Antioxidant properties of selected pigmented and white long grain rice varieties of Sri Lanka at market available polishing rates


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INTRODUCTION

Non-communicable diseases (NCDs) are the world’s leading cause of deaths and it accounts for nearly 41 million deaths each year worldwide (Anonymous 2018). Approximately 71% of the world’s total deaths are due...
to NCDs. The leading NCDs in the world are cardiovascular diseases, cancers, diabetes mellitus and chronic respiratory diseases (Anonymous 2018). Excessive generation of free radicals which is known as the oxidative stress is reported to play a vital role in the initiation and propagation of NCDs (Birben et al. 2012, Rahman et al. 2012). These free radicals are generated from either oxygen or nitrogen and are known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Birben et al. 2012, Rahman et al. 2012, Phaniendra et al. 2015). ROS and RNS alter biological macromolecules such as DNA, proteins, lipids and carbohydrates leading to a range of NCDs namely cancers, cardiovascular diseases, diabetes mellitus, and inflammatory diseases (Rahman et al. 2012, Kalam et al. 2015). Therefore, in the prevention and management of NCDs, the management of oxidative stress is very important (Birben et al. 2012, Rahman et al. 2012). Antioxidants are compounds that can act against oxidative stress and thereby preventing oxidation of biological macromolecules in the human body (Krishnaiah et al. 2011, Pandey & Rizvi 2009). Recent research findings have clearly shown that naturally derived dietary antioxidants can play a vital role in the prevention and management of NCDs through the management of oxidative stress (Krishnaiah et al. 2011, Pandey & Rizvi 2009).

Rice (Oryza sativa L.) is one of the highly consumed cereals and it is the staple food of more than half of the world’s population (Prasad et al. 2011). There are over 40,000 rice varieties cultivated worldwide (Anonymous 2019a). Rice varieties can be classified based on physical attributes of the rice grain such as grain length as long grain, medium grain and short grain and color of the pericarp as pigmented and non-pigmented rice (Senarath & Samaraweera 2017). Consumer preference for rice varieties depends on variety of factors including physical attributes of the rice grain (Anang et al. 2011, Cuevas et al. 2016). Currently, there is a huge demand for long grain specially Basmati or Basmati type rice varieties among the rice consumers world over (Suwannaporn & Linnemann 2008, Asante et al. 2013, Senarath & Samaraweera 2017). Long grain rice varieties can be either pigmented or non-pigmented (Anonymous 2019b). Rice varieties with the pigment have many health benefits including high antioxidant properties compared to non-pigmented rice varieties (Tian et al. 2004, Sompong et al. 2011, Deng et al. 2013, Premakumara et al. 2013, Gunaratne et al. 2013, Pengkumsri et al. 2015, Abeyesekera et al. 2015a,b, Thitipramote et al. 2016, Abeyesekera & Premakumara 2016, Abeyesekera et al. 2017a,b,c, Abeyesekera et al. 2018). Therefore, long grain pigmented Basmati or Basmati type rice varieties may have a higher demand in the market compared to non-pigmented long grain rice varieties as these varieties may have potential in utilizing in prevention and management of NCDs (Suwannaporn & Linnemann 2008, Asante et al. 2013, Senarath & Samaraweera 2017).

In Sri Lanka, rice is the staple food (Walisinghe & Gunaratne 2012) and there are many rice varieties cultivated island wide including some long grain varieties (Anonymous 2019a). Antioxidant properties of a range of Sri Lankan rice varieties have been studied and it has been shown that pigmented rice varieties of Sri Lanka had the highest antioxidant properties compared to non-pigmented rice varieties (Gunaratne et al. 2013, Premakumara et al. 2013, Abeyesekera & Premakumara 2016, Abeyesekera et al. 2017a,b,c.). However, antioxidant properties of long grain Basmati type quality rice varieties of Sri Lanka have been very limitedly studied. In addition, antioxidant properties of these rice varieties at market available polishing rates are not reported to date. Therefore, the present study was carried out to evaluate the antioxidant properties of locally developed long grain red and white pericarp Basmati type quality rice varieties and a black pericarp rice variety of Sri Lanka at market available polishing rates.

**MATERIALS AND METHODS**

**Rice samples**

CIC Red Fragrant, CIC White Slender having Basmati type grain qualities and CIC Black rice were obtained from the CIC Agribusinesses (Pvt.) Ltd, Sri Lanka. Pakistan White Basmati rice was purchased from a local retail shop.

**Chemicals and reagents**

Folin-ciocalteu phenol reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Gallic acid, 2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazine (DPPH), 2, 2’-azobis (2-amidino-propane) dihydrochloride (AAPH), 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate, ferric chloride, fluorescein, sodium acetate, sodium carbonate were purchased from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade.

**Sample preparation and extraction**

Rough rice grains of all selected rice varieties were de-hulled using a rice de-huller (THU-35B, Satake, Hiroshima, Japan) and polished using a laboratory polisher (TM-05C, Satake, Hiroshima, Japan) to obtain rice
samples at market available polishing rates (100%, 40%, and 0% or whole grain). Thereafter, polished rice grains were milled using a laboratory milling machine (Universal mill PE 402, Bauermeister, Germany) with a 0.5 mm sieve to obtain rice flour. Rice flour was then extracted in 10 times the sample weight of 70% ethanol-water (v/v) overnight at room temperature (28±2°C). Samples were then centrifuged (825 g) for 10 min, filtered through 0.45 μm nylon filters and evaporated to dryness under vacuum in a rotary evaporator and freeze dried. Freeze dried samples were used in determination of antioxidant properties using following antioxidant assays.

Evaluation of antioxidant properties of selected rice varieties

Total phenolic content: Total phenolic content (TPC) of freeze dried extracts of all selected rice samples were tested according to the method described by Singleton et al. (1999) with some minor modifications using 96-well micro-plates. Hundred and ten microliters of freshly prepared, 10 times diluted folin-ciocalteu reagent was added to 20 μl of freeze dried rice extracts (1 mg ml⁻¹). Then, 70 μl of sodium carbonate solution was added to each reaction mixture and incubated at room temperature (30±2°C) for 30 min. Absorbance of samples was measured at 765 nm using a micro plate reader (SpectraMax Plus, Molecular Device, USA). Gallic acid was used as the standard antioxidant. Results were expressed as mg Gallic Acid Equivalent (GAE) g⁻¹ extractables.

Ferric reducing antioxidant power: Ferric reducing antioxidant power (FRAP) of rice samples were evaluated as per the method described by Benzie & Szeto (1999a) with some modifications using 96-well microplates. Forty mill molar HCl solution was used to prepare 10 mM TPTZ solution. Working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM FeCl3.6H2O and 10 mM TPTZ solution at a ratio of 10:1:1 just before use and incubated at 37°C for 10 min. Twenty microliters of samples (1 mg ml⁻¹) were added to each reaction volume containing 30 μl of acetate buffer and 150 μl of freshly prepared working FRAP reagent and it was incubated at room temperature (30±2°C) for 8 min and the absorbance was measured at 600 nm using a micro plate reader. As the standard antioxidant, Trolox was used and results were given as mg Trolox Equivalents (TE) g⁻¹ extractables.

Oxygen radical absorbance capacity: The oxygen radical absorbance capacity of rice samples were studied as per the method described by Ou et al. (2001) with some modifications using 96-well micro-plates. AAPH solution (40 mg ml⁻¹), fluorescein (4.8 μM) and Trolox (1.5 and 0.75μg ml⁻¹) were prepared in phosphate buffer (75 mM, pH 7.4). Fifty microliters of rice samples and 100 μl of 4.8 μM fluorescein were mixed and pre-incubated at 37°C for 10 min. Then, 50 μl of 40 mg ml⁻¹ AAPH was added to the reaction volume to initiate the reaction. Fluorescence was measured using a fluorescent micro plate reader (SPECTRAMax-Gemini EM, Molecular Devices, USA) at excitation and emission wave lengths of 494 and 535 nm, respectively. The decaying of fluorescein was recorded for 35 min at 1 min intervals. The net area under the curve of fluorescein decay of blank and samples were used to calculate the ORAC values. Results were given as mg TE g⁻¹ extractables.

DPPH radical scavenging activity: DPPH radical scavenging activity of rice samples were determined according to the method of Blois (1958) with modifications using 96-well micro-plates. Fifty microliters of different concentrations (18.75, 37.5, 75, 150, 300 and 600 μg ml⁻¹) of rice samples were added to 150 μl of 125 μM DPPH radical. Then samples were kept at room temperature (25±2°C) for 15 min and the absorption was recorded at 517 nm using a micro-plate reader. Trolox was used as the standard antioxidant (0.78, 1.56, 3.125, 6.25 and 12.5 μg ml⁻¹). Results were expressed as mg TE g⁻¹ extractables.

ABTS radical scavenging activity: ABTS+ radical scavenging activity of rice samples were tested as per the method of Re et al. (1999) using 96-well micro-plates. Fifty microliters of different concentrations (6.25, 12.5, 25, 50, 100 and 200 μg ml⁻¹)of rice samples were mixed with 150 μl of 40 μM ABTS radical and the mixture was incubated at room temperature (25±2°C) for 10 min. Then, the absorbance was measured at 734 nm using a micro-plate reader. Trolox was used as the standard antioxidant. Results were expressed as mg TE g⁻¹ extractables.

Statistical analysis: SAS version 6.12 was used in statistical analysis of data. One way analysis of variance (ANOVA) was performed to analyze data. Duncan’s Multiple Range Test (DMRT) was used for mean comparisons.

RESULTS & DISCUSSION

Pakistan and Indian Basmati rice are very popular and highly priced long grain rice varieties in the retail markets of Sri Lanka. People in Sri Lanka are used to consume these rice for special functions in the country as
for day to day consumption it is not affordable. Therefore, identification and promotion of rice varieties with Basmati type grain quality developed in Sri Lanka will be of high value as an alternative to Pakistan and Indian Basmati rice. In addition, rice being the staple food in Sri Lanka, if such rice varieties are functionally sound there may be a huge demand among the health-conscious consumers in the country as functional dietary staples can be effectively used as vehicles to improve the health status of the people (Pandey & Rizvi 2009, Krishnaiah et al. 2011).

In the present study two long grain rice varieties with Basmati type grain qualities namely CIC Red Fragrant and CIC White Slender developed by the CIC Agri Businesses (Pvt.) Ltd, Sri Lanka were used as those long grain rice varieties are currently very popular among the rice consumers in many parts of the country (Senarath & Samaraweera 2017). CIC Black rice was used because generally Black rice has the highest antioxidant properties compared to the other rice varieties world over (Sompong et al. 2011, Yodmanee et al. 2011, Walter et al. 2013). Further, to compare Sri Lankan Basmati type long grain rice varieties with the other worldwide well known long grain Basmati rice, Pakistan White Basmati rice sample collected from the Sri Lankan market was used.

Antioxidant properties of rice depends on several factors such as genotype, storage, post-harvest processes and particularly the degree of milling (Abeysekera et al. 2017c, Tong et al. 2019). Most of the phytochemicals which have antioxidant properties are concentrated in the outer layers of the rice grain named as the rice bran (Ravichanthiran et al. 2018). Thus, milling process can lead to reduction of antioxidant compounds in the milled rice grain (Zhou et al. 2004). In the present study selected rice varieties were studied for antioxidant properties at different polishing rates are currently available in the market.

Phenolic compounds are widely found in plants and are reported to have multiple biological activities including antioxidant activity (Tungmunnithum et al. 2018). Cereals including rice especially pigmented rice varieties are reported to have high amounts of phenolic anti-oxidants (Yodmanee et al. 2011, Gunaratne et al. 2013, Premakumara et al. 2013, Walter et al. 2013, Abeysekera & Premakumara 2016, Abeysekera et al. 2017a,b,c, Abeysekera et al. 2018). TPC of selected rice varieties at different polishing rates are presented in table 1. TPC of selected rice samples were ranged from 6.83±0.76 to 27.67±1.26 mg GAE g⁻¹ extract. Whole grains of CIC Black rice (27.67±1.26 mg GAE g⁻¹ extract) and whole grains of CIC Red Fragrant (26.00 ± 3.12 mg GAE g⁻¹ extract) showed significantly high (p <0.05) TPC compared to the other rice samples tested. Most of the studies on antioxidant properties of different rice varieties world over have shown that black rice processes the highest phenolic antioxidants compared to the red rice (Yodmanee et al. 2011, Sompong et al. 2011, Walter et al. 2013,). However, in the study we observed that whole grains of CIC Red Fragrant had comparable TPC to that of CIC Black rice. This is in agreement with the findings of some recent studies (Sompong et al. 2011, Jun et al. 2012) that the differences in phenolic contents of different rice varieties are due to the varietal differences irrespective of red or black colour of the rice grain.

Table 1. Total phenolic content, ferric reducing antioxidant power, and oxygen radical absorbance capacity of selected rice varieties of Sri Lanka and Pakistan White basmati rice at market available polishing rate.

<table>
<thead>
<tr>
<th>Rice varieties and type</th>
<th>Total phenolic content (mg GAE g⁻¹ extract)</th>
<th>Ferric reducing antioxidant power (mg TE g⁻¹ extract)</th>
<th>Oxygen radical absorbance capacity (mg TE g⁻¹ extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIC Black rice (Whole grains or at 0% polishing rate)</td>
<td>27.67±1.26a</td>
<td>35.58±1.03a</td>
<td>107.58±4.60a</td>
</tr>
<tr>
<td>CIC Red Fragrant (Whole grains or at 0% polishing rate)</td>
<td>26.00±3.12a</td>
<td>33.21±0.57b</td>
<td>65.9±8.38c</td>
</tr>
<tr>
<td>CIC Red Fragrant (40% polishing rate)</td>
<td>14.33±0.29b</td>
<td>28.11±0.45c</td>
<td>88.99±8.93d</td>
</tr>
<tr>
<td>CIC Red Fragrant (100% polishing rate)</td>
<td>11.33±0.33b</td>
<td>23.00±2.61d</td>
<td>71.07±7.51b,c</td>
</tr>
<tr>
<td>CIC White Slender (100% polishing rate)</td>
<td>10.67±1.04bc</td>
<td>23.72±0.53d</td>
<td>56.41±2.71c</td>
</tr>
<tr>
<td>Pakistan White Basmati (100% polishing rate)</td>
<td>6.83±0.76c</td>
<td>15.84±1.87c</td>
<td>48.54±9.85c</td>
</tr>
</tbody>
</table>

Note: Mean values in a column superscripted by different letters are significantly different at (p<0.05); GAE- Gallic Acid Equivalents; TE- Trolox Equivalents.

The increasing polishing rate had an inverse relationship with the TPC of rice grains. This is because the outer layer of the rice grain (rice bran) contains high amount of antioxidant compounds and increasing in the degree of polishing results in reduction in such compounds in the milled rice (Zhou et al. 2004). Interestingly,
all the selected Sri Lankan rice varieties at tested polishing rates showed high TPC compared to that of Pakistan White Basmati rice at 100% polishing rate (6.83±0.76 mg GAE g⁻¹ extract).

FRAP assay measures the reducing capacity of samples (Benzie & Strain 1999b). FRAP of rice samples tested are presented in table 1. Significant differences (p <0.05) among the selected rice varieties at different polishing rates for FRAP were observed. The FRAP of rice samples studied ranged from 15.84±1.87 to 35.58±1.03 mg TE g⁻¹ extract. The highest FRAP was observed in whole grains of CIC Black rice followed by whole grains of CIC Red Fragrant. This clearly showed that pigmented rice had the highest FRAP compared to the non-pigmented white rice varieties. A recent study on antioxidant properties of 29 Sri Lankan rice varieties have clearly shown that red rice varieties had significantly high (p <0.05) FRAP compared to white rice varieties tested (Abeysekera et al. 2017a). Further, similar observations have been made for many rice varieties worldwide (Thitipramote et al. 2016, Samyor et al. 2016). The present study also demonstrated that increased degree of polishing caused a reduction in FRAP of milled rice grains. This is in agreement with the findings of some recent studies by Rosnaini & Abdullah (2016) and Prom-U-Thai et al. (2016).

Free radicals namely ROS and RNS are generated in the human body due to various biological reactions. These radicals play vital roles in generation of ATP from ADP in the mitochondrial oxidative phosphorylation, detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes), apoptosis of defective cells, killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes (Devasagayam et al. 2004), physiological roles in cellular responses to noxia, such as defense against infectious agents, functions in the number of cellular signaling pathways, and the induction of a mitogenic response (Valko et al. 2007). However, excess production of ROS and RNS are now well known to alter biological macromolecules leading to variety on NCDs (Halliwell 2009, Lobo et al. 2010). Therefore, compounds which can scavenge the free radicals are of great importance in the prevention and management of chronic diseases (Halliwell 2009, Kalam et al. 2015). Antioxidants are compounds which can manage the free radicals induced damage to biological macromolecules (Karadag et al. 2009). Artificial antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are widely used in the food industry. However, such antioxidants have shown potential health risks in comparison to naturally derived antioxidants from plant sources (Kahl & Kappus 1993). In the present study, free radical scavenging activity of selected rice samples were evaluated using a physiological radical based antioxidant assay, ORAC and two artificial free radicals based antioxidant assays namely DPPH and ABTS radical scavenging assays (Gupta 2015).

ORAC assay measures the radical scavenging activity of peroxyl radical and is reported as more biological relevant assay to measure the antioxidant activity of a sample (Ou et al. 2001). Rice samples at market available polishing rates showed significant differences (p <0.05) among the samples (Table 1). ORAC of rice samples varied from 48.54±9.85 to 107.58±4.60 mg TE g⁻¹ extract. The highest ORAC was observed for whole grains of CIC Black rice while CIC White Slender at 100% polishing rate and Pakistan White Basmati at 100% polishing rate had the lowest.

### Table 2.
Screening of selected pigmented and white long grain rice varieties of Sri Lanka and Pakistan White basmati rice at market available polishing rates for DPPH and ABTS radical scavenging activities.

<table>
<thead>
<tr>
<th>Rice varieties and type</th>
<th>DPPH radical scavenging activity (% inhibition)</th>
<th>ABTS radical scavenging activity (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIC Red Fragrant (Whole grains or at 0% polishing rate)</td>
<td>46.60±6.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.52±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIC Black rice (Whole grains or at 0% polishing rate)</td>
<td>33.46±1.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.71±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIC Red Fragrant (40% polishing rate)</td>
<td>24.05±7.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.37±2.34&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIC White Slender (100% polishing rate)</td>
<td>16.67±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>64.25±1.48&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIC Red Fragrant (100% polishing rate)</td>
<td>7.05±1.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>53.76±1.05&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pakistan White Basmati (100% polishing rate)</td>
<td>2.36±0.72&lt;sup&gt;f&lt;/sup&gt;</td>
<td>47.00±1.85&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Mean values in a column superscripted by different letters are significantly different at (p<0.05); DPPH and ABTS radical scavenging activity • % inhibition at 200 µg ml⁻¹ concentration.

DPPH and ABTS radical scavenging assays are widely used in evaluation of antioxidant activity of variety of food and natural products (Gülçin 2012). The % inhibition of DPPH and ABTS radicals at 200 µg ml⁻¹ of selected rice varieties are presented in table 2. Significant differences among the rice samples for both DPPH
and ABTS radical scavenging activities were observed. Percent inhibition of DPPH and ABTS radicals at 200 µg ml\(^{-1}\) concentration ranged from 2.36±0.72 to 46.60±6.35 and 47.00±1.85 to 97.52±0.04, respectively. Highest inhibitory activities for both DPPH and ABTS radicals were observed for whole grains of CIC Black rice, whole grains of CIC Red Fragrant followed by CIC Red Fragrant at 40% polishing rate. Dose-response relationships of whole grains of CIC Black rice, whole grains of CIC Red Fragrant and CIC Red Fragrant at 40% polishing rate for DPPH and ABTS radical scavenging activities are shown in figure 1 and figure 2. Further, IC50 values and mg TE g\(^{-1}\) extract of whole grains of CIC Black rice, whole grains of CIC Red Fragrant and CIC Red Fragrant at 40% polishing rate are presented in table 3. All the selected rice samples had good dose response relationships in dose response studies for both DPPH and ABTS radical scavenging activities.

![Figure 1](image1.png)

**Figure 1.** Dose response relationship of whole grains of CIC Black rice (WGBR), whole grains of CIC Red Fragrant (WGRB) and CIC Red Fragrant at 40% polishing rate (RB40PR) for DPPH radical scavenging activity.

![Figure 2](image2.png)

**Figure 2.** Dose response relationship of whole grains of CIC Black rice (WGBR), whole grains of CIC Red Fragrant (WGRB) and CIC Red Fragrant at 40% polishing rate (RB40PR) for ABTS radical scavenging activity.

A recent study conducted on physicochemical and nutritional properties using the same three rice varieties at the same polishing rates used in the present study has shown that there were significant losses in nutritional properties (crude fat, total dietary fiber and total ash contents) with the increasing polishing rate (Samaranayake et al. 2018). The increasing polishing rate in the present study resulted in significant reduction in the antioxidant properties. Therefore, selected whole grain rice varieties especially CIC Black rice and CIC Red Fragrant rice have to be consumed as whole grains to obtain high nutritional and antioxidant properties which may be important in the prevention and management of NCDs.
### Table 3. DPPH and ABTS radical scavenging activities (IC50 values and Trolox equivalent values) of selected Sri Lankan rice varieties.

<table>
<thead>
<tr>
<th>Rice varieties and type</th>
<th>DPPH radical scavenging activity</th>
<th>ABTS radical scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (µg ml⁻¹)</td>
<td>mg TE g⁻¹ extract</td>
</tr>
<tr>
<td>CIC Red Fragrant (Whole grains or at 0% polishing rate)</td>
<td>173.00±7.60b</td>
<td>19.96±0.88a</td>
</tr>
<tr>
<td>CIC Black rice (Whole grains or at 0% polishing rate)</td>
<td>207.35±0.90b</td>
<td>16.64±0.07b</td>
</tr>
<tr>
<td>CIC Red Fragrant (40% polishing rate)</td>
<td>326.46±30.16a</td>
<td>10.61±0.98c</td>
</tr>
</tbody>
</table>

**Note:** Results expressed as mean ± SD (n=3) on % dry weight basis; Mean values in a column superscripted by different letters are significantly different at (p<0.05); TE- Trolox Equivalents.

### CONCLUSIONS

Different long grain rice varieties with Basmati type grain qualities available at market available polishing rates in Sri Lanka, showed different levels of antioxidant properties. Whole grains of CIC Red Fragrant and whole grains of CIC Black rice showed the highest antioxidant properties among the rice samples tested. Although higher the grain polishing rate lower the antioxidant properties, even 40% polished Red Fragrant showed high levels of antioxidant properties. Interestingly, antioxidant properties of 100% polished CIC Red Fragrant and CIC White Slender of Sri Lanka are either superior or comparable to that of Pakistan White Basmati rice at the same polishing rate.

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### REFERENCES


