

# UNVEILING THE PROXIMATE COMPOSITION OF PIGMENTED AND NON-PIGMENTED RICE BRAN

**Comment [L1]:** what is the name of the rice cultivar

## Abstract

This present study aimed to evaluate the proximate composition of pigmented and non-pigmented rice. We have evaluated the proximate components of 16 rice bran genotypes, 9 pigmented rice bran, and 7 NP rice bran, grown and cultivated in the research fields. The proximate composition (moisture, ash, crude fat, crude proteins, crude fiber) were determined using proximate analysis methods. Raising awareness regarding rice's bran nutritional quality and health benefits is important. The results shown that black rice genotypes exhibited high range of almost all the parameters. Crude protein in black rice genotypes, ranged from 18.02-21.01%, crude fat content in black rice genotypes, ranged from 19.83-21.73% and ash content in the black rice genotypes, ranged from 14.08-16.80%, ash in black rice genotypes, ranged from 11.28-12.35%, crude fibre The result exhibited a significant difference in the content of proximate composition. The study concludes that the significant distinctiveness and difference in nutritional and is use full in rice bran products. The results suggest that the black rice genotypes have better nutrition as compared to NP rice genotypes. The results showed great variations in each parameter among different rice bran varieties.

**Comment [L2]:** please separate the abstract; aims, methods, results

**Key Words:** Pigmented rice, NP-non pigmented rice, crude protein, and ash

## Introduction

Rice (*Oryza sativa* L.) is a significant and staple food among all the major cereal crops consumed by over 50 % of the total world's population. It is fundamental to Asian societies not just as a food source, but also in an array of different social, financial and religious activities (Hedge *et al* 2013, Sathya 2013). Different layers of rice have known to have varying amounts of nutrients (Sharif *et al* 2014). In contrast to the aleurone layer, which is deficient in vital nutrients, the bran layer is rich in dietary fibre, minerals, and vitamin B complex (Park *et al* 2017, Devi *et al* 2021). When compared to germ and bran, the rice endosperm consists of low concentrations of proteins, lipids, and fibre but an increased amount of carbohydrates (Kennedy *et al* 2002, Ma *et al* 2020). Most of the bioactive compounds are present in bran and germ fractions. Tocopherols (vitamin E), phenolics, carotenoids (lutein,  $\beta$ -cryptoxanthin, zeaxanthin, and  $\beta$ -carotene), as well as compounds containing nitrogen and organo-sulphur are among the phytochemicals found in whole grains of rice (Usman *et al* 2017, Ravichanthiran *et al* 2018). Most of the phytochemicals are highly concentrated in the outer layer (pericarp) and testa or the bran part of the rice kernel, which results in rice with different colours *i.e.*, purple, black and red.

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Rice is naturally brown after harvesting, but once the nutrient-rich outer layer of bran is removed, it is white in color. Red, black, and purple rice, all feature unique pigmentation in the bran.

Rice bran contains 12-20 % protein of excellent quality which can be a source of dietary protein in human

nutrition (Adebiyi *et al* 2007). Among cereals, rice bran protein has the highest nutritional value due to its high content of lysine and threonine (limiting amino acids), which are generally low in other cereals. Rice bran protein (RBP) contains approximately 3-4% lysine, an essential amino acid, which is highest among other cereal proteins (Shih *et al* 1999). Protein digestibility of rice bran protein is greater than 90% and the protein efficiency ratio (PER) values of rice bran concentrate is relatively high, around 2.0 to 2.5, whereas casein is 2.5 (Wang *et al* 1999). The RBP can be used as a source of protein in infant food formula and restricted food for children with allergies, due to its hypoallergenicity. After hydrolysis of RBP with protease enzyme, small peptides with higher antioxidative activity were obtained by Adebiyi *et al* (2009b).

## Materials and methods

### Collection of samples

Rice crop was harvested at maturity. After drying the paddy to moisture content of 14%, cleaned it thoroughly to get rid from impurities. Cleaned paddy was stored in cloth bags at ambient temperature for two months. Paddy was dehulled on Satake Rice Machine (Kitashiba electronics Co.ltd., Japan) to obtain brown rice and then milled on Satake Test Mill (Kitashiba electronics Co.ltd., Japan) to obtain rice bran. The bran which obtained after milling was sieved through Standard Test Sieve (425 MICS) and analyzed for various biochemical parameters by standard procedures reported in literature.

Comment [L4]: Where rice plants from?

### Moisture content (AACC 2000)

Weighed rice fractions (10 g) were dried in a hot air oven at  $130 \pm 1^\circ\text{C}$  for one hour and the content of moisture was calculated in percent from the loss in weight of the sample

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### Crude protein (Micro Kjeldahl Method AOAC 2000)

#### Reagents

- i. Digestion mixture ( $\text{CuSO}_4: \text{K}_2\text{SO}_4, 1:9$ )
- ii. Concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ )
- iii. 1 M sodium hydroxide (NaOH)
- iv. 0.01N hydrochloric acid (HCl)
- v. 4% Boric acid ( $\text{H}_3\text{BO}_3$ )
- vi. Indicator: 0.5 g bromocresol green and 0.1g methyl red dissolve in 100 ml of ethanol (95%)

#### Procedure

Took 100 mg rice powder in digestion tubes and added 10 ml concentrated  $\text{H}_2\text{SO}_4$  along with 5 g digestion mixture. Digestion was done till a clear solution was obtained. Digestion tubes were allowed to

cool to room temperature and the content was transferred to 100 ml volumetric flask, quantitatively and made to volume. An aliquot of 20 ml was distilled using Kjeldahl distillation unit (FOSS Kjeltec 2100) after the addition of 50 ml 1 N NaOH. Distillate was collected in 25 ml 4% H<sub>3</sub>BO<sub>3</sub> and titration was done against 0.01 N HCl till grey end point. Percent nitrogen content (%N) was calculated as follows:

$$\text{Percent nitrogen (\% N)} = \left[ \frac{(X - \text{Blank}) \times 0.00014}{Y} \right] \times 100$$

$$\text{Crude protein content} = \text{Percent nitrogen (\% N)} \times 5.95$$

Where X= Titre value (ml)

Y = Sample weight (g)

#### **Crude fat (AACC 2000)**

##### **Reagent**

Petroleum ether

##### **Procedure**

SOCS plus apparatus (Pelican Equipment's, Chennai, India) was used for the extraction of crude fat in the samples. Thimble containing 5 g rice powder sample was placed in the pre-weighed extraction beaker containing 70 ml petroleum ether. Beakers were loaded in the chambers of the apparatus. Extraction was carried out for 1 hour 30 minutes after which the extraction beakers were allowed to cool. The extraction beakers were oven dried (60- 65°C). The solvent was recovered. The crude fat was calculated as percentage from the increase in weight of the extraction beakers:

#### **Ash content (AACC 2000)**

##### **Procedure**

Rice powder (5g) was taken in pre- weighed crucible and charred completely on a hot plate. The crucibles were placed in the muffle furnace for 5 hours at 550°C. The crucibles were allowed to cool to room temperature and were weighed accurately. Content of ash was calculated from the increase in the weight of the crucibles and expressed in percentage.

#### **Estimation of crude fibre (AACC 2000)**

##### **Reagents**

- i. 0.12 N H<sub>2</sub>SO<sub>4</sub>
- ii. 1 N NaOH

##### **Procedure**

The sample (2 g) was boiled with 250 ml H<sub>2</sub>SO<sub>4</sub> solution for 30 min. Filtered it through sintered glass crucibles and washed thoroughly with hot water. The residue was boiled again with 250 ml 1 N NaOH for 30 min followed by filtration and washing with hot water until a clear filtrate was obtained. The residue was dried and ashed in muffle furnace at 600° C. The crude fibre content was calculated from the loss in weight of samples and expressed as a percentage.

## Results and Discussion

### Moisture content:

Moisture content in bran ranged similar in all the three-color groups. In bran fraction, in black rice genotypes, the moisture content ranged from 7.41-9.59%, in red rice genotypes, it ranged from 7.35-10.49%, and in NP rice genotypes it ranged from 6.87-8.71%. The moisture content of all three colour groups were lower than 14%, Champagne *et al* (2004) stated that the 14% moisture content was considered safe for storing grains to avoid damage and deterioration of seed quality. The difference in the moisture content might be due to climatic conditions where the crop is cultivated and the difference in the genetic makeup of an individual genotype. Ebuehiet *al* (2007) reported that the moisture content plays a significant role in determining the shelf life of foods. Xheng and Lan (2007) reported that moisture influences the milling characteristics and the taste of cooked rice (Table 1).

### Crude protein

Crude protein content in bran fraction, in black rice genotypes, ranged from 18.02-21.01%, in red rice genotypes, it ranged from 16.21-20.45%, and in NP rice genotypes it ranged from 15.78-21.06% (Table 1). The average crude protein content was found to be higher in pigmented rice groups compared to NP rices. Simonelli *et al* (2016) stated that proteins play a crucial role in cooked rice texture because they form a complex with starch that restricts the starch granule swelling. Suwannaporn *et al* (2007) reported that high protein rice is less sticky and has a harder texture of cooked rice. Kennedy *et al* (2002) reported that the second major component next to starch are proteins which influence the eating quality and the nutritional quality of rice. The dietary supply of rice per person per day is 207.9 g, which provides about 24.1% of the required dietary protein.

### Crude fat

Crude fat refers to the crude mixture of fat-soluble material present in a sample and is also known as the ether extract or the free lipid content. The lipid materials may include free fatty acids, fat-soluble vitamins, monoglycerides, diglycerides, triglycerides, phospholipids, steroids, carotene pigments, chlorophylls, etc. Juliano *et al* (1986) and Tanaka *et al* (1978) Starch lipids present in rice are composed of monoacyl lipids (fatty acids and lysophosphatides) complexed with amylose (Choudhury *et al* 1980). In the present study crude fat content in bran fraction, in black rice genotypes, ranged from 19.83-21.73%, in red rice genotypes, it ranged from 15.25-20.97%, and in NP rice genotypes it ranged from 17.38-22.39%. Among three colours, the mean crude fat content was found to be the highest in black rices while red rices and NP rice genotypes exhibited overlapping content. It has been reported that the lipids or fats in rice are mainly confined to the rice bran (20%, dry basis) as lipid bodies in the aleurone layer and bran. Priya *et al* (2019) reported that the amount of crude fat present in pigmented rices was high in red

rices.

#### **Ash content**

The ash content in bran fraction of the black rice genotypes, ranged from 14.08-16.80%, in red rice genotypes, it ranged from 10.72-14.75% and in NP rice genotypes it ranged from 10.45-15.40%. In the bran fraction of the black rice genotype CP, red rice genotype YKM, and NP rice genotype Bas 386 displayed higher amounts of ash that is; 16.80, 14.35, and 15.4%. Black rice possessed a high amount of ash followed by NP rice and red rice genotypes. Kalpanadevi *et al* (2018) reported that the bran from the pigmented rice (Jyothi) possessed marginally higher ash content (10.2–14.2%). Deepa *et al* (2008) reported that most minerals are abundant in the pigmented varieties. Aoet *al* (2012) stated that the ash content of different cultivars of rice may be due to genetic factors or the mineral content of the soil. Ramaiah and Rao (1953) reported that the zinc and iron content of red rice is two to three times higher than that of white rice. The most common minerals found in rice includes calcium, magnesium, iron, and zinc. Resurreccion *et al* (1979) reported that the outer layers (bran) of the rice grain had the highest concentration of ash content and it decreases towards the centre of the grain.

#### **Crude fibre**

Crude fibre is the organic residue that remains after the food sample has been treated under the standardized condition with boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution, and alcohol. The fibre in the diet increases the bulk of faeces, which has a laxative effect on the gut (Oko *et al* 2019). In the present study crude fibre content in bran fraction, in black rice genotypes, ranged from 11.28-12.35%, in red rice genotypes, it ranged from 5.93-10.72%, and in NP rice genotypes it ranged from 5.91-10.24%(Table 1). In bran fraction, black rice genotype FCP, red rice genotype BKG and NP rice PB1121 possessed the highest crude fibre content of 12.35,10.72 and 10.24 respectively. Red rice is known to be rich in iron and zinc, while black and purple rice is especially high in protein and crude fibre. Priya *et al* (2019) reported that among pigmented rice varieties, Chak-hao amubi (Manipur black rice) has a significantly lower content of crude fibre. in the current study, we found high crude fibre content in black rice genotypes in all three fractions followed by red and NP rice genotypes. In all the nutritional components determined in the three colours, the black rice genotypes possessed the highest amount of ash, crude protein, crude fat, and crude fibre content. We didn't find distinct differentiation in the nutritional components among the red and NP rice groups.

**Table 1: Proximate composition of the bran of pigmented and non-pigmented rice genotypes**

Genotype	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)
<b>Black rices</b>					
FCP	9.59 ± 0.12 <sup>a</sup>	15.41 ± 0.29 <sup>b</sup>	20.1 ± 0.46 <sup>a</sup>	19.96 ± 0.23 <sup>b</sup>	12.35 ± 0.04 <sup>a</sup>
FCAP	8.36 ± 0.03 <sup>b</sup>	14.08 ± 0.26 <sup>c</sup>	18.02 ± 0.4 <sup>b</sup>	19.83 ± 0.11 <sup>b</sup>	11.28 ± 0.02 <sup>c</sup>
CP	7.41 ± 0.06 <sup>c</sup>	16.80 ± 0.16 <sup>a</sup>	21.01 ± 0.34 <sup>a</sup>	21.73 ± 0.14 <sup>a</sup>	11.75 ± 0.01 <sup>b</sup>
Mean	<b>8.45<sup>A</sup></b>	<b>15.43<sup>A</sup></b>	<b>19.71<sup>A</sup></b>	<b>20.51<sup>A</sup></b>	<b>11.79<sup>A</sup></b>
Range	<b>7.41-9.59</b>	<b>14.08-16.80</b>	<b>18.02-21.01</b>	<b>19.83-21.73</b>	<b>11.28-12.35</b>
C.V.	<b>1.65</b>	<b>2.73</b>	<b>3.54</b>	<b>1.43</b>	<b>0.38</b>
P-Value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Red rices</b>					
GSL	6.82 ± 0.13 <sup>c</sup>	12.93 ± 0.06 <sup>b</sup>	19.23 ± 0.07 <sup>bc</sup>	20.97 ± 0.16 <sup>a</sup>	8.33 ± 0.02 <sup>c</sup>
DMI	8.46 ± 0.01 <sup>c</sup>	14.75 ± 0.01 <sup>a</sup>	20.45 ± 0.47 <sup>a</sup>	17.62 ± 0.12 <sup>b</sup>	9.04 ± 0.02 <sup>b</sup>
KMA	9.65 ± 0 <sup>b</sup>	10.72 ± 0.04 <sup>c</sup>	19.87 ± 0.1 <sup>ab</sup>	15.25 ± 0.08 <sup>d</sup>	7.0 ± 0.04 <sup>d</sup>
BKG	10.49 ± 0.11 <sup>a</sup>	13.06 ± 0.25 <sup>b</sup>	16.21 ± 0.28 <sup>d</sup>	16.76 ± 0.07 <sup>c</sup>	10.72 ± 0.06 <sup>a</sup>
MSTY	9.65 ± 0.21 <sup>b</sup>	12.87 ± 0.29 <sup>b</sup>	18.47 ± 0.11 <sup>c</sup>	15.49 ± 0.06 <sup>d</sup>	6.94 ± 0.02 <sup>d</sup>
YKM	7.35 ± 0.07 <sup>d</sup>	14.35 ± 0.07 <sup>a</sup>	20.36 ± 0.47 <sup>a</sup>	17.63 ± 0.04 <sup>b</sup>	5.93 ± 0.08 <sup>c</sup>
Mean	<b>8.73<sup>A</sup></b>	<b>13.11<sup>B</sup></b>	<b>19.10<sup>A</sup></b>	<b>17.29<sup>B</sup></b>	<b>7.99<sup>B</sup></b>
Range	<b>6.82-10.49</b>	<b>10.72-14.75</b>	<b>16.21-20.45</b>	<b>15.25-20.97</b>	<b>5.93-10.72</b>
C.V.	<b>2.25</b>	<b>2.13</b>	<b>2.72</b>	<b>0.97</b>	<b>0.97</b>
P-Value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Non-pigmented rices</b>					
PR 114	8.46 ± 0.06 <sup>b</sup>	10.45 ± 0 <sup>c</sup>	18.29 ± 0.34 <sup>c</sup>	18.61 ± 0.08 <sup>cd</sup>	6.4 ± 0.03 <sup>c</sup>
PR 121	6.92 ± 0.15 <sup>d</sup>	12.35 ± 0.01 <sup>c</sup>	21.06 ± 0.37 <sup>a</sup>	22.39 ± 0.14 <sup>a</sup>	5.91 ± 0.02 <sup>d</sup>
PR 126	8.91 ± 0.04 <sup>a</sup>	10.69 ± 0.11 <sup>c</sup>	16.27 ± 0.32 <sup>de</sup>	19.84 ± 0.18 <sup>b</sup>	7.66 ± 0.03 <sup>d</sup>

BAS 370	7.82 ± 0.02 <sup>c</sup>	13.76 ± 0.07 <sup>b</sup>	19.36 ± 0.19 <sup>b</sup>	18.72 ± 0 <sup>ad</sup>	7.91 ± 0 <sup>c</sup>
BAS 386	8.71 ± 0.11 <sup>a</sup>	15.4 ± 0.06 <sup>d</sup>	15.78 ± 0.03 <sup>c</sup>	19.02 ± 0 <sup>c</sup>	8.17 ± 0.16 <sup>b</sup>
Pb Bas7	8.02 ± 0.08 <sup>c</sup>	11.27 ± 0.19 <sup>d</sup>	17.02 ± 0.39 <sup>d</sup>	18.28 ± 0.18 <sup>d</sup>	8.33 ± 0.05 <sup>b</sup>
PB 1121	6.87 ± 0.05 <sup>d</sup>	10.49 ± 0.04 <sup>e</sup>	16.02 ± 0.19 <sup>c</sup>	17.38 ± 0.3 <sup>c</sup>	10.24 ± 0.1 <sup>a</sup>
Mean	<b>7.96<sup>b</sup></b>	<b>12.06<sup>b</sup></b>	<b>17.69<sup>b</sup></b>	<b>19.18<sup>ab</sup></b>	<b>7.80<sup>b</sup></b>
Range	<b>6.87-8.91</b>	<b>10.45-15.40</b>	<b>15.78-21.06</b>	<b>17.38-22.39</b>	<b>5.91-10.24</b>
C.V.	<b>1.78</b>	<b>1.31</b>	<b>2.83</b>	<b>1.45</b>	<b>1.69</b>
P-Value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Average (Overall)	<b>8.34</b>	<b>13.09</b>	<b>18.60</b>	<b>18.72</b>	<b>8.62</b>
Range (Overall)	<b>6.82-10.49</b>	<b>10.45-16.80</b>	<b>15.78-21.06</b>	<b>15.25-22.39</b>	<b>5.91-12.35</b>

Each value represents the mean ± SD of three independent replications.

The results are presented as percentage, and values in each column with different capital letters are significantly different ( $P < 0.001$ ) means of the three coloured groups, while the different small letters in each column are significantly different ( $P < 0.001$ ) proximate composition like moisture (%), ash (%), crude protein (%), Crude fat (%) and crude fiber (%) of bran in each coloured group

discuss

**Comment [L6]:** I haven't seen any discussion yet

add

**Comment [L7]:** where is difference of pigmented and non-pigmented rice

### Conclusion

The primary interest of this study is to unveil the proximate composition of various pigmented rice which in turn serves as an alternative food for the malnourished. Therefore, bran which by-product of rice milling that was observed appeared to have an excellent nutrition, among the three colour groups black rice bran has identified best.

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