



## ***In Vitro* Glycemic Response of Indigenous Pigmented Rice Cultivars from South India and Influence of Different Carbohydrate Components**

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### **Abstract**

Staple diet patterns such as white rice consumption play an important part in the occurrence of chronic lifestyle-related disease like diabetes. This study intended to identify pigmented rice cultivars from India as an alternative to white rice. Nine carbohydrate components were quantified in six red and two black pigmented rice varieties. *In vitro* starch digestibility was also analyzed and the predicted glycemic index (pGI) was estimated. The relationship between the carbohydrate components and the pGI of the rice varieties was analyzed. The rice varieties, Kattuyanam and Chennangi exhibited high levels of insoluble dietary fibre, total dietary fibre and amylose. High soluble dietary fibre contents were observed in Poonghar and Aruvadam kuruvai. The levels of resistant starch and slowly digestible starch were found to be the highest in Karupakavuni. The results indicated Kattuyanam, Chennangi, Karungkuruvai, and Poonghar to be low pGI rice varieties (< 55). The three varieties, Kesari, Karupakavuni, and Aruvadam kuruvai were categorized under the medium pGI category (56-69). Mapillai samba had a high pGI of 70 which could be due to processing (partially milled and parboiled), but had significantly lower pGI than the white rice, Sona masuri (GI – 76). The dietary fibre components, resistant starch, slowly digestible starch, and the amylose content were negatively correlated with the pGI. Among these components, the insoluble dietary fibre, total dietary fibre, and resistant starch with significantly high ( $p \leq 0.01$ ) correlation were found to be the major determining factors of pGI in the studied pigmented rice cultivars. The inclusion of the identified pigmented rice varieties with low to medium glycemic response in the diet could become a key role in the dietary management of diabetes, especially among the rice-eating population.



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## Introduction

Rice is produced and consumed all over the world, especially in Asia. It is considered as the staple food for the majority of the world's population. However, in recent times higher consumption of white rice has been implicated in increasing the risk of certain chronic lifestyle-related diseases such as type 2 diabetes.<sup>1</sup> The suitability of carbohydrate-based foods for diabetic subjects has been assessed using glycemic index studies. 'Glycemic index (GI) is defined as the area under the curve of blood glucose level, within 2 hour period, after consuming 50 g of a carbohydrate based test food in comparison with the area following the ingestion of 50 g of carbohydrate-based reference food, usually white bread or glucose.<sup>2</sup> The standardized method of measuring GI with human feeding trials is validated but not practical when it comes to measuring the GI of a large number of samples. It is a resource-intensive method and hence as an alternative, *in vitro* methods that are simple, reliable, and inexpensive have become popular for the screening of low GI foods.<sup>3</sup> A high correlation has been reported between the *in vivo* glucose response and *in vitro* pGI based on starch digestibility.<sup>3,4,5</sup>

A common *in vitro* starch digestibility technique uses controlled enzymatic hydrolysis of starch-based foods to simulate the release of glucose over time and its absorption in the intestinal tract to identify starch fractions such as rapidly digested starch (RDS) and slowly digested starch (SDS) fractions.<sup>6</sup> Another method<sup>4</sup> uses starch digestibility to calculate the hydrolysis index (HI) of a carbohydrate-based test food and a reference food such as white bread or glucose and predict the glycemic index of the test food.

Low-GI foods have an advantageous effect on glycemic control and lower HbA1c and fructosamine levels in the body.<sup>7</sup> Glycemic index of foods is influenced by many factors. Processing, dietary fibre, amylose content and resistant starch content appear to be the decisive factors among others.<sup>8,9</sup> The average GI values of white rice varieties irrespective of the type of processing, cooking time, and amylose content are reported to be high.<sup>10</sup> Therefore, it poses a public predicament for the large populations that depend largely on rice for sustenance. Hence,

there is a compelling need to screen, identify and scientifically validate low GI rice varieties.

Some have reported lower starch digestibility in pigmented rice varieties from Thailand in comparison with white rice.<sup>11,12,13,14</sup> One study has reported the resistant starch content and starch digestibility (*in vitro*) of two red rice varieties and one white rice variety from India<sup>15</sup> and another study has reported the amylose content and *in vivo* GI of four pigmented rice varieties.<sup>16</sup>

Cultivation and consumption of traditional pigmented rice varieties are being revived in India as their nutritional awareness is increasing. In this study, eight pigmented rice varieties cultivated in Southern regions of India and steadily gaining market value have been selected for the study. This is one of the first composite studies to report different carbohydrate components relevant to the glycemic response along with *in vitro* starch digestibility and pGI in Indian pigmented rice varieties to the best of our knowledge. This study has also determined the correlation between the different carbohydrate components and the *in vitro* glycemic response of the pigmented rice varieties providing insights into the role of these components in carbohydrate metabolism.

## Materials and Methods

### Sample Collection and Storage Conditions

Five rice varieties (Karungkuruvai, Poonghar, Aruvadam Kuruvai, Karupa Kavuni, and Kattuyanam) were collected directly from rice farms in Tamil Nadu, India. Mapillai Samba (parboiled and partially milled) was procured from Nammanellu, Centre for Indian Knowledge Systems, Tamil Nadu, India. Chennangi and Kesari were procured from Timbaktu, Dharani Farms, Andhra Pradesh, India. The samples codes, bran colour and morphological classification of the rice varieties are given in Table 1. Sona masuri (WSm) a medium and slender polished white rice, commonly consumed in Southern parts of India was procured from the local market in Andhra Pradesh, India. It was considered as a control for this investigation.

The rice samples were cleaned and dehusked manually. Whole rice grains (including the bran) were

pulverized using an electric blender and sieved using a 0.5 mm mesh to obtain a homogenous sample. The samples were freeze-dried, vacuum packaged and stored at – 20 °C until analysis. No physical, biological changes or contamination was observed during storage.

**Table 1: Samples codes, bran colour and morphological classification of pigmented rice**

| S. No. | Pigmented rice varieties | Sample code | Bran colour | Morphological classification* |
|--------|--------------------------|-------------|-------------|-------------------------------|
| 1.     | Kattuyanam               | BKa         | Black       | Short, bold                   |
| 2.     | Chennangi                | RChe        | Red         | Short, bold                   |
| 3.     | Karungkuruvai            | RKku        | Red         | Short, round                  |
| 4.     | Poonghar                 | RPo         | Red         | Long, slender                 |
| 5.     | Kesari                   | RKe         | Red         | Short, round                  |
| 6.     | Karupakavuni             | BKk         | Black       | Medium, slender               |
| 7.     | Aruvadam kuruvai         | RAk         | Red         | Short, bold                   |
| 8.     | Mapillai samba           | RMs         | Red         | Short, round                  |

\*As per National Post Harvest Institute for Research and Extension (NAPHIRE), Department of Agriculture, Philippines

**Dietary Fibre Components**

The dietary fibre components including total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) were analyzed using an enzymatic and gravimetric modified procedure<sup>17</sup> standardized in our laboratory<sup>18</sup> using a dietary fibre kit (TDA-100A kit) from Sigma-Aldrich, USA.

$$SDF \text{ and } IDF = \frac{[R1 + R2] - B - P - A}{M} \times 100$$

$$TDF \% = SDF + IDF$$

Where, B – Blank, R1 – Weight of residue used for protein analysis, R2 – Weight of the residue used for ash analysis, M – Weight of sample, P – Protein, A – Ash

**Amylose Estimation**

Starch was isolated from the experimental rice flours by following the method given by Lim *et al.*,<sup>19</sup> The amylose content was analyzed using the 6-well plate iodine binding assay standardized by Kaufman *et al.*,<sup>20</sup> The absorbance values of the samples were recorded at 620 nm using Thermo Scientific Varioskan LUX multimode microplate reader. Amylose standard curve with concentrations ranging from 25 to 500 µg/ ml was built and a regression equation was determined for calculating concentrations in the samples.

**Total Starch, Resistant Starch, and Digestible Starch**

The total starch (TS) content of rice was determined using the enzymatic method (amylase and amyloglucosidase) standardized by de Guzman *et al.*,<sup>21</sup> The absorbance was measured at 510 nm. Total starch was calculated as glucose (mg) and multiplied by the conversion factor 0.9 to give the starch content.

The resistant starch (RS) content was analysed by the method given by Goñi *et al.*,<sup>22</sup> The resultant glucose was quantified using the glucose oxidase-peroxidase method. RS was calculated as glucose (mg) and multiplied by the conversion factor 0.9 to give the starch content.

Digestible starch (DS) was computed by subtracting the RS from TS as reported by Frei *et al.*, (2003).<sup>23</sup>

**In vitro Starch Digestion and Starch Kinetics**

*In vitro* starch digestion was carried out according to the protocol of Goñi *et al.*,<sup>4</sup> with modifications. In this study, the dynamic continuous flow dialysis method, to mimic the small intestinal phase and the diffused digesta, was used for estimating the hydrolyzed starch and predict the glycemic index. The chewing stage was replicated by macerating cooked rice (0.2 g) in a mortar and pestle with 400 µl of

$\alpha$ -amylase (80 U in phosphate buffer saline, pH 7.4), 2 ml distilled water for 2 min followed by 5 ml of 0.1 M phosphate buffer (pH 6.9). The contents were transferred to a boiling tube and the pH was reduced to 2.5 using 10 % phosphoric acid. The gastric digestion was followed according to the method by Kumar *et al.*,<sup>39</sup> From the digesta, 500  $\mu$ l aliquots were taken at 0, 20, 30, 60, 90, 120, 150 and 180 min. The aliquots were further diluted to 5 ml with double distilled water. The glucose contents were estimated using glucose oxidase-peroxidase reagent and the absorbance was determined at 510 nm. The starch hydrolyzed was calculated as glucose (mg) and multiplied by the conversion factor 0.9 to give the starch content.

The starch hydrolysis reactions were fitted to the first-order rate equation<sup>4</sup>:

$$C_t = C_{\infty} (1 - e^{-kt})$$

$C_t$  = quantity of starch hydrolyzed at time  $t$

$C_{\infty}$  = quantity of starch hydrolyzed at the endpoint of the reaction

$k$  = digestibility rate constant or first-order rate constant, which was calculated by the equation<sup>4</sup>:

$$k = (\ln(C_t/C_{\infty})) / (-t)$$

$$k = \frac{\ln\left(\frac{C_t}{C_{\infty}}\right)}{-t}$$

$t$  = time taken for digesting the starch in minutes

#### Rapidly Digestible Starch and Slowly Digestible Starch

The RDS and SDS contents were computed according to the equations given by Englyst *et al.*,<sup>6</sup>: RDS =  $(G_{20} - \text{FSG}) \times 0.9$  and SDS =  $(G_{120} - G_{20}) \times 0.9$ , where,  $G_{20}$  and  $G_{120}$  correspond to the available glucose after 20 min and 120 min, respectively. Fasting glucose (FSG) represents glucose available at 0 min. The glucose values were multiplied by the conversion factor 0.9 to give the starch content.

#### Hydrolysis Index

The hydrolysis index (HI) was derived as the ratio between the areas under the curve of the hydrolysed test samples (pigmented rice and white rice) and

the reference sample (i.e. glucose). The areas under the curves were calculated using Sigma plot V14.0, USA.

$$\text{HI} = (\text{Area under the curve of test sample}) / (\text{Area under the curve of glucose}) \times 100$$

#### Predicted Glycemic Index

The HI determined in the experiment was used to compute the pGI applying the equation given by Goñi *et al.*,<sup>4</sup>: pGI = 39.7 + 0.548 HI.

#### Statistical Analysis

The results were presented as mean  $\pm$  standard deviation of three replicates. The data were subjected to one-way analysis of variance (ANOVA) and Tukey HSD at a significance level of  $p \leq 0.05$  to detect significant differences among the pigmented rice varieties and the control. Pearson's correlation coefficient was used to ascertain relationships between the carbohydrate components and the pGI at  $p \leq 0.05$  and  $p \leq 0.01$  levels. All statistical analyses were performed using SPSS® 16 software, student version.

#### Results

##### Dietary Fibre Components

The total dietary fibre (TDF), soluble dietary fibre (SDF) and (insoluble dietary fibre) IDF contents of the samples are presented in Table 2. The TDF content of the experimental samples ranged from 6.66 to 8.53 g/ 100 g. The highest TDF content was found in BKa and the least in RMs. The sample BKa had significantly higher ( $p \leq 0.05$ ) TDF content than the other samples. All the experimental samples had significantly higher TDF content compared to WSm.

The SDF content was found to be the highest in RPo (1.55 g/ 100 g) followed closely by RAK (1.49 g %) and RKe (1.48 g %). All the pigmented rice varieties recorded significantly higher SDF than WSm (0.48 g %). The IDF content was highest in BKa (7.04 g/ 100 g) followed by RChe (7.02 g/ 100 g) and least in RMs (5.22 g/100 g) among the pigmented rice varieties. These values were significantly higher ( $p \leq 0.05$ ) compared to WSm.

##### Amylose Content

The amylose content (Table 2) was found to be the highest (29.22 g/ 100 g DW) in RAK. However, this was not significantly different from the other

pigmented rice varieties except RMs which recorded a lower value of 16.42 g/ 100 g DW. All the experimental samples fall under the high amylose content category (> 25 %) except RMs which belong to low amylose content category (10-20 %) according

to the classification given by Kumar and Khush.<sup>24</sup> However, all the eight pigmented rice varieties had significantly higher ( $p \leq 0.05$ ) amylose content than WSm (12.26 g %).

**Table 2: Dietary fibre profile and amylose content of pigmented rice cultivars and white rice**

| Sample | TDF (g/100 g)            | SDF (g/100 g)             | IDF (g/100 g)             | Amylose content (g/ 100 g) |
|--------|--------------------------|---------------------------|---------------------------|----------------------------|
| BKa    | 8.53 <sup>f</sup> ± 0.08 | 1.42 <sup>c</sup> ± 0.09  | 7.04 <sup>f</sup> ± 0.04  | 28.42 <sup>c</sup> ± 0.1   |
| RChe   | 8.29 <sup>e</sup> ± 0.51 | 1.27 <sup>b</sup> ± 0.45  | 7.02 <sup>ef</sup> ± 0.31 | 27.12 <sup>c</sup> ± 0.09  |
| RKku   | 7.40 <sup>c</sup> ± 0.18 | 1.48 <sup>c</sup> ± 0.09  | 6.15 <sup>d</sup> ± 0.05  | 27.14 <sup>c</sup> ± 0.22  |
| RPo    | 8.24 <sup>e</sup> ± 0.41 | 1.55 <sup>cd</sup> ± 0.04 | 6.79 <sup>e</sup> ± 0.29  | 27.22 <sup>c</sup> ± 0.06  |
| RKe    | 7.92 <sup>d</sup> ± 0.82 | 1.25 <sup>b</sup> ± 0.13  | 6.44 <sup>de</sup> ± 0.18 | 27.86 <sup>c</sup> ± 0.06  |
| BKk    | 7.84 <sup>d</sup> ± 0.15 | 1.45 <sup>c</sup> ± 0.25  | 6.29 <sup>d</sup> ± 0.24  | 26.72 <sup>c</sup> ± 0.17  |
| RAk    | 6.76 <sup>b</sup> ± 0.03 | 1.49 <sup>cd</sup> ± 1.42 | 5.34 <sup>c</sup> ± 0.24  | 29.22 <sup>c</sup> ± 0.12  |
| RMs    | 6.66 <sup>b</sup> ± 0.31 | 1.44 <sup>bc</sup> ± 0.05 | 5.22 <sup>b</sup> ± 0.17  | 16.42 <sup>b</sup> ± 0.18  |
| WSm    | 2.26 <sup>a</sup> ± 0.16 | 0.48 <sup>a</sup> ± 1.62  | 1.78 <sup>a</sup> ± 0.25  | 12.26 <sup>a</sup> ± 0.08  |

BKa - Kattuyanam, RChe - Chennangi, RKku - Karungkuruvai, RPo - Poonghar, RKe - Kesari, BKk - Karupakavuni, RAK - Aruvadam Kuruvai, RMs - Mapillai Samba and WSm - Sona Masuri (control).

IDF - Insoluble dietary fibre, SDF - Soluble dietary fibre, TDF - Total dietary fibre  
Data are Mean ± SD (n = 3). Values in columns with different letters denote statistically significant differences, according to Tukey HSD test ( $p \leq 0.05$ ).

**Table 3: Total starch, resistant starch and digestible starch contents of pigmented rice cultivars and white rice**

| Sample | TS (g/100 g)               | RS (g/100 g)              | DS (g/ 100 g)              |
|--------|----------------------------|---------------------------|----------------------------|
| BKa    | 74.39 <sup>c</sup> ± 0.04  | 2.28 <sup>de</sup> ± 0.07 | 72.11 <sup>ab</sup> ± 0.03 |
| RChe   | 73.36 <sup>ab</sup> ± 0.06 | 2.13 <sup>d</sup> ± 0.08  | 71.86 <sup>a</sup> ± 0.13  |
| RKku   | 74.41 <sup>c</sup> ± 0.09  | 2.05 <sup>d</sup> ± 0.05  | 72.35 <sup>ab</sup> ± 0.03 |
| RPo    | 73.30 <sup>a</sup> ± 0.08  | 1.52 <sup>b</sup> ± 0.10  | 71.78 <sup>a</sup> ± 0.05  |
| RKe    | 79.29 <sup>e</sup> ± 0.03  | 2.37 <sup>d</sup> ± 0.15  | 76.92 <sup>d</sup> ± 0.06  |
| BKk    | 74.03 <sup>c</sup> ± 0.14  | 2.54 <sup>f</sup> ± 0.27  | 71.49 <sup>a</sup> ± 0.22  |
| RAk    | 73.97 <sup>b</sup> ± 0.15  | 1.75 <sup>cd</sup> ± 0.10 | 72.22 <sup>b</sup> ± 0.04  |
| RMs    | 75.36 <sup>d</sup> ± 0.04  | 1.68 <sup>c</sup> ± 0.07  | 73.68 <sup>c</sup> ± 0.35  |
| WSm    | 78.79 <sup>e</sup> ± 0.05  | 0.23 <sup>a</sup> ± 0.11  | 78.56 <sup>e</sup> ± 0.15  |

BKa - Kattuyanam, RChe - Chennangi, RKku - Karungkuruvai, RPo - Poonghar, RKe - Kesari, BKk - Karupakavuni, RAK - Aruvadam Kuruvai, RMs - Mapillai Samba, and WSm - Sona Masuri (control).

TS – Total starch, RS – Resistant starch, DS – Digestible starch.

Data are Mean ± SD (n = 3). Values in columns with different letters denote statistically significant differences, according to Tukey HSD test ( $p \leq 0.05$ ).

### Total Starch, Resistant Starch, and Digestible Starch

The total starch (TS), resistant starch (RS), and digestible starch (DS) contents of the pigmented rice varieties are given in the Table 3. The percentage of TS in the rice varieties varied from 73.30 to 79.29 g/ 100 g. The RS content was found to be the highest (2.54 g/ 100 g) in BKK. All the pigmented rice varieties had significantly ( $p \leq 0.05$ ) higher RS content than WSm. The DS content of the pigmented rice samples ranged from 71.49 to 76.92 g/ 100 g, with the highest and least content in RKe and BKK, respectively. The control, WSm, had significantly higher ( $p \leq 0.05$ ) DS than the other samples.

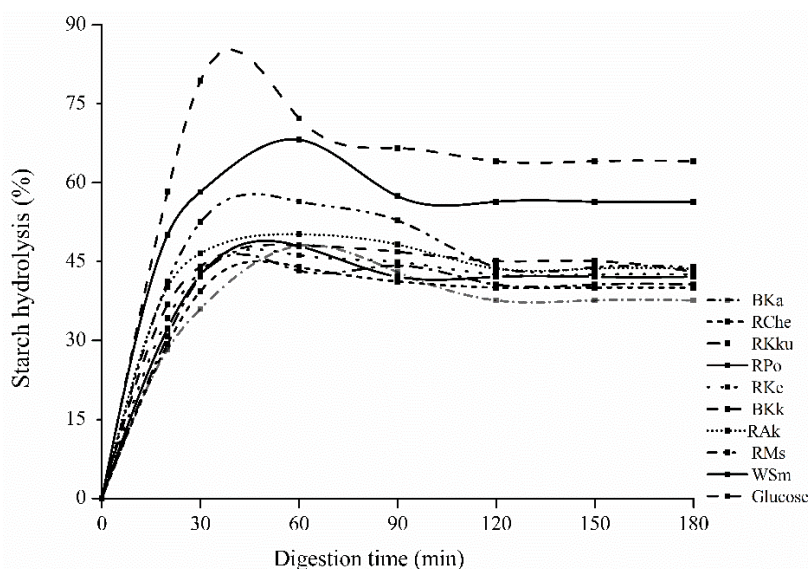
### Starch Digestibility, Kinetics, and Digestible Fractions

The starch hydrolysis curves for the pigmented rice varieties, WSm and glucose are given in Figure 1. The equilibrium concentration ( $C_{\infty}$ ) and kinetic constant ( $k$ ) are shown in Table 4. The highest equilibrium concentration ( $C_{\infty}$ ) was observed in glucose (64.11 %) and the least  $C_{\infty}$  was found in BKa (37.63 %). The sample BKa had significantly lower  $C_{\infty}$  than the other pigmented rice samples. All experimental samples had significantly lower ( $p \leq 0.05$ )  $C_{\infty}$  compared to WSm (56.38 %). With

respect to the rate of hydrolysis, RPo reached a plateau of 42 % at 90 min while other samples reached equilibrium after 120 min. The starch hydrolysis peak occurred at 30 min for glucose, whereas it occurred at 60 min for all the experimental samples and WSm. The starch hydrolysis peak was also lower in all experimental samples compared to WSm and glucose. The digestibility rate constant ( $k$ ) was found to be the least in BKa ( $0.055 \text{ min}^{-1}$ ) and highest in RKe ( $0.084 \text{ min}^{-1}$ ). WSm had significantly higher ( $p \leq 0.05$ )  $k$  ( $0.1 \text{ min}^{-1}$ ) than pigmented rice samples.

The fractions of digested starch (RDS and SDS) of the cooked pigmented rice varieties are presented in Table 3. The amount of RDS in the pigmented rice cultivars was in the range of 28.31 to 36.86 g/ 100 g. Significantly lower ( $p \leq 0.05$ ) contents of RDS were found in RChe and BKa among the pigmented rice samples. Significantly higher ( $p \leq 0.05$ ) amounts of RDS (40.12 g/ 100 g) was noticed in WSm compared to all the pigmented rice varieties.

The SDS content of pigmented rice samples ranged from 7.89 in RKe to 14.23 g/ 100 g in BKa. WSm had a significantly lower ( $p \leq 0.05$ ) SDS content (6.26 g/ 100 g) compared to the pigmented rice samples.



**Fig 1: Starch hydrolysis curves of pigmented rice cultivars and white rice**

BKa - Kattuyanam, RChe - Chennangi, RKku - Karungkuruvai, RPo - Poonghar, RKe - Kesari, BKK - Karupakavuni, RAK - Aruvadham Kuruvai, RMs - Mapillai Samba, and WSm - Sona Masuri (control)

**Hydrolysis Index and Predicted Glycemic Index**

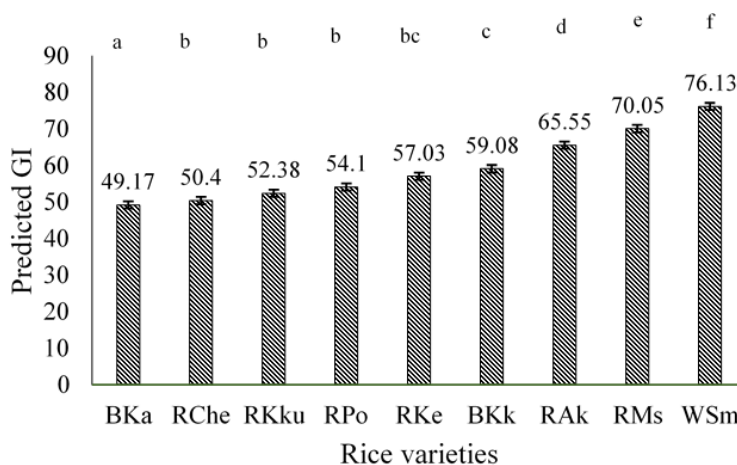
The HI and pGI values of the experimental samples are given in Table 4 and Figure 2, respectively. BKA exhibited the least HI (17.27) and pGI (49.17) values. The highest HI (55.38) and pGI (70) values were found in RMs among the pigmented rice varieties.

According to the classification given by Brand Miller *et al.*,<sup>53</sup> the experimental samples belonging to the low GI category (< 55) include BKA, RChe, RKku, and RPo; the medium GI category (55 – 69) includes RKe, BKK, and RAK; and the high GI category (70 and above) includes RMs.

**Table 4: Equilibrium concentration of hydrolyzed starch, starch hydrolysis rate, rapidly and slowly digestible starch and hydrolysis index of pigmented rice cultivars and white rice**

| Sample | C <sub>∞</sub> (%)          | k (min <sup>-1</sup> )      | RDS (g/ 100 g)             | SDSn (g/ 100 g)            | HI                         |
|--------|-----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| BKa    | 37.63 <sup>a</sup> ± 0.04   | 0.055 <sup>a</sup> ± 0.004  | 28.31 <sup>a</sup> ± 0.07  | 14.23 <sup>g</sup> ± 0.16  | 17.27 <sup>a</sup> ± 0.51  |
| RChe   | 40.09 <sup>ab</sup> ± 0.16  | 0.068 <sup>b</sup> ± 0.007  | 29.81 <sup>a</sup> ± 0.12  | 10.28 <sup>de</sup> ± 0.06 | 19.53 <sup>b</sup> ± 0.41  |
| RKku   | 40.64 <sup>bc</sup> ± 0.11  | 0.066 <sup>b</sup> ± 0.018  | 32.31 <sup>b</sup> ± 0.03  | 11.63 <sup>ef</sup> ± 0.06 | 23.13 <sup>c</sup> ± 0.04  |
| RPo    | 42.12 <sup>bcd</sup> ± 0.21 | 0.073 <sup>c</sup> ± 0.051  | 31.21 <sup>b</sup> ± 0.16  | 9.43 <sup>d</sup> ± 0.08   | 26.28 <sup>cd</sup> ± 0.04 |
| RKe    | 42.54 <sup>cd</sup> ± 0.16  | 0.084 <sup>d</sup> ± 0.009  | 34.23 <sup>bc</sup> ± 0.21 | 7.89 <sup>b</sup> ± 0.17   | 31.62 <sup>d</sup> ± 0.26  |
| BKk    | 43.12 <sup>bcd</sup> ± 0.07 | 0.059 <sup>a</sup> ± 0.031  | 30.19 <sup>ab</sup> ± 0.09 | 13.49 <sup>f</sup> ± 0.06  | 35.36 <sup>e</sup> ± 0.55  |
| RAK    | 43.68 <sup>d</sup> ± 0.05   | 0.076 <sup>c</sup> ± 0.013  | 29.32 <sup>a</sup> ± 0.03  | 8.31 <sup>c</sup> ± 0.14   | 47.18 <sup>f</sup> ± 0.11  |
| RMs    | 43.94 <sup>d</sup> ± 0.06   | 0.078 <sup>cd</sup> ± 0.014 | 36.86 <sup>c</sup> ± 0.22  | 8.26 <sup>bc</sup> ± 0.18  | 55.38 <sup>g</sup> ± 0.02  |
| WSm    | 56.38 <sup>e</sup> ± 0.04   | 0.100 <sup>e</sup> ± 0.011  | 40.12 <sup>d</sup> ± 0.54  | 6.26 <sup>a</sup> ± 0.22   | 66.47 <sup>h</sup> ± 0.46  |

BKa - Kattuyanam, RChe - Chennangi, RKku - Karungkuruvai, RPo - Poonghar, RKe - Kesari, BKk - Karupakavuni, RAK - Aruvadam Kuruvai, RMs - Mapillai Samba, and WSm - Sona Masuri (control). C<sub>∞</sub> - equilibrium concentrations, k - Digestibility rate constant, RDS - Rapidly digestible starch, SDS - Slowly digestible starch, HI - hydrolysis index, Data are Mean ± SD (n = 3). Values in columns with different letters denote statistically significant differences, according to Tukey HSD test (p ≤ 0.05).



**Fig 2: Predicted glycemic index of pigmented rice cultivars and white rice**

BKA - Kattuyanam, RChe - Chennangi, RKku - Karungkuruvai, RPo - Poonghar, RKe - Kesari, BKk - Karupakavuni, RAK - Aruvadam Kuruvai, RMs - Mapillai Samba, and WSm - Sona Masuri (control) Values are Mean ± SD (n = 3). Letters above the bars indicate homogeneous groups and denote statistically significant differences, according to Tukey HSD test (p ≤ 0.05)

**Correlation among Carbohydrate Components**

The results of the correlation analysis among the different carbohydrate components are presented in Table 5. A highly significant positive correlation (at  $p \leq 0.01$ ) was observed between RS and all dietary fibre components, TDF ( $r = 0.889$ ), SDF ( $r = 0.857$ ), and IDF ( $r = 0.876$ ). A strong positive correlation was also observed between amylose and dietary fibre contents at  $p \leq 0.01$ . RS also had a significant ( $p \leq$

0.05) positive correlation with amylose content. With respect to digestible starch fractions, RDS had a significant negative correlation with amylose content, dietary fibre components and RS. Rapidly digestible starch had significant ( $p \leq 0.05$ ) positive correlation with the total starch content. A significant negative correlation was observed between total DS and TDF as well as IDF ( $r = 734$ ;  $p \leq 0.05$ ).

**Table 5: Pearson's correlation coefficient between different carbohydrate components**

|            | <i>TDF</i> | <i>SDF</i> | <i>IDF</i> | <i>AC</i> | <i>TS</i> | <i>RS</i> | <i>DS</i> | <i>RDS</i> | <i>SDS</i> |
|------------|------------|------------|------------|-----------|-----------|-----------|-----------|------------|------------|
| <i>TDF</i> | 1.000      |            |            |           |           |           |           |            |            |
| <i>SDF</i> | 0.916**    | 1.000      |            |           |           |           |           |            |            |
| <i>IDF</i> | 0.996**    | 0.879**    | 1.000      |           |           |           |           |            |            |
| <i>AC</i>  | 0.878**    | 0.947**    | 0.845**    | 1.000     |           |           |           |            |            |
| <i>TS</i>  | -0.577     | -0.483     | -0.585     | -0.507    | 1.000     |           |           |            |            |
| <i>RS</i>  | 0.889**    | 0.858**    | 0.877**    | 0.784*    | -0.384    | 1.000     |           |            |            |
| <i>DS</i>  | -0.734*    | -0.660     | -0.734*    | -0.650    | 0.966**   | -0.599    | 1.000     |            |            |
| <i>RDS</i> | -0.837**   | -0.870**   | -0.811**   | -0.906**  | 0.747*    | -0.724*   | 0.838**   | 1.000      |            |
| <i>SDS</i> | 0.655      | 0.621      | 0.648      | 0.563     | -0.588    | 0.691     | -0.706*   | -0.722*    | 1.000      |

TDF - Total dietary fibre, SDF - Soluble dietary fibre, IDF - insoluble dietary fibre, AC - Amylose content, TS - Total starch, RS - Resistant starch, DS - Digestible starch, RDS - Rapidly digestible starch, SDS - Slowly digestible starch

Correlation significant at \* $p \leq 0.05$ , and \*\*  $p \leq 0.01$ .

**Table 6: Pearson's correlation coefficient of carbohydrate components with hydrolysis index and predicted glycaemic index**

| Variables                 | Hydrolysis index | Predicted glycaemic index |
|---------------------------|------------------|---------------------------|
| Total dietary fibre       | -0.841**         | -0.840**                  |
| Soluble dietary fibre     | -0.595*          | -0.593                    |
| Insoluble dietary fibre   | -0.891**         | -0.891**                  |
| Amylose                   | -0.732           | -0.742                    |
| Total starch              | 0.565            | 0.564                     |
| Resistant starch          | -0.865**         | -0.865**                  |
| Digestible starch         | 0.614            | 0.613                     |
| Rapidly digestible starch | 0.593            | 0.589                     |
| Slowly digestible starch  | -0.753*          | -0.745*                   |

Correlations are significant at \* $p \leq 0.05$ , and \*\*  $p \leq 0.01$ .



### Correlation between Carbohydrate Components and pGI

The correlation results between *in vitro* glycemic response, pGI and carbohydrate components are presented in Table 6. A highly significant ( $p \leq 0.01$ ) negative correlation was observed for pGI with IDF ( $r = -0.891$ ), RS ( $r = -0.865$ ) and TDF ( $r = -0.84$ ). SDS too showed a significant ( $p \leq 0.05$ ) negative correlation ( $r = -0.745$ ) with pGI. A non-significant negative correlation was observed for pGI with amylose content ( $r = -0.742$ ) and SDF ( $r = -0.593$ ).

### Discussion

#### Carbohydrate Components

#### Dietary Fibre Components

Dietary fibre has been reported to exert several physiological health benefits such as loss of weight, laxative effect, lowering blood cholesterol levels, dietary management during diabetes, and improving gut health among others.<sup>25</sup> The TDF and IDF values of the experimental pigmented samples were similar to the range reported by Deepa *et al.*,<sup>26</sup> i.e. TDF - 5.82 to 8.08 g/ 100 g and IDF - 5.39 to 7.56 100 g/100 g, but the SDF content of the experimental samples was slightly higher than the reported value (0.43 to 0.52 g/ 100 g) for two red rice varieties, Jyothi and Njavara from India. However, the SDF contents of the experimental samples were similar to the content reported (0.90 to 1.25 g/ 100 g) by Savitha and Singh<sup>27</sup> for four other red rice varieties from India. The experimental samples had higher TDF content than those reported (2.82 to 4.51 g/100 g) in nine pigmented rice varieties from Thailand, Sri Lanka and China.<sup>28</sup> The bran of pigmented rice is reported to contain 2 to 35 % higher dietary fibre content than bran of white rice varieties.<sup>29</sup> Cellulose, arabinoxylans and pectin are the main classes of dietary fibre reported to be present in rice bran layer.<sup>30</sup> The partially milled and parboiled sample, RMs had the least TDF and IDF content among the pigmented rice samples. It was reported by Savitha and Singh<sup>27</sup> that on parboiling, 5 to 11% reduction of dietary fibre content was observed in 4 red rice varieties from India.

#### Amylose Content

Amylose has a lot of industrial importance in terms of medical treatments, improving food product properties, films and packaging development,

textiles and paper making, optical fibres, printed circuit boards, electronic chips, etc.<sup>31</sup> Amylose contents of the experimental samples were similar to the values reported by Thuengtung *et al.*,<sup>11</sup> for red and black rice varieties (20 to 28 g/100 g) from Thailand. However, the experimental samples had higher amylose content than the values reported (22.7 and 22.9 g/100 g) for two other red rice varieties from India<sup>26</sup> which could be due to varietal differences. The high amylose pigmented rice varieties identified in this study can have an important nutritional role by slowing the rate of starch digestibility and hence glucose release in the body, beneficial particularly to diabetics. Ingestion of high amylose rice in human volunteers have been earlier reported to significantly delay digestion and/ absorption of carbohydrates and to reduce serum glucose and insulin response.<sup>32</sup>

#### Rice Starch Components

Total starch and digestible starch contents of the experimental samples were slightly lower than the values reported (TS – 79.56 to 84.30 g/ 100 g, DS – 79.16 to 83.96 g/ 100 g) by Deepa *et al.*,<sup>15</sup> for two red rice varieties from India. The differences could be due to genotypic variation. However, Thuengtung *et al.*,<sup>11</sup> reported similar TS content ranging from 71.44 to 73.76 g/ 100 g for three pigmented rice cultivars from Thailand.

Among all the starch components, Resistant starch (RS) has gained a lot of importance for its functional properties such as its lower influence on the sensory quality of food in comparison with traditional fibre sources. Its physicochemical properties like gel forming ability, swelling ability, viscosity, and water-binding capacity are more desirable, making it a beneficial ingredient in the food industry. More importantly, RS is reported to beneficially impact the working of the gastrointestinal tract, gut microbiota, the blood cholesterol level, and the glycemic index thereby, assisting in the regulation of glycaemic response, and hence, management of diabetes.<sup>33</sup> The RS content of the experimental pigmented samples were within the range (0.35 to 2.57 g/ 100 g) reported in Jyothi and Njavara, two red rice varieties from India,<sup>15</sup> but were higher than the values reported (0.14 to 0.39 g/ 100 g) by Thuengtung *et al.*,<sup>11</sup> for pigmented rice varieties from Thailand.

To understand the nutritional importance of starch, the concept of slowly digestible starch and rapidly digestible starch has been evolved. The positive health effects of SDS are related to regulated glucose metabolism, diabetes management, and satiety.<sup>34</sup> The SDS contents of the experimental pigmented samples were higher than the range reported (0.3 to 6.2 %) for commercial white rice cultivars of different sizes from Canada, U.S, Italy, and Thailand.<sup>35</sup> The RDS starch content of the pigmented rice varieties was lower than reported values (79.4 to 87.9 %) in the same study. The SDS content of the experimental samples were slightly lower than the reported value (48 %), and the RDS content was similar to the reported value (24.8 %) for Riceberry, a red rice from Thailand.<sup>14</sup>

#### **Correlation among Carbohydrate Components**

The strong positive correlation observed between resistant starch (RS) and amylose level were in agreement with the literature.<sup>36</sup> Amylose content has been classified as a type of resistant starch, hence rice varieties having higher amylose content have been known to contain higher resistant starch.<sup>37</sup> Several reasons have been speculated for the positive correlation between resistant starch and amylose content. The main enzyme accountable for amylose synthesis is granule bound starch synthase1 (gbssl) in the starch biosynthetic pathway. The RS content of rice grain is facilitated by soluble starch synthase enzyme which in turn requires high levels of gbssl expression,<sup>38</sup> hence leading to high amylose and RS content. Resistant starches are of different types. Type 5 RS is a stable complex formed between amylose and lipid.<sup>8,34</sup> The high amylose content recorded in the experimental samples could have contributed to the formation of type 5 RS, and overall a higher content of RS in the pigmented rice samples. A study reported that twenty rice varieties showed a positive correlation between RS and amylose content and confirmed that amylose could be used as a determining factor to correlate with RS in rice.<sup>39</sup>

The positive correlation between RS and DF could be because RS is one of the many components that comprise dietary fibre. Resistant starches have also been reported to function similarly to dietary fibre in the large intestine.<sup>40</sup> Deepa *et al.*,<sup>15</sup> also reported a positive but insignificant correlation between RS and IDF ( $r = 0.624$ ), and TDF ( $r = 0.6$ ).

#### **In Vitro Starch Digestibility and Hydrolysis Index**

Cereals having low digestibility are desirable especially in the dietary management of metabolic conditions like diabetes.<sup>41</sup> The  $C_{\infty}$  of the experimental pigmented rice samples were lower than the values reported (61.5 and 65.2 %) for two red rice varieties from India,<sup>15</sup> but were higher than the reported values (9.52 to 13.25 %) for two red and two purple rice varieties from Thailand.<sup>13</sup> Similar to the current study, this study also reported a lower  $C_{\infty}$  in Thai pigmented rice varieties than the white rice, Hom Mali. The digestibility rate constant values of all the studied pigmented rice samples were similar to the values ( $k = 0.073$  to  $0.095$ ) reported by Deepa *et al.*, for two other red rice varieties from India (Njavara and Jyothi).<sup>15</sup> The hydrolysis indices of the studied pigmented samples were lower than the reported values (60.9 to 63.9) in two Indian red rice varieties,<sup>15</sup> and similar to the range reported (37.46 to 47.03) in pigmented rice varieties from Thailand.<sup>13</sup>

The lower starch digestibility of experimental pigmented rice varieties could be due to the presence of high RS. The digestibility of starchy foods like cereals has been reduced considerably due to the presence of RS which cannot be hydrolyzed by amylase enzymes.<sup>42</sup> The dietary fibre,  $\alpha$ -cellulose present in pigmented rice bran has been reported to inhibit  $\alpha$ -amylase activity and slow the starch digestion rate through competitive inhibition due to the greater affinity of  $\alpha$ -amylase for  $\alpha$ -cellulose than starch.<sup>30</sup> The role of amylose in slowing starch digestibility reported previously<sup>43</sup> could be confirmed by the negative correlation observed between amylose and hydrolysis index in the present study. Low amylose content results in low hydrogen bonds formed between chains which induces weak arrangement of the crystalline starch structure, which could result in a greater vulnerability to enzyme hydrolysis and an increase in the kinetic rate constant.<sup>44</sup> The significantly lower resistant starch and amylose content in WSm could have contributed to its rapid starch digestion rate.

#### **In Vitro Glycemic Response and Influence of Carbohydrate Components**

With very few exceptions, majority of the studies to date have reported pigmented rice varieties having moderate to high GI. The pGI values indicative of *in vitro* glycemic response of the experimental

pigmented rice samples were lower than the values reported (pGI – 73.1 to 74.8) by Deepa *et al.*,<sup>15</sup> and lower or similar to the values reported (pGI – 60.79 to 70.36) by Kumar *et al.*,<sup>38</sup> for different pigmented and brown rice varieties from Southern and Eastern regions of India, respectively. Majority of the samples in the present study also recorded lower pGI values compared to those (pGI 65 to 83) reported in ten pigmented rice cultivars from Thailand.<sup>45,14</sup> The pGI values of the experimental samples BKa and BkK were similar to the *in vivo* GI values of the same varieties (BKa – 47 and BkK – 56) reported by Meera *et al.*,<sup>16</sup> indicating the robustness of the *in vitro* method used. Two other varieties in the same study have recorded GI greater than 60. In an *in vivo* study on four Sri Lankan pigmented rice<sup>46</sup> only one variety (Weddaheenati) was reported to have a low GI (52.5), whereas the other three rice varieties were reported to have medium GI values (56.3 – 64). The higher pGI of RMs in this study could be because the sample was partially milled and had relatively lower levels of dietary fibre and amylose content. Somaratne *et al.*,<sup>47</sup> reported that the *in vivo* GI of red rice basmati varieties, CIC-RB and CIC-RF increased by 11% and 17.6% respectively, when the rice milling level was increased from 10 % to 100 %. The low pGI experimental pigmented rice varieties could be used as a gluten free healthier alternative, especially in the global dietary management of diabetes. Studies are also underway to confirm their antihyperglycemic potential using *in vivo* studies and antidiabetic potential using several mechanistic assays.

Among all the starch fractions, RS was observed to be one of the key factors affecting the glycemic index of the experimental rice samples. Kumar *et al.*,<sup>38</sup> in their study on 24 rice genotypes also observed a strong correlation between RS and pGI. Van Hung *et al.*,<sup>48</sup> also reported a significant negative correlation ( $r = -0.747$ ;  $p \leq 0.01$ ) between RS and predicted GI in a study on white rice varieties from Vietnam. The other carbohydrate fractions with a major influence on glycemic response were IDF and TDF. In line with this observation, Wolever<sup>49</sup> also reported a significant correlation ( $r = -0.461$ ;  $p \leq 0.05$ ) for TDF and IDF from different carbohydrate-based foods with their *in vivo* GI values. Similar to our study, Odenigbo *et al.*,<sup>50</sup> reported a negative correlation between SDS and pGI in a study on starch digestibility of fried sweet potato.

Amylose content has been reported to negatively correlate with glycemic index<sup>9</sup> as observed in the present study. However, in the present study it was not found to be a significant correlation. This could be because all the experimental pigmented rice varieties except RMs had similar high amylose content. The significant differences in the predicted glycemic index among all the experimental samples having similar amylose content indicate that factors other than amylose content, such as granule size, physicochemical properties, and degree of crystallinity could also play a major role in affecting the starch digestibility,<sup>51</sup> hence the resultant glycaemic response. A recent review has reported non-starch components such as dietary fibre to have a greater influence on the starch digestibility of rice than factors such as amylose content<sup>52</sup> as observed in the present study. The role of phytochemicals such as phenolics acids, flavonoids, and anthocyanins on starch digestibility in pigmented rice is another aspect that is being explored by the research team.

### Conclusions

This study has helped to identify four pigmented rice varieties from the southern part of India having low, and three having medium *in vitro* GI which could be beneficial to the huge rice-eating population. The study indicates that the levels of IDF, TDF and RS could be used to get a preliminary assessment of the glycemic response of pigmented rice cultivars as they were found to be significantly inversely related to pGI. The study revealed that processing (milling and parboiling) of pigmented rice sample affect the GI and needs to be considered while evaluating their glycemic response. Some of the experimental samples exhibiting high levels of resistant starch, dietary fibre, amylose content and slowly digestible starch content could also provide several other physiological health benefits making them a choice ingredient in designing functional foods.

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#### Conflict of interest

The authors declare no conflict of interest.

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