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Dynamics of Antioxidant Capacity in Pigmented and Non-Pigmented Rice Varieties: Influence on Pericarp Colour Strength Before and After Milling and Cooking

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ABSTRACT

Antioxidants help to mitigate oxidative stress, prevent non-communicable diseases, and promote health. Rice (*Oryza sativa* L), a staple food, contains antioxidants and comes in various varieties locally. This study assessed the antioxidant capacity of pigmented and non-pigmented rice varieties grown in Sri Lanka, focusing on the relationship between pericarp colour strength and antioxidant levels before and after milling and cooking. Forty rice varieties, including improved, traditional, and exotic types, were cultivated, harvested, processed, and sampled concurrently. The DPPH assay was used to assess the free radical scavenging capacity of rice at three processing stages: whole, milled, and cooked. The effective concentration (EC_{50}), defined as the concentration required to scavenge 50% of DPPH radicals, was calculated. The reciprocal of EC_{50} was expressed as the antiradical power (ARP). Additionally, the total antioxidant capacity was measured using Ferric Reducing Antioxidant Power (FRAP) assay. Colour values for lightness, redness, and yellowness of whole, milled, and cooked rice were recorded with a CR-20 colorimeter. Both methods revealed significantly higher antioxidant capacity in pigmented whole rice ($p \leq 0.05$), with traditional varieties such as *Masuran* and *Nonabokra* and improved varieties such as Ld 368 and Ld 408 demonstrating promising antioxidant levels. A significant decline in antioxidants was observed after milling and cooking ($p \leq 0.05$), with a greater loss occurring from whole to milled rice than from milled to cooked rice. Cooked pigmented rice had six times more antioxidants than non-pigmented rice. Milling resulted in a substantial reduction in both antioxidant capacity and redness, while cooking caused a comparatively smaller loss ($p \leq 0.05$). Overall, antioxidant activity was reduced by up to 96% as a result of milling and cooking. Although processing reduces redness and increases lightness in pigmented rice varieties, a deeper red colour does not necessarily indicate

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higher antioxidant levels. Positive correlations between antioxidant capacity and pericarp colour values suggest that lighter reddish-yellow rice had significantly greater antioxidant capacities.

Keywords: Antioxidants, Improved varieties, Pigmented rice, Traditional varieties

INTRODUCTION

Antioxidants reduce excessive oxidative stress in cells, offering numerous health benefits and lowering the risk of various non-communicable diseases, as shown in previous studies (Hongcheng *et al*., 2014). In Sri Lanka and many South Asian countries, rice is the main cereal and staple food (Rajapakse *et al*., 2000; Gunaratne *et al*., 2013). It serves as a potential source of natural antioxidants that act as free radical scavengers in the body.

Cereals and cereal-based foods have been fundamental to the human diet since ancient times. Current global dietary guidelines recommend whole grain cereals for their health benefits that extend beyond basic nutrition (WHO and FAO, 2003). These benefits are largely due to various phytochemicals, including naturally derived antioxidants (Muntana and Prasong, 2010). Previous studies indicate that pigmented rice varieties have high antioxidant content (Sompong *et al*., 2011; Saikia *et al*., 2012; Hongcheng *et al*., 2014). Sri Lanka is a leading producer of pigmented rice and boasts a diverse collection of both pigmented and non-pigmented varieties, including traditional and improved cultivars popular in the country.

In white, red, and black rice, secondary metabolites such as phenolic acids and anthocyanins are mostly concentrated in rice bran compared to other parts of the grain, including whole grains, embryos, and endosperm. Several studies reported that secondary metabolites present in rice bran have diverse bioactivities such as antioxidant, antimicrobial, cancer preventive, antidiabetic, and hypolipidemic etc. (Zhang *et al*., 2010; Goufo and Trindade, 2014; Iqbal *et al*., 2021; Andriani *et al*., 2022). Phenolic compounds are bound to macromolecules like hemicellulose, cellulose, structural proteins, arabinoxylan, and pectin, making them insoluble and difficult to extract (Shahidi and Hossain, 2023). Consequently, recent studies have focused on releasing these bound phenolic compounds to enhance antioxidant activity (Chandrasekara and Shahidi, 2011; Shao *et al*., 2014; Chen *et al*., 2019; Xu *et al*., 2021; Lu *et al*., 2022).

Further, rice is known to contain other compounds such as anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, which significantly contribute to enhancing its antioxidant capacity (Gaufo and Trinidale, 2014). While proanthocyanidin is responsible for red pericarp colour (Mbanjo, 2020). Most people prefer milled or polished rice, whereas unpolished whole rice from pigmented varieties is typically consumed locally. Polishing or milling removes the pericarp, the outermost layer of the rice kernel, resulting in milled rice that has diminished nutritional content. Cooking can further decrease the availability of these nutrients due to rinsing and heat (Kalita *et al*., 2021).

The health benefits of pigmented and brown rice are debated, yet rice is often milled, rinsed, and cooked before consumption. Pigmented rice typically has higher antioxidant levels and stronger colour, suggesting greater nutraceutical value. However, the colour intensity of pigmented varieties varies, and the impact of milling on colour loss is unpredictable. Understanding the antioxidant capacities and dynamics of whole, milled, and cooked pigmented and non-pigmented rice varieties, along with their colour strength, aids in selecting rice that offers optimal health benefits and culinary qualities.

This study examined the antioxidant content of pigmented and non-pigmented whole and milled rice in both raw and cooked states, while comparing their colour intensities and the relationship between antioxidant levels and colour contrasts. Given the limited scientific information on these various forms of the same rice varieties, these findings will benefit rice consumers as well as food industry.

MATERIALS AND METHODS

Sample Preparation

Seeds of twenty pigmented and 20 non-pigmented rice varieties were cultivated in Ambalantota, Sri Lanka using proper agricultural practices, and then they were harvested, processed and sampled simultaneously and de-husked to obtain unpolished whole grain rice. A portion of the whole rice was subjected to abrasive milling for 30s to obtain polished rice which removed the rice bran approximately to 5±2%. A portion of polished rice was cooked using the procedure given here; first, washing twice followed by adding a definite amount of water which was calculated based on the gelatinization temperature as measured by alkali spreading score of each rice variety and wet cooking was performed in a mini rice cooker. Cooked rice was oven dried at 65^{o}C for 3.5 hrs. and antioxidant capacity of whole, milled and cooked rice varieties was determined using 2,2-

diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams, 1995; Abeysuriya *et al*., 2020) and Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996; Abeysuriya *et al*., 2020).

Sample Preparation for Antioxidant Determination

Antioxidant capacities of whole, milled and cooked rice were determined with a sample of five grams (5 g) of finely ground rice weighed in a 100 ml volumetric flask. Twenty milliliters of 80% methanol was added and stirred for 30 min in an electrical stirrer (Nemke UL 61010-1, Italy) and then allowed 5 min for separation.

DPPH (2,2′-Diphenyl-1-Picrylhydrazyl Radical) Radical Scavenging) Assay

The antioxidant capacity was determined using the DPPH radical scavenging assay, following methods described by Brand-Williams *et al*. (1995), with modifications adopted from Marinova and Batchvarov, (2011), Mishra *et al*. (2012), Saikia et al. (2012) and Abeysuriya *et al*. (2020).

With the above rice extract, a dilution series was prepared in 5 ml volumetric flasks. Each of the above solutions in methanol (100 µl) was added to freshly prepared 3.9 mL of 6×10^{-5} mol/L methanol DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (Brand-Williams *et al*., 1995) and left for 20 min in the dark, and the absorbance of each sample was measured under the 515 nm wavelength (Marinova and Batchvarov, 2011) using the UV spectrophotometer (Genesis-10S Vis). Percentage inhibition = $(A_o - A_1) / A_o \times 100$, where A_o is absorbance of control blank, and A_1 is absorbance of sample extract. Effective concentration (EC_{50}) required to decrease the initial DPPH concentration by 50% (Brand-Williams et al., 1995) was calculated using the graph. For the easiness of interpretation, $1/EC_{50}$ (inverse of EC_{50}) was used and denoted as antiradical power (ARP), as explained by Mishra *et al*. (2012).

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was used to measure the total antioxidant capacity of rice varieties. The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. The resultant solution was incubated at 37 °C for 10 min in a water bath. The assay was carried out by adding100 µL of rice extract (prepared in 80% methanol) to a test tube containing 3 mL of the freshly prepared FRAP reagent. The mixture was incubated at room temperature for approximately 10 min in the dark, and the absorbance was measured at 593 nm. Results were expressed as Ferric Reducing Antioxidant Power (FRAP) using Fe^{3+} as the standard (Benzie and Strain, 1996; Benzie and Szeto, 1999; Abeysuriya *et al*., 2020). Antioxidant capacity was reported in µM Fe^{2+} equivalents per mL.

Colour Measurement (L, a, b)

The colour values of all rice samples (whole, milled and cooked) were measured using the spectrophotometer CR-20 (colorimeter) (Minolta Co., Ltd., Osaka, Japan) with spectra magic version 3.6 software. The colour was expressed as the L, a*, and b* colour space coordinates, where L, +a*, -a*, +b*, and -b* represent lightness (100 white to 0 black), redness, greenness, yellowness, and blueness respectively (*Zhang et al*., 2015).

Data Analysis

Analysis of variance and Pearson correlation analysis was performed with STAR for Windows version 2.0.1 (IRRI, 2014) and cluster analysis and matrix plots were accomplished through Minitab 17 version for Windows.

RESULTS AND DISCUSSION

DPPH· (2,2′-Diphenyl-1-Picrylhydrazyl Radical) Radical Scavenging) Assay

DPPH radical scavenging activity of different rice varieties tested as reciprocals of EC_{50} values as antiradical power (Ereifej *et al*., 2016) are given in Table 1. Low EC_{50} values denote high antioxidant capacity and vice versa. Mean anti radical power (ARP) values of whole, milled and cooked rice were significantly ($p \leq 0.05$) higher in pigmented red pericarp varieties compared to non-pigmented white pericarp varieties (Table 1). Saikia *et al*. (2012) also reported significantly higher mean values in whole and milled rice of pigmented rice compared to the non-pigmented rice ($p \leq 0.05$). This could be due to the colour pigments which comprise anthocyanins and proanthocyanidins in red rice varieties (Goufo and Trindade, 2014). Gunaratne *et al.* (2013) have found proanthocyanidin as the dominant antioxidant in red varieties, including *Kaluheenati, Behethheenati* and *Madathwalu*. ARP values of 20 whole, milled and cooked pigmented rice varieties varied from 7.87×10^{-3} to 37.93×10^{-3}, 4.48×10^{-3} to 16.62×10^{-3} and 0.86×10^{-3} to 2.07×10^{-3} ml/mg respectively. Among the twenty red rice varieties studied, the ARP scores of whole grain rice (greater than 27 mL/mg) were significantly higher ($p \leq 0.05$) in two traditional varieties, *Masuran* and

Nonabokra, and two improved varieties, Ld 368 and Ld 408.

Milled rice had lower ARP values than whole rice. The reason could be due to the removal of bran in milled rice where the colour pigments are concentrated. Although partial removal of rice bran through abrasive milling for 30 s significantly ($p \leq 0.05$) reduced the antioxidant content of rice varieties, a considerable amount of antioxidants remained, with levels varying among varieties.

The percentage ARP reduction of red and white rice varieties due to milling ranged from 11 to 70% and 2 to 48% respectively. ARP values of all rice varieties decreased further upon cooking (reduction ranged from 19 to 92%). This is due to further removal of rice bran through washing and the effect of heat during cooking. Yamuangmorn e*t al*. (2018) found higher retention in both anthocyanin and antioxidant capacity with wet cooking using an electric rice cooker without presoaking the grain than steaming. However, the current study also showed that anti-oxidant property remained even after wet cooking using an electric rice cooker without presoaking. Similarly, significantly higher mean ARP values of more than 13 ml/mg were obtained by traditional as well as improved milled rice varieties of *Murungakayan,* Bw 272-6B, Ld 368, At 311, *Masuran, Mass, Beheth heenati,* and Ld 408. Whole rice with higher antioxidant capacities also showed a considerable reduction in those values after milling and cooking. Antioxidant content was reduced by up to 96% from the whole rice to the cooked state According to these findings, total available antioxidants in whole rice becomes partially unavailable after milling and then cooking. However, on average cooked pigmented rice contain six times more antioxidants than non-pigmented rice. A study done by Mackon *et al*. (2021) reported nearly three times higher anthocyanins in red than brown rice represented in cyanidin 3-O-glucoside equivalent. In the same study proanthocyanidin content denoted through catechin acid equivalent was significantly higher in red compared to white, black and purple rice. *Kaluheenati, Rathsuwandel, Nonabokra* and Bw 272-6B showed significantly high antioxidant capacities ($p \leq 0.05$) exceeding 1.5 mg/ml. These values are higher than those of most non-pigmented whole rice varieties (Table 1). Unfortunately, the high-yielding and most popularly cultivated pigmented rice variety At 362 (Agstat, 2023), showed low antioxidant values.

Ferric Reducing Antioxidant Power (FRAP) Assay

The reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) is commonly used to assess the total antioxidant capacity of natural products, including rice. This reaction produces a blue-coloured complex, the intensity of which varies depending on the antioxidant capacity of the sample (Benzie and Strain, 1996; Benzie and Szeto, 1999; Abeysuriya *et al*., 2020).

Table 2, shows the mean FRAP values of whole, milled and cooked pigmented red pericarp rice varieties. These values were significantly ($p \leq 0.05$) higher than those of non-pigmented white pericarp varieties.

Antioxidant strengths of the same set of pigmented and non-pigmented rice showed similar trends as in DPPH method. Abeysekara *et al*. (2017) showed that red rice bran has comparatively higher antioxidant capacity than the white rice. Reduction of molecule complexes of 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3.6H2O$) which are almost colourless, and eventually become slightly brownish with oxidizing of rice seed-based ingredients such as anthocyanins, oligomeric, proanthocyanidin and phenolic compounds. Accordingly, the colour strengths shown as FRAP values in Table 2 indicate the level of antioxidant availability across the 40 rice varieties examined.

FRAP values of the 20 pigmented rice varieties ranged from 8.79×10^2 to 30.42×10^2 µM/mL in whole grain rice, 2.46×10^2 to 20.52×10^2 µM/mL in milled rice, and 0.37×10^2 to 2.20×10^{-3} µM/mL in cooked rice. Significantly higher FRAP values ($p \leq 0.05$), exceeding 20.67×10^2 µM/mL, were observed in whole grains of several pigmented traditional varieties such as *Masuran* and *Nonabokra,* as well as improved varieties such as Ld 368 and Ld 408. Other varieties with high FRAP values included three traditional types (*Pokkali, Kaluheenati,* and *Herath Banda*), one improved variety (Bw 361), and one exotic type (*Mass*), as shown in Table 2.

Milling, which involves partial removal of the rice bran over 30 s, resulted in a significant reduction in antioxidant content ($p \leq 0.05$). Nonetheless, several improved and traditional varieties retained relatively high FRAP values even after milling, consistent with the DPPH results. White pericarp varieties generally exhibited much lower antioxidant capacity, though a few, Bg 358, Basmathi 442, Bg 94-1, Bg 352, and Bg 360, demonstrated relatively high FRAP values (≥ 1.80 µM/mL), indicating notable antioxidant potential within this group.

Antioxidants-rich rice varieties can be selected based on whole grain antioxidant values; however, what actually consumed is milled and cooked rice. Nevertheless, people mostly consume milled rice after cooking. Therefore, selecting varieties based on the antioxidant capacity of cooked rice may be more appropriate; alternatively, the consumption of whole grain rice is advisable. Among the tested varieties cooked *Masuran* retained comparatively high antioxidant levels in both antioxidant assays. Other varieties including *Nonabokra, Kaluheenati, Beheth heenati, Rathsuwandel*, Bw 272-6B, Ld 368, and *Mass* also retained considerable amount of antioxidants after cooking.

Cluster Analysis for Total Antioxidant Contents

Clustering was performed through Minitab 17 for windows over the whole and milled rice antioxidants considering together (Figure 1). The clustering clearly distinguishes the genotypes according to pigmentation and processing status, reflecting the variation in antioxidant potential across different rice types. Figure 1A and 1B represent pigmented while 1C and 1D denotes non-pigmented rice varieties which were analyzed through DPPH and FRAP methods respectively. The varieties grouped within the clusters in Figures 1A and 1B differed, with maximum distances reaching up to 9.33 and 8.93, respectively. The multivariate hierarchical cluster analysis grouped 20 pigmented red rice varieties mainly into three clusters (Figure 1A). The majority of varieties (90%) were grouped into two clusters, which were divided into sub-clusters. The third cluster includes two varieties, *Nonabokra* and *Masuran,* which show similar ARP values. Similarly, Ld 368 and Ld 408, both bred at the Rice Research Station, Labuduwa (RRDI, 2024), are grouped together in a sub-cluster of the first cluster due to their comparable ARP values.

Figure 1B, which represents FRAP antioxidant analysis, shows four distinct clusters. The majority of the rice varieties (80%) were grouped within a single large cluster, further divided into three sub-clusters. The remaining four varieties; *Pokkali, Nonabokra, Masuran,* and Ld 368, were distributed among three separate clusters. *Pokkali* and *Masuran* each form individual clusters, while *Nonabokra* and Ld 368 were grouped together. These four varieties exhibited the highest FRAP antioxidant levels in whole rice, as shown in Table 1. Likewise, *Nonabokra*, Ld 368, and *Pokkali* also showed the highest antioxidant levels in milled rice based on FRAP values.

Dendrograms in Figures 1C and 1D reveal that Bg 358 forms a unique cluster, reflecting its exceptionally high antioxidant content measured by both methods, as detailed in Tables 1 and 2. In contrast, the remaining white, non-pigmented varieties were grouped into different clusters.

Colour Measurements (L, a, b)

Colour (lightness, redness and yellowness) values of tested pigmented varieties (Table 3) were significantly **($p \leq 0.05$)** different among whole rice as well as milled rice. There was a significant decline in redness of pigmented varieties after the milling process. Further decline in redness was observed after cooking which removes the bran further losing the colour more through washing.

Comparative to whole rice, redness decreased by 38% in milled and 61% in cooked rice respectively. The "a" value, which measures redness, ranged from 12.56 to 16.34, from 9.84 to 14.10 and from 7.2 to 13.12 in whole, milled for 30s and cooked rice separately. Redness values of whole rice of red pericarp (pigmented) varieties *Masuran, Nonabokra,* Ld 368 and Ld 408 which had the highest antioxidants compared to other 36 varieties ranged between 12.56 and 14.46. In contrast, higher redness values were also revealed in some other red varieties representing traditional, improved and exotic varieties (Table 3). Contrasting to the declining trend found in redness of pigmented varieties, lightness increased by 12% after milling and by a further 30% after cooking. The removal of the pericarp in pigmented rice varieties led to a gradual increase in grain lightness due to the reduction of red pigments.

Table 4 presents the lightness, redness, and yellowness values of non-pigmented rice varieties. Lightness values ranged from 33.33 to 50.53 in whole rice, 32.97 to 49.73 in milled rice, and 31.45 to 48.48 in cooked rice. Among the varieties, the improved variety Bg 369 consistently showed the highest lightness across all processing stages: whole, milled, and cooked. In contrast, the three traditional non-pigmented varieties: *Suwanda Samba, Suduru Samba*, and *Suwandel,* exhibited significantly lower lightness values ($p \leq 0.05$) compared to the other varieties.

Correlation Analysis for Colour Values and Total Antioxidants (DPPH and FRAP)

Antioxidant contents expressed via DPPH and FRAP assays were positively correlated ($p \leq 0.01$) in some occurrences as stated below (Table 5). Whole rice antioxidant capacities showed strong positive ($p \leq 0.01$) correlations with milled ($r = 0.55$) and cooked rice ($r = 0.49$) antioxidant contents which were assessed through DPPH method. Similarly, strong positive relationship was observed between DPPH-WR and FRAP methods of antioxidant assay in whole ($r = 0.81$), milled ($r = 0.58$) and cooked ($r = 0.46$) rice.

Similarly, whole rice antioxidants obtained by FRAP also exhibited positive correlations with milled ($r = 0.35$) and cooked ($r = 0.42$) rice antioxidant contents expressed by DPPH process. Antioxidant contents conveyed in milled rice and whole rice through FRAP methods also had a significantly ($p \leq 0.01$) positive association ($r = 0.6$). Above relationships proves the validity of interpreted antioxidant values through both methods. Milled rice antioxidant strengths analyzed via DPPH method exhibited slightly negative correlation with lightness (L). Correlation between yellowness and lightness among the forty rice varieties was highly positive ($r = 0.85$) and significant at the 99% probability level. A positive correlation between a and b ($r = 0.46$) suggests that increases in redness were moderately associated with increases in yellowness, with statistical significance at the 99% probability level. Redness, yellowness, and whiteness are often correlated because they were all measured within the same colour space, specifically the CIELAB colour space, and are mathematically related to each other. Changes in whiteness can be linked to changes in yellowness, and redness can be a contributing factor to both. Nevertheless, antioxidant levels in cooked rice, as determined by DPPH and FRAP methods, showed no correlations with those in milled rice measured by the same methods. The reduction in antioxidant content after cooking may be due to two factors: the removal of rice bran during washing and the impact of heat. Unlike the controlled milling process (30 seconds), bran removal through washing lacks uniformity and consistency. This may explain the absence of correlation between the antioxidant contents of milled and cooked rice.

Matrix plot diagram for colour values against antioxidant of whole, milled and cooked rice

Figure 2, illustrates the data points of matrix plot diagram for lightness (L), redness (a) and yellowness (b) against antioxidant strengths of whole, milled and cooked rice. Figure 2 A and B illustrate the diagrams of pigmented and C and D of non-pigmented rice. Figure 2 A and B show the similar pattern of data point gathering for whole, milled and cooked rice. All three status of rice grain demonstrate similar pattern with colour values (L, a, b). Most probably data points were drifted to one direction for lightness (L), redness (a) and yellowness (b). It was observed that light reddish yellow pigmentation is most common for high antioxidant capacity which stands for pigmented rice. Most of red rice varieties were inclined to light reddish yellow with high antioxidant capacity in both DPPH and FRAP methods. Similar results were found for barley where the purple barley grain had reported reddish to yellow colour value with high antioxidant level among black and yellow barley grain (Bellido and Beta, 2009).

Table 1. DPPH scavenging antiradical power (ARP) of forty pigmented and non-pigmented rice varieties.

Rice Variety (Pigmented/red)		Mean ARP value (mg/mL)			Rice Variety (non-pigmented/white)		Mean ARP value (mg/mL)		
Variety	Origin	Whole rice $\times10^{-3}$	30s milled rice $\times10^{-3}$	Cooked rice $\times10^{-3}$	Variety	Origin	Whole rice $\times10^{-3}$	30 s milled rice $\times10^{-3}$	Cooked rice $\times10^{-3}$
Murungakayan	T	18.69^{de}	16.62^{a}	1.29^{cde}	Suwanda samba	T	1.68^{efg}	1.65^{c}	0.73^{a}
Pokkali	T	20.38^{cde}	9.04^{ef}	1.21^{cde}	Suduru samba	T	1.74^{efg}	1.57^{cd}	0.20^{bc}
Nonabokara	T	36.10^{a}	8.76^{ef}	1.52^{abc}	Suwadel	T	1.9^{de}	1.30^{def}	0.23^{bc}
Pachchaperumal	T	20.76^{cde}	9.65^{e}	1.29^{cde}	H7	I	1.89^{de}	1.19^{efg}	0.18^{bc}
Kaluheneti	T	23.63^{bc}	8.98^{ef}	1.53^{abc}	H5	I	1.24^{j}	0.92^{g}	0.30^{b}
Behethheniti	T	23.14^{cd}	14.16^{bc}	1.92^{ab}	At 405	I	1.04^{cd}	1.36^{cdef}	0.25^{bc}
Herathbanda	T	9.71^{gh}	5.22^{hi}	1.16^{cde}	Bg 369	I	1.32^{hij}	0.97^{g}	0.18^{bc}
Madathawalu	T	24.33^{bc}	7.21^{g}	1.40^{bcde}	Bw 367	I	1.58^{fgh}	1.37^{cdef}	0.29^{bc}
Rathsuwadel	T	13.20^{fg}	5.79^{h}	1.58^{abc}	Bg 94-1	I	1.80^{defg}	1.51^{cde}	0.17^{bc}
Masuran	T	37.79^{a}	13.65^{bc}	2.07^{a}	At 306	I	0.93^{k}	0.87^{g}	0.71^{a}
H4	I	16.15^{ef}	11.58^{d}	0.92^{de}	Bg 358	I	3.13^{a}	1.61^{cd}	0.18^{bc}
At 85-2	I	11.90^{fgh}	4.91^{hi}	0.93^{de}	Bg 380	I	1.77^{efg}	1.49^{cde}	0.15^{bc}
At 311	I	20.22^{cde}	14.39^{b}	1.20^{cde}	At 354	I	1.55^{ghi}	1.19^{efg}	0.14^{c}
At 362	I	7.87^{h}	4.48^{i}	0.86^{e}	Bg 352	I	1.84^{def}	1.41^{cde}	0.28^{bc}
At 402	I	13.82^{fg}	5.03^{hi}	1.29^{cde}	Bg 300	I	1.77^{efg}	1.06^{fg}	0.16^{bc}
Bw 361	I	16.48^{ef}	8.13^{fg}	1.46^{bcd}	Bg 360	I	2.29^{bc}	2.15^{a}	0.30^{b}
Bw 272-6B	I	20.34^{cde}	16.12^{a}	1.51^{abc}	Bg 379-2	I	1.80^{defg}	1.35^{cdef}	0.26^{bc}
Ld 408	I	27.81^{b}	13.03^{c}	1.30^{cde}	Basmathi 370	E	1.30^{ij}	1.20^{efg}	0.24^{bc}
Ld 368	I	28.06^{b}	15.64^{a}	1.03^{cde}	Basmathi 442	E	2.42^{b}	1.98^{b}	0.23^{bc}
Mass	E	24.33^{bc}	13.47^{bc}	1.90^{ab}	Bg 250	I	1.25^{j}	0.95^{g}	0.30^{b}

*Note: T-traditional rice, I-improved rice, E-exotic rice. *Means with the same letter within the column are not significantly different*

Table 2: FRAP antioxidant capacity of forty pigmented and non-pigmented rice varieties

Varietal characters (pigmented/red)		Mean FRAP value (μM/mL)			Varietal characters (Non-pigmented/white)		Mean FRAP value (μM/mL)		
Variety name or identity	Origin	Whole rice $\times 10^2$	30s milled rice	Cooked rice$\times 10^2$	Variety name or Identity	Origin	Whole rice $\times 10^2$	30s milled rice	Cooked rice $\times 10^2$
Murungakayan	T	16.80^{k}	3.40^{k}	1.77^{de}	Suwanda samba	T	3.94^{cde}	2.97^{e}	0.81^{cd}
Pokkali	T	25.50^{b}	13.72^{b}	1.26^{k}	Suduru samba	T	4.02^{cd}	2.40^{g}	0.80^{cd}
Nonabokara	T	30.48^{a}	20.52^{a}	1.55^{i}	Suwadel	T	3.71^{e}	1.70^{j}	0.79^{cd}
Pachchaperumal	T	16.40^{kl}	13.30^{c}	1.63^{gh}	H7	I	3.83^{de}	3.43^{d}	0.44^{j}
Kaluheneti	T	20.72^{ef}	10.41^{e}	1.86^{c}	H5	I	3.71^{e}	1.40^{k}	0.73^{ef}
Behethheniti	T	17.57^{j}	9.65^{f}	1.84^{cd}	At 405	I	2.54^{fg}	1.90^{i}	0.36^{k}
Herathbanda	T	21.55^{d}	4.40^{i}	1.18^{l}	Bg 369	I	4.14^{c}	1.93^{i}	0.82^{cd}
Madathawalu	T	19.65^{h}	10.69^{e}	1.84^{cd}	Bw 367	I	4.77^{e}	3.72^{c}	1.03^{a}
Rathsuwadel	T	11.62^{o}	2.52^{l}	1.68^{fg}	Bg 94-1	I	2.35^{fg}	2.21^{h}	0.85^{c}
Masuran	T	30.42^{a}	4.21^{ij}	2.20^{a}	At 306	I	1.22^{h}	0.93^{l}	0.86^{c}
H4	I	15.93^{l}	3.06^{k}	1.24^{kl}	Bg 358	I	4.42^{b}	7.63^{a}	0.82^{cd}
At 85-2	I	15.06^{m}	2.46^{l}	1.34^{j}	Bg 380	I	3.72^{e}	3.41^{d}	0.83^{c}
At 311	I	18.79^{i}	5.52^{h}	1.72^{ef}	At 354	I	4.18^{bc}	1.65^{i}	0.32^{k}
At 362	I	8.79^{p}	2.05^{m}	1.09^{m}	Bg 352	I	2.49^{fg}	1.60^{j}	0.46^{ij}
At 402	I	12.61^{n}	4.05^{j}	1.78^{cde}	Bg 300	I	2.58^{f}	1.41^{k}	0.52^{hi}
Bw 361	I	20.07^{gh}	9.36^{fg}	0.88^{kl}	Bg 360	I	3.81^{de}	4.38^{b}	0.19^{l}
Bw 272-6B	I	18.43^{i}	9.11^{g}	0.37^{o}	Bg 379-2	I	4.05^{cd}	2.70^{f}	0.77^{de}
Ld 408	I	20.39^{fg}	5.24^{h}	1.58^{hi}	Basmathi 370	E	1.46^{h}	1.56^{jk}	0.65^{g}
Ld 368	I	23.67^{c}	20.38^{a}	2.14^{a}	Basmathi 442	E	4.16^{bc}	1.66^{j}	0.69^{fg}
Mass	E	21.20^{de}	11.37^{d}	1.95^{b}	Bg 250	I	2.27^{g}	1.05^{j}	0.55^{h}

*Note: T- traditional rice, I-improved rice, E-exotic rice, R-red rice, W-white rice. *Means with the same letter within the column are not significantly different*

Table 3: Mean colour values (L, a, b) of whole, 30 milled and cooked red pigmented rice varieties

	Mean colour value of whole rice			Mean colour value of 30s milled rice			Mean colour value of cooked rice		
Rice variety	L	a	b	L	a	b	L	a	b
Murungakayan	29.08^{gh}	13.44^{cde}	12.60^{g}	38.20^{g}	13.44^{bc}	14.46^{gh}	46.72^{abcd}	11.34^{d}	15.20^{cde}
Pokkali	32.08^{abcd}	16.34^{a}	16.58^{abc}	45.14^{bcd}	12.00^{efg}	14.44^{h}	52.98^{ab}	09.28^{h}	14.12^{hi}
Nonabokara	31.74^{abcde}	14.30^{cd}	15.48^{bcdef}	43.08^{bcde}	13.56^{abc}	15.72^{cde}	50.94^{abc}	10.24^{ef}	14.62^{defghi}
Pachchaperumal	32.04^{abcde}	13.58^{cde}	16.42^{abc}	45.36^{bc}	11.58^{fg}	15.24^{defg}	54.84^{ab}	08.34^{i}	14.26^{fghi}
Kaluheneti	31.80^{abcde}	12.72^{de}	15.30^{bcdef}	45.92^{ab}	09.84^{i}	14.58^{gh}	52.78^{abc}	07.62^{j}	14.18^{ghi}
Behethheniti	32.46^{abcd}	13.38^{cde}	15.20^{cdef}	42.40^{de}	11.36^{g}	15.12^{efgh}	46.04^{abcd}	09.66^{gh}	14.66^{defghi}
Herathbanda	32.08^{abcd}	14.26^{cd}	16.26^{abc}	45.48^{abc}	11.96^{efg}	15.04^{efgh}	54.92^{ab}	08.34^{i}	14.08^{i}
Madathawalu	31.30^{bcdef}	13.40^{cde}	14.66^{ef}	42.12^{e}	12.52^{de}	15.38^{def}	49.08^{abc}	10.34^{ef}	14.96^{cdefgh}
Rathsuwadel	33.14 ^{ab}	12.72^{de}	16.84^{a}	41.78^{ef}	12.60^{de}	18.34^{a}	51.40^{abc}	10.26^{ef}	18.60^{a}
Masuran	33.44^{a}	14.46^{bc}	15.50^{bcde}	40.94^{f}	12.46^{de}	14.58^{gh}	42.06^{cd}	09.98^{fg}	14.18^{ghi}
H4	29.92^{efg}	14.02^{cde}	14.90^{def}	43.92^{bcde}	12.40^{de}	15.06^{efgh}	47.52^{abcd}	09.26^{h}	14.36^{efghi}
At 85-2	32.68^{abc}	14.60^{bc}	16.00^{abcd}	42.96^{cde}	12.34^{de}	14.88^{fgh}	53.04^{ab}	09.28^{h}	14.98^{cdefg}
At 311	29.30^{fgh}	13.50^{cde}	15.40^{bcdef}	48.28^{a}	10.68^{h}	15.74^{cde}	57.80^{a}	07.20^{j}	15.10^{c}
At 362	27.40 ^{hi}	13.06^{cde}	12.64^{g}	43.48^{bcde}	12.00^{efg}	16.56^{b}	51.24^{abc}	08.24^{i}	15.40^{bcd}
At 402	31.88^{abcde}	16.02^{ab}	17.08^{a}	43.36^{bcde}	12.18^{ef}	15.82^{bcde}	52.16^{abc}	9.30^{h}	15.46^{bcd}
Bw 361	31.42^{abcdef}	13.30^{cde}	15.96^{abcde}	41.62^{ef}	12.62^{de}	15.40^{def}	52.64^{abc}	10.74^{e}	16.08^{bcdf}
Bw 272-6B	30.36^{defg}	14.24^{cd}	14.80^{def}	39.14^{fg}	14.10^{ab}	15.92^{bcd}	44.30^{bcd}	12.76^{b}	16.18^{b}
Ld 408	25.78^{i}	12.98^{de}	11.62^{g}	42.74^{cde}	12.56^{cd}	16.26^{bc}	51.48^{abc}	08.52^{i}	15.02^{cdefg}
Ld 368	26.68^{i}	14.33^{cd}	12.78^{g}	31.18^{h}	13.82^{b}	14.80^{fgh}	36.26^{d}	13.28^{a}	15.54^{bc}
Mass	30.62^{cdefg}	14.30^{cd}	14.16^{f}	41.80^{ef}	12.24^{ef}	14.88^{fgh}	44.54^{bcd}	12.04^{c}	16.16^{b}

Note: Means with the same letter within the column are not significantly different

Table 4: Mean colour values (L, a, b) of whole, 30s milled and cooked non- pigmented rice

	Mean colour values of whole rice			Mean colour values of 30s milled rice			Mean colour values of cooked rice		
Rice variety	L	a	b	L	a	b	L	a	b
Suwanda samba	41.27^{f}	4.70^{b}	18.67^{a}	40.47^{e}	3.47^{d}	18.37^{a}	39.03^{e}	3.37^{d}	17.18^{a}
Suduru samba	34.10^{h}	3.97^{c}	13.77^{d}	32.97^{h}	4.20^{c}	12.87^{bc}	31.45^{h}	3.27^{d}	14.23^{c}
Suwadel	38.30^{g}	4.93^{ab}	16.10^{ab}	35.33^{g}	4.70^{ab}	16.01^{b}	33.90^{g}	4.03^{c}	17.8^{a}
H7	50.27^{ab}	3.73^{d}	13.57^{d}	47.13^{c}	2.27^{g}	9.97^{c}	45.64^{c}	1.87^{h}	9.12^{j}
H5	45.93^{d}	3.45^{f}	14.40^{c}	44.93^{d}	2.62^{f}	12.87^{bc}	43.43^{d}	2.33^{e}	12.23^{ef}
At 405	48.67^{bc}	5.40^{a}	16.80^{ab}	48.07^{b}	3.07^{e}	12.80^{bc}	46.65^{b}	2.87^{e}	11.83^{g}
Bg 369	50.23^{ab}	4.77^{b}	16.80^{ab}	49.73^{a}	3.30^{d}	12.93^{bc}	48.26^{a}	2.98^{e}	13.36^{d}
Bw 367	43.00^{e}	3.63^{e}	11.03^{e}	48.13^{b}	4.70^{ab}	10.07^{c}	46.77^{b}	4.12^{ab}	9.15^{j}
Bg 94-1	45.13^{d}	4.70^{b}	16.53^{ab}	37.83^{f}	2.29^{g}	9.90^{c}	36.36^{f}	1.87^{h}	9.33^{i}
At 306	33.33^{h}	4.77^{b}	14.67^{c}	34.47^{g}	3.73^{cd}	14.47^{bc}	33.80^{g}	3.43^{d}	14.35^{c}
Bg 358	45.70^{d}	3.67^{e}	15.47^{b}	45.07^{d}	4.97^{a}	12.57^{bc}	43.68^{d}	4.47^{a}	11.42^{g}
Bg 380	49.33^{ab}	3.80^{c}	14.97^{c}	47.33^{c}	2.31^{g}	12.73^{bc}	45.46^{c}	1.67^{j}	12.16^{ef}
At 354	38.80^{g}	3.82^{c}	15.63^{b}	35.80^{g}	2.60^{f}	15.37^{abc}	34.33^{g}	2.11^{g}	14.80^{c}
Bg 352	48.03^{bc}	5.50^{a}	15.80^{b}	47.00^{c}	3.70^{cd}	13.40^{bc}	45.52^{c}	3.32^{d}	12.83^{e}
Bg 300	53.53^{a}	4.47^{b}	16.53^{ab}	47.17^{c}	3.07^{e}	12.13^{bc}	45.53^{c}	2.57^{e}	11.56^{f}
Bg 360	49.93^{ab}	5.43^{a}	14.90^{c}	49.45^{a}	4.77^{ab}	15.50^{abc}	48.48^{a}	4.27^{ab}	14.93^{c}
Bg 379-2	48.30^{bc}	4.43^{b}	16.00^{ab}	42.70^{d}	2.60^{f}	12.00^{bc}	41.25^{d}	2.21^{f}	11.43^{g}
Basmathi 370	48.73^{bc}	4.73^{b}	15.83^{b}	48.87^{ab}	4.90^{a}	15.38^{abc}	47.03^{ab}	4.34^{a}	15.09^{b}
Basmathi 442	49.33^{ab}	5.03^{ab}	18.19^{a}	42.40^{d}	4.53^{b}	13.83^{bc}	40.91^{d}	4.03^{c}	13.26^{d}
Bg 250	48.57^{bc}	4.99^{ab}	17.00^{ab}	40.43^{d}	3.40^{d}	12.17^{bc}	39.92^{d}	2.39^{e}	10.60^{h}

Note: Means with the same letter within the column are not significantly different

Table 5: Correlation coefficients of antioxidant (DPPH and FRAP) activities and colour value

	DPPH WR	DPPH MR	DPPH CR	FRAP WR	FRAP MR	FRAP CR	L	a
DPPH WR	1							
DPPH MR	0.55**	1						
DPPH CR	0.49**	0.24	1					
FRAP WR	0.81**	0.35*	0.42**	1				
FRAP MR	0.58**	0.24	0.12	0.60**	1			
FRAP CR	0.46**	0.08	0.1	0.27	0.13	1		
L	- 0.05	- 0.40*	0.48**	0.13	- 0.07	- 0.00	1	
a	- 0.15	- 0.22	- 0.06	0.1	0.05	- 0.20	0.34*	1
b	*-0.24*	*0.51***	*0.18*	*- 0.01*	*- 0.00*	*- 0.07*	*0.85***	*0.46***

Note: WR – whole rice, MR - milled rice, CR - cooked rice, L - lightness, a - redness, b - yellowness.

** Significant at 0.05, ** significant at 0.01*

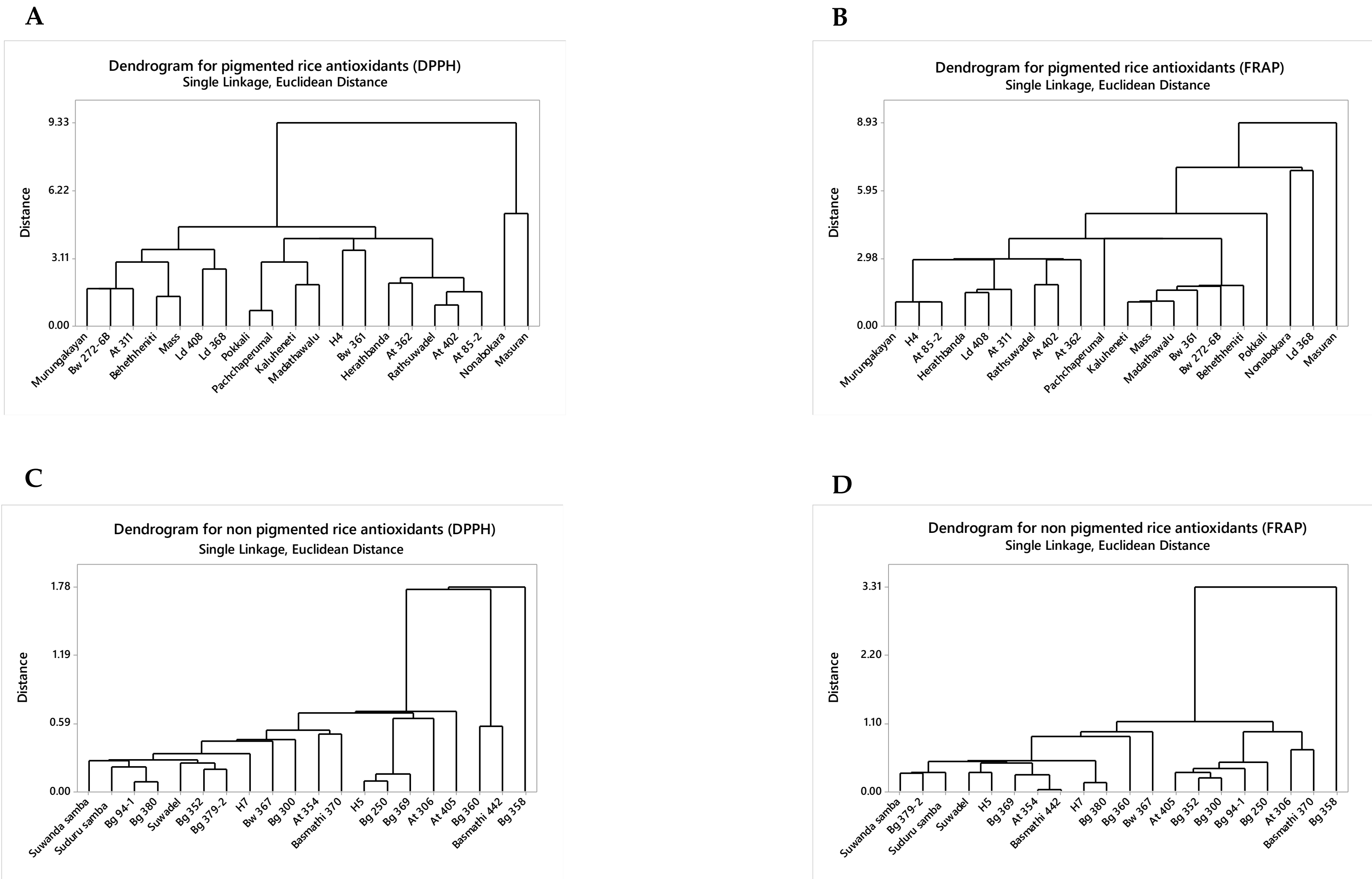

Figure 1: Dendrogram of 20 red pigmented (A, B) and 20 white non-pigmented (C, D) rice varieties derived by Euclidean Single Linkage method of cluster analysis for total antioxidants.

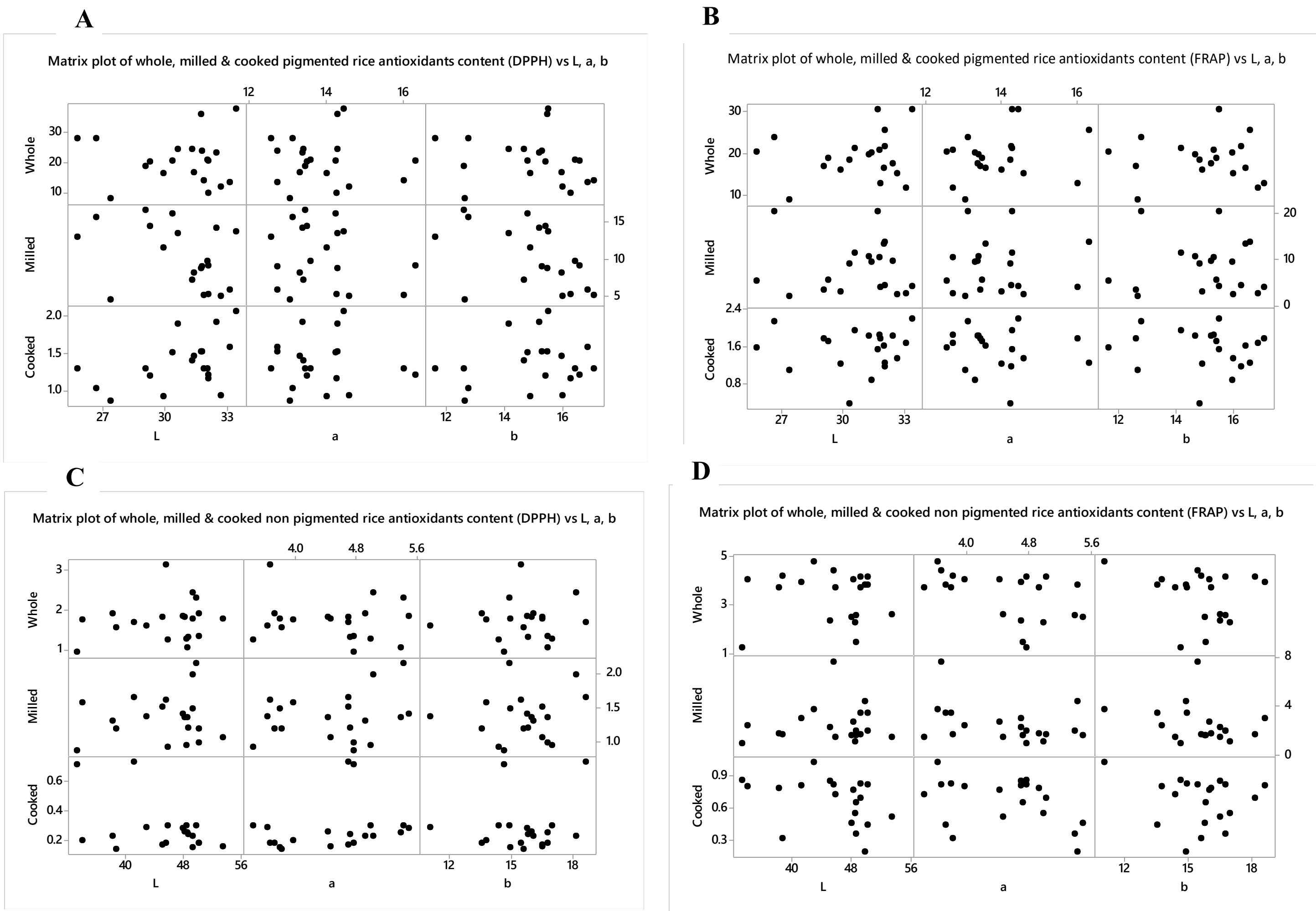

Figure 2: Matrix plot diagrams for lightness (L), redness (a) and yellowness (b) against antioxidant capacities of whole, milled and cooked rice captured in pigmented (A, B) and non- pigmented (C, D) rice through DPPH and FRAP methods chronologically

CONCLUSION

It can be concluded that antioxidant content varies among whole, milled, and cooked rice in improved, traditional, and exotic varieties. Pigmented rice had higher antioxidant levels than non-pigmented varieties. Some improved rice varieties also show increased antioxidant content. Although milling and cooking reduce antioxidant activity, pigmented cooked rice contains six times more antioxidants than non-pigmented rice, highlighting its role as a vital antioxidant-rich staple. A positive correlation exists between antioxidant content and colour values, with lighter reddish-yellow rice exhibiting significantly higher antioxidant capacities.

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