

Grain Colour and Type as Determinants of Glycemic Response in Medicinal and Aromatic Rice Varieties of Kerala

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Abstract

This study aimed to estimate the Glycemic Index (GI) of medicinal and aromatic rice varieties from Kerala and compare them with Uma, a high-yielding, non-medicinal cultivar. Medicinal varieties exhibited relatively higher resistant starch (RS) content; however, cooking significantly reduced RS across all samples. The varieties represented diverse grain colours (brown, red, and white), yet grain colour alone was not a reliable predictor of GI. Notably, the released rice variety Uma (red) showed the highest GI, exceeding that of the white aromatic types, whereas the Njavara Black variety consistently exhibited the lowest GI. These findings highlight that biochemical attributes, particularly amylose and RS content, play a more decisive role in modulating glycemic response than visual traits such as grain colour. The estimated glycemic index values obtained from the *in vitro* digestion model provide preliminary estimates of glycemic potential and should not be considered direct evidence of clinical glycemic responses or as a basis for specific dietary recommendations for individuals with diabetes. Further validation using broader rice germplasm, diverse cooking and processing conditions, and controlled *in vivo* and human postprandial studies is warranted. The study underscores the importance of selecting rice based on functional and nutritional properties for potential dietary and therapeutic applications. Integrating traditional knowledge with scientific evaluation can facilitate the identification of rice varieties with potentially favourable health benefits.

Categories: Agricultural and food economics, Food security and sustainability, Food biochemistry

Keywords: resistant starch, digestible starch, total starch, hydrolysis index, estimated glycemic index, *in vitro* starch digestion

Introduction

Lifestyle modification, especially dietary regulation and regular physical activity, has become central to the prevention and management of type 2 diabetes mellitus (T2DM), reducing dependence on pharmacological interventions. Among dietary factors, the quality of carbohydrates, rather than their absolute quantity, plays a significant role in determining the postprandial glycemic response. The concept of the Glycemic Index (GI) is defined as the incremental area under the blood glucose response curve over 2 hours following consumption of a fixed carbohydrate portion relative to a reference food [1]. Frequent consumption of such high-GI foods may contribute to the development of type 2 diabetes and associated complications. By the year 2030, it is estimated that about 630 million people from both developed and developing countries may be diagnosed with type 2 diabetes, underscoring the urgency of identifying staple foods with improved glycemic profiles [2].

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Rice (*Oryza sativa* L.) constitutes the primary dietary carbohydrate source for much of the Asian population. Despite its cultural and nutritional importance, polished white rice is often categorized as a high-GI food due to its rapid digestibility. However, rice is not metabolically homogeneous. Substantial variation exists among cultivars in starch composition, structural organization, and non-starch bioactive constituents, all of which influence glycemic response. The state of Kerala is well known for several indigenous rice varieties that have been under cultivation for centuries. Indigenous rice landraces of Kerala represent a valuable but underexplored genetic resource in this context. Several of these varieties, such as Njavara, Jeerakasala, Gandhakasala, Pokkali, Palakkadan Matta, and Kaipad, are traditionally recognized for their nutritional richness, elevated fiber content, and perceived lower glycemic impact compared with polished white rice. The medicinal variety Njavara, in particular, holds a distinct position in Ayurvedic practice, including Panchakarma therapy, suggesting unique compositional attributes with potential metabolic relevance.

The glycemic behaviour of rice is primarily governed by starch quantity and structural organization [3]. Starch consists of two major polymers: amylose, a predominantly linear α -(1 $\rightarrow$ 4)-linked glucan, and amylopectin, a highly branched molecule with α -(1 $\rightarrow$ 6) linkages. The amylose-to-amylopectin ratio strongly influences starch physicochemical properties, enzymatic susceptibility, and postprandial glucose release. High-amylose rice typically exhibits increased formation of ordered double helices and retrograded starch structures, limiting enzymatic accessibility and slowing hydrolysis. As a result, higher amylose content is generally associated with reduced starch digestibility, lower hydrolysis index (HI), and decreased estimated glycemic index (eGI).

Mechanistically, starch digestibility can be partitioned into rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS) [4]. Elevated amylose content promotes the formation of RS and SDS fractions, which resist or delay enzymatic breakdown in the small intestine. Resistant starch escapes digestion and undergoes colonic fermentation to produce short-chain fatty acids, initiating additional metabolic benefits, including improved insulin sensitivity and glycemic stability [5-7].

Thus, amylose content serves not merely as a compositional trait but as a structural determinant influencing glycemic response through modulation of starch digestibility kinetics. However, amylose content alone does not fully predict GI. Rice varieties with comparable amylose levels may exhibit distinct glycemic responses due to differences in starch granule morphology [8], crystalline architecture, degree of polymerization, protein-starch interactions, lipid complexes, and the presence of phenolic components [9,10]. Grain pigmentation (red, brown, or white) may further influence glycemic behaviour through associated bioactive compounds, although colour does not necessarily reflect starch functionality. Therefore, an integrated evaluation of starch fractions, amylose content, and *in vitro* digestibility is essential to accurately estimate glycemic potential.

While *in vivo* GI determination remains the gold standard [11], it is constrained by ethical considerations, inter-individual variability, cost, and methodological inconsistencies across laboratories [12-14]. An enzyme-based *in vitro* digestion method that simulates oral, gastric, and intestinal phases was developed [15], which provides a reproducible and cost-effective [16] alternative for estimating HI and eGI. These systems sequentially employ salivary and pancreatic α -amylases followed by amyloglucosidase to replicate physiological starch breakdown, enabling kinetic assessment of glucose release.

In this context, the present study systematically evaluates the *in vitro* starch digestibility, HI, and eGI of selected medicinal and aromatic rice varieties of Kerala in both raw and cooked forms. Resistant starch, non-resistant starch, total starch, and amylose content were quantified to elucidate their structural and functional interrelationships. By comparing varieties with similar grain pigmentation but contrasting functional attributes, such as the high-yielding released variety Uma (red) and the medicinal Njavara Black rice, this study investigates whether visual classification correlates with metabolic behaviour. Together, this integrated compositional and functional approach establishes a mechanistic framework linking amylose content to starch hydrolysis kinetics and eGI, thereby supporting the identification and development of nutritionally superior rice genotypes with potentially favourable glycemic characteristics.

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Materials And Methods

Rice varieties: Seven rice varieties including medicinal, aromatic, and released rice varieties were used for the study. The properties of the rice varieties selected were shown in Table 7.

Name of the variety	Type of rice variety	Grain colour	Maturation time (days)	Collection site
Njavara Black	Medicinal	Red	70–75	Njavara Eco Farm, Chittoor, Palakkad, Kerala
Njavara Yellow	Medicinal	Red	70–75	Njavara Eco Farm, Chittoor, Palakkad, Kerala
Raktasali	Medicinal	Red	110	Farmer's plot, Chirayinkil, Thiruvananthapuram, Kerala
Mullan Kaima	Aromatic	Straw-coloured	150–180	Thirunelly Agro Producer Company, Wayanad, Kerala
Jeerakasala	Aromatic	Straw-coloured	150–180	Thirunelly Agro Producer Company, Wayanad, Kerala
Gandhakasala	Aromatic	Straw-coloured	150–180	Thirunelly Agro Producer Company, Wayanad, Kerala
Uma (MO16)	High-yielding released variety	Semi-polished red rice	120–180	Rice Research Station, Kerala Agricultural University, Moncombu, Alappuzha, Kerala

TABLE 1: Properties of rice varieties used for the study

All rice samples were analysed in the form in which they are traditionally consumed to ensure physiological and dietary relevance of the findings. The medicinal rice varieties, including Njavara Black and Yellow (Glume colour), were evaluated in their unpolished (raw) form, as these landraces are customarily consumed without milling in traditional therapeutic and dietary practices (Figure 7), Uma and the aromatic rice varieties such as Jeerakasala and Gandhakasala were analysed in their parboiled form, consistent with prevailing post-harvest processing and consumption patterns in Kerala.



FIGURE 1: Rice varieties selected for the study

Sample preparation

Rice flour: The rice was ground well using liquid nitrogen in a mortar and pestle. The flour was passed through a 60-mesh sieve under laboratory conditions.

Cooked rice: All samples were cooked following the traditional boiling method. Rice varieties were soaked in water for 1 hour and cooked in a 50 ml test tube for 30 minutes in a boiling water bath. All the samples reached a soft consistency, and the aromatic rice varieties were sufficiently flavoured. The test tubes were removed, and excess water was decanted. The cooking and soaking time was standardized in multiple trials.

Estimation of amylose content

Amylose content in all rice varieties was determined using the iodine colorimetric method. Briefly, 100 mg of powdered sample was gelatinized using ethanol and sodium hydroxide. The gelatinized samples were treated with iodine, which is absorbed by the helical coils of amylose and forms a starch-iodine complex, and the colour was read at 620 nm in triplicates using a spectrophotometer. Amylose content of the rice varieties was determined from the standard curve expressing the data as percent amylose content. The standard curve was made by gelatinizing 40 mg of purified potato amylose (Sigma) and preparing a series of standard solutions. The absorbance of the standard solutions was read at 620 nm after treating with the iodine solution. The absorbance values were plotted at 620 nm against the concentration of anhydrous amylose (mg) and the conversion factor was determined. The dilution factor of 20 for the samples is included in the conversion factor.

Determination of resistant, digestible, and total starch in rice varieties

The resistant starch and non-resistant or digestible starch contents were estimated using the Megazyme Resistant Assay Kit (Megazyme Ltd, Ireland) following the manufacturer's instructions with few modifications.

Resistant Starch Hydrolysis and Solubilization

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A total of 100 mg of sample (Cooked and Raw powdered rice) was placed in a 15 ml polyethylene tube, mixed with 4 ml of enzyme solution (10 mg/ml pancreatic alpha amylase and 3 U/ml AMG. The sample and enzyme solutions were vortexed and incubated at 37°C for exactly 16 hours under constant shaking (200 strokes/minutes). At the 16th hour, the mixture was mixed with 4 ml absolute ethanol and mixed well. The tubes were uncapped and centrifuged at 1,500 g for 10 minutes. The supernatant was collected in a separate 50 ml tube and the pellet was resuspended by adding 2 ml of 50% ethanol and vortexed. In all, 6 ml of 50% ethanol was added to the vortexed mixture and centrifuged at 1,500 g for 10 minutes. The supernatant was again collected in the 50 ml tube and the pellet was resuspended by adding 2 ml of 50% ethanol and vortexed for 5 minutes and recentrifuged at 1,500 g for 10 minutes. The supernatant was again collected in the 50 ml tube, and the pellet was dried slightly to remove the excess ethanol (done by inverting the tube on an absorbent paper). The supernatants collected during the centrifugation can be utilized for digestible or non-resistant starch determination.

Quantification of Resistant Starch

The residual pellet obtained after the starch hydrolysis was used to determine the RS content. The pellet was initially dissolved in 2 ml potassium hydroxide (2M KOH) by stirring vigorously for 20 minutes in an ice-cold water bath (do not vortex and preferably stir it by placing it over a magnetic stirrer). The pellet was dissolved well to prevent the formation of starch lumps. After dissolving the pellet, 8 ml of sodium acetate (1.2M NaOAc, pH 3.8) was added with continuous stirring over a magnetic stirrer. Immediately, 100 µl of AMG (3,300 U/ml) was added, mixed well, and incubated at 50°C for 30 minutes (vortexed briefly every 5 minutes during the incubation period). The mixture was then centrifuged at 1500 g for 10 minutes. The volume of the supernatant was 10.5 ml. In all, 100 µl aliquot in triplicates from the tube was taken in a 15 ml tube and 3 ml of GOPOD reagent (glucose oxidase peroxidase) was added and the mixture was incubated at 50°C for 20 minutes. A blank reagent solution (mixing 100 µl of 0.1M sodium acetate buffer (pH 4.5) and 3 ml GOPOD reagent) was also maintained. A standard glucose solution was also prepared in triplicates by mixing 100 µl of glucose standard (1-10 mg/ml) with 3 ml GOPOD reagent and incubated at 50°C for 20 minutes. After incubation, the absorbance of the samples and standard solutions were measured at 510 nm against the reagent blank in a Genesys 180, UV-Vis spectrophotometer (ThermoFisher Scientific, USA). RS content was calculated as explained in the Megazyme Resistant Starch Assay Kit on the basis of dry weight of starch [17].

$$\% \text{Starch} = \frac{\Delta A \times F \times 100 \times 1}{0.1 \times 100 \times W} \times \frac{162}{180} = \frac{\Delta A \times F}{W} \times 90$$

ΔA = Change in absorbance (Sample absorbance – Blank absorbance)

F = Factor obtained from the glucose standard curve

W = Weight of the sample taken (mg or g, depending on protocol)

100 = Conversion factor to express the value as percentage

1/0.1 = Correction factor for aliquot volume used in the assay

162/180 = Conversion factor of glucose to starch

Molecular weight of glucose = 180

Molecular weight of anhydroglucose unit in starch = 162

Quantification of Digestible or Non-Resistant Starch

The supernatant pooled after starch hydrolysis at 16 hours was used to estimate the digestible fraction of starch. The combined supernatant solution was made up to 100 ml using 100 mM sodium acetate buffer (pH 4.5). A total of 100 µl in triplicates from the diluted sample was collected in a 15 ml tube and 10 µl of AMG solution (300 U/ml in 100 mM sodium maleate buffer, pH 6.0) was added at 50°C for 20 minutes. In all, 3 ml of GOPOD reagent was added to the tube and the

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mixture was incubated at 50°C for 20 minutes. The absorbance was measured at 510 nm against the reagent blank using a Genesys 180, UV-Vis spectrophotometer (ThermoFisher Scientific, USA). The percentage of non-resistant starch was calculated as mentioned in the Megazyme Resistant Starch kit [4].

Quantification of Total Starch

The total starch (TS) content of both cooked and raw powdered rice samples was calculated as

Total Starch = Resistant Starch + Non-Resistant Starch

Estimation of In-vitro Starch Hydrolysis and Estimated Glycemic Index Prediction

A total of 100 mg of sample was ground well to a fine paste using 10 ml of HCl-KCl buffer (pH 1.5) and transferred to a 15-ml polyethylene tube. Immediately, 200 µl of pepsin solution (Pepsin stock: 1 g pepsin dissolved in 10 ml of HCl-KCl buffer at pH 1.5) was added. The mixture was stirred well and incubated at 40°C for 1 hour under continuous shaking at a medium speed. After incubation, the volume of the mixture was adjusted to 20 ml using Tris-Maleate buffer (pH 6.9). To the diluted solution, 5 ml of alpha-amylase solution (40 mg/ml Tris-Maleate buffer) was added, and the mixture was incubated at 37°C with continuous shaking at medium speed.

From the mixture, 100 µl aliquots in triplicate were collected at every 30-minute interval (30, 60, 90, 120, 150, and 180 minutes) during the incubation period. At each collection time, the aliquots were placed in a 100°C water bath for 5 minutes to block the enzyme activity and cooled rapidly. The cooled aliquots were kept at 4°C till the end of the incubation. The process continued for all the samples collected at each time period. After collecting the digested starch at each time period, the tubes were added with 400 µl of sodium acetate buffer (0.4 M, pH 4.5) and 3 µl of AMG (3,300 U/ml). The mixture was incubated at 50°C for 30 minutes to hydrolyze digested starch to free glucose. Then, 3 ml of GOPOD reagent was added to the mixture, and the mixture was incubated at 50°C for 20 minutes. A reagent blank was also prepared. The absorbance of each solution was read at 510 nm using a Genesys 180 UV-Vis spectrophotometer (ThermoFisher Scientific, USA). The average value was calculated and used to plot curves.

The amount of starch present in each time period was calculated using the percentage of starch as described by Englyst [4]. The starch digestion rate was expressed as the percentage of hydrolyzed starch (estimated at 30-minute intervals for 3 hours) using the following equation:

Percentage of Starch hydrolyzed = Percentage of starch at specific time point/ Percentage of Total Starch × 100

The percentage of starch hydrolyzed was plotted against the corresponding time period (in min) and the area under curve (AUC) for both cooked and raw powdered samples was estimated utilizing the trapezoid method (Microsoft Excel 2021). The HI was calculated to check how rapidly the starch was digested during the 3-hour digestion relative to the reference food, white bread.

Percentage of Hydrolysis Index (HI%) = AUC of sample/AUC of reference food × 100

The eGI of the selected rice varieties in cooked and raw powdered form was determined according to the calculated HI percentage. The calculation followed to predict eGI [17] is shown below

$$eGI = 39.71 + (0.549 \times HI)$$

Statistical analysis

All the data were analyzed in triplicate and expressed as mean ± standard deviation (SD). The 95% confidence intervals (CIs) were calculated using the two-sided Student's t-distribution for the mean, because there are only three replicates (n = 3) and the population SD is unknown. Here, df = 2 and t = 4.303. The data were statistically analysed using the GraphPad PRISM software version 7 (GraphPad Software Inc., California, USA).

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Results And Discussion

The association between white rice consumption and the risk of type II diabetes mellitus is studied and explained well in many reports [18-21]. Although rice remains a major staple food contributing significantly to India’s food and nutritional security, its contribution to total calorie intake has slightly declined in recent years due to changing dietary patterns and diversification of food habits, particularly in urban populations [22]. The state of Kerala has a rich diversity of traditional rice varieties including scented, medicinal, brown, red, and black rice. In the present study, we have done the *in vitro* starch digestion of two medicinal rice varieties, Njavara (Black and Yellow glume coloured) and Raktasali, three scented or aromatic rice varieties, Jeerakasala, Gandhakasala, and Mullankaima, and a released variety, Uma (Released from RRS, Kerala Agricultural University, Moncombu), and predicted the estimated glycemic index. The medicinal rice varieties are widely used in Kerala for Ayurvedic preparations. Njavara varieties are used for neurological disorders, and Raktasali is used as an immune booster and for blood purification. The aromatic rice varieties such as Jeerakasala and Gandhakasala are most popular and used in culinary purposes on special occasions and are priced high. Mullankaima rice is highly aromatic and used for preparing special dishes and also consumed as raw. Uma (MO16), the red released rice variety of Kerala, covers 80% of the paddy fields in Kerala, and was taken for the study to check the relation of a popular red rice variety with medicinal and aromatic traditional rice varieties.

Amylose content of medicinal and aromatic rice varieties

The amylose concentration was determined by comparing with a standard curve plotted using potato amylose. The amylose content of Njavara Black, Njavara Yellow, Raktasali, Jeerakasala, Gandhakasala, Mullankaima, and Uma was determined through iodine colorimetric method (Table 2).

Rice variety	Amylose content (%)	AC type
Njavara Black	22.90 ± 0.005	High
Njavara Yellow	22.70 ± 0.026	High
Raktasali	22.10 ± 0.095	High
Jeerakasala	16.60 ± 0.028	Low
Gandhakasala	11.50 ± 0.011	Low
Mullankaima	17.80 ± 0.017	Intermediate
Uma	19.80 ± 0.011	Intermediate

TABLE 2: Amylose content and amylose classification of the selected rice varieties

Amylose content varied significantly between the medicinal and aromatic rice varieties, which had a good impact on their eating and cooking qualities. Eating and cooking qualities are considered among the most significant traits in rice to meet the market demands and also for breeding programmes. Previous studies have identified traditional Kerala rice varieties such as Kuruva rice and Pokkali rice as high-amylose rice cultivars, characterized by slower starch digestibility and a comparatively lower glycemic response [23]. Based on the classification criteria, the rice varieties were classified on the basis of amylose content into types as: High amylose content (>22% amylose), Intermediate (17-22% amylose), Low

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(10-17% amylose) and very low (<10% amylose). The amylose content of the medicinal rice varieties was observed to be high, while aromatic rice varieties such as Jeerakasala and Gandhakasala have low amylose content. Among the aromatic rice varieties, Mullankaima rice and the released rice variety Uma have intermediate amylose content. Njavara Black and Yellow glumed medicinal rice varieties were considered the same with varying glume colour, but they also have slight variation in the amylose content. The amylose content of Raktasali rice is lower when compared to that of the Njavara varieties. The high and intermediate amylose rice varieties will digest slowly and may increase the resistant starch levels and lower the glycemic load of the rice after consumption. A comparative evaluation of traditional Kerala rice varieties with two widely cultivated high-yielding varieties demonstrated that the traditional landraces predominantly exhibited intermediate to high amylose content, indicating their potential nutritional and functional significance. Resistant, digestible, and total starch content in medicinal and aromatic rice varieties [24].

Resistant, digestible, and total starch content in medicinal and aromatic rice varieties

The rate of resistant and digestible starch affects the starch hydrolysis rate and consequently relates to the amount of glucose released. Measuring the starch fractions could predict the rate of starch digestion pattern in the small intestine of humans. RS plays an important role in regulating postprandial glucose response by slowing starch digestion and limiting glucose release, thereby reducing the glycemic load of foods [25]. In contrast, rapidly digestible starch is hydrolyzed quickly during digestion, leading to rapid glucose release and a higher glycemic response after consumption. Therefore, the proportion of RS present in rice is considered a significant nutritional parameter influencing starch digestibility, glycemic index, and the potential health benefits associated with rice consumption. Digestible and total starch levels were determined in both raw and cooked conditions of medicinal, aromatic, and released rice varieties selected for the study. Different starch hydrolysis rates were observed, and there was significant variation among and between the medicinal and aromatic rice varieties (Table 3).

Rice variety	RS content (%) (Raw)	DS content (%) (Raw)	Total starch (%) (Raw)	RS content (%) (Cooked)	DS content (%) (Cooked)	Total starch (%) (Cooked)
Njavara Black	2.630 ± 0.000	44.900 ± 0.000	47.497 ± 0.006	1.029 ± 0.001	48.293 ± 0.012	49.297 ± 0.006
Njavara Yellow	2.793 ± 0.006	45.200 ± 0.000	47.907 ± 0.012	1.649 ± 0.001	49.097 ± 0.006	50.703 ± 0.006
Raktasali	2.747 ± 0.006	46.800 ± 0.000	49.597 ± 0.006	0.987 ± 0.006	49.290 ± 0.010	50.303 ± 0.006
Jeerakasala	0.730 ± 0.000	68.907 ± 0.012	69.603 ± 0.006	0.453 ± 0.006	73.967 ± 0.058	74.297 ± 0.006
Gandhakasala	0.790 ± 0.000	69.300 ± 0.000	70.097 ± 0.006	0.593 ± 0.006	72.407 ± 0.006	72.897 ± 0.006
Mullankaima	1.080 ± 0.000	61.403 ± 0.006	62.500 ± 0.000	0.990 ± 0.001	64.590 ± 0.010	65.497 ± 0.006
Uma	1.170 ± 0.000	58.800 ± 0.000	59.897 ± 0.006	0.577 ± 0.006	62.407 ± 0.006	62.907 ± 0.006

TABLE 3: RS, DS, and TS content of tested rice varieties

*Values are expressed as mean ± SD (n = 3). RS, Resistant Starch; DS, Digestible Starch; Total Starch = RS + DS

Comparatively, the resistant starch levels were lower in the cooked form when compared to the raw powdered form in all the rice varieties selected. Among the medicinal rice varieties, the resistant starch levels were high in Njavara Yellow followed by Raktasali and Njavara Black and the similar trend was also observed with the digestible or non-resistant

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starch content. In Njavara Black, the RS levels were reduced from 2.63% to 1.03% while in Njavara Yellow, RS was reduced from 2.79% to 1.65% and Raktasali rice showed a reduction of RS percentage from 2.75% to 0.98% upon cooking. Similarly, the digestible starch levels and total starch levels were increased in the tested rice varieties upon cooking. The total starch content of Njavara Black and Yellow varieties increased by about 3.7% and 2.9% respectively upon cooking. The medicinal rice Raktasali also showed a 2.2% increase in total starch and Uma showed a much higher 5% increase in total starch content after cooking. The RS, DS, and TS levels of Raktasali rice variety were higher when compared to Njavara Black and Yellow rice varieties. The RS value of cooked medicinal rice ranged from 0.98% to 1.65% with a mean of 1.22% and the RS content of its counterpart raw powdered form ranged from 2.63% to 2.79% with a mean of 2.72%.

Resistant starch level was lower in aromatic rice varieties in both cooked and raw powdered states when compared to the medicinal and released rice variety Uma. Generally, in all studied rice varieties RS levels were lowered upon cooking and digestible starch levels were increased. The non-medicinal, non-aromatic rice Uma has a good resistant starch percentage of 1.17 for raw powdered form and 0.58% for cooked forms and similarly the digestible starch levels for cooked and raw samples were 62.4% and 58.8%, respectively. Mullankaima rice variety exhibited a comparatively better RS level (0.99% for cooked and 1.08% for raw samples) among the aromatic rice varieties. The RS content was very low in Jeerakasala rice (0.73%), followed by Gandhakasala (0.79%) and Mullankaima (1.08%) with a mean of 0.86% in raw powdered. The RS level of cooked aromatic rice varieties was reduced to 0.45%, 0.59%, and 0.99% in Jeerakasala, Gandhakasala and Mullankaima respectively with a mean of 0.67%. The DS and TS content were also increased in cooked aromatic rice varieties, there was observed 6.75% increase of TS in Jeerakasala, 4.8% in Mullankaima, and 3.9% in Gandhakasala after cooking. When comparing all the rice varieties under study Njavara Yellow showed higher RS content in both forms and Jeerakasala have the lower RS content in cooked as well as raw conditions. Even though the medicinal rice variety Raktasali showed RS level higher (2.75%) than Njavara Black (2.63%) in raw powdered form, its RS level in cooked condition varied, in cooked form the RS level was high in Njavara Black when compared with Raktasali. The total starch content also varied in all studied rice varieties and the similar trend was observed in RS and digestible starch levels of medicinal and aromatic rice varieties. Among the medicinal rice varieties, Njavara Yellow showed higher starch content in cooked samples and Raktasali has higher starch content in raw powdered samples. In both cooked and raw powdered rice samples, the total starch content was higher for Jeerakasala and the lower starch content was observed for Njavara Black. In raw powdered samples the DS and TS level was high in Gandhakasala (69.30% and 70.09%, respectively) and in cooked samples the DS and TS content was high in Jeerakasala (73.96% and 74.29%, respectively).

***In vitro* starch hydrolysis index and estimated GI prediction in rice varieties**

The *in vitro* starch digestion process estimated how the starch content in the selected rice varieties was broken down into glucose by mimicking the human digestion process and also predicted the amount of glucose released. The HI is the percentage of starch which was hydrolyzed over a period of 3 hours (typically at 30 minutes intervals) for both raw and cooked forms of medicinal, aromatic and released rice varieties (Figure 2).

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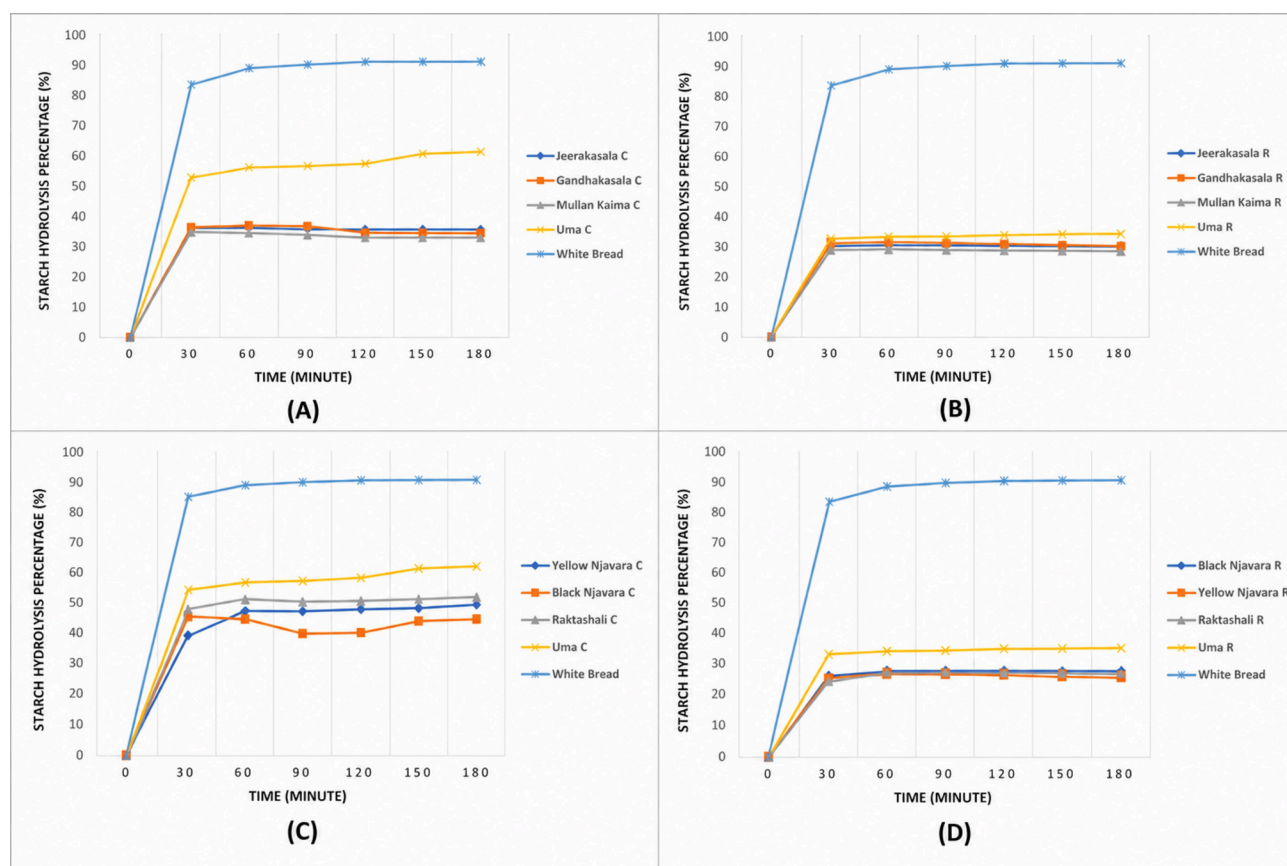


FIGURE 2: In vitro starch digestibility pattern during 3 hours of tested rice varieties with reference food, white bread (A): starch hydrolysis percentage of cooked form of medicinal rice, (B): starch hydrolysis percentage of cooked form of aromatic rice, (C): starch hydrolysis percentage of raw form of medicinal rice, (D): starch hydrolysis percentage of raw form of aromatic rice

HI was calculated for both raw and cooked forms based on the plots for predicting the AUC of each sample and comparing it with the AUC of the reference food (white bread). The percentage of HI was considered for predicting the glycemic index of the selected rice varieties which ranked the rice varieties as high GI or low GI rice. The HI and eGI of all raw powdered and cooked forms of medicinal, aromatic and released rice varieties were shown in Table 4.

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Rice Variety	HI Raw	95% CI	HI Cooked	95% CI	eGI Raw	95% CI	eGI Cooked	95% CI
Njavara Black	32.703 ± 0.006	32.688–32.719	34.903 ± 0.006	34.888–34.919	56.897 ± 0.006	56.882–56.912	58.203 ± 0.006	58.189–58.218
Njavara Yellow	32.033 ± 0.058	31.890–32.177	34.393 ± 0.006	34.378–34.409	56.500 ± 0.010	56.475–56.525	57.597 ± 0.006	57.582–57.611
Raktasali	32.397 ± 0.006	32.382–32.412	35.093 ± 0.006	35.078–35.109	56.703 ± 0.006	56.688–56.719	58.197 ± 0.006	58.182–58.211
Jeerakasala	38.603 ± 0.006	38.588–38.619	40.100 ± 0.010	40.075–40.125	60.193 ± 0.006	60.178–60.209	61.067 ± 0.115	60.780–61.354
Gandhakasala	39.803 ± 0.006	39.788–39.819	40.033 ± 0.058	39.890–40.177	60.797 ± 0.006	60.782–60.812	60.967 ± 0.058	60.823–61.110
Mullankaima	36.600 ± 0.010	36.575–36.625	37.803 ± 0.006	37.788–37.819	59.100 ± 0.010	59.075–59.125	59.697 ± 0.006	59.682–59.711
Uma	41.070 ± 0.058	40.927–41.213	41.907 ± 0.006	41.892–41.922	61.003 ± 0.006	60.988–61.019	62.033 ± 0.058	61.890–62.177

TABLE 4: HI and estimated glycemic index predicted for selected rice varieties

*Values are expressed as mean ± SD (n = 3). The 95% confidence interval (CI) was calculated using the two-sided Student's t-distribution with 2 degrees of freedom. HI: Hydrolysis Index, eGI: Estimated Glycemic Index

The HI and eGI of medicinal, aromatic and released rice varieties in cooked and raw forms varied consistently. It was observed that during cooking, the starch was hydrolysed to sugars at high temperature which increased the concentration of total sugar in the tested cooked samples.

During the 3-hour incubation period, except for the reference food, there was no sudden increase in the amount of glucose released from the rice varieties tested in either the raw powdered or cooked forms. The raw powdered medicinal rice varieties showed a HI ranging from 32.03% to 32.70%, while the cooked medicinal rice varieties showed an HI ranging from 34.39% to 35.09%. The raw powdered aromatic rice varieties exhibited an HI ranging from 36.60% to 39.80%, whereas the cooked aromatic rice varieties showed an HI ranging from 37.80% to 40.10%. The eGI values of the raw powdered rice varieties ranged from 56.50% to 61.00%, while those of the cooked rice varieties ranged from 57.60% to 62.03%.

The cooked form of the released rice variety Uma showed the highest starch hydrolysis percentage (41.9%). But still, the highest percentage of starch hydrolyzed was not close to the starch hydrolysis pattern observed for the reference food and the lowest percentage of starch digestibility was observed with the raw powdered form of medicinal rice variety Njavara Yellow (32.03%). All the medicinal and traditional aromatic rice varieties selected for the study showed a slow digestion pattern from 30 minutes and were steady after 120 minutes. No rice varieties selected for the study had a similar digestibility pattern to the reference food and this might be due to the traditionally believed comparatively lower GI of traditional and red rice varieties.

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The HI and eGI of medicinal rice varieties were low when compared to those of aromatic and released varieties. Two Njavara varieties, Black glumed and Yellow glumed, were considered to be the same in their nutrient composition other than their glume colour differences, but they showed slight variation in the hydrolysis index in both raw and cooked rice samples. The raw powdered Njavara Black and Njavara Yellow exhibited HI values of 32.70% and 32.03%, respectively, whereas the corresponding cooked samples showed HI values of 34.90% and 34.39%, respectively. The difference between Njavara Black and Njavara Yellow was 0.67 percentage points in the raw form and 0.51 percentage points in the cooked form, corresponding to relative variations of approximately 2.1% and 1.5%, respectively. Among the medicinal rice varieties, Raktasali exhibited HI values close to those of Njavara Black, with values of 32.40% in the raw form and 35.09% in the cooked form, compared with 32.70% and 34.90%, respectively, for Njavara Black. In the case of aromatic rice varieties selected, Jeerakasala and Gandhakasala, the most demanded and popular scented varieties in Kerala, have the highest HI in both raw and cooked forms. In Jeerakasala raw powdered form, the starch hydrolysis was observed as 38.60% and after cooking, the starch hydrolysis rate was increased to 40.10%. Similarly, Gandhakasala also exhibited the same pattern of starch hydrolysis; in raw form, the HI was 39.80% and in cooked form, the HI increased to 40.03%. Among the traditional scented varieties, Mullankaima rice showed slightly lower HI which was 36.60% in raw condition and the HI increased slightly after cooking (37.80%). The released rice variety taken for the study, Uma, is a high-yielding variety which has the highest HI in both raw (41.07%) and cooked form (41.90%). Uma was compared with both medicinal rice varieties and aromatic rice varieties. From the results, it was evident that the starch hydrolysis rate and glycemic index significantly increased upon cooking. The narrow 95% CIs for most measurements indicate low variation among the three replicates, while the slightly wider intervals for some measurements, such as cooked eGI of Jeerakasala, reflect greater replicate variability. This might be due to the hydrolysis of starch to sugars upon boiling at high temperature which increases the total soluble sugar concentration in cooked rice samples. However, the percentage of starch hydrolysis variation among the medicinal rice samples and aromatic rice samples was very low and was not more than 1 0% between and among the samples.

In the case of medicinal rice varieties, black Njavara has a 7.0% increase in the starch hydrolysis rate upon cooking. Similarly, yellow Njavara has a 7.6% increase and Raktasali has an 8.3% increase in HI when cooked under the boiling method. On the other hand, the aromatic rice varieties such as Jeerakasala and Mullankaima showed 3.8% and 3.2% increases in starch hydrolysis from raw powdered state to cooked state. Another aromatic rice variety, Gandhakasala, showed a slight change of 0.5% in HI upon cooking even though Jeerakasala and Gandhakasala showed nearly similar HI percentages (40.10% and 40.03%) in cooked state. The released rice variety, Uma, also followed the same pattern of starch hydrolysis with an increase of 4.48% from raw powdered condition to cooked condition.

GI is usually taken as a scale which ranks food from 0 to 100 based on the rapid rise of blood sugar in blood. By simulating the starch digestion in humans, eGI was predicted for both medicinal, aromatic and a released rice variety in both raw powdered form and cooked form. The cooking was done by the boiling method only following similar conditions for all rice varieties. As observed with the hydrolysis rate, the GI was lower for raw samples in all rice varieties compared with cooked samples. Njavara Yellow has the lowest estimated glycemic index of 56.50 in raw form and 57.59 in cooked form with 2.3% variation from raw to cooked state. Among the rice varieties, Uma has the highest GI of 62.03 in cooked rice and 61 .00 in raw form followed by Gandhakasala (60.79 in raw and 60.96 in cooked rice), Jeerakasala (60.19 in raw and 61 .06 in cooked form), and Mullankaima (59.10 in raw and 59.69 in cooked rice). Njavara Black and Raktasali, the two medicinal rice varieties, have nearly same GI (58.20 and 58.19) in cooked rice samples, while in raw powdered form there was a slight variation of 0.3% (56.89 for black Njavara and 56.70 for Raktasali) in the two varieties. Among the medicinal rice varieties, Njavara Yellow showed a better glycemic response followed by Raktasali and Njavara Black. Similarly, among aromatic rice varieties, Mullankaima has the lowest eGI followed by Gandhakasala and Jeerakasala. The released variety Uma has the highest GI among the tested rice varieties but can be considered as lower when compared to the GI of white rice varieties and the reference food (white bread in the current study).

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Correlation between *in vitro* starch digestibility pattern RS and amylose content

From the correlation analysis, significant correlations were noted between the RS, DS and TS levels with HI and eGI. It was observed that there was a strong positive correlation between amylose content and RS level (0.851) and also a strong negative correlation was observed with the DS content (-0.919) and TS content (-0.924). Moreover, the resistant starch content has a strong negative correlation with digestible starch level (-0.976) and total starch levels (-0.971). The HI and eGI showed a perfect correlation (0.998), stating that they are comparable in their correlation across the tested rice varieties.

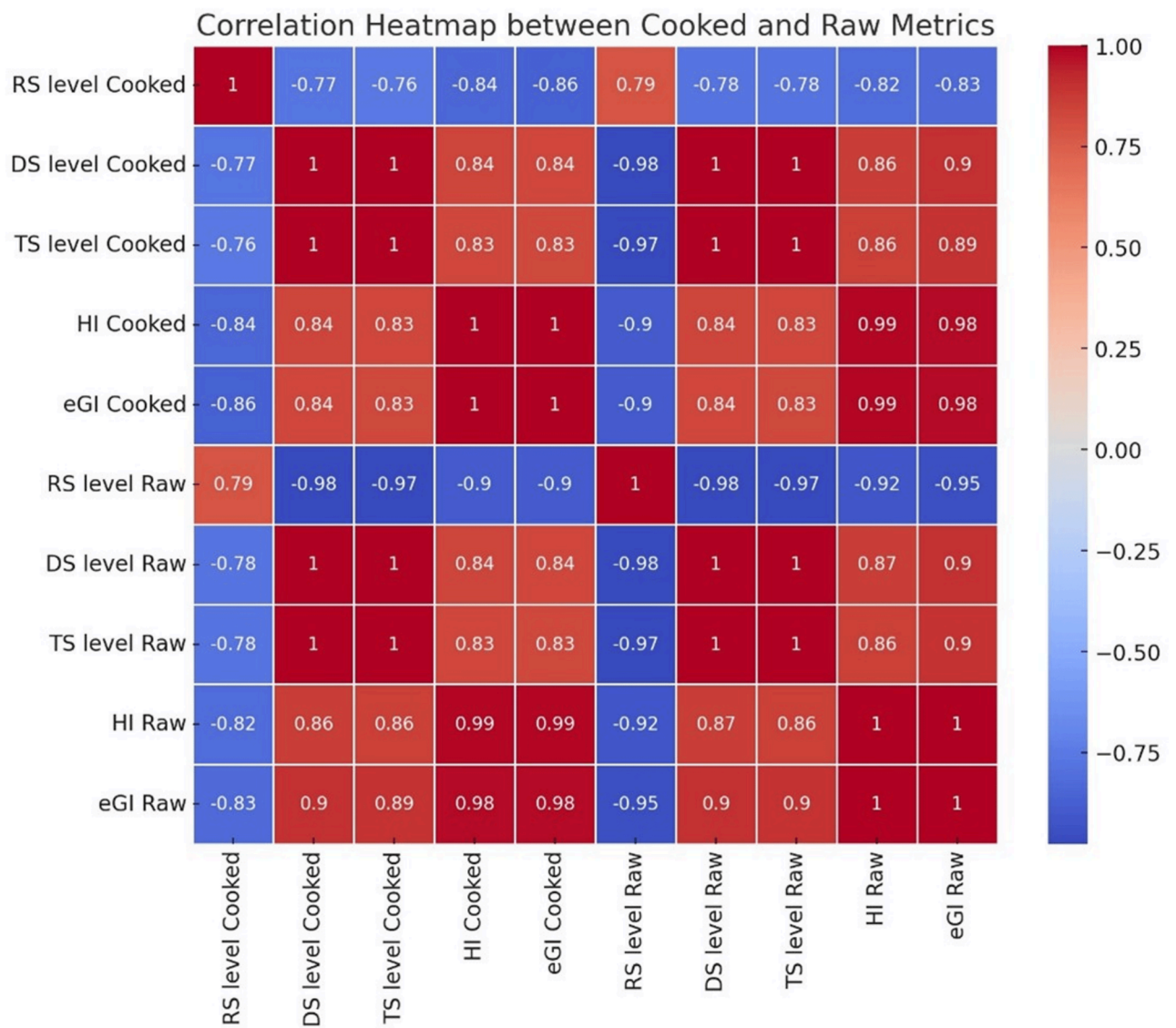


FIGURE 3: Correlation heat map of variables in tested medicinal and aromatic rice varieties between the cooked and raw metrics. Strong positive correlations are shown in deep red colour. RS: Resistant Starch Level, DS: Digestible Starch Level, TS: Total Starch Level, HI: Hydrolysis Index, eGI: Estimated Glycemic Index

The correlation heatmap between the raw and cooked sample matrices (Figure 3) shows a strong positive correlation between the two forms, particularly for digestible starch (DS), total starch (TS) and eGI. Negative correlations were observed between a few matrices, such as cooked RS content with raw DS and TS content. The study also identified the

correlation between the variables observed in cooked samples of medicinal and aromatic rice varieties and the matrices (RS, HI and eGI).

In medicinal rice varieties, RS content is strongly negatively correlated with HI (-0.98) and eGI (-1.0), and the HI is positively correlated with eGI (0.96). Similarly, in aromatic rice varieties, a negative correlation of RS level with HI (-0.98) and eGI (-0.98) was observed, and HI and eGI have a strong positive correlation (0.999). The heatmap demonstrates the relationships depicting stronger correlations in dark shades (Figure 4).

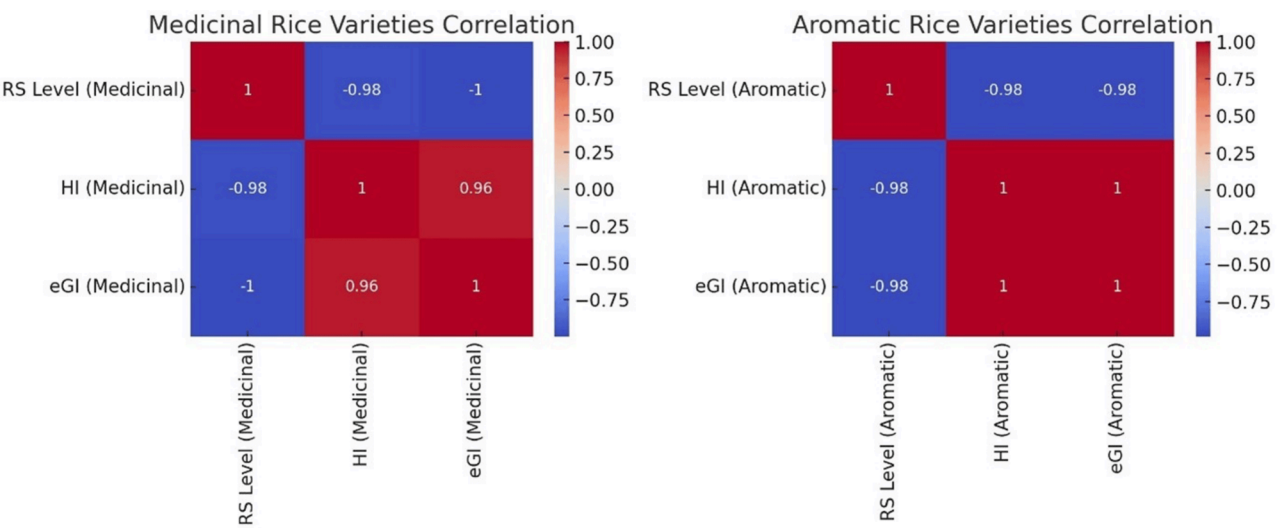


FIGURE 4: Correlation of RS level, HI and eGI in aromatic and medicinal rice varieties

RS: Resistant Starch Level, HI: Hydrolysis Index, eGI: Estimated Glycemic Index

Several studies showed that the amylose content is inversely proportional to GI [26,27]. The correlation between amylose content and eGI for both cooked and raw samples of tested rice varieties showed that there was a moderate negative correlation between eGI and amylose content (-0.68), which indicates that higher amylose content is significantly associated with lower GI. The stronger negative correlation between eGI (-0.79) and amylose content in raw samples of tested varieties was also inversely proportional, suggesting a highly reliable relationship between these two variables (Figure 5).

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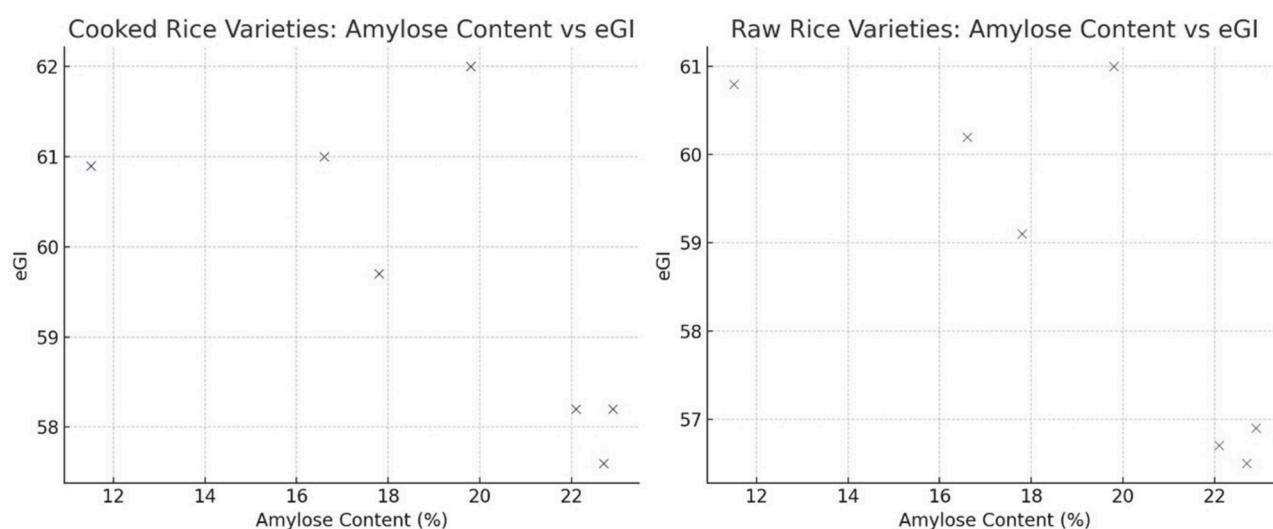


FIGURE 5: Scatter plots with trend lines for each metric showing the inversely correlation between amylose content and estimated glycemic index (eGI)

The eGI of both cooked and raw samples of aromatic and medicinal rice varieties tested in the study may not be exactly similar to that observed *in vivo* studies or clinical trials; however, a significant positive correlation was observed with the variables studied. The relatively lower eGI observed for Njavara compared with the released and aromatic rice varieties evaluated showed that its starch characteristics may be associated with slower carbohydrate hydrolysis and glucose release during *in vitro* digestion. However, these findings represent the *in vitro* estimates and should not be interpreted as evidence of a clinically lower glycemic response in humans, as *in vitro* models do not fully recapitulate the physiological processes leading to digestion, glucose absorption, insulin secretion, gastric emptying, and postprandial glucose regulation. The observed differences in resistant starch, starch digestibility, hydrolysis index, and eGI therefore provide preliminary evidence of variation in carbohydrate-digestibility characteristics among the rice varieties, warranting further validation through controlled *in vivo* and human post-prandial studies. From a nutritional and breeding perspective, the starch-related traits identified in Njavara may serve as potential targets for further investigation; however, their relevance to glycemic management and their application in breeding programs should be considered exploratory until supported by physiological and clinical evidence.

A few limitations of the present study should also be considered when interpreting the findings of the study. First, the use of an *in vitro* simulated digestion model rather than human clinical trials provides predictive rather than clinically definitive estimates of glycemic response. Yet, this approach enabled reproducible, cost-effective, and standardized comparison of starch digestibility among the selected rice varieties. The inclusion of raw rice powder allowed assessment of intrinsic biochemical and starch digestibility characteristics independent of cooking-related variation and provided a baseline for evaluating the effects of heat treatment. Although raw rice powder is not directly representative of normal dietary consumption, it facilitated standardized comparison of varietal differences. The relatively small number of seven rice varieties, selected to represent diverse regional, medicinal, pigmented, aromatic, and improved rice types, limits the generalizability of the findings. However, the significant differences observed among varieties enabled meaningful comparative evaluation of the measured traits. In addition, a single cooking method was employed to maintain consistency with the traditional cooking practice commonly followed for these rice varieties in the region, thereby minimizing variation arising from different cooking procedures. However, cooking conditions can influence starch gelatinization, resistant starch formation, and carbohydrate digestibility, and the present results may therefore not represent all possible preparation methods. Future studies incorporating a broader and genetically diverse rice panel,

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multiple cooking and processing methods, and controlled human postprandial studies are warranted to validate and extend these findings. Therefore, the findings may not fully reflect the effects of different household or processing conditions.

Conclusions

In vitro starch digestion was performed on seven traditionally cultivated rice varieties of Kerala, including medicinal, aromatic, and a released high-yielding variety, in both raw and cooked forms. Amylose content was estimated in the raw samples of all varieties and used to examine its correlation with the eGI. The study revealed significant variation in RS, DS, and TS levels between raw and cooked forms. Cooking was found to markedly reduce RS content and increase eGI across all varieties, indicating that thermal processing enhances starch gelatinization and accelerates glucose release. The digestion patterns were used to predict the eGI of each variety. The three medicinal rice varieties analyzed exhibited high amylose content and intermediate glycemic indices. Among the aromatic rice types, only Mullankaima showed intermediate amylose content, while the others displayed low levels. The widely cultivated red released rice variety Uma, which accounts for nearly 80% of Kerala's paddy fields, was found to have intermediate amylose but showed the highest GI (61.0 in raw and 62.03 in cooked form). In contrast, Njavara Yellow, a medicinal rice, exhibited the lowest eGI (56.50 in raw and 57.59 in cooked form). Among the aromatic rice varieties, Gandhakasala displayed the highest eGI (60.79 in raw and 60.96 in cooked rice).

Correlation analysis indicated strong associations between DS, TS, HI and eGI, suggesting these variables collectively influence the glycemic response of rice. Notably, all medicinal rice varieties in the study had lower eGI values compared to the aromatic and released types, indicating their potential suitability for diabetic diets and glycemic management in Kerala. Importantly, the study also concluded that grain colour, whether red, white, or black, did not correlate with GI. For example, although both Njavara and Uma are brown rice varieties, they exhibited the lowest and highest GI values, respectively. This indicates that grain colour alone is not a reliable predictor of glycemic behaviour in rice. These findings provide preliminary evidence of varietal differences in carbohydrate digestibility characteristics and identify Njavara as a promising candidate for further investigation of rice varieties with potentially favourable glycemic properties. However, the eGI values reported here are derived exclusively from an *in vitro* digestion model and should not be interpreted as direct evidence of clinical glycemic responses or as a basis for specific dietary recommendations for individuals with diabetes. Further studies involving a broader rice germplasm, different cooking and processing methods, metabolomic profiling of potentially bioactive compounds, and controlled *in vivo* and human postprandial studies are required to validate these findings and clarify their nutritional and breeding relevance.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen.

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with [the ICMJE uniform disclosure form](#), all authors declare the following:

- **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work.
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

All data generated or analyzed during this study are included in this published article and/or its appendices.

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