Grain Dimension, Nutrition and Nutraceutical Properties of Black and Red Varieties of Rice in India

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Abstract

Traditional colored rice varieties in India are the source of carbohydrates, phytochemicals and minerals. They facilitate the growth of probiotics in intestine and protect human from many chronic diseases. The present study investigated the nutritional properties such as total sugars, digestible sugars, resistant sugars, hydrolysis index, glycemic index and total proteins of thirteen colored varieties of rice in India. Nutraceutical properties like anti diabetic and prebiotic activity were investigated by standard methods. Chak hao poreiton and mappillai samba grains were 6.3 mm in length. Lowest length of 5.1 mm was recorded in 60 m kuruvai. Among the rice varieties, mappillai samba has high concentration of digestible starch of 91% and chak hao poreiton had low concentration of 62%. Resistant starch was 38% in chak hao poreiton and 8% in mappillai samba. Lowest glycemic index of 52 and 53 were recorded in karuthakar poha and Chak hao poreiton respectively. Anthocyanin extracted from Chak hao poreiton inhibited 24% of human pancreatic $\alpha$-amylase activity. It significantly increased the probiotic number from 0.15 CFU/mL to 1.95 CFU/mL. The study revealed that the black rice variety, Chak hao poreiton was rich in resistant starch and exhibited low glycemic index. The anthocyanins from Chak hao poreiton possessed significant antidiabetic and prebiotic activity. Molecular docking studies revealed the interaction of anthocyanin with pancreatic $\alpha$-amylase, $\beta$-glucosidase and GLUT1.

Introduction

India is the global capital of diabetes with approximately 69 million people suffering from diabetes. Pre-diabetic people are in danger of developing insulin resistant diabetes. Uncontrolled diabetes leads to many other chronic diseases like heart, liver, kidney, stroke, nervous disorders etc.¹ The major driving force for the rapid emergence of
diabetes is the consumption of energy dense food, less intake of fruits, vegetables and omega 3 fatty acids. Rice is the staple food in many countries.\textsuperscript{2} Asia produces the 95\% needs of rice globally.\textsuperscript{3} About 60\% of caloric needs of Asian people are met by rice. Some rice varieties have low concentration of readily digestible sugars and high concentration of resistant sugars. The resistant sugars are not digested by the human gastrointestinal tract. The probiotic population of intestine digests them. Rice varieties rich in resistant starch have low glycemic index (GI). GI is an indicator of the rate at which postprandial blood sugar level is increased within 90 minutes of digestion. The GI of rice varies widely from 40 to 121.\textsuperscript{4} GI of rice is classified into low (<55), medium (55) and high (>70). Understanding the hydrolysis index (HI) and GI of rice varieties are of prime importance to prevent diabetes mellitus. Rice with higher GI values induce diabetes.\textsuperscript{5,6} and those with low GI value does not impose stress on pancreas to secrete insulin. One powerful strategy to control diabetes is to limit the release of glucose from food by inhibiting the activity of $\alpha$-amylase and glucosidase.\textsuperscript{7} One of the promising strategies to reduce the complications and counteract the metabolic alterations of diabetes is to use inhibitors of $\alpha$-amylase and glucosidase. Synthetic inhibitors of amylase and glucosidase such as acarbose and miglitol induce gastrointestinal complications and hepatic injury. Side effects associated with these drugs limit their success.\textsuperscript{8} Hence, research towards the identification of natural inhibitors becomes prime importance.

Red, purple and black rice were used since ancient time in India.\textsuperscript{9} They are good sources of resistant starch, minerals and nutraceuticals.\textsuperscript{10} Colored rice varieties available in India includes Mattai, Kiarali, Black Kavuni, Chak hao poreiton, Thavala kanna matta, Onamatta, Mapillai samba, Navara, Red Kavuni, Kuruvaikar, Poonkar, Kuzhiyadichan etc. Recently these rice varieties were evaluated for their nutraceutical properties such as antioxidants, antidiabetic etc.\textsuperscript{11} The color and aroma of rice plays a key role in consumer preference.\textsuperscript{12} The color of the endosperm distinguishes rice into black red, brown and white.\textsuperscript{13} Different anthocyanins contribute to the color of rice. Red varieties are unique with high mineral concentration while the black varieties are rich in minerals, protein and crude fiber.

Changing the food practice based on the consumption of functional foods and foods with pharmaceutical value are complimentary approaches to mitigate diabetes and chronic diseases mapped with diabetes.\textsuperscript{14-16} Nutritional therapy aims to use foods with nutraceutical value. Nutraceuticals are products which provide nutrition and pharmaceutical value to the consumer. Many nutraceuticals play a vital role in protection against diabetes, cardiovascular diseases,\textsuperscript{17-18} neurological and nephrological disorders.\textsuperscript{19} They block the absorption of cholesterol and decrease the harmful effects of atherosclerosis.\textsuperscript{20} They function as potent antioxidants and anti-inflammatory agents.\textsuperscript{21} Due to increasing awareness among people about the side effects of synthetic drugs used to increase the glucose uptake in tissues\textsuperscript{22} and the benefits of consuming functional foods, research is focused on natural compounds as therapeutic agents. The anthocyanin in rice are chemically flavonoids which serves as an antioxidant, antidiabetic, antihyperlipidemic and anti-ageing agents.\textsuperscript{23-25} Recently the correlation between disease and gut microbiome is unraveled. Establishing the probiotics in gut is essential for maintaining health.

There is only scarce scientific information on the grain properties, resistant starch and nutraceutical value of different colored varieties of rice available in India. Hence, the present study was aimed to analyze the grain characteristics, GI, resistant starch, total proteins, anti-diabetic and prebiotic activity of few colored rice varieties available in India.

Materials and Methods
Collection of Rice Varieties
Thirteen unique traditional rice varieties originated in different states of India like Tamil Nadu, Manipur and Kerala, were gifted by Spirit of earth, Chennai, Tamil Nadu, India.

Grain Dimensions
Length, Width, Thickness and Aspect ratio
Length (L) and width (W) of the rice samples were measured. Thickness (T) was measured with Vernier caliper of accuracy 0.01mm. Aspect ratio ($R_a$) and sphericity ($S_p$) of the rice were determined using the following formula,
Based on the FAO standards, the grains are classified as long, medium and short as per the following criteria:

Long: Kernel length > 6 mm and length/width ratio 3 and above

Medium: Kernel length between 5.2 mm and 6 mm, L/W ratio < 3

Short: Kernel length <5.2 mm and L/W ratio <2

Equivalent Diameter
The equivalent diameter of the rice was calculated using the following formula followed by Haq et.al.26

\[ D_e = (LBT)^{\frac{1}{3}} \]

Thousand Kernel Weight
Thousand kernels were counted and weighed in digital balance with an accuracy of 0.001g. The measurement was replicated thrice and the mean value was recorded.27

Nutritional Properties
Estimation of Total Starch, Readily Digestible Starch and Resistant Starch
Total sugars, readily digestible sugars and resistant sugars were estimated by Glucose Oxidase- Peroxidase (GOD-POD) method.

Estimation of Total Starch
The concentration of total starch in the rice samples were estimated using porcine pancreatic amylase.28 Rice samples were powdered, sieved and homogenized with 5 mL of distilled water. To the sample, 1 mL of 100 U amylase (pancreatin) was added and incubated at 37°C for 15 min. The volume was made up to 25 mL with distilled water. To 1 mL of the digested sample, 10 µL of (14 U) amyloglucosidase and 2 mL of phosphate buffer (pH 4.75) were added and incubated in shaker for 60 min. It was diluted to 5 mL and the glucose was estimated using GOD-POD. The available starch was calculated using the formula,

\[ \text{Available Starch} \% = \left( \frac{\mu g \text{ of glucose} \times 0.9 \times 25}{\text{sample weight (mg, dry basis)}} \right) \]

Estimation of Readily Digestible and Slowly Digestible Starch
Rice sample (1g) was homogenized with 50 mL of phosphate buffer (pH 6.9). 1 mL of 100 U amylase (Pancreatin) was added and incubated at 37°C in shaker. The glucose concentration was estimated at a regular interval of 30 min up to 90 min using GOD-POD.29

Resistant Starch
The concentration of resistant starch in rice varieties was determined after complete digestion of digestible starch.19 Powdered sample (100 mg) was solubilized with 10 mL of 0.2M phosphate buffer and incubated with 1 mL of 100 U amylase (Pancreatin) for 16 h to completely hydrolyze the digestible starch. The sample was centrifuged at 10,000 rpm for 10 min and the residue was solubilized with 2.0M KOH. Glucose was estimated using GOD-POD method. The obtained value of glucose was multiplied by a factor of 0.9 to calculate the resistant starch.

Digestible Starch
The digestible starch was calculated using the following formula,

\[ \text{Digestible starch} = \text{Total Starch} - \text{Resistant Starch} \]

Hydrolysis Index (HI)
HI Index was determined from the ratio of area under the hydrolysis curve (AUC) of test sample and bread as the reference sample.19 AUC was constructed using the concentration of starch hydrolyzed until 90 min as described in the readily digestible starch.

\[ \text{HI} = \left( \frac{\text{AUC of test sample}}{\text{AUC of bread}} \right) \times 100 \]

Glycemic Index (GI)
The GI of rice samples was calculated from the formula given by Goni et al.20

\[ \text{GI} = 39.71 + 0.549 \times \text{HI} \]
Estimation of Total Proteins
The concentration of total proteins in the rice samples were estimated by Biuret method. 30 500 mg of powdered rice samples were extracted with 10 mL of NaOH at 60°C for 90 min. The samples were cooled to room temperature and centrifuged at 4000 g at 4°C for 30 min. To 100 µL of the supernatant, 1 mL of Biuret reagent, was added and incubated at room temperature for 5 min. The optical density was recorded at 546 nm.

Nutraceutical Properties
Extraction of Anthocyanins from Rice Samples
Anthocyanin was extracted using methanol acidified with 1.5N HCl (70:30) as per the protocol of Sun et al. 31 The procedure consisted of dissolving 1mg/mL of the rice powder in two different buffer solutions of hydrochloric acid-potassium chloride buffer (pH 1.5) and sodium acetate buffer (pH 4.5). The extract was dried in a rotary evaporator at 40°C.

Antidiabetic Activity
Antidiabetic activity of rice extract was quantified by their ability to inhibit porcine pancreatic amylase that releases reducing sugars from starch. 32 500 µL of amylase (16 U/mg) and 500 µL of the anthocyanin extract of rice varieties (1mg/mL) were incubated at 25°C for 10 min. To the mixture, 500 µL of starch (0.05% in phosphate buffered saline of pH 6.9) was added and incubated at room temperature for 30 min. 500 µL of the reaction mixture was withdrawn for the determination of reducing sugars released by amylase. A control was maintained simultaneously with enzyme and substrate without the rice extract. To the sample 1000 µL of dinitro salicylic acid was added and heated in a boiling water bath for 10 min. The tubes were cooled to room temperature and the color was read at 540 nm. Inhibition of amylase was calculated using the formula,

\[
\text{Inhibition of Amylase (\%)} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

Probiotic Activity of Anthocyanin Extracted from Rice
The prebiotic activity of anthocyanins was evaluated by viable plate count method. The probiotic organisms were purchased from Bifilac (300 million cells/100 mg powder). The bacilli were activated in MRS broth for 24 hr. The To 50 mL of MRS medium 1mL of activated probiotics (1x10^8 CFU/mL) and anthocyanin powder (1 mg/mL) were added and incubated for 36 hr. A tube without anthocyanin extract was used as control. The sample was diluted and plated in MRS agar plate to count the viable colonies. 33

Statistical Analysis
All experiments were replicated thrice, and the data represented are the mean of three replicates. Graphs were plotted with standard error of the mean using Graph Pad Prism 5.

Molecular Docking of Anthocyanin
Preparation of Receptor
Crystal structure of human pancreatic α-amylase (2QMK), human cytosolic β-glucosidase (2JFE) and human glucose transporter-GLUT1(4PYP), were retrieved from protein data bank. The bound ligands, cofactors and solvent molecules associated with the protein structures were removed and saved in .pdb format as an input format for PyRx and Discovery Studio Visualizing software.

Preparation of the Ligands
As the black rice variety was superior to other varieties in its resistant sugar concentration and nutraceutical properties, docking study was restricted to bioactive compounds in black rice only. The major anthocyanin pigments from black rice such as cyanidin, cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside were selected to evaluate their interaction with amylase, glucosidase and GLUT1. To compare the efficiency of anthocyanins, three clinically available drugs such as acarbose, miglitol and voglibose were used. The ligands were retrieved from PubChem database in .sdf format and then converted to .pdb format using Open Babel GUI tool. The ligands were loaded into the PyRx software.

Molecular Docking
The ligands and proteins were docked using Autodock Vina incorporated in Pyrx software and their binding affinities were obtained. The docked results were compiled, visualized and the receptor-ligand interactions were analyzed using Discovery Studio Visualizer.
Results and Discussion

Grain Dimension

There is very less scientific information about the grain dimensions of the colored rice varieties available in India. The rice varieties collected in the present study is represented in Figure 1. The figure clearly depicts the difference in the color between the rice varieties. The difference is attributed to the variation in the composition of phytochemicals. The color of the rice is due to the flavonoid group of compounds called anthocyanins, which are potent antioxidants.\(^{31, 34}\) The dimensions of rice grains are represented in Table 1. Physical dimensions of rice grains are critical in screening, grading and discriminating the quality. Size based classification is essential for further processing of agro products in machines.\(^{34}\) Length of the rice ranged from 5.1 mm to 6.3 mm. Width of the rice varieties varied between 2.5 mm and 3 mm. Lower most length (5.1 mm) and width (2.6 mm) were recorded in 60m kuruvai. Mappillai samba variety showed highest length (6.3 mm) and width (2.9 mm). The results agree with the observations of Rui et al.\(^{35}\) and Yang et al.\(^{36}\) Difference in the grain size among the different varieties of rice is due to the difference in the genetic composition of the seed and soil quality. Similar results were reported in the previous studies.\(^{37}\) Positive correlation between the morphological parameters like area, perimeter, length, diameter width, roundness, thickness and grain weight are reported earlier.\(^{38}\) Grain morphology is related to milling quality. The overall appearance of rice is dependent on the uniformity in size, shape and thickness.\(^{39}\)

Table 1: Grain Dimensions of Rice

<table>
<thead>
<tr>
<th>S.No</th>
<th>Rice Variety</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>L/W</th>
<th>De(mm)</th>
<th>Aspect ratio (%)</th>
<th>S(_p)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chak hao poreiton</td>
<td>6.3</td>
<td>2.5</td>
<td>1.9</td>
<td>2.52</td>
<td>3.1</td>
<td>39.68</td>
<td>49.20</td>
</tr>
<tr>
<td>2</td>
<td>Mapillai samba (raw)</td>
<td>6</td>
<td>3</td>
<td>2.1</td>
<td>2</td>
<td>3.36</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>Mapillai samba (boiled)</td>
<td>6.3</td>
<td>2.9</td>
<td>1.8</td>
<td>2.17</td>
<td>3.2</td>
<td>46.03</td>
<td>50.79</td>
</tr>
<tr>
<td>4</td>
<td>60m kuruvai</td>
<td>5.1</td>
<td>2.6</td>
<td>1.9</td>
<td>1.96</td>
<td>2.93</td>
<td>50.9</td>
<td>57.45</td>
</tr>
<tr>
<td>5</td>
<td>Karuthakar poha</td>
<td>5.7</td>
<td>2.8</td>
<td>1.8</td>
<td>2.03</td>
<td>3.06</td>
<td>49.12</td>
<td>53.68</td>
</tr>
<tr>
<td>6</td>
<td>Kothamalli samba (raw)</td>
<td>5.5</td>
<td>2.7</td>
<td>2</td>
<td>2.03</td>
<td>3.09</td>
<td>49.09</td>
<td>56.18</td>
</tr>
<tr>
<td>7</td>
<td>Kothamalli samba (boiled)</td>
<td>5.1</td>
<td>2.7</td>
<td>1.8</td>
<td>1.88</td>
<td>2.92</td>
<td>52.94</td>
<td>57.25</td>
</tr>
<tr>
<td>8</td>
<td>Kuzhiyadichan</td>
<td>5.8</td>
<td>2.7</td>
<td>2.1</td>
<td>2.14</td>
<td>3.2</td>
<td>46.55</td>
<td>55.17</td>
</tr>
<tr>
<td>9</td>
<td>Navara boiled</td>
<td>5.5</td>
<td>2.5</td>
<td>1.7</td>
<td>2.2</td>
<td>2.86</td>
<td>45.45</td>
<td>52</td>
</tr>
<tr>
<td>10</td>
<td>Perungar</td>
<td>6.2</td>
<td>3</td>
<td>2</td>
<td>2.06</td>
<td>3.34</td>
<td>48.38</td>
<td>53.87</td>
</tr>
<tr>
<td>11</td>
<td>Kullakar (raw)</td>
<td>5.3</td>
<td>2.6</td>
<td>1.7</td>
<td>2.03</td>
<td>2.86</td>
<td>49.05</td>
<td>53.96</td>
</tr>
<tr>
<td>12</td>
<td>Onamatta</td>
<td>6.1</td>
<td>3</td>
<td>1.9</td>
<td>2.03</td>
<td>3.26</td>
<td>49.18</td>
<td>53.4</td>
</tr>
<tr>
<td>13</td>
<td>Thavalakannamatta</td>
<td>5.5</td>
<td>2.5</td>
<td>1.6</td>
<td>2.2</td>
<td>2.8</td>
<td>45.45</td>
<td>50.90</td>
</tr>
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</table>

Significant variation was observed in the aspect ratio and roundedness among rice varieties. Lower roundedness indicates the cylindrical nature of rice.\(^{40}\) Chak hao poreiton variety has less aspect ratio of 39.68 and kothamalli samba (boiled) has the highest aspect ratio of 52.94. Wide variations in the grain dimension was reported by Correa et al.\(^{41}\) and Shittu et al.\(^{42}\) Lower roundedness indicates the cylindrical nature of rice.\(^{40}\) The results corroborate well with the total starch concentration presented in Figure 3. The total starch in kothamalli samba was higher than chak hao poreiton. The results clearly confirm that large grain favors dry matter accumulation than small grain relative to their hull weight. Grain dimension is useful in deciding the sieve during milling process.\(^{25}\) Size, shape, sphericity, aspect ratio and color are some of the grain parameters that govern the quality of rice. Grain weight increases with grain size and the duration of grain filling which in turn depend on the panicles.\(^{43}\)
Weight of thousand kernels is represented in Figure 2. Thousand kernel weight was 18g in chak hao poreiton and 28g in perungar. Weight of grain was less in boiled variety of mappillai samba than raw variety. It is an indicator of seed quality and it is due to the milling process. Consumer preference and pricing of rice is based on the grain dimension and quality.
Nutritional Profile of Rice

Total Starch

Starch in cereals is composed of repeated glucose units arranged as straight chain in amylose and branched chain in amylopectin. Total starch concentration present in the rice varieties was assessed and the results are shown in Figure 3. The concentration of total starch in rice varied from 61% (kullakar -raw) to 85% (mapillai samba-boiled). The concentration of total starch was 78% in kothamalli samba (raw) and it was slightly higher in processed sample (81%). Similarly, a slight increase in total starch was observed in boiled variety of mapillai samba than raw variety. During processing conditions, the starch in the rice varieties were solubilized which leads to increased starch content. The present results corroborate with the reports of Omar et.al. which documented the variation in starch concentration between 81-92% of total constituents. Starch from rice is different from other plant sources by their small size and the presence of hypoallergenic protein. The concentration of amylose in the starch determines the rate of digestion of starch. Compactness of amylose reduces its exposed surface area for digestion by salivary and pancreatic amylase.

Fig.3: The concentration of total starch in rice

Digestible Starch

The concentration of digestible starch present in the rice varieties are shown in Figure 4. The concentration of digestible starch in Chak hao poreiton, Kullakar (raw) and bread were 62%, 90% and 97% respectively. The digestibility of the starch...
varies widely due to processing conditions, genetic modification in the seeds and storage conditions. Bread is easily digestible because of the exposure of amylose and amylopectin to hydrolyzing enzymes. Many colored varieties have low digestibility because of the complex structure of amylose and amylopectin, which are not easily accessible to the enzymes. Significant portion of starch was not digestible in chak hao poreiton. The concentration of readily digestible starch increases the burst release of glucose into blood stream.

**Resistant Starch**
Resistant starch is defined as “the fraction of starch that escapes digestion in the small intestine and that is fermented in the large intestine”. It is an example of complex carbohydrate that resists digestion in the stomach, and they function as soluble fiber. The concentration of resistant starch present in the rice is depicted in Figure 5. It varied widely between varieties with 38% in chak hao poreiton and 8% in mapillai samba. The resistant starch is fermented by the microflora in the large intestine. Among the rice varieties used, chak hao poreiton, has the highest concentration of resistant starch, which reduces its GI. 60m kuruvai has second highest concentration of 19%. Boiled mapillai samba (14%) showed high concentration than raw variety (8%). Richness of colored varieties of rice with resistant starch was supported by the observations of Deepa et.al. Resistant starch decrease the post prandial glucose and improves insulin sensitivity. Rice rich in amylose content are resistant to digestion by amylase. Resistant starch increases the growth of beneficial bacteria in colon by supplying the free fatty acids, acetate, propionic acid and butyric acid. These short chain fatty acids increase the absorption of minerals in the bowel and prevent bowel related disorders.

**Hydrolysis Index**
Hydrolysis of starch determines the body’s response to metabolic conditions such as hyperglycemia and hyperlipidemia. The hydrolysis of starch at different time intervals is represented in Figure 6. Starch hydrolysis was very rapid in bread where more than 90% of starch was hydrolyzed within 90 min. In chak hao poreiton only 20% of starch was hydrolyzed in 90 min. Kothamalli samba variety rapidly digested 90% of starch within 90 min. Other varieties of rice were intermediate between bread and Chak hao poreiton. The Hydrolysis Index of the food sample is calculated from the starch hydrolysis curve with bread as the reference food and the results are shown in Figure 7. The HI of bread was 100. Karuthakar poha and chak hao poreiton have low HI of 22 and 25 respectively. This low HI may be due to the fact that the rice samples may contain non-starch polysaccharides. Boiled mapillai samba has lower hydrolysis index than raw variety. The results are in accordance with the observations of Perera. Variation in the hydrolysis of starch among the different sources of plant and within the varieties were supported by the observations of starch digestibility both among plant sources and within a single variety.
Glycemic Index

GI determines the rate at which the body converts starch into a reducing sugar. It is directly proportional to HI value. The GI values of rice varieties are presented in Figure 8. GI of rice varieties varied significantly between 83 and 52. Higher glycemic index of bread is attributed to the solubility of starch and its susceptibility to amylase action which
releases glucose rapidly. Studies by Miller et al.\textsuperscript{4} reported the wide variation in GI value of rice from 62 to 93. Raw Mapillai samba has the highest GI (83) compared to boiled variety (52). Still higher GI of 102 was reported by Frei et al.\textsuperscript{59} Karuthakar Poha and chak hao poreiton have low GI of 52 and 53 respectively. The observed variation in the GI among the rice varieties is due to the difference in the composition of starch, particle size, the concentration of other nutrients such as lipids, fiber and proteins.\textsuperscript{13} Varieties like mapillai samba and kullakar rice with higher GI values release high concentration of glucose.\textsuperscript{60} Therefore, it leads to hyper insulinemia and culminates insulin receptor down regulation.\textsuperscript{61} Moreover, foods with higher glycemic index triggers hunger rapidly and induce over eating and obesity.\textsuperscript{62} American Diabetes Association describes the significance of controlling blood sugar to prevent the worst the consequence of diabetes mellitus.\textsuperscript{31,63} Rice with low GI value is preferred recently due to increased awareness about the relationship between the GI and diabetes. Low glycemic index diets enhance insulin sensitivity and improve metabolic and cardiovascular risk factors. Foods with low glycemic index protect people from obesity, type II diabetes,\textsuperscript{64-66} breast cancer, gall bladder disease\textsuperscript{67} and cardiovascular disease.\textsuperscript{68, 69} Low glycemic food reduces the absorption of glucose and imposes no stress to pancreas to secrete insulin, gastric inhibitory peptide and glucagon like peptide. Low GI food reduces obesity and diseases linked with obesity.\textsuperscript{70}

![Fig.8: Glycemic Index of rice](image)

### Total Proteins

The total proteins present in the rice varieties is shown in Figure 9. Rice is an important source of protein. The protein concentration of almost all the rice samples is less than 20%. High concentration of protein (16%) was observed in chak hao poreiton followed by mapillai samba raw (11%). The results agree with the previous records.\textsuperscript{71} Rice varieties with high concentration of proteins decrease the access of enzyme for starch hydrolysis.\textsuperscript{71} As the concentration of protein was high in Chak hao poreiton, the hydrolysis index and GI are also low in the same variety.

### Antidiabetic Activity of Anthocyanin

Inhibition of amylase by anthocyanins is evident from Figure 10. Inhibition of amylase by anthocyanin of chak hao poreiton was highest with 24% followed by kothamalli samba (14%). Lower most inhibition of 4% was exhibited by mapillai samba variety. The results are similar to the observation of Stephen et al.\textsuperscript{72} The significant difference in the amylase activity was correlated to the difference in the concentration of anthocyanins in the rice varieties. Anthocyanins from different food sources were reported to possess inhibition towards amylase and glucosidase.\textsuperscript{73-75} Inhibition of amylase activity
reduces the release of sugars into the blood thus reducing the burden of diabetes. Anthocyanins compete for the catalytic sites of amylase and glucosidase by mimicking the structure of starch. Anthocyanin glucosides mimic the transition state complex of enzyme substrate complex and block the enzyme activity. Other studies supported that the bulky structure of anthocyanin masks the active site like a cap and prevent the binding of carbohydrates to the hydrolyzing enzyme. Few other studies described the inhibition of amylase and glucosidase by noncompetitive and mixed inhibition. Anthocyanins exhibit antidiabetic activity, not only by inhibiting the hydrolytic enzymes but also inhibiting the glucose transporters that are essential for the transport of glucose. So, anthocyanins are potent in regulating the glucose level by multiple ways. Hence, the traditional colored varieties of rice are nutritionally and pharmaceutically significant.

Prebiotic Activity of Anthocyanin
Gut microbiome plays a vital role in the health and diseased status of human being. The biological effect of any active ingredient of food or pharmaceutical formulation depends on the colonic microbiome. Hence in the present study, the effect of anthocyanin in maintaining the gut microbiome was assessed and the results are presented in Figure 11. The number
of viable probiotic bacteria increased significantly in the medium supplemented with anthocyanin. In control without anthocyanin, the number of colonies was log 0.15CFU/mL. It was significantly increased to log 1.95 CFU/mL in Chak hao poreiton. Growth promoting effect of anthocyanin was supported by the observations of Sun et al.\textsuperscript{31} and Diaz-Rizzolo et al.\textsuperscript{82} The anthocyanin in Chak hao poreiton is primarily glycosylated derivatives. These glycosyl units are hydrolyzed by glucosidases synthesized by probiotics. The released glucose is used for the growth of probiotics.\textsuperscript{83} The growth promoting activity of Karuthakar poha increase the bacterial count to log 1.0 CFU/mL. Growth inducing effect of anthocyanin on \textit{Bifidobacterium bifidum}, \textit{Bifidobacterium adolescentis}, \textit{Bifidobacterium infantis} and \textit{Lactobacillus acidophilus} were supported in previous studies.

![Fig.11: Prebiotic activity of anthocyanins](image)

Firmicutes/bacteroidetes which are abundant in the gut of hyperlipidemic and obese human were reversed by the supplementation of anthocyanin extract from black rice. Supplementation of anthocyanin increased the bacteria from phylum such as \textit{Lactobacillus}, \textit{Bifidobacterium}, \textit{Parabacteroides}, \textit{Oscillospira}, \textit{Akkermansia}, \textit{Ruminococcus}, and \textit{Butyricimonas} and simultaneously decreased \textit{Clostridium} and \textit{Desulfovibrio}.\textsuperscript{84-87}

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amylase</td>
</tr>
<tr>
<td>Acarbose</td>
<td>- 7.5</td>
</tr>
<tr>
<td>Miglitol</td>
<td>- 5.7</td>
</tr>
<tr>
<td>Voglibose</td>
<td>- 8.1</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>- 7.9</td>
</tr>
<tr>
<td>Cyanidin-3-glucoside</td>
<td>- 9.9</td>
</tr>
<tr>
<td>Cyanidin-3-rutinoside</td>
<td>-10.8</td>
</tr>
<tr>
<td>Peonidin-3-glucoside</td>
<td>- 6.8</td>
</tr>
<tr>
<td>Peonidin-3-rutinoside</td>
<td>- 6.8</td>
</tr>
</tbody>
</table>
Molecular Docking of Anthocyanin

The interaction of major anthocyanins of black colored rice with pancreatic $\alpha$-amylase, $\beta$-glucosidase and GLUT1 was analyzed by molecular docking. The results were compared with clinically available drugs such as acarbose, miglitol and voglibose.

The free energy of binding of the ligands and controls with the target proteins are shown in Table 2. The table reveals the strong affinity between the anthocyanins and proteins than the control drugs. Druggability analysis of cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside showed more than 2 violations in Lipinski rule. Cyanidin has good drug likeliness with the binding affinity of -7.9 kcal/mol, -4.3 kcal/mol and -8.5 kcal/mol against amylase, glucosidase and GLUT1 respectively. The binding affinity of cyanidin was greater than acarbose and miglitol against $\alpha$-amylase Druggability $\beta$-glucosidase. Affinity to GLUT1 was equal to voglibose.

![2D interaction view of the ligands against pancreatic $\alpha$-amylase](Fig.12)
Based on the results of drug likeliness, only cyanidine was used for further analysis. Interaction of acarbose, miglitol, voglibose and cyanidin with amylase, glucosidase and GLUT 1 are shown in Figure 12, 13, and 14 respectively. Voglibose binds with Asp197, Glu 300 and Asp 233 which are the active site residues of pancreatic $\alpha$-amylase. Similarly, cyanidin also binds with Glu 233 and Asp 300 confirming the competitive inhibition. The binding of cyanidine with the active site residues are supported by the observations of Xu et al. Acarbose and miglitol binds with $\alpha$-amylase in many sites other than the active sites. Oudjeriouat, et al. reported the uncompetitive inhibition of barely $\alpha$-amylase by acarbose.
Active site of glucosidase is comprised of Glu 165, Glu 273 and Gln 307. Hydrophobic amino acids such as Phe 179, Phe 121, Phe 225, Phe 433, Tyr 308, Tyr 309, Trp 345 and Trp 425 covers the substrate.  

Active site binding was not observed in controls and cyanidin confirming the absence of competitive inhibition. Cyanidin binds with glucosidase through vanderwaals forces and hydrogen bond. Strongest affinity of cyanidin to glucosidase than acarbose corroborates with the results of Chen et al. GLUT1 mobilizes glucose to tissues and reduces the concentration of postprandial glucose. Hence, in the present study, the binding of cyanidin with
GLUT1 was evaluated. Control drugs and cyaniding revealed similar binding sites as represented in Figure 14. Trp 388 and Trp 412 are absolutely essential for transport of glucose through GLUT1. Acarbose and cyanidine interact with both residues. Trp 388 was bound by all controls and cyanidin. Similar interaction was documented by Son and Lee. In addition to the essential sites, the interaction involved many residues which contributed to higher binding affinity. The results confirm the interaction of cyanidin with α-amylase, β-glucosidase and glucose transporter to reduce the postprandial glucose level.

Conclusion
Among the rice varieties used, chak hao poreiton has low concentration of total starch, high concentration of resistant starch, lowermost, GI and high concentration of proteins. The variety is the richest source of anthocyanins that possess antidiabetic and probiotic activity. Molecular docking revealed the binding of cyanidin from Chak hao poreiton with pancreatic α-amylase, α-glucosidase and GLUT 1 with key residues essential for their function.

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Author’s contributions
Mala Rajendran conceived the concept and designed the experimental plan and Keerthana Ravi Chandran executed the experiments.

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Conflict of Interest
The authors do not have any conflict of interest.

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