, with the latter showing significant reduction. In both GB and PB, TPC was decreased to half and the flavonoids decreased 4.4-4.7-times, as compared to the unpolished seeds. SW polished seeds showed comparatively smaller loss, viz., 1.8-times less TPC and 3.4-times less flavonoids than unpolished seeds. The reduction in TPC and flavonoids in the polished seeds of all the cultivars was statistically significant at $P \leq 0.05$. SW polished seeds accumulated more of these two antioxidants (1.7-times higher TPC than both GB and PB polished seeds and 1.2-times higher flavonoids than both GB and PB) (Table 1).

Total antioxidant capacity and reducing power ability

The total antioxidant capacity decreased significantly ($P \leq 0.05$) in the polished seeds of all the rice cultivars. However, this decrease was found to be slightly lesser in both SW and PB (1.2-times less than unpolished seeds) as compared to GB (1.4-times less). The maximum antioxidant capacity was recorded in SW polished seeds and the minimum in GB. Even in the unpolished seeds, SW showed the highest antioxidant potential (1.9-times of GB and 1.3-times of PB) (Fig. 1A).

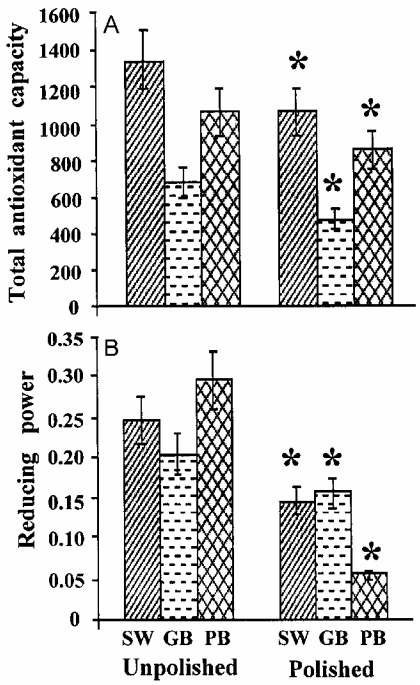

Fig. 1—Total antioxidant capacity (A) and reducing power ability (B) of the unpolished and polished seeds of SW, GB and PB [Total antioxidant capacity represented as μg mg^{-1} of plant material and reducing power as absorbance at 700 nm. The data represent mean of three observations (n = 3). The vertical bar at the top represents standard error (SE) in each case. * (asterisk) mark above the bar indicates statistically significant decrease at $P \leq 0.05$]

Table 1—Estimation of sucrose, starch, amylose, total phenolics and flavonoid content

[Values expressed as mean ± SE (n = 3). The decrease in the level of each carbohydrate, TPC and flavonoid in all the cultivars was found to be statistically significant at $P \leq 0.05$]

	Unpolished seeds			Polished seeds		
	SW	GB	PB	SW	GB	PB
Sucrose (mg g^{-1})	177.1 ± 0.01	146.2 ± 0.02	311.3 ± 0.01	90.8 ± 0.01	111.6 ± 0.02	109.4 ± 0.02
Starch (mg g^{-1})	33.3 ± 0.01	75.9 ± 0.02	103.7 ± 0.01	13.5 ± 0.01	47.4 ± 0.02	59.8 ± 0.01
Amylose (mg g^{-1})	66.2 ± 0.02	81.4 ± 0.02	63.3 ± 0.02	56.2 ± 0.01	48.8 ± 0.02	47.7 ± 0.01
Phenolics (mg g^{-1})	0.9 ± 0.01	0.6 ± 0.01	0.6 ± 0.01	0.5 ± 0.01	0.3 ± 0.01	0.3 ± 0.01
Flavonoids (mg g^{-1})	46.8 ± 0.06	51.7 ± 0.14	52.1 ± 0.10	13.6 ± 0.05	11.8 ± 0.12	11.2 ± 0.07

A significant reduction ($P \leq 0.05$) in the generation of reducing power was observed in the polished seeds of all the cultivars; the maximum decrease was noted in PB (5.4-times), while much lesser in SW (1.7-times) and GB (1.3-times). The polished seeds of SW and GB were identical in their reducing power ability (Fig. 1B). The extracts of polished SW and particularly GB seeds demonstrated better electron donor properties or neutralization efficiency of free radicals. However, unpolished seed extracts of all the three cultivars provided efficient reducing capacity, thereby acting as efficient reductones.

Free radical scavenging activity

The unpolished seeds of PB exhibited the maximum of DPPH, superoxide and hydroxyl radical scavenging abilities, followed by SW and GB. However, after polishing of seeds, PB showed a significant decrease in scavenging activity, especially the DPPH scavenging activity (5.6-times). The reduction in superoxide and hydroxyl scavenging was comparatively lesser, viz., 1.5-1.6-times. In case of polished seeds of SW and GB, the decrease in DPPH and superoxide scavenging was similar (1.4-1.7-times), while hydroxyl scavenging capability was reduced in SW (2.2-times) as compared to GB (1.5-times). While the DPPH scavenging was recorded the highest in the polished seeds of SW, the superoxide and hydroxyl scavenging were the maximum in PB polished seeds (Fig. 2A, B and C). GB seeds (unpolished or polished) gave the higher NO scavenging potential and hardly had any alteration due to polishing as compared to SW and PB (1.3-times less than unpolished). The decrease in scavenging capacity with polishing was statistically significant in all the cultivars ($P \leq 0.05$). The NO scavenging potential of SW and PB polished seeds was 1.6-times and 1.3-times less, respectively than GB polished seeds (Fig. 2D).

Fe(II) chelation capacity

Minimizing Fe(II) may afford protection against oxidative damage by inhibiting the production of ROS and lipid peroxidation. Thus, the extent of chelation of ferrous sulfate by the antioxidants in seed extracts of the three rice cultivars was examined. In this assay, SW showed the highest activity with both unpolished and polished seeds. However, a significant decrease ($P \leq 0.05$) in the chelation capacity was noted in the polished seeds of all the cultivars; the maximum

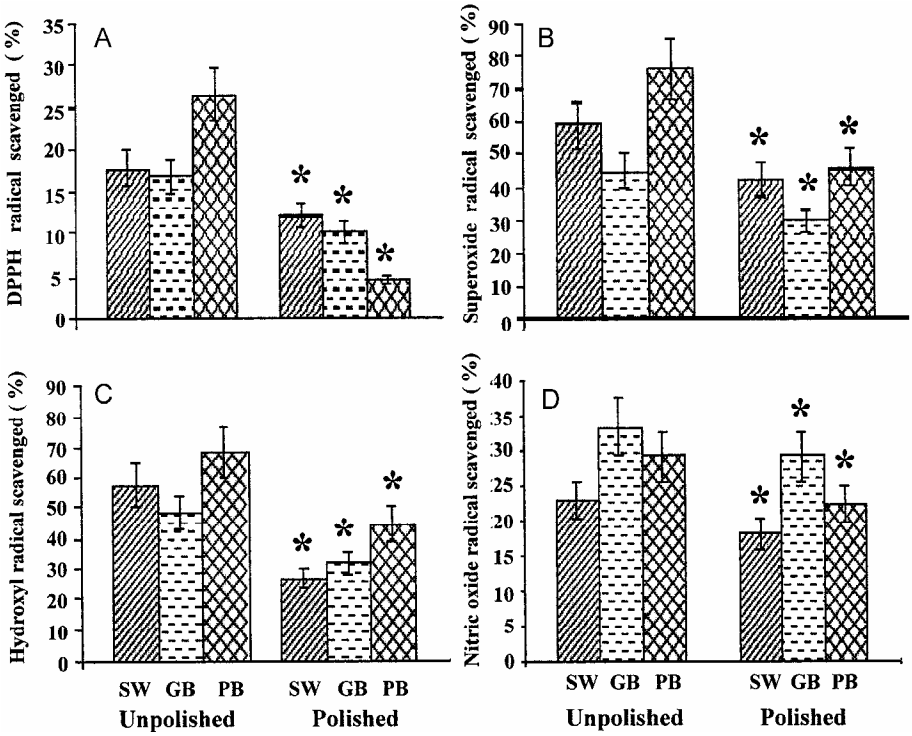

Fig. 2—Percentage of DPPH radical scavenging activity (A), superoxide radical scavenging activity (B), hydroxyl radical scavenging activity (C) and NO scavenging activity (D) of the unpolished and polished seeds of SW, GB and PB [Values expressed as mean ± SE (n = 3). * (asterisk) mark above the bar indicates statistically significant decrease at $P \leq 0.05$]

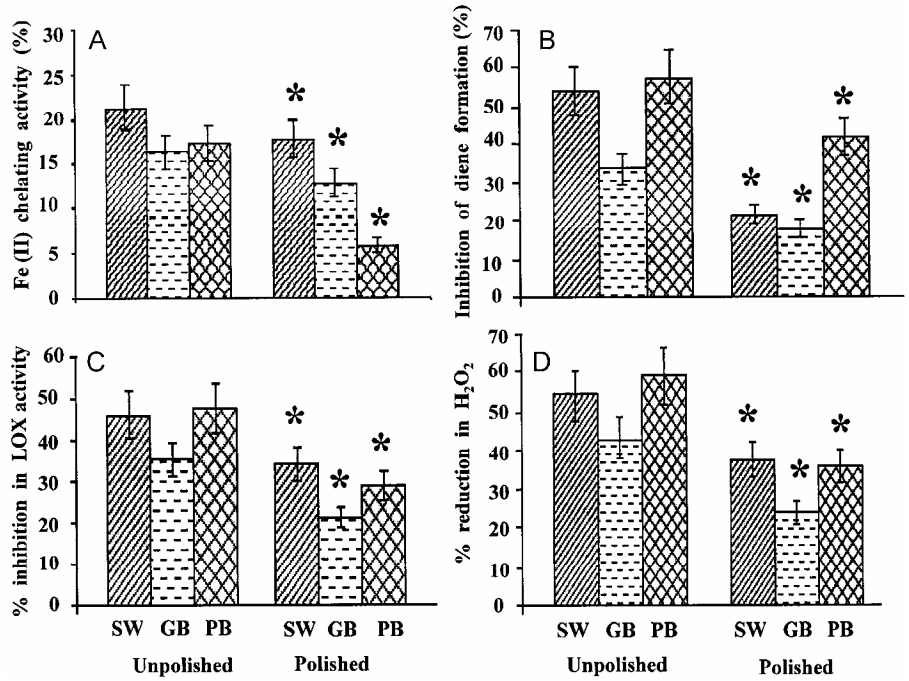

Fig. 3—Percentage of Fe(II) chelation activity (A), inhibition of conjugated diene formation (B), inhibition in LOX activity (C) and reduction in H_2O_2 concentration (D) in the unpolished and polished seeds of SW, GB and PB [Values expressed as mean ± SE (n = 3). *(asterisk) mark above the bar indicates statistically significant decrease at $P \leq 0.05$]

decrease was observed in PB (3.0-times), while lesser in SW and GB (1.2-times). The PB polished seeds showed significantly less chelation efficiency compared to SW or GB polished seeds (Fig. 3A).

Inhibition of conjugated dienes formation in linoleic acid emulsion

Enhancement of conjugated dienes inhibition percentage may be an indicator of prooxidant production. Among the unpolished seeds, PB and SW showed the highest percentage inhibition of diene formation (57.5% and 54%, respectively), while GB showed the least (33.6%). However, after polishing SW showed the maximum and significant ($P \leq 0.05$) decrease in inhibition percentage, viz., 2.5-times, followed by GB (1.9-times) and lesser for PB (1.4-times). PB polished seeds were more potent in the inhibition of conjugated diene formation, being 2.0-2.3-times more efficient than SW and GB polished seeds (Fig. 3B). The inhibition of conjugated dienes formation due to polishing was statistically significant ($P \leq 0.05$) in all the cultivars.

Estimation of LOX inhibition and H_2O_2 scavenging potential

The unpolished seeds of PB and SW exhibited almost similar LOX inhibition capacity (47.8% and 46.1%, respectively) and H_2O_2 scavenging potential (59.1% and 54.2%, respectively), while GB was the least efficient (43.1%) in this regard. After polishing, the LOX inhibition percentage decreased significantly ($P \leq 0.05$) in PB and GB seeds (1.7-times). For SW, the decrease was lesser (1.3-times), though significant ($P \leq 0.05$). SW polished seeds showed the highest LOX inhibition power (34.1%), while GB the least (21.2%) (Fig. 3C). A similar pattern was noted in case of H_2O_2 scavenging potential (Fig. 3D). Following polishing, H_2O_2 scavenging potential decreased significantly ($P \leq 0.05$) in all the cultivars, viz., 1.8-times in GB, 1.6-times in PB and 1.4-times in SW. The scavenging potential was the highest in SW (37.5%), followed by PB (36.1%) polished seeds and the least (24.2%) in GB polished seeds.

Discussion

The higher antioxidant activity in various plants[7] has a considerable role in the prevention of various degenerative diseases[31] by reducing free radical-induced oxidative damage. This has stimulated research to investigate the antioxidative potential of various plant sources. Rice constitutes a staple food item for a bulk of global population. The polished rice, which is mainly used for human consumption has lower nutritional value compared to non-polished rice, due to the commercial processing of the removal of the germ or outer layers. Thus, in this study, we

investigated the effect of polishing on the carbohydrate content and antioxidant potential of whole grains of three edible indica rice cultivars.

Mature storage tissues, such as cereal endosperm consist primarily of starch and a minor pool of soluble sugars like sucrose, glucose and fructose[32]. The endosperm sucrose pool represents an alternative storage form for incoming photosynthate. Rice sweetness may be important in the flavor formation during cooking or processing. Starches mainly contain different amounts of amylose and amylopectin, which influence physicochemical properties, such as gelatinization, retrogradation, water absorption and paste viscosity. Rice starch is usually digested quite rapidly, as compared to other starch foods. The amylose content in rice is also considered to be one of the most important traits related to the taste and cooking quality and determines the viscosity of cooked rice. There exists a relationship (positive or negative) between amylose content and the appearance quality traits (AQT) of rice[33]. All these factors make quantification of carbohydrates like sucrose, starch and amylose from different rice cultivars so important. Our observation suggested that both the sucrose and starch content were the maximum in PB seeds (both unpolished or polished), whereas the amylose content was the highest in SW polished seeds.

Earlier studies have shown that many flavonoids, phenolic acids, anthocyanins and related polyphenols contribute significantly to the total antioxidant activity[34], as their extensive, conjugated electron systems allow ready donation of electrons or H atoms from the hydroxyl moieties to free radicals. Most polyphenols are effective scavengers of hydroxyl and peroxyl radicals, can stabilize lipid oxidation and act as chelators of metals. The cereal grains contain special phenolic compounds, such as ferulic acid esters of phytosterols (steryl ferulates) and triterpene alcohols, which have been identified in rice, corn, wheat, rye and barley. The aleurone layer has been shown to exhibit the highest antioxidant activity, TPC and phenolic acids[35]. In our study, SW polished seeds accumulated more of the total phenolics and flavonoids than both GB and PB polished seeds.

The reducing power of bioactive compounds is generally associated with the presence of reductones[36], which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom[37]. The DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants[38]. When DPPH radicals encounter a proton-donating substance, such as an antioxidant present in plant extract, the radicals are scavenged. The superoxide radical is considered to play an important role in the peroxidation of lipids[39]. Amongst the oxygen radicals, the hydroxyl radicals in particular are the most reactive and severely damage almost any adjacent biological molecule it touches[40]. The most common ROS molecule is H_2O_2, which is mainly produced by mitochondria as a byproduct of oxidative metabolism. Because a high level of H_2O_2 is cytotoxic and can modify the protein conformation or alter protein function, the issue of H_2O_2 scavenging is also important. In addition to ROS, NO is also implicated in several pathological conditions[41]. Fe(II) chelation may render important antioxidative effects by suppressing metal-catalyzed oxidation[42]. Rice extracts have been reported to possess components acting as electron donors, which can terminate LP chain reactions, possibly through conversion of lipid peroxy radicals to more stable products[43]. The enhanced LOX activity is also associated with oxidative damage accompanied with the increased accumulation of reactive aldehyde-like toxic degradation products.

The TPC and antioxidant activities have been compared earlier in Thai white, red and black rice bran extracts[44]. The high levels of antioxidant activity as shown by DPPH radical scavenging and inhibition of LP assays or scavenging capacity of intracellular ROS in cell-based assays have been found to be closely correlated to their flavonoid and phenolic contents[45]. The crude methanolic extract from Njavara rice bran is shown to contain significantly high polyphenolic compounds with superior antioxidant activity, as evidenced by scavenging of free radicals, including DPPH and NO. The extract has also shown high reducing power activity, indicating that Njavara rice could be exploited as one of the potential sources for plant-based pharmaceutical products[46]. Increase in radical scavenging, lowering in LP levels and formation of vitamin E isomers as antioxidants, have been observed in transgenic soybean overexpressing the gene encoding rice homogentisate geranylgeranyl transferase (HGGT)[47].

Conclusion

A major aim of this study was the determination of rice cultivars with high antioxidative capacity.

Our work demonstrated substantial loss in the carbohydrate content and antioxidants in the polished rice seeds, the extent of losses varied in the three edible rice cultivars. Amongst the three cultivars, SW proved to be the best in terms of higher amylose content, total antioxidant capacity, DPPH radical scavenging, Fe(II) chelation potential and reducing power ability, as well as the highest LOX inhibition or H_2O_2 scavenging potential, accompanied with the maximum accumulation of total phenolics and flavonoids, the two important antioxidants. The higher antioxidant level or free radical scavenging property might be one of the mechanisms, making SW an important foodstuff with medicinal properties, thereby demanding its increased consumption to prevent oxidative damage. The PB polished seeds showed higher sucrose and starch content and were more potent in superoxide and hydroxyl scavenging capacity . However, NO scavenging was better in GB. The reducing power ability was, however, identical in both SW and GB polished seeds. Further investigation of individual compounds, their *in vivo* antioxidant activities, and their participation in different antioxidant mechanisms is warranted, including in other edible indica rice cultivars.

Acknowledgement

Financial assistance from Bose Institute, Kolkata, and Department of Biotechnology, Government of India, New Delhi, is gratefully acknowledged.

References

1 Valko M, Rhodes C J, Moncol J, Izakovic M & Mazur M (2006) *Chem-Biol Interact* 160, 1-40
2 Halliwell B & Whiteman M (2004) *Br J Pharmacol* 142, 231-255
3 De B R & Van L N (2004) *Mutagenesis* 19, 169-185
4 Finkel T & Holbrook N J (2000) *Nature* 408, 239–247
5 De la Fuente M & Victor M (2000) *Immunol Cell Biol* 78, 49–54
6 Prior R L, Cao G, Martin A & O'Brien C (1998) *J Agric Food Chem* 46, 2686-2689
7 Velioglu Y S, Mazza G, Gao L & Oomach B D (1998) *J Agric Food Chem* 46, 4113–4117
8 Lin K-H, Chao P-Y, Yang C-M, Cheng W-C, Lo H-F & Chang T-R (2006) *Bot Studies* 47, 417-426
9 Basu S, Roychoudhury A, Saha P P & Sengupta D N (2010) *Plant Growth Regul* 60, 51–59
10 Smith C W & Dilday R H (2003) Rice: origin, history, technology, and production, p. 642, John Wiley & Sons, Inc, New York
11 Lloyd B J, Siebenmorgen T J & Beers K W (2000) *Cereal Chem* 77, 551-555
12 Adom K K & Liu R H (2002) *J Agric Food Chem* 50, 6182–6187
13 Asamarai A M, Addis P B, Epley R J & Krick T P (1996) *J Agric Food Chem* 44,126–130
14 Montilla P, Espejo I, Muñoz M C, Bujalance I, Muñoz-Castañeda J R & Tunez I (2006) *Clin Nutr* 25, 146-153
15 Roychoudhury A, Basu S, Sarkar S N & Sengupta D N (2008) *Plant Cell Rep* 27,1395-1410
16 Sellappan K, Datta K, Parkhi V & Datta S K (2009) Plant Sci 177, 557-562
17 Van Handel E (1968) *Anal Biochem* 22, 280–283
18 Mccready R M, Guggolz J, Silviera V & Owens H S (1950) *Anal Chem* 22, 1156
19 Sood D R (2006) *J Dairy Foods Home Sci* 25, 1-7
20 Jayaprakasha G K, Singh R P & Sakariah K K (2001) *Food Chem* 73, 285–290
21 Jia Z, Tang M & Fu J (1999) *Food Chem* 64, 555–559
22 Prieto P, Pineda M & Aguilar M (1999) *Anal Biochem* 269, 337–341
23 Kumaran A & Karunakaran R J (2006) *Food Chem* 97, 109–114
24 Martinez A C, Marcelo E L, Marco A O & Moacyr M (2001) *Plant Sci* 160, 505–515
25 Halliwell B, Gutteridge J M C & Grootveld M (1987) *Methods Biochem Anal* 33, 59–90
26 Marcocci L, Packer L, Droy-Lefaix M T, Sekaki A & Gardes-Albert M (1994) *Methods Enzymol* 234, 462–475
27 Carter P (1971) *Anal Biochem* 40, 450–458
28 Mitsuda H, Yasumodo K & Iwami K (1966) *Eiyo Shokuryo* 19, 210-214
29 Shobana S & Naidu K A (2000) *Prostaglandins Leukot Essent Fatty Acids* 62, 107–110
30 Ruch R J, Cheng S-J & Klaunig E (1989) *Carcinogenesis* 10, 1003–1008
31 Hu F B & Willett W C (2002) *J Am Med Assoc* 2888, 2569–2578
32 Singh R & Juliano B O (1977*) Plant Physiol* 59, 417-421
33 Chauhan J S, Chauhan V S & Lodh S B (1995*) Indian J Genet Plant Breed* 55, 6-12
34 Luo X D, Basile M J & Kennelly E J (2002) *J Agric Food Chem* 50, 1379–1382
35 Zhou Z, Robards K, Helliwell S & Blanchard C (2004) *Food Chem* 87, 401-406
36 Pin-Der-Duh X (1998) *J Am Oil Chem Soc* 75, 455–461
37 Juntachote T & Berghofer E (2005) *Food Chem* 95, 193–202
38 Oyaizu M (1986) *Jap J Nutr* 44, 307–315
39 Dahl M K & Richardson T (1978) *J Dairy Sci* 61, 400–407
40 Aruoma O I (1998) *J Am Oil Chem Soc* 75, 199–211
41 Moncada A, Palmer R M J & Higgs E A (1991) *Pharmacol Rev* 43, 109–142
42 Kehrer J P (2000) *Toxicology* 149, 43–50
43 Tsuchihashi H, Kigoshi M, Iwatsuki M & Niki E (1995) *Arch Biochem Biophys* 323,137–147
44 Muntana N & Prasong S (2010) *Pak J Biol Sci* 2010 13, 170-174
45 Srisawat U, Panunto W, Kaendee N, Tanuchit S, Itharat A, Lerdvuthisopon N & Hansakul P (2010) *J Med Assoc Thai* 93, S83-S91; Erratum in: *J Med Assoc Thai* (2011) 94, 896
46 Rao A S, Reddy S G, Babu P P & Reddy A R (2010) *BMC Complement Altern Med* 10, 4
47 Kim Y H, Lee Y Y, Kim Y H, Choi M S, Jeong K H, Lee S K, Seo M J, Yun H T, Lee C K, Kim W H, Lee S C, Park S K & Park H M (2011) *J Agric Food Chem* 59, 584-591