RESEARCH ARTICLE https://doi.org/10.58297/FBJS8734

Starch Kinetics, Cooking Quality and Phytochemical Composition of Geographical Indication (GI) Tagged Rice of Kerala

Chitra Pillai[1], Faseela KV[2] and Harikumaran Thampi[1*]

[1] Department of Life Sciences, University of Calicut-673635, Kerala, India.

[2]Department of Plant Breeding and Genetics, Regional Agricultural Research Station (Central zone), Kerala Agricultural University, Mele Pattambi-679306, Kerala

*Corresponding author Email: drhari@uoc.ac.in

Received: 30th September, 2024; Accepted: 5th November, 2024

Abstract

Geographical Indication (GI) tagged traditional rice of Kerala namely the red pigmented Palakkadan Matta and non-pigmented aromatic rice - Wayanadan Gandhakasala and Wayanadan Jeerakasala are well known for their cooking and eating quality, but are underutilized for their nutritional significance. The objective of this study was to classify these varieties based on their starch digestibility, biochemical composition and cooking characteristics. Results indicated significant ($p < 0.05$) variation among rice varieties for all the parameters evaluated. *In vitro* starch digestibility studies found significantly low glycemic index (< 68) but higher RS content for Wayanadan Gandhakasala and Chettadi. Aromatic varieties had lower cooking time (< 25 min) than Palakkadan Matta varieties. High water uptake ratio and elongation ratio was also noted for Wayanadan Gandhakasala. Total phenolic content correlated positively to total flavonoid content. Over all, Palakkadan Matta varieties had significantly higher free phenolic, free flavonoid and total flavonoid content than non-pigmented varieties. The study indicates that these GI tagged traditional rice varieties can thus be utilised in the functional food industry based on their intermediate glycemic index, desirable cooking qualities and the presence of beneficial bioactive components.

Key words: Geographical indication, Palakkadan Matta, Aromatic varieties, Glycemic Index, Cooking Properties, Total phenolic content

Introduction

Rice (*Oryza sativa* L.) is one of the most studied cereal crops of the world and forms the central part of the Indian diet. Rice gained popularity from the fact that it can be grown under diverse climatic conditions and can be cooked in many different ways. Therefore, quality of rice is a topic for which there can be different answers. Different countries or regions within a country prefer eating rice in different ways and therefore the definition of rice quality differs accordingly (Bhattacharya, 2009). The most commonly used quality traits include physical appearance, nutrition, milling and cooking characteristics. Numerous scientific studies suggest that both genetic and environmental factors can affect quality traits in rice grain (Chen et al., 2012; Custodio *et al.,* 2019; Krishnamrutha *et al.,* 2023). Some rice varieties are well known for their unique qualities because of the particular region in which they are grown. These special varieties found reference in historical scripts and were assigned with a unique status or tag known as geographical indication (GI) under the Geographical Indications of Goods

(Registration and Protection) Act, 1999 (Blakeney *et al.,* 2020). GI status gives them a unique status among other varieties and provides more market and export value.

Among the agriculture commodities, 12 GI tags are recorded for rice in India, six of which are cultivated in the state of Kerala. These include Palakkadan Matta, Kaipad rice, Navara rice, Pokkali rice, Wayanadan Gandhakasala and Wayanadan Jeerakasala. Among these varieties, Navara is the most popular and has the highest market income, which could be due to its medicinal properties (Radhika *et al.,* 2018). Majority of scientific literature available on traditional rice in Kerala has focused on Navara. Palakkadan Matta find reference in Tamil classic 'Thirukkural' and was consumed by the royal families of Chola and Chera dynasty. Under the GI registry, Palakkadan Matta include ten different varieties. However, only four varieties are most commonly cultivated in Palakkad, which include Thavalakannan, Chenkayama, Chettadi and Chitteni. They have bold grain with red pericarp and is known for their unique earthy flavour which is supposed to be due to the heavy soil they are grown in, which is rich in clay and silt. These varieties are mostly consumed in parboiled form and used in the preparation of a wide range of rice based dishes and snacks.

Wayanadan Gandhakasala and Wayanadan Jeerakasala are non-pigmented fine aromatic rice cultivated organically in high altitudes of Wayanad by the Kurichiya tribe, an agricultural based community in Kerala. Owing to their distinct aroma when cooked, they are known as 'Kerala Basmathi' and fetch higher price in the market than other varieties (Krishnankutty *et al.,* 2021; Soam, 2005). Agronomical features of the varieties taken for this study are listed in **Table 1.**

Table 1: Agronomical features of rice varieties

Sl. No.	Popular name	Crop maturation (days)	Agronomical features
Palakkadan Matta Varieties			
1	Chenkazhama	110- 120	Tall *indica* variety.
2	Chettadi	130 -160	Photosensitive and drought resistant.
3	Chitteni	130	Highly tolerant to Bacterial leaf blight and pests like Brown plant hopper, Gall fly and green leaf hopper.
4	Thavalakannan	130	Less prone to lodging, good straw yield and tolerant to Gall fly and Foot rot. Highly adaptive to adverse soil conditions and shows strong resistance to green leaf hopper.
Aromatic varieties			
5	Wayanadan Gandhakasala	160 - 180	Aromatic, highly tolerant to pest and disease, High priced, suitable as fodder (Blakeney 2020)
6	Wayanadan Jeerakasala	160 - 180	Aromatic, high priced

Even though these varieties are known for their unique properties, very few scientific studies are available on the nutritional and cooking properties of these GI rice varieties. The selected varieties are not explored for their phytochemical composition as most studies have focused on their agro morphological characteristics. The objective of this study is to evaluate and classify selected GI tagged rice varieties for their nutritional, phytochemical and cooking characteristics.

Materials and Methods

Rice samples

Six rice samples including four most commonly cultivated Palakkadan Matta rice varieties namely Chenkazhama, Chettadi, Chitteni and Thavalakannan and two aromatic varieties namely Wayanadan Gandhakasala and Wayanadan Jeerakasala were collected from Regional Agricultural Research Stations (Mele Pattambi and Ambalavayal), Kerala and Abhayam, Pattambi, Kerala. Varieties Wayanadan Jeerakasala and Wayanadan Gandhakasala were non-pigmented aromatic rice and all others were red pigmented varieties.

The dried paddy samples were kept at 4 °C in airtight plastic containers until used. The samples were dehulled manually and homogenised into fine rice flour (60 mesh sieve) freshly before analysis. For the determination of glycemic index and starch fractions, 50 mg rice flour was homogenised in 5 ml distilled water and cooked for 30 minutes.

Chemicals and Reagents

Glucose oxidase / peroxidase reagent, amyloglucosidase solution (from *Aspergillus niger)*, heat stable α-amylase (from *Bacillus licheniformis)*, standards of gallic acid and (+) - catechin were purchased from Sigma-Aldrich, USA. HPLC grade chemicals namely methanol, acetonitrile and pepsin from porcine stomach mucosa were purchased from HiMedia. All other chemicals used were of analytical grade.

Determination of Total Starch (TS) and its fractions

The TS content was determined following the method reported by Goñi *et al*., (1997)to calculate the glycemic index (GI). Briefly, 50 mg brown rice flour was added to 2M KOH (6 ml) and energetically shaken for 30 minutes at room temperature. To the mixture, added 0.4 M Sodium acetate buffer, pH-4.75 (3 ml) and amyloglucosidase (60 µl) and incubated for 45 minutes in a shaking water bath at 60 °C. Starch content was measured as glucose using glucose oxidase-peroxidase reagent and a factor of 0.9 was used for the conversion of glucose to starch.

Resistant starch (RS) content was measured following the enzymatic method described by Goñi *et al*., (1997)to calculate the glycemic index (GI. Briefly, to the brown rice flour (100 mg), added KCl-HCl buffer (10 ml), pH 1.5 and homogenized in 50 ml centrifuge tube. For the removal of proteins, the mixture was treated with freshly prepared pepsin solution containing 0.02 g pepsin in 0.2 ml KCl-HCl buffer at 40 °C for 60 minutes. Then added 9 ml Tris-Maleate buffer, pH 6.9 and the suspension was hydrolysed with solution containing α-amylase (40 mg) at 37 °C for 16 hours. The suspension was centrifuged and the residue was then incubated with 80 µl amyloglucosidase solution for 45 minutes at 60 °C with constant shaking. The hydrolysate was centrifuged and the supernatant was collected for the estimation of RS content, as described earlier. For the estimation of digestible starch (DS), the difference between TS and RS was calculated.

In vitro starch hydrolysis and determination of glycemic index

The rate of rice starch hydrolysis was studied following the *in vitro* method suggested by Goñi *et al*., (1997) to calculate the glycemic index (GI. Briefly, 50 mg brown rice flour was added to 10 ml of KCl- HCl buffer and pH adjusted to 1.5. To this suspension, 0.2 ml of freshly prepared Pepsin solution was added and kept for 1 hour at 40 °C in a shaking water bath. The volume was completed to 25 ml with Tris maleate buffer. The solution was then hydrolysed with α- amylase solution (2.6 units in Tris-Maleate buffer) and kept in a shaking water bath at 37 °C. One ml hydrolysate solution was drawn every 30 minutes for a total of 180 minutes.

The aliquots were then heated in boiling water bath followed by rigorous shaking in a vortex mixer to inactivate their enzyme activity. To these aliquots,

added 0.4 M sodium acetate buffer, pH 4.75 (3 ml) and amyloglucosidase (60 µl), mixed and incubated at 60 °C for 45 minutes in shaking water bath. The glucose content was determined by glucose oxidase/ peroxidase reagent and the amount of starch was calculated. The results were then expressed as the per centage of TS hydrolysed at a given time interval.

The data obtained was used for plotting starch hydrolysis curve and the area under curve (AUC) was calculated. A first order non-linear reaction equation followed for the starch hydrolysis as provide by Goñi *et al*., (1997)to calculate the glycemic index (GIto calculate the glycemic index (GI: $C=C_{\infty}(1-e^{-kt})$,

where C - Concentration at time t, C∞- Equilibrium concentration, k - kinetic constant and t - chosen time.

Parameters C∞, k and AUC were determined from the experimental data using software SYSTAT (Sigma plot 14), MS office version. The hydrolysis index (HI) was expressed as per centage and calculated using the formula:

Glycemic Index was determined by the following equation:

Glycemic Index = 39.71 + 0.549 HI

Determination of cooking characteristics

Cooking characteristics of rice varieties were determined following the method suggested by Singh *et al*., (2005).

Minimum cooking time

Brown rice kernels (1g) were added to 10 ml distilled water and heated in a boiling water bath. The rice grains were removed every 2 minutes during cooking and pressed between two glass slides. The time (min) at which no white residue was left in the glass slides was taken as the minimum cooking time.

Water uptake ratio

After cooking rice kernels for their minimum cooking time as described above, the water was drained and the cooked samples were weighed after pressing in filter paper. The difference in weight of cooked sample was calculated as the water uptake ratio.

Elongation ratio

Elongation ratio was obtained by dividing the average length of cooked rice kernels by the average length of uncooked rice kernels (n=10).

Cooked length-breadth ratio

The average length of cooked rice kernels was divided by the average breadth of cooked kernels and termed as l/b ratio (n=10).

Extraction of free form phenolic compounds

Free form phenolic compounds were extracted from brown rice flour using the method of Gong *et al.,* (2017). Briefly, 500 mg of whole grain flour was blended with 80% chilled Ethanol (5 ml) for 15 minutes. After centrifuging the mixture (5000 rpm) for 10 minutes, the supernatant was pooled and the residue was extracted twice. The pooled extract was then subjected to evaporation at 45 ºC until the extract was reduced to 3 ml. Reconstituted the extract with distilled water to 6 ml and kept at -40 ºC until use.

Extraction of bound form phenolic compounds

The residue obtained after extracting free form phenolics, was treated with 2M NaOH solution (10 ml) at room temperature for 1 hour under nitrogen gas. The mixture was then acidified with 2 M HCl solution (10 ml) until pH 2 was obtained. The acidic solution was then extracted with hexane (10 ml). The final solution was then extracted with Ethyl acetate (10 ml) thrice. The extracts were pooled, dried and reconstituted in 5 ml of distilled water. The extract was then stored at -40 ºC until use (Gong *et al.,* 2017).

Determination of phenolic content

The total phenolics content was estimated by the colorimetric method reported by Sompong et al., (2011). Briefly, extract solution (120 µl) was treated

with freshly prepared Folin-Ciocalteu reagent diluted 10-folds (600 µl) and incubated for 2 minutes. To the mixture, added 960 µl NA_2CO_3 solution (75 g/l) and kept at 50ºC for another 2 minutes. The blue colour developed was then read at 760 nm. Gallic acid was taken as the standard and the results were expressed as mg gallic acid equivalent (GAE) per 100 g brown rice.

Determination of flavonoid content

The total flavonoid content was determined following the modified colorimetric method of Dewanto *et al*., (2002). The extract solution (300 µl) was initially diluted with 1.5 ml of distilled water in a test tube. Then added 5% $NaNO_2$ solution (90 µl) and kept at room temperature for 6 minutes followed by the addition of 10% $AlCl_3.6H_2O$ solution (180 µl) and incubated for 5 minutes. To the mixture, finally added 1 M NaOH (600 µl) and the volume was made up to 3 ml with distilled water. The colour developed was measured at a wavelength of 510 nm and compared with the standard + (-) Catechin solution. Total flavonoid contents were expressed in terms of mg + (-) catechin equivalents (CE) per 100 g of brown rice.

Statistical analysis

All the analytical assays were performed thrice (n-3) and reported as mean ± standard deviation on fresh weight basis. The results were analysed by Analysis of Variance (ANOVA) SPSS version 20 using Duncan's Multiple Range Test (DMRT) to compare means at $p < 0.05$ significance level. Pearson's correlation analysis was performed for calculating the relationship between different variables.

Results and Discussions

TS and its fractions

TS, RS and DS contents of rice samples are shown in **Table 2.** The amount of TS in rice samples ranged from 72.99% in Wayanadan Jeerakasala to 83.68% in Chenkazhama. All the varieties were subjected to same cooking method for TS determination. Higher TS content in rice could result from leaching of starchy fragments during cooking because of different degree of damage to the grain structure (Ahmed and Urooj, 2003). RS and DS content were in the range of 0.56 - 0.69% and 72.35- 76.01% respectively. Wayanadan Jeerakasala had significantly lower TS and DS content among all varieties, which is a desired parameter for managing blood glucose response. The values obtained were comparable to other studies by Deepa *et al*., (2010) and Hu *et al*., (2004).

Table 2: Total starch, resistant starch and digestible starch content of rice varieties (%)

Sample	Total Starch	Resistant Starch	Digestible Starch
Chenkazhama	83.68 ±2.06^{a}	0.64 ±0.01^{b}	83.03 ±2.07^{a}
Chettadi	77.75 ±2.33^{b}	0.69 ±0.02^{a}	77.10 ±2.33^{b}
Chitteni	76.58 ±1.83^{bc}	0.56 ±0.03^{d}	76.01 ±1.82^{c}
Thavalakannan	73.86 ±1.96^{c}	0.63 ± 0.02^{c}	73.22 ±1.96^{d}
Wayanadan Gandhakasala	74.60 ±0.36^{bc}	0.64 ±0.0^{bc}	73.96 ±0.36^{d}
Wayanadan Jeerakasala	72.99 ±2.5^{c}	0.63 ±0.03^{c}	72.35 ±2.53^{e}
Mean	76.57	0.63	75.94
CV	2.76	0.38	2.77
CD (0.05)	3.85	0.00	3.08

Values with the same letters in a column are not significantly different ($p < 0.05$). TS, total starch; RS, resistant starch; DS, digestible starch

Rice mainly comprises Type 1 RS, which is the physically inaccessible starch normally found in whole grains or Type 5, which is formed by amylose-lipid complexes (Sajilata *et al.,* 2006). Amylose content is predicted as a major factor affecting the RS content. Retrogradation of amylose was found to be the

primary mechanism for the formation of RS (Deepa *et al.,* 2010; Berry, 1986) . Lehmann and Robin, (2007) and Sajilata *et al.,* (2006) reported a positive correlation between RS and amylose content in rice. In contrast, some of the intermediate amylose varieties found in the study had higher RS than high amylose varieties (reported elsewhere) (Pillai *et al.,* 2020). In a similar study by Hu *et al.,* (2004) rice cultivars with similar amylose content had different RS content due to difference in planting seasons and varietal factors. His study also characterized highamylose varieties with high RS content to be associated with low RVA (Rapid visco analyser) parameters like peak viscosity, hot plate viscosity and cool paste viscosity.

Cooking characteristics

Significant differences were observed for cooking characteristics of rice varieties as shown in **Table 3**. Minimum cooking time ranged between 23.83 to 35.50 min. Wayanadan Gandhakasala and Wayanadan Jeerakasala had significantly lower cooking time than Matta varieties. Highest cooking time was observed in Thavalakannan, which also had significantly higher protein content (11.4 g/100 g) among the varieties analysed (Pillai *et al.,* 2020). This was similar to the observation made by Juliano *et al.,* (1965) that high protein rice varieties require longer cooking time. However, no positive correlation was found between protein content and cooking time of the rice varieties. Protein content of Chenkazhama, Chettadi, Chitteni, Gandhakasala and Jeerakasala were 6.75, 8.01, 7.77, 10.67 and 8.16 g/100g respectively (reported elsewhere) (Pillai *et al.*, 2020). Longer cooking times can lead to low acceptability of brown rice among consumers (Adebamowo *et al.,* 2017).

Table 3: Cooking properties of rice varieties

Rice varieties	Minimum cooking time (min)	Water uptake ratio	Elongation ratio	Cooked l/b ratio
Chenkazhama	26.46±0.41^{d}	1.76±0.01^{d}	1.15±0.00^{c}	1.82±0.02^{f}
Chettadi	33.5±0.50^{b}	2.03±0.00^{b}	1.10±0.01^{d}	2.44±0.04^{e}
Chitteni	32.4±0.36^{c}	2.01±0.02^{c}	1.18±0.02^{b}	3.04±0.03^{c}
Thavalakannan	35.5±0.50^{a}	2.15±0.01^{a}	1.16±0.03^{c}	3.35±0.04^{a}
Wayanadan Gandhakasala	24.4±0.36^{e}	2.16±0.01^{a}	1.29 ±0.01^{a}	2.84±0.04^{d}
Wayanadan Jeerakasala	23.83±0.28^{e}	2.01±0.01^{c}	1.11±0.02^{d}	3.18±0.02^{b}
Mean	29.35	2.02	1.17	2.78
CV	1.21	0.51	1.07	1.10
CD (0.05)	0.65	0.02	0.02	0.05

Values with the same letters in a column are not significantly different ($p < 0.05$)

Water uptake ratio is a measure of volume expansion of rice during cooking and was significantly higher in Gandhakasala (2.16) and Thavalakannan (2.15). ER refers to the length wise elongation of rice after cooking and is the most desirable cooking traits especially in varieties like Basmathi. Significantly higher ER was observed in Wayanadan Gandhakasala (1.29) followed by Chitteni and Thavalakannan whereas Wayanadan Jeerakasala had significantly lower ER value. Wayanadan Gandhakasala had better price and demand than Wayanadan Jeerakasala in the market (Radhika *et al.*, 2018), which could

also be attributed to its better cooking qualities. Results obtained for ER and water uptake ratio were comparable to the observations made by Rajesh *et al*, (2018) and Nirmala Devi *et al.,* (2015). Cooked l/b ratio ranged from 1.82 to 3.35 and was higher for Thavalakannan which could be due to its higher water uptake ratio.

In vitro starch digestion

More than 50% of the TS was digested within the first 30 minutes of hydrolysis except for Wayanadan Gandhakasala and Chettadi. When the rate of starch hydrolysis was plotted against time, the curve obtained reached a plateau after 60 minutes of digestion as shown in **Figure 1.** According to Goñi *et al.,* (1997), rate of starch hydrolysis after 90 minutes (H 90 Experimental) was found to be the best hydrolysis value for determining the in vivo glycemic response. H 90 values were also calculated theoretically (H 90 Theory) as shown in **Table 4**. There was good agreement between H 90 Exp and H 90 Theory values. Rate of starch hydrolysis after 90 min was highest for Wayanadan Jeerakasala (62.56%) followed by Chitteni (59.74%) which suggests that they get digested more rapidly than other varieties and could elicit a high glycemic response. Significantly lower H 90 value was observed in Chettadi (51.41%) and Wayanadan Gandhakasala (52.16%). From 120 to 150 min, rate of starch hydrolysis was slow.

Table 4: Rate of starch hydrolysis (%) at 90 min

Sl. No.	Rice variety	H_{90} Exp*	H_{90} Theory**
1.	Chenkazhama	56.35±0.23^{c}	56.78 ±0.13^{d}
2.	Chettadi	51.41 ±0.22^{d}	53.02 ±0.34^{e}
3.	Chitteni	59.74 ±0.71^{b}	60.20 ±0.61^{b}
4.	Thavalakannan	56.46 ±3.56^{c}	59.09 ±1.68^{c}
5.	Gandhakasala	52.16 ±1.07^{d}	53.14 ±0.85^{e}
6.	Jeerakasala	62.56 ±0.43^{a}	64.91 ±0.32^{a}

*based on experimental results; **based on the equation, C = C∞ (1-e-kt)

Glycemic index

There were significant variations in C ∞ values of rice varieties which refers to the amount of starch hydrolysed after a prolonged time (180 minutes) as depicted in **Table 5**. C ∞ values of all varieties suggests that starch hydrolysis terminated before 180 min and significantly lower values were observed for Chettadi and Wayanadan Gandhakasala. The k value ranged between 0.05-0.10. Itdetermines the rate of starch digestion and absorption in the body. The C ∞ and k values are good predictors of glycemic index (Edwards *et al.,* 2019).

Table 5: Starch kinetics parameter of rice varieties

Rice variety	k	C_{∞}	AUC*	HI	GI
Chenkazhama	0.10	56.79±0.13^{c}	9293 ±17^{c}	55.93 ±0.10^{c}	70.41±0.06^{c}
Chettadi	0.06	53.16±0.44^{d}	8505 ±٣٦^{d}	51.19 ±0.21^{d}	67.81±0.12^{d}
Chitteni	0.07	60.32±0.49^{b}	9717±173^{b}	58.48±1.04^{b}	71.81±0.57^{b}
Thavalakannan	0.05	59.80±1.68^{b}	9365±230^{c}	56.36±1.39^{c}	70.65±0.76^{c}
Wayanadan Gandhakasala	0.07	53.30±0.93^{d}	8525±116^{d}	51.30±0.70^{d}	67.87±0.38^{d}
Wayanadan Jeerakasala	0.06	65.22±0.32^{a}	10373±44^{a}	62.43±0.27^{a}	73.98±0.15^{a}

Values with the same letters in a column are not significantly different ($p < 0.05$)

AUC of glucose (reference food) was calculated as 16,616

The HI values ranged between 51.19- 62.43%. HI refers to the proportion of starch that is theoretically digestible and is a predictor of glycemic index. Glycemic Index values ranged from 67.81 to 73.98. Brand-Miller *et al.,* (2009) classified food based on their glycemic index values as low (55 or less), intermediate (56 to 69) or high (70 or more). Accordingly, Wayanadan Jeerakasala, Chitteni, Thavalakannan and Chenkazhama had high glycemic index whereas Chettadi and Wayanadan Gandhakasala were varieties with intermediate glycemic index.

Some of the physico-chemical factors that can affect the starch digestibility in rice include amylose and amylopectin ratio, size of starch granules, presence of fiber, natural amylase inhibitors, starch- protein interactions and formation of lipid-amylose complexes (Panlasigui *et al.,* 1991 and Sagum and Arcot, 2000).

Total phenolic content

Table 6 provides the total phenolic content of brown rice flour along with its fractions namely free form and bound form phenolics. The free form phenolic content ranged from 34.45 mg GAE/100 g in Wayanadan Gandhakasala to 132.82 mg GAE/100 g in Chettadi. A range of 49.76 to 103.77 mg GAE/100g was found for bound form phenolics. Palakkadan Matta varieties had significantly higher free phenolic content than non-pigmented varieties. However, the highest amount of bound phenolics was recorded in Wayanadan Jeerakasala followed by Chitteni, Wayanadan Gandhakasala, Chenkazhama, Chettadi and Thavalakannan.

Table 6: Phenolics and flavonoid content of brown rice flour

Variety	Phenolics content (mg GAE / 100 g)			Flavonoid content (mg CE/ 100 g)		
	Free form	Bound form	Total	Free form	Bound form	Total
Chenkazhama	109.98±0.71^{c}	74.30±0.87^{c}	184.29±0.66^{c}	272.50±4.44^{b}	9.74±0.64^{d}	282.24±4.90^{b}
Chettadi	132.82±4.07^{a}	66.36±2.47^{d}	199.19±5.38^{b}	388.50±9.5^{a}	3.37±0.21^{f}	391.87±9.73^{a}
Chitteni	116.77±4.30^{b}	94.43±0.58^{b}	211.20±4.88^{a}	264.66±8.5^{b}	14.37±0.37^{a}	279.04±8.81^{b}
Thavalakannan	57.14±2.64^{d}	49.76±1.26^{e}	106.90±2.95^{e}	101.00±1.5^{c}	6.49±0.12^{e}	107.49±1.43^{c}
Wayanadan Gandhakasala	34.45±1.28^{e}	72.89±0.47^{c}	107.34±1.23^{e}	81.33±3.01^{d}	11.49±0.12^{c}	92.83±2.94^{d}
Wayanadan Jeerakasala	36.94±1.03^{e}	103.77±0.95^{a}	140.71±0.30^{d}	85.66±2.51^{d}	13.33±0.06^{b}	98.99±2.58^{cd}
Mean	81.35	76.91	158.27	198.94	9.80	208.74
CD (0.05)	5.41	2.18	6.31	11.20	0.61	11.68
CV	3.66	1.56	2.19	3.09	3.45	3.07

Values with the same letters in a column are not significantly different ($p < 0.05$).

The free phenolic content of the Palakkadan Matta varieties was greater than their respective bound form phenolics. This was consistent with the observation made by Sumczynski *et al.,* (2016) for red and black rice varieties. Non-pigmented rice varieties on the other hand, had higher content of bound form phenolics than their free form phenolic content, as also observed by Goufo and Trindade (2014). Therefore, pigmented and non-pigmented rice varieties can be good sources of free and bound form phenolics respectively and their distribution in rice kernel might be related to their bran colour.

Free and bound form phenolics perform different physiological functions in the body. Free form phenolics are readily absorbed in the small intestine and exhibit inhibitory action against LDL cholesterol oxidation whereas bound form phenolics are released by enzymatic or microbial fermentation in the colon, have anti-inflammatory properties and provide protection against colon cancer (Chandrasekara and Shahidi, 2011; Shao and Bao, 2015).

Total phenolic content ranged between 106.90 mg GAE/100g in Thavalakannan to 211.20 mg GAE/100g in Chitteni. The values obtained falls within the range of 79.18 to 691.37 mg GAE/100g reported for red and black rice varieties by Sompong *et al.,* (2011). Three out of four Palakkadan Matta varieties namely Chettadi, Chitteni and Chenkazhzma had higher total phenolic content than non-pigmented varieties. However, another red rice variety Thavalakannan had significantly less total phenolic content than non-pigmented variety Wayanadan Jeerakasala. This could be explained by the observation made by Sumczynski *et al.,* (2016) that total phenol content is more of a cultivar specific property rather than a colour dependent trait. Their study further suggested that phenolic compounds are secondary metabolites, significantly affected by stress conditions like wounding, extreme temperatures as well as environmental factors like cultivation techniques, altitude and use of fertilizers.

Total flavonoid content

Flavanoids are phenolic compounds with wide range of biological activities. They are potent antioxidants, antimicrobial and anti-inflammatory compounds. They are primarily known for their antioxidant or radical scavenging activities (Pietta, 2000). Free form flavonoid content of rice varieties exhibited significant variations with high CD value of 11.20 (p <0.05) as shown in **Table 6**. The range obtained was 81.33 in Wayanadan Gandhakasala to 388.50 mg CE/100g in Chettadi with a mean value of 198.94 mg CE/100g. Free form flavonoids content was significantly higher in Palakkadan Matta varieties than non-pigmented aromatic varieties, as also observed for free form phenolic content.

Bound form flavonoids ranged from 3.37mg CE/100g (Chettadi) to 14.37 mg CE/100g (Chitteni) and constituted a maximum of 5.15% towards total flavonoid content. This suggests that the flavonoids found in free form are the major contributor towards total flavonoid content (> 95%). This was in line with the observation made by Sumczynski *et al.,* (2016) for red and black rice varieties.

Total flavonoid content was significantly higher in Palakkadan Matta varieties (Chettadi> Chenkazhama > Chitteni > Thavalakannan) than Wayanadan Jeerakasala and Wayanadan Gandhakasala. The width of variation between the lowest (92.83) and highest value (391.87) of total flavonoid content was high (299.04 mg CE/100g). High CD value was observed for total flavonoid content, suggesting high varietal variation among rice varieties. The results obtained for total flavonoid content and total phenolic content in the present study was comparable to the data reported by Goufo and Trindade (2014). Total flavonoid content and total phenolic content of rice (r- 0.76, p-0.001) were found to be positively correlated, as also reported in a study done by Zhang *et al.,* (2010) (**Table 7**). Their study also found significant correlation between total phenolic content and total flavonoid content of black rice bran with its total antioxidant activity. Therefore, rice varieties with elevated levels of total phenolic content and total flavonoid content can be used for their antioxidant properties in developing rice based functional foods.

Table 7: Correlation among different characteristics studied in rice varieties

	Total phenolic content	Total flavonoid content	Total starch	Resistant starch	Glycemic index
Total phenolic content	1				
Total flavonoid content	0.760**	1			
Total starch	0.301	0.484	1		
Resistant starch	0.126	0.348	-0.042	1	
Glycemic index	0.210	-0.363	-0.311	-0.329	1

** Correlation is significant at 0.01 probability level

Conclusion

GI tagged rice varieties of Kerala were evaluated for various grain quality parameters and significant differences (p & lt; 0.05) were observed for every parameter studied. All varieties had high TS content (& gt; 70%) however, their rate of starch digestibility was different. For all varieties except Wayanadan Gandhakasala and Chetttadi, more than 50% of the TS was digested within 30 min and the hydrolysis reaction terminated before 180 min. Wayanadan Gandhakasala was also noted for significantly lower cooking time but higher water uptake and elongation ratio. Palakkadan Matta varieties had comparatively higher free phenolic, free flavonoid and total flavonoid content than the non-pigmented varieties. Pearson's correlation analysis revealed significant positive correlation between total phenolic and total flavonoid content. The width of variation for lowest and highest flavonoid content was high amongst varieties suggesting the necessity of screening rice varieties for their phytochemical composition. The study suggests that GI tagged varieties, which are known for their eating and cooking qualities, can also prove to be a potent ingredient for the development of functional food.

Acknowledgements

Authors acknowledge the Associate Director of Research, Kerala Agricultural University for providing rice samples and the Director, Central Sophisticated Instrumentation Facility, University of Calicut for laboratory support.

References

Adebamowo SN, Eseyin O, Yilme S, Adeyemi D, Willett WC, Hu FB, Spiegelman D and Adebamowo CA. 2017. A Mixed-Methods Study on Acceptability, Tolerability and Substitution of Brown Rice for White Rice to Lower Blood Glucose Levels among Nigerian Adults. *Frontiers in Nutrition*, 4(33): 1-9.

Ahmed F and Urooj A. 2003. *In vitro* starch digestibility and nutritionally important starch fractions in processed roots and tubers. *Starch/ Stärke*, 55(2): 94-99.

Bhattacharya KR. 2009. Physicochemical basis of eating quality of rice. *Cereal Foods World*, 54(1): 18-28.

Berry CS. 1986. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Journal of Cereal Science*, 4(4): 301-314.

Blakeney M, Krishnankutty J, Raju RK and Siddique KHM. 2020. Agricultural Innovation and the Protection of Traditional Rice Varieties: Kerala a Case Study. *Frontiers in Sustainable Food Systems*, 3(1): 1-11.

Brand-Miller JC, Stockmann K, Atkinson F, Petocz P and Denyer G. 2009. Glycemic index, postprandial glycemia and the shape of the curve in healthy subjects: Analysis of a database of more than 1000 foods. *American Journal of Clinical Nutrition*, 89(1): 97-105.

Chandrasekara A. and Shahidi F. 2011. Bioactivities and antiradical properties of millet grains and hulls. *Journal of Agricultural and Food Chemistry*, 59(1): 9563-9571.

Chen Y, Wang M and Ouwerkerk PBF. 2012. Molecular and environmental factors determining grain quality in rice. *Food and Energy Security*, 1(2): 111-132.

Custodio MC, Cuevas RP, Ynion J, Laborte AG, Velasco ML and Demont M. 2019. Rice quality: How is it defined by consumers, industry, food scientists and geneticists? *Trends in Food Science and Technology*, 92: 122-137.

Deepa G, Singh V and Naidu KA. 2010. A comparative study on starch digestibility, glycemic index and resistant starch of pigmented ("Njavara" and 'Jyothi') and a non-pigmented ('IR 64') rice varieties. *Journal of Food Science and Technology*, 47(6): 644-649.

Dewanto V, Xianzhong W, Adom KK and Liu RH. 2002. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, 50(10): 3010-3014.

Edwards CH, Cochetel N, Setterfield L, Perez-Moral N and Warren FJ. 2019. A single-enzyme system for starch digestibility screening and its relevance to understanding and predicting the glycaemic index of food products. *Food and Function*, 10(8): 4751-4760.

Gong ES, Luo SJ, Li T, Liu CM, Zhang GW, Chen J, Zeng Z C and Liu RH. 2017. Phytochemical profiles and antioxidant activity of brown rice varieties. *Food Chemistry*, 227: 432-443.

Goñi I, Garcia-Alonso A and Saura-Calixto F. 1997. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, 17(3): 427-437.

Goufo P and Trindade H. 2014. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ -oryzanol and phytic acid. *Food Science and Nutrition*, 2(2): 75-104.

Hu P, Zhao H, Duan Z, Linlin Z and Wu D. 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science*, 40(3): 231-237.

Juliano BO, Oñate LU and del Mundo AM. 1965. Relation of starch composition, protein content and gelatinization temperature to cooking and eating qualities of milled rice. *Food Technology*, 19(6): 116-121.

Krishnamrutha TS, Sai Prasad SV, Sameer Kumar CV and Ramesh T. 2023. Study on Genetic Variability and Correlation Coefficient for Grain Quality Parameters in South Indian Rice (*Oryza sativa* L.) Varieties. *Journal of Rice Research*, 16(2): 66-72.

Krishnankutty J, Blakeney M, Raju RK and Siddique KHM. 2021. Sustainability of traditional rice cultivation in Kerala, India-a socio-economic analysis. *Sustainability (Switzerland)*, 13(2): 1-16.

Lehmann U and Robin F. 2007. Slowly digestible starch - its structure and health implications: a review. *Trends in Food Science and Technology*, 18(7): 346-355.

Nirmala Devi G, Ravindra Babu V, Padmavathi G and Sunitha T. 2015. Genetic Diversity in Grain Quality Traits of Rice Genotypes. *Journal of Rice Research*, 8(1): 6-14.

Panlasigui LN, Thompson LU, Juliano BO, Perez CM, Yiu SH and Greenberg GR. 1991. Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. *American Journal of Clinical Nutrition*, 54(5): 871-877.

Pietta PG. 2000. Flavonoids as Antioxidants. *Journal of Natural Products,* 63(7), 1035-1042.

Pillai C, Faseela K V and Thampi H. 2020. Nutritional composition of selected traditional rice varieties of Kerala. *Journal of Tropical Agriculture*, 58(1), 33-43.

Radhika AM, Jesy Thomas K and Anil Kuruvila R. 2018. Assessing the impact of Geographical Indications on well-being of rice farmers in Kerala. *International Journal of Intellectual Property Rights*, 9(2): 1-11.

Rajesh T, Radhakrishnan V, Pressana Kumari K, Francies R, Sreenivasan E, Ibrahim K and Latha A. 2018. Grain quality characteristics of two-line rice hybrids in Kerala. *Journal of Rice Research*, 11(1): 19-25.

Sagum R and Arcot J. 2000. Effect of domestic processing methods on the starch, non-starch polysaccharides and *in vitro* starch and protein digestibility of three varieties of rice with varying levels of amylose. *Food Chemistry*, 70(1): 107-111.

Sajilata MG, Singhal RS and Kulkarni PR. 2006. Resistant Starch - A Review. *Comprehensive Reviews in Food Science and Food Safety*, 5(1): 1-17.

Shao Y and Bao J. 2015. Polyphenols in whole rice grain: Genetic diversity and health benefits. *Food Chemistry*, 180, 86-97.

Singh N, Kaur L, Singh Sodhi N and Singh SK. 2005. Physicochemical, cooking and textural properties of milled rice from different Indian rice cultivars. *Food Chemistry*, 89(2): 253-259.

Soam SK. 2005. Analysis of Prospective Geographical Indications of India. *The Journal of World Intellectual Property*, 8(5): 679-704.

Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G and Berghofer E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry*, 124(1): 132-140.

Sumczynski D, Kotásková E, Družbíková H and Mlček J. 2016. Determination of contents and antioxidant activity of free and bound phenolics compounds and *in vitro* digestibility of commercial black and red rice (*Oryza sativa* L.) varieties. *Food Chemistry*, 211: 339-346.

Zhang MW, Zhang RF, Zhang FX and Liu RH. 2010. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *Journal of Agricultural and Food Chemistry*, 58(13): 7580-7587.