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## COMPARISON OF ANTI-OXIDANT COMPOUNDS AND ANTIOXIDANT ACTIVITY OF NATIVE AND DUAL MODIFIED RICE FLOUR

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### Keywords:

Black rice, Anti-oxidant compounds, Total phenolic content, Total flavonoid content, Total anthocyanins, Anti-oxidant activity

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**ABSTRACT:** Rice (*Oryza sativa* L.) is one of the most important cereal crops consumed in Asia and the primary staple food being consumed by nearly half of the world's population. Black rice is getting popular recently and is consumed as a functional food. Black rice contains relatively high anthocyanin in the pericarp layer which gives the dark purple color. Black rice has a higher content of phenolic compounds as compared to white rice. The total phenolic content was four times higher in pigmented rice than in non-pigmented varieties. Heat Moisture Treatment (HMT) and enzymatic modification are essential processes prior to absorption in the human body as they affect digestibility. On the basis of these considerations, the dual modification applied in this study was a combination of heat-moisture treatment (HMT) and enzymatic modification. The samples used for the study were native and dual modified rice flour of selected white and black rice variety. The samples were termed as Native White Rice Flour (NWRf), Dual Modified White Rice Flour (DMWRf), Native Black Rice Flour (NBRf) and Dual Modified Black Rice Flour (DMBRf). The objective of this study was to determine and compare antioxidant compounds such as total phenolic content, total flavonoid content, total anthocyanins and antioxidant activity such as DPPH radical scavenging activity of native and dual modified rice flour. Results revealed that NBRf had higher total phenolic content ( $167.13 \pm 12.90$  mg GAE/100 g) and total flavonoid content ( $109.81 \pm 10.3$  mg QE/100g) when compared with other selected rice flour. Anthocyanins were not detected in white rice flour. Total anthocyanins in NBRf and DMBRF were  $78.85 \pm 8.42$  and  $72.87 \pm 2.40$  mg CGE per 100g respectively. It was observed that the anti-oxidant compounds decreased drastically on dual modification. Studies have shown that thermal treatment generally causes a significant reduction in the phenolic content and antioxidant capacity. Temperature and heating time had a significant impact on total anthocyanin content and total antioxidant activity. The drastic decrease in total phenolic content, flavonoid content and total anthocyanins may be due to the thermal degradation of phenolic and flavonoid compounds, as these compounds are very sensitive to heat treatments. A higher percentage of antioxidant activity was exhibited by NBRf ( $25.13 \pm 1.92$ ) and followed by that DMBRF ( $16.9 \pm 1.29$ ). A decline in antioxidant activity in DMBRF may be due to loss of anti-oxidant compounds in the water, thermal decomposition and potential interaction with other components.

**INTRODUCTION:** Rice (*Oryza sativa* L.) is one of the most important cereal crops consumed in Asia and the primary staple food being consumed by nearly half of the world's population <sup>1</sup>.

In addition to common white-rice varieties, the other types are colored varieties such as black, red and purple rice <sup>2</sup>. White rice is obtained by removing the husk and outer layer from the entire grain, whereas colored rice is obtained by eliminating the husk and only a small amount of the outer layer that contains some pigment.

Their name refers to the kernel color (black, red or purple) which is formed by deposits of anthocyanin in different layers of the pericarp, seed coat and aleurone layer <sup>3</sup>. Black rice contains higher levels

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of proteins, vitamins and minerals than common white rice<sup>4</sup>. Compared to white rice, black rice is relatively richer in mineral contents such as iron, zinc, magnesium and phosphorous<sup>5,6</sup>. Black rice is getting popular recently and is consumed as a functional food. Black rice contains relatively high anthocyanin in the pericarp layer which gives the dark purple color<sup>7</sup>. Pigments, which are located in the aleurone layer of a rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids<sup>8</sup>. Anthocyanins, particularly cyanidin3-glucosidase and peonidin3-glucosidase, are responsible for the color of black rice<sup>9,10</sup>. These bioactive compounds were reported to have strong free radical scavenging and antioxidant effects<sup>11,12</sup>. Their important bioactivities include anti-inflammatory activity<sup>13</sup>, enhancement of the immune system<sup>14</sup>, prevention of cardiovascular disease, glycemic control, prevention of diabetes and inhibit tumor promotion<sup>15</sup>.

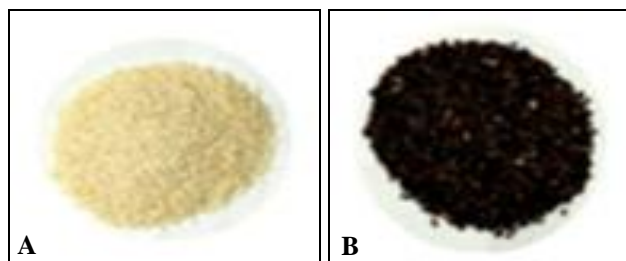
Black rice has a higher content of phenolic compounds as compared to white rice<sup>16</sup>. The total phenolic content was four times higher in pigmented rice than in non-pigmented varieties<sup>17</sup>. Researchers have demonstrated a positive correlation between the concentration of phenolic compounds and the antioxidant activity (AOA)<sup>18</sup>. The type and concentration of polyphenols in the rice grain vary among genotypes and are related mainly to the pericarp color. Normally, grains with red and black pericarp colors have a higher concentration of phenolic compounds compared to those with a light brown pericarp color. Furthermore, the concentration of these compounds is also affected by processing<sup>19</sup>.

Studies have shown that thermal treatment generally causes a significant reduction in the phenolic content and antioxidant capacity of the cereals mainly due to their release from bound forms, degradation, polymerization, oxidation and conversion to Maillard reaction products<sup>20</sup>. A decrease of phenolic compounds was observed, with a higher reduction in pigmented varieties<sup>21</sup>. Anthocyanins are unstable compounds. Its stability is affected by factors such as pH, storage temperature, heat, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, proteins and metallic ions<sup>22</sup>.

Since, black rice contains a high amount of anthocyanins, they are not stable and can be easily destroyed by these factors. Temperature and heating time had a significant impact on total anthocyanin content and total antioxidant activity<sup>23</sup>. Heat Moisture Treatment (HMT) and enzymatic modification are essential processes before absorption in the human body as they affect digestibility<sup>24</sup>. On the basis of these considerations, the dual modification applied in this study was a combination of two processes such as heat-moisture treatment (HMT) and enzymatic modification. The objective of this study was to determine and compare antioxidant compounds such as total phenolic content, total flavonoid content, total anthocyanins and antioxidant activity of native and dual modified rice flours.

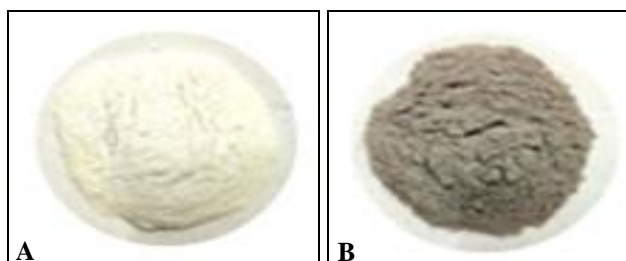
## MATERIALS AND METHODS:

**Raw Materials:** Two varieties of rice (*Oryza sativa* L.) such as Samba-White Ponni **Fig. 1A** and Black Kavuni rice **Fig. 1B** was used in this study. The rice grains were purchased from Organic Shop at Trichy, Tamil Nadu, India.



**FIG. 1: A: SAMBA-WHITE PONNI, B: BLACK KAVUNI RICE**

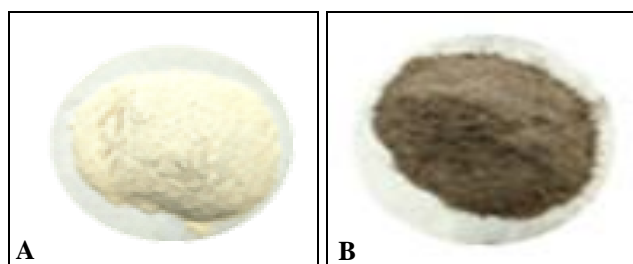
**Rice flour preparation:** Each of rice variety was ground in an analytical mill and then passed through 100 mesh (150  $\mu$ m) sieve. The rice flours were collected and stored in plastic bags at 4 °C. These samples were termed as Native White Rice Flour (NWRf) **Fig. 2A** and Native Black Rice Flour (NBRf) **Fig. 2B**.



**FIG. 2: A: NATIVE WHITE RICE FLOUR (NWRf), B: NATIVE BLACK RICE FLOUR (NBRf)**

**Dual Modified Rice Flour Preparation:** The dual modified rice flour was prepared by applying the method of (Isara, 2018)<sup>25</sup> with slight modifications. The selected rice varieties were separately steeped in water for 3 h and then wet-milled to produce 10% (w/v) rice flour slurry. The flour slurry was adjusted to pH 4.5 with a 0.1 M sodium acetate buffer. The enzyme alpha-amylase (0.2 g) was added into the flour suspension (110 g) to obtain a concentration of 20 ASPU/ g of flour (dry basis). The solution was incubated at 55 °C for 8, 24 and 36 h in a shaking water bath. The solution was centrifuged (3000 g) for 10 min; the precipitate was washed twice with distilled water and collected by centrifugation<sup>26</sup>.

The precipitate was oven-dried at 40 °C until the target HMT moisture content (25%) was obtained. The rice flour sample was then put in a sealed screw-cap container and equilibrated at room temperature for 24 h. The equilibrated containers were then placed in a hot air oven (100 °C) for 1 h. After that, the treated flour was taken out and dried in a hot air oven (40 °C) until 12% moisture content was obtained. The obtained sample or “dual modified” rice flour was milled and sieved to a particle size of 100 mesh (150 µm) sieve, sealed in a plastic bag and kept at 4 °C. These samples were termed as Dual Modified White Rice Flour (DMWRF) **Fig. 3A** and Dual Modified Black Rice Flour (DMBRF) **Fig. 3B**.



**FIG. 3: A: DUAL MODIFIED WHITE RICE FLOUR (DMWRF), B: DUAL MODIFIED BLACK RICE FLOUR (DMBRF)**

**Extraction Procedure:** Total phenolics were extracted from the samples by the method of (Moore, 2006)<sup>27</sup> with slight modifications. Each flour samples (2.0 g) were mixed with 16 ml of methanol containing 1% HCl for 24 h at 24 °C. The procedure was repeated twice. The methanol extracts were centrifuged at  $4000 \times 9.81 \text{ (ms}^{-2}\text{)}$  for 15 min, and the resulting supernatants were pooled and stored at 4 °C.

### **Determination of Anti-oxidant Compounds:**

**Determination of Total Phenolic Content:** The Folin-Ciocalteu colorimetric method (Bao, 2005)<sup>28</sup> was used to determine the total phenolic content. 200 µl of extractions was oxidized with 1 ml of 0.5 N Folin-Ciocalteu reagents and then the reaction was neutralized with 1 ml of the saturated sodium carbonate (75 g/l). The absorbance of the resulting blue color was measured at 760 nm with a Spectrophotometer after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent (mg GAE) per 100 g of rice flour.

**Determination of Total Flavonoid Content:** Total phenolic content was determined by the method (Kusirisin, 2009)<sup>29</sup>. 150 µl of 5% sodium nitrite was mixed with 2 ml of distilled water and 500 µl of extract and incubated at room temperature for 5 minutes. This was followed by the addition of 150 µl of 10% aluminum chloride hexahydrate solution and incubated again for 6 min at room temperature. 1 ml of 1 M sodium hydroxide was added and the total volume came up to 5 ml using deionized water which was later incubated at room temperature for 10 min after appropriate mixing. After incubation, absorbance was measured at 510 nm and the total flavonoid content was denoted as mg quercetin equivalent (mg QE) per 100 g of rice flour.

### **Determination of Total Anthocyanin Content:**

Determination of total anthocyanin content was done using the method reported by Abdel Aal (1999)<sup>30</sup>. Anthocyanins were extracted with acidified methanol (methanol and 1 M HCl, 85:15, v/v) with a solvent to sample ratio of 1:10. Absorbance was measured after centrifugation at 525 nm against a reagent blank. Cyanidin3-glucoside-chloride was used as a standard pigment, and total anthocyanin content was expressed as mg cyanidin3-glucoside equivalent per 100 g flour.

### **Determination of Antioxidant Activity:**

**Determination of 2, 2-Diphenyl-1-Picrylhydrazyl Free Radical Scavenging Activity (DPPH Radical Scavenging Activity):** DPPH radical scavenging ability was evaluated according to the procedure reported by Brand Williams, Cuvelier and Berset (1995)<sup>31</sup>. A volume of 3.9 ml of diluted DPPH (1:10) was added to the samples (0.1 ml).

The DPPH solution was added to the black rice extracts and stirred, and afterward, each mixture was maintained in the dark for 30 min. The decrease in the absorbance (A) was measured spectrophotometrically at 515 nm. The antioxidant activity was expressed as the percentage of DPPH radical scavenging activity according to the following equation<sup>32</sup>.

$$\text{DPPH radical-scavenging activity (\%)} = [1 - \text{absorbance of sample} / \text{absorbance of control}] \times 100$$

Statistical analysis: All the analyses were performed in triplicates and presented as mean  $\pm$  standard deviation. Data were subjected to One Way Analysis of Variance (ANOVA) using MS Excel - 2007. Comparisons within the sample set were carried out at the 5% significance level using Duncan's multiple range tests.

**TABLE 1: ANTIOXIDANT COMPOUNDS IN RICE FLOUR**

Rice flour	Total Phenolic Content (mg GAE / 100 g)	Total Flavonoid Content (mg QE / 100 g)	Total Anthocyanin Content (mg QE / 100 g)
NWRF	25.64 $\pm$ 0.106 <sup>b</sup>	2.60 $\pm$ 0.41 <sup>b</sup>	***
DMWRF	23.29 $\pm$ 0.36 <sup>a</sup>	2.59 $\pm$ 0.08 <sup>a</sup>	***
NBRF	167.13 $\pm$ 12.90 <sup>d</sup>	109.81 $\pm$ 10.30 <sup>d</sup>	78.85 $\pm$ 8.42 <sup>b</sup>
DMBRF	146.72 $\pm$ 10.23 <sup>c</sup>	95.78 $\pm$ 4.93 <sup>c</sup>	72.87 $\pm$ 2.40 <sup>a</sup>

NWRF (Native White Rice Flour), DMWRF (Dual Modified White Rice Flour), NBRF (Native Black Rice Flour), DMBRF (Dual Modified Black Rice Flour), \*\*\* (Not detected) the total phenolic content were expressed in milligram of gallic acid equivalent (GAE) per 100 g of rice flour. The total flavonoid content was expressed in milligram of quercetin equivalent (QE) per 100 g of rice flour. The total anthocyanin content was expressed in mg of cyanidin3-glucoside (CGE) equivalent per 100 g rice flour. Values followed by the different superscripts in the same column are significantly different ( $p < 0.05$ )

Results revealed that the Native Black Rice Flour (NBRF) had higher total phenolic content (167.13  $\pm$  12.90 mg GAE / 100 g) and total flavonoid content (109.81  $\pm$  10.3 mg QE / 100 g) when compared to other rice flours. It was also noted that there was a decrease in total phenolic and flavonoid content in dual modified rice flour of both white and black rice flour. Polyphenols are the most effective anti-oxidative constituents in plant products consumed<sup>32</sup>. Black rice contains a high amount of phenolic compounds that have anti-oxidant properties. Flavonoids are the major class of phenolic compounds.

On exposure to heat or temperature, phenolic compounds have a tendency of breaking down into smaller stable forms which may or may not show antioxidant activity. In the case of rice, the maximum amount of the phenolic compounds are present in the bound form. On cooking, the cellular breakdown facilitates the release of these bound

Statistical significance was considered for  $p < 0.05$ . Pearson correlation (r) was also calculated using statistical package for social sciences (SPSS version 16.0) to know the correlation of coefficient between anti-oxidant compounds and antioxidant activity of rice flour.

## RESULTS AND DISCUSSION:

**Determination of Anti-oxidant Compounds:** The anti-oxidant compounds namely, total phenolic content, total flavonoid content, and total anthocyanins were studied in the four rice flour samples such as Native White Rice Flour (NWRF), Dual Modified White Rice Flour (DMWRF), Native Black Rice Flour (NBRF), Dual Modified Black Rice Flour (DMBRF). Anti-oxidant compounds in rice flour varieties are presented in **Table 1**.

phenolics. There is a possibility that these phenolics could replace the free phenolics, the maximum of which got destroyed during the cooking process<sup>33</sup>. Anthocyanins are responsible for the purple, blue or red pigmentation of various plants. The dark purple color of black rice results from the high content of anthocyanins in the pericarp layers. A recent study showed that anthocyanin had a high positive correlation with total phenolic compounds and antioxidant activity. Anthocyanin is known for being an active compound that can readily reacting with a constituent or simply degrading by the action of oxygen, light, enzyme pH modification and high-temperature processing<sup>34</sup>. Anthocyanins were not detected in native and dual modified white rice flour variety.

Total anthocyanins in native and dual modified black rice flour were 78.85  $\pm$  8.42 and 72.87  $\pm$  2.40 mg CGE per 100 g respectively. It was observed

that the anti-oxidant compounds were decreased drastically on dual modification. The drastic decrease in total phenolic content, flavonoid content and total anthocyanins may be due to the thermal degradation of phenolic and flavonoid compounds, as these compounds are very sensitive to heat treatment <sup>35</sup>.

**Determination of Anti-oxidant Activity:** DPPH is a free radical that accepts an electron or hydrogen radical to form a more stable compound. DPPH assay is based on the decrease in purple color of the DPPH solution when the nitrogen atom in DPPH is reduced upon receiving a hydrogen atom from an anti-oxidant. DPPH assay is a method used for screening the antioxidant activity of the sample extract since it measures the antioxidant abilities to scavenge the stable radical DPPH <sup>36</sup>.

**TABLE 2: ANTI-OXIDANT ACTIVITY OF RICE FLOUR**

Rice flour	DPPH radical scavenging activity (%)
NWRF	3.08 ± 0.83 <sup>b</sup>
DMWRF	2.66 ± 1.06 <sup>a</sup>
NBRF	25.13 ± 1.92 <sup>d</sup>
DMBRF	16.90 ± 1.29 <sup>c</sup>

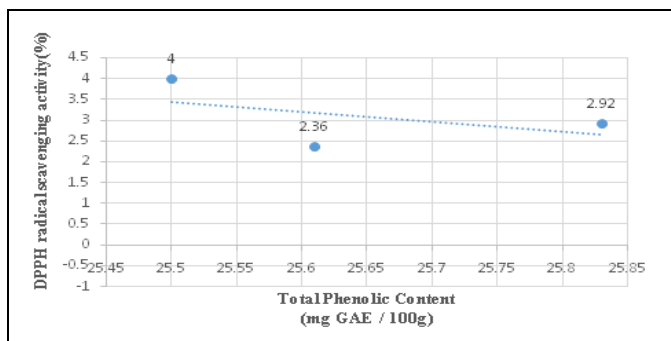
NWRF (Native White Rice Flour), DMWRF (Dual Modified White Rice Flour), NBRF (Native Black Rice Flour), DMBRF (Dual Modified Black Rice Flour), Values followed by the different superscripts in the same column are significantly different (p<0.05)

The anti-oxidant activity of rice flour varieties is shown in **Table 2**. Higher percentage of antioxidant activity was exhibited by Native Black Rice Flour (NBRF) (25.13 ± 1.92), followed by that Dual Modified Black Rice Flour (DMBRF) (16.9 ± 1.29). A decline in antioxidant activity in Dual Modified Black Rice Flour (DMWRF) may be due to loss of anti-oxidant compounds in the water, thermal decomposition and potential interaction with other components. It was reported that raw black rice contains significantly high DPPH radical scavenging activity (88.72%) as compared to other processed black rice <sup>37</sup>. The concentration of total phenolics in the rice grain has been positively correlated with the antioxidant activity <sup>38</sup>.

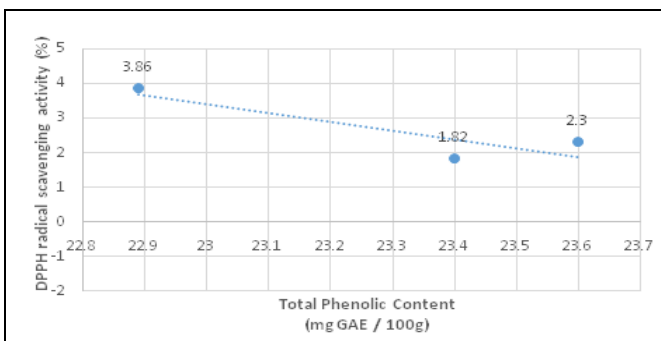
**TABLE 3: RELATIONSHIP BETWEEN ANTI-OXIDANT COMPOUNDS AND ANTI-OXIDANT ACTIVITY OF RICE FLOUR**

Rice flour	Relationship between		
	TPC and AOA	TFC and AOA	TAC and AOA
NWRF	- 0.49	- 0.92	***
DMWRF	- 0.87	+0.93	***
NBRF	+0.43	+ 0.82	- 0.98
DMBRF	- 0.99	-0.79	+0.11

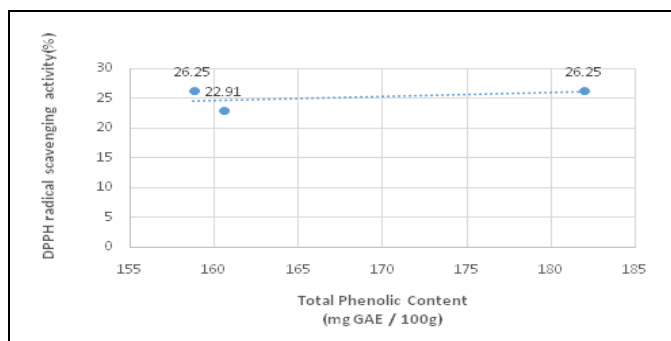
\*\*\* (Anthocyanins not detected) TPC - Total Phenolic Content, TFC - Total Flavonoid Content, TAC - Total anthocyanin Content, AOA - Antioxidant Activity



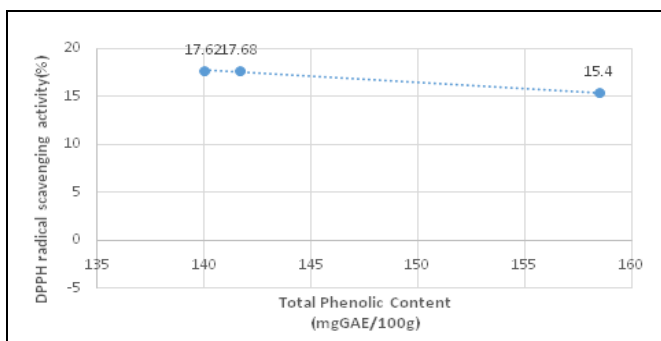
**A. NWRF**



**B. DMWRF**



**C. NBRF**



**D. DMBRF**

**FIG. 4: (A, B, C, D) RELATIONSHIP BETWEEN TOTAL PHENOLIC CONTENT AND DPPH RADICAL SCAVENGING ACTIVITY (ANTI-OXIDANT ACTIVITY)**

A positive correlation ( $r = 0.4383$ ) was found between total phenolic content and DPPH radical scavenging activity **Fig. 4** in NBRF. A negative correlation was found in other rice flours between total phenolic content and DPPH radical scavenging activity (Anti-oxidant activity). Rice grains with higher phenolic compounds are responsible for the antioxidant activity<sup>39</sup>. DMWRF and NBRF showed a positive correlation between total flavonoid content and antioxidant activity. Anthocyanins were not detected in white rice flour. The calculated coefficient of correlation between total anthocyanin content and antioxidant activity in NBRF was negative and in DMBRF it was positive. Black rice flour can be used as an ingredient for gluten-free cereal products with higher nutritional value<sup>39</sup>.

There is a need to preserve the anthocyanin content during thermal processing, as well as prolonged storage and shelf-life periods. This bioactive compound is very reactive and can be simply degraded to colorless or brown-color compounds. The stability of such substances in foods is influenced by a number of factors, including processing and storage conditions, physical and chemical properties of foods, the presence of copigments and metallic ions<sup>40,41,42</sup>.

**CONCLUSION:** Rice contains different compounds with anti-oxidant activity, including polyphenols, but variations are observed in the concentration of these compounds due to genotype, pericarp color and effect of processing. It was proved in this study, pigmented variety such as Native Black Rice Flour (NBRF) had higher total phenolic content and total flavonoid content. It was observed that the antioxidant compounds decreased drastically on dual modification.

The drastic decrease in total phenolic content, flavonoid content and total anthocyanins may be due to the thermal degradation of anti-oxidant compounds. Native Black Rice Flour (NBRF) exhibited a higher percentage of DPPH radical scavenging activity. But after dual modification decline in antioxidant activity was observed in Dual Modified Black Rice Flour (DMBRF). A positive correlation was demonstrated between the concentration of phenolic compounds and the antioxidant activity of NBRF.

Hence, the study may be concluded that Native Black Rice Flour (NBRF) had higher total phenolic, flavonoid and anthocyanin content and also exhibited a higher level of DPPH radical scavenging activity. The antioxidant compounds and antioxidant activity were decreased drastically on dual modification; however the starch digestibility may be enhanced by dual modification.

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