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Evaluation of Total Phenolic Content and Antioxidative Properties of Unpolished and Polished Black, Red and Brown Rice Varieties from India

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Abstract—*Rice is considered to be among one of the most consumed cereals and it is consumed in large quantities across the globe, therefore its nutrient content has a significant role in maintaining our health. Phenolic content of the rice is low but it is of prime concern as it is taken in significant quantity. Phenolic contents which are accountable for major antioxidant activities (AOAs) are basically responsible for antimutagenic and anticarcinogenic effects in organisms. In this study, polished and unpolished black, red and brown color rice grains were analyzed for total soluble phenolic content (TSPCs) and AOAs. Solvent acetone/water and methanol/water (70:30, 80:20 v/v respectively) were used for extraction. Result obtained, showed the average value of TSPCs as black rice> red rice > Brown rice ($P < 0.05$). The highest TSPCs in unpolished black rice was 539.46mg GAE 100 g^{-1} and lowest in brown polished rice 28.17 mg GAE 100 g^{-1}. After processing, TSPCs observed for all three rice variants, unpolished grain had significantly higher TPCS as compared to polished grain. A DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed to determine AOAs of all rice variants. AOAs showed positive correlation ($r = 0.99$) with TSPCs. The result shows that in all the genotypes higher percentage of TSPCs was present in pericarp and therefore AOAs are mainly associated with pericarp. A positive correlation observed between TSPCs and AOAs which depict that the phenolic content is mainly responsible for antioxidant activities of rice grain. Processing of rice decreases both TSPCs and AOAs significantly.*

Keywords*: Rice, Total Soluble phenolic content (TPCSs), Antioxidant activities (AOAs), Methanol*

1. INTRODUCTION

Rice is considered among one of the most consumed cereals and it has a significant role in maintaining human health. Several epidemiological studies and research recommended that there is lesser incidence of chronic diseases in those regions where rice grains are consumed more [26]. This might be due to the antioxidant compounds present in rice. Although rice has a low concentration of antioxidant it is taken in large quantity so useful antioxidants may be consumed by the organisms in large amount.

Vegetables and fruits are considered as prominent phenolic compounds source, but various significant study and research has established that consumption of cereals is also increase the intake of phenolic compounds [21]. These Phenolic compounds exert several beneficial physiological effects in humans, as these are responsible for platelet aggregation inhibition [3], it also prevents oxidative damage of low-density lipoproteins as well as lipids [15], and diminish the risk of cancer and coronary heart disease [17]. Phenolic compounds are secondary metabolites of plants and its distribution is universal in the plant kingdom and they have effective antioxidant properties as well as free radical scavenging capabilities [13, 22]. It has been demonstrated by several research that a positive correlation exists between the phenolic compounds concentration and AOAs [27, 4]. Several compounds responsible for antioxidant activities have already been recognized in rice, among them most plentiful is anthocyanins and phenolic acids [27, 2].

Several genotypes of rice is present on the globe which is having different grain color among them white rice cultivar is widely consumed. However there are several other rice cultivars which contains color pigments, and they are named on basis of kernel color as black, red and brown rice. The deposition of anthocyanins in different layers of the aleurone, pericarp and seed coat is responsible for the difference in kernel color [25, 1]. Different genotypes of rice have different concentration and types of polyphenols and they are significantly responsible for grain color. It has been reported by many researchers that pigmented cultivar of rice shows enhanced antioxidant activities than non-pigmented one [14, 29]. Now a day's these pigmented grain are in high demand, as for instance, red rice grains are very popular in Japan as functional food as it has higher anthocyanin and polyphenols composition [8]. The Pigmented whole grains are significant phenolic compounds source but, consumption of the phenolic compounds present in it is at present neglected. The main reason behind this is that the pericarp is rich in phenolic compound and other nutrients are removed during the processing of whole grain to obtain polished grain [23, 28]

Polishing of grain leads to loss of nutrient content and thus reduces the rice quality. In this study three variants of rice that is black, red and brown grain were considered for study. Their TSPCs and AOAs were estimated and compared in unpolished and polished rice, to study the effect of processing (polishing) on the concentration of phenolic compounds and antioxidant properties of rice. Thereafter amount of TSPCs in pericarp was also evaluated to estimate the phenolic content in polished rice which is largely consumed by consumer all over the world and how change in dietary pattern could increase the antioxidant intake

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

Six samples two of each three variants of rice that is red and brown were collected from IARI, Delhi and black rice from Manipur farmer's field. Grains were dried at 30^oC then dehulled to obtain brown, red and black grains and they were polished to remove bran as rice grains have to be evaluated both in polished and unpolished form. Before estimation of TSPCs and AOAs of rice, grains were properly washed to ensure no dust and soil particles present. After that these grains were shade dried and ground into fine flour powder. This powder of rice was stored in airtight container for further analysis.

2.2 Extraction of TSPCs

For extracting TSPCs 1g of rice flour of each variant was weigh and kept in Falcon. For extracting phenolic compounds experiments were performed in three steps. 20 ml methanol solvents (80%) were added in each Falcon in the first step of extraction. These Falcons were covered with foil and parafilm, and then they were left on the shaker for overnight at 200 rpm at 40^oC. After that Falcons were taken out and shaken for ten minutes on vortex and shaken vigorously then it centrifugation was done at 10000 rpm and supernatant was taken in an amber bottle. Again 10ml methanol (pH-2, concentration 80%) was added and kept on the shaker for an hour at 40^oC after completion it was taken out and shaken vigorously on vortex and centrifuge for 10 minutes at 10000 rpm. The supernatant was transferred into an amber bottle and this step was repeated again with 20 ml of acetone (70%).

2.3 Total Soluble Phenolic contents (TSPCs) determination

The TSPCs of rice grain extracts were estimated by modified Folin-Ciocalteu method [30]. In 1ml rice extracts, five ml of phenol's reagent was added in test tube. This phenol reagent was diluted beforehand in a ratio of 1:10 (v/v) using water. 4ml of Na_2CO_3 (concentration-75 g/l) was added and were shaken on vortex for 10-15 seconds and it was kept aside for an hour at 40^oC in BOD for color development. After that, the absorbance was measured at 765nm wavelength. TSPCs were estimated in extracts of all rice cultivar using the standard curve of GA and results were expressed as milligram of Gallic acid equivalent (GAE) per 100 gram of sample. All experiment was performed in triplicate

2.4 Determination of AOAs using DPPH assay

This assay was performed following a modified method of Liyana et al. [12]. 0.135 milliMolar DPPH solution was prepared using solvent methanol. For analysis 1ml of the extract was added into an equal volume of DPPH solution. After that it was kept in dark at RT for 40 minutes and absorbance was taken at 517nm. O.D. of the control sample was also taken.

Trolox was used as a standard. The AOAs were calculated using standard curve of Trolox and AOAs of all the samples were expressed as mM (TE) Trolox equivalent per gram.

3. RESULT AND DISCUSSION

3.1 TSPCs and AOAs estimation

In this study acidified methanol (80%) and acetone (70%), solvent was used to extract phenolic content at 40^oC temperature. The phenolic compounds in plants are having different polarities, thus different solvents and a mixture of solvents are used to isolate them. Water, acetone, ethanol, and methanol are commonly used solvents for extraction of the phenolic compound. The yield of the phenolic compound and AOAs of the extract is basically dependent upon the type of solvent which is used during extraction of phenolic compound [5]. The efficacy of extraction and phenolic compound especially anthocyanin stability is depends mainly on temperature and pH of extraction solution [10; 20]. In a study, it was reported that antioxidant ability of litchi extract was significantly higher when incubated for 30 minutes at 45°C as compared to 55 °C [20]. Naczk and Shahidi [16], stated that anthocyanins higher concentration could be extracted from plant samples when acidified solvent is used, most common organic acidified solvent used is methanol.

Results of TSPCs estimation showed that phenolic compounds concentration differs significantly ($p < 0.05$) in rice extracts which is having different pericarp color. TSPCs was evaluated for all sample i.e unpolished and polished grain and it was noticed the concentration follows the order Black

Unpolished>Red Unpolished >Brown Unpolished (**Figure1**). The highest TSPCs was in black unpolished B rice 539.46 ± 7.05 mg GAE per 100g grain flour and in Brown polished A rice sample concentration was lowest 26.56 ± 3.95 mg GAE per 100g grain flour. The difference observed in TSPCs in the different cultivar of same color pericarp as well. This kind of variation within the samples having same color pericarp was also reported by Shen et al. [23]. Variation in concentration ranges black unpolished rice- 546.17 to 449.97, red unpolished rice 402.71 - 441.97 and for brown unpolished rice it was 63.87-68.98 mg GAE/100 mg grain, similarly, for polished grains it ranges for red rice 36.93-42.82, black rice 39.88-44.12 and for brown polished that is white rice was 24.81-31.97 mg GAE/100 mg grain. The average value of all samples represented in **Table1**. These values predict that processing (polishing) of rice noticeably decreases the TSPCs content.

Table1 TSPCs (Total Soluble Phenolic contents) in different rice genotypes

Sample Name	mg GAE 100 g−1
Black Polished A	42.62 ±1.31
Black Polished B	40.88 ±1.61
Red Polished A	40.14 ±2.96
Red Polished B	39.21 ±2.53
Brown Polished A	26.56 ±1.57
Brown Polished B	28.17 ±3.95
Black Unpolished A	458.15 ±7.48
Black Unpolished B	539.46 ±7.05
Red Unpolished A	429.99 ±10.9
Red Unpolished B	410.92 ±8.93
Brown Unpolished A	64.16 ±1.21
Brown Unpolished B	66.99 ±2.06

Table 2 Showing TSPCs for all rice cultivar in Bran and Seed in mg GAE /100g

Sample Name	Bran	Seed
Black Rice A	415.53	42.62
Black Rice B	498.58	40.88
Red Rice A	389.85	40.14
Red Rice B	371.71	39.21
Brown Rice A	37.6	26.56
Brown Rice B	38.82	28.17

To evaluate the loss of phenolic compound during polishing and amount of phenolic compound present in pericarp above data was analyzed (**Table 2, Figure 2**). Black and red rice showed 90%-92% phenolic compound in their pericarp while brown rice has approximately 58-59% phenolic compound in their pericarp. Result obtained showed that major part of the phenolic compound in rice grain is associated with pericarp and polishing remove this and makes the grain less healthy. The similar studies were also conducted by Hu et al. [7], in which bran account for 70%-90% content of phenolic acid in brown rice grains and in black rice grains around 85% anthocyanins content was observed. Walter et al., 2013 [27] also observed similar result, 92-97% phenol in bran of pigmented black and red rice and in brown rice around it was around 62%.

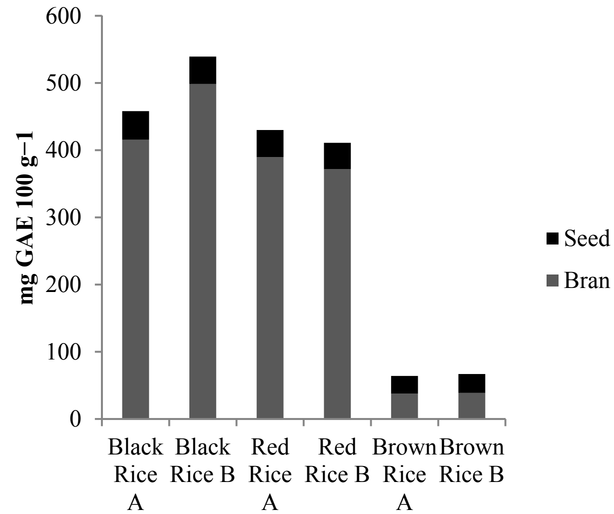

Figure 2 Graph showing TSPCs from all variants of rice samples in their seed and pericarp

Antioxidant activities of all rice samples were tested through DPPH method. The antioxidant activities followed a similar trend as observed in TSPCs that is Black Unpolished Rice> Red Unpolished Rice>

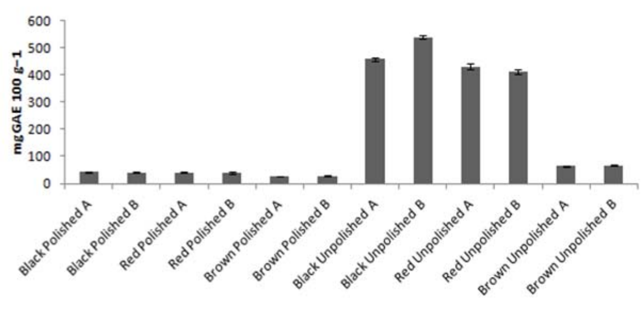

Figure 1 TSPCs of all rice samples (Polished/Unpolished) expressed as mg GAE 100 g-1

Brown Unpolished Rice, but in polished rice samples result was different Polished Black rice ≥ Polished

Red rice> Polished brown rice. Antioxidant compounds generally exist in phenolic form. The

DPPH radical scavenging activities rice extract samples were estimated using graph drawn using different concentrations of Trolox. It was noticed from that extracts of unpolished black rice and red rice had higher antioxidant activities than brown rice this result of DPPH compliments the result of TSPCs (**Figure 3**). On basis of this study, it could be predicted extracts have a proton-donating ability and it performs free radical scavenging activities, these extract must have a compound which acts as a primary antioxidant. It was observed that AOAs of the extract was positively correlated with their total TSPCs (r= 0.994). Previous literature sources also showed a positive correlations existence between phenolic compounds concentration and antioxidant activities [9, 11]. While on the other hand there are also few of the literatures reported that there is not any positive correlation [6, 18]. On basis of our result, it can be predicted that a positive correlation exists in between total antioxidant activities and phenolic substances composition. The antioxidant activity is determined and established in vitro models, using ABTS methods, nitric oxide method, DPPH method, DMPD method etc [19]. Total antioxidant activities, DPPH radical scavenging activities, metal chelation, reducing power as well as activities destructive to active oxygen species are widely used for this purpose [24]. The DPPH method is usually preferred as it is easy, fast and reliable, it does not necessitate a special device or reaction. DPPH is a synthetic, stable radical which never disintegrate in methanol, ethanol or water. Therefore stable free radical DPPH is commonly used for determining antioxidative properties.

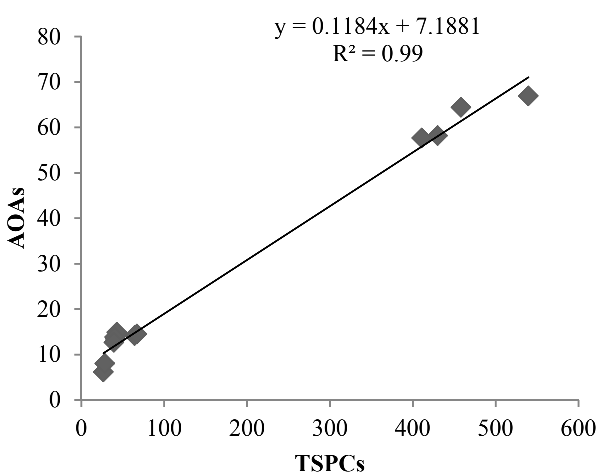

Figure 3 Correlation curve drawn between the TSPCs and AOAs in black rice, red rice and brown rice grains. TSPCs and AOAs were expressed as mg GAE/100g and mM TE/gram rice, flour respectively.

4. CONCLUSION

Phenolic compounds a secondary metabolite present in the plant is responsible for the prevention of various health issues like cancer and cardiovascular diseases. In this study, the TSPCs and AOAs of three variants of rice were determined and possible ranges of them in rice varieties were estimated. Significant differences observed in the concentration of antioxidant properties and phenolic content of rice varieties possess different pericarp color. This study represents rice especially red and black as one of the good source of antioxidants as it is taken daily in large amount. The study might help in increasing awareness among population regarding rice health benefits. It could also aid in understanding which process should be followed and how that it will not diminish and decrease phenolic compound present in rice grain.

5. ACKNOWLEDGEMENT

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