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Physicochemical properties, eating and cooking quality and genetic variability: a comparative analysis in selected rice varieties of South India

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Abstract

The physicochemical characteristics and digestibility properties of rice are greatly influenced by its genetic variability. In this study, we attempt to understand the overall quality of five traditional and popularly consumed rice varieties of Kerala. The major traits affecting the eating and cooking quality of rice such as amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) were determined and correlated with the expression of the starch-synthesis-related genes (SSRGs). The *Wx* is a major SSRG, which modulates the amylose content and the eating as well as cooking qualities of rice. The rice varieties including, Jaya, Matta and Rakthashali were found to have intermediate amylose content (Wx^a allele) while Kuruva and Pokkali were found to have high Amylose Content (Wx^a allele). The glycemic index (GI) of all varieties was found to be in the range of 51.0–58.6. Among the screened rice varieties, Pokkali was found to have the lowest glycemic index and digestibility with appreciable eating and cooking qualities. Pokkali rice, with its low GI (51.0), could be a recommended variety for diabetes management. Furthermore, the rice eating and cooking qualities, and plant breeding techniques coordinated by the expression of the starch synthesis-related gene (*Wx*), could be a novel approach to improve the valuable germplasm.

Keywords Waxy allele, Gelatinization temperature, Gel consistency, Amylose content, Glycemic index

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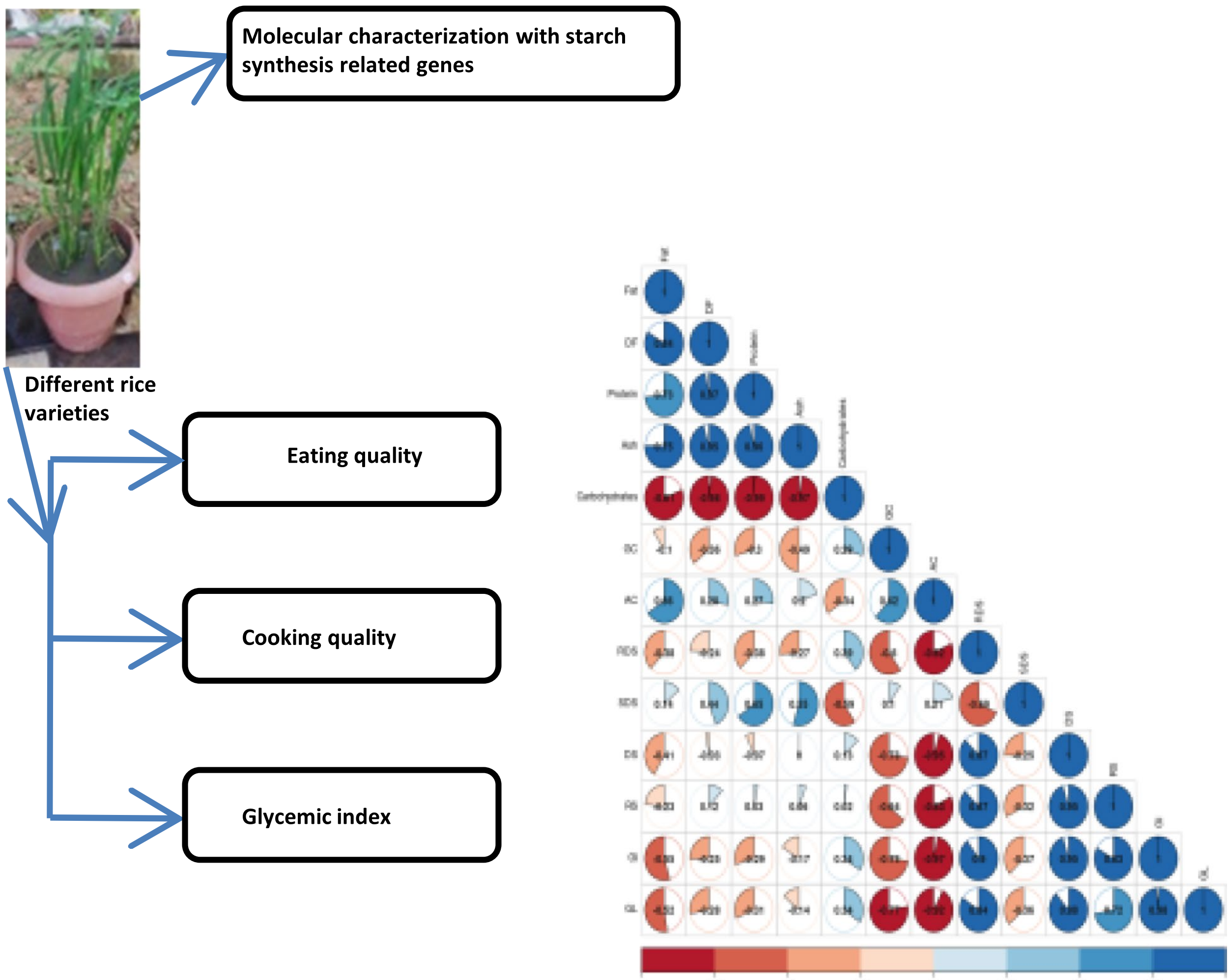
Graphical Abstract



Introduction

Rice is a major staple diet worldwide, and its yield has been greatly increased over recent decades. Rice grain quality plays a key role in consumer acceptance and includes eating and cooking quality (ECQ) (Zhou et al. 2020), nutritional quality (Yang et al. 2019), grain appearance (Zhao et al. 2018) and milling quality. Among these, the ECQ is the foremost priority among consumers and breeders. Starch, accounts for > 80% of the edible part of milled rice and is the primary determinant of rice ECQ. It influences the palatability, appearance, hardness, stickiness and digestibility of cooked rice. Starch, a semi-crystalline polysaccharide, consists of linear amylose and highly branched amylopectin (Rodriguez-Garciaet et al. 2021). The starch composition and structure determine the physicochemical properties; and therefore, ECQ that includes amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) or alkali spreading value (ASV). Among these traits, AC is the most important factor affecting the ECQ of rice. Rice varieties with high AC (> 25%) are dry, separate, less tender, and become hard upon cooling. Intermediate AC (20–25%) remains soft and flaky whereas those with low (12–20%) are glossy, soft, and sticky (Biselli et al. 2014). Previous studies have demonstrated that rice varieties with low to intermediate AC tend to show better palatability, stickiness and hardness than high AC types and glutinous rice varieties (Zhang et al. 2019, Huang et al. 2020). Rice varieties with intermediate amylose content are most preferred by consumers of South Asia. Apart from quality, people nowdays have more concerns about their health, especially among the diabetic subjects. Carbohydrate rich foods are generally classified based on Glycemic Index (GI), which indicates their postprandial glycemic effect. GL gives an idea about the amount of ingested carbohydrate for a serving (Vlachos et al. 2020). Studies have been conducted to understand the effect of low GI foods on blood glucose level, and it has been found to be useful for glycemic control in people with diabetes (Zafar et al. 2019, Ojo et al. 2018). The AC, GC and GT in rice varieties could be positively correlated with starch synthesis related genes (SSRGs) including genes encoding ADP glucose pyrophosphorylase (AGPase), soluble starch synthase (SS), granule-bound starch synthase (GBSS), starch branching enzyme (SBE) and starch debranching enzyme (DBE) (Jeon et al. 2010). The *Waxy* (*Wx*) gene encoding granule-bound starch synthase I (GBSSI) is the major determinant of AC and GC and minor determinant for GT (Tian et al. 2009, Zhang et al. 2019). The*Wx*is located on chromosome 6 and is usually selected during rice domestication. A strong correlation has been observed between amylose content, GI and waxy locus (Fitzgerald et al. 2011).

Three important allelic variants of the *Wx* gene: Wx_a, Wx_b and *wx*, identified based on the G/T SNP at the splice site of the first intron, accounts for 80–90% of variations observed with respect to AC in non-waxy rice varieties (Yang et al. 2018, Zhang et al. 2019). A spontaneous mutation caused by a 23-bp duplication inserted in exon 2 resulted in the loss of function of *Wx*, which corresponds to *wx* allele. This leads to the inactivation of GBSSI and hence, very low or no AC (Zhou et al. 2015). GLU 23 marker has been used widely to identify this type of mutation (Wanchana et al. 2003). Wx_ballele-specific to *japonica* varieties carries a substitution mutation at the 5′ splice site of the 1st intron and has a TT sequence at the 59th splice junction of the first intron. Wx^aallele, specific to *indica* varieties, has GT in the same position (Bligh et al. 1998). A single nucleotide polymorphism on exon 6 (exon 6 SNP A/C), results in another allele Wx^{in}that is associated with intermediate AC content up to 22% (Larkin et al. 2003). PCR based markers have been used to distinguish the rice varieties based on the type of *waxy* locus allele present and correlated it with the amylose content (Wanchana et al. 2003, Ferdous et al. 2018).

The current study was carried out to evaluate the grain quality characteristics including physicochemical properties and digestibility characteristicsof selected rice varieties of Kerala and to understand its functional correlation with GI and molecular correlation with the *Waxy* gene. The determination of cooking characteristics would be an additional information to understand ECQ. This could be a promising approach for generating and maintaining the novel improved germ plasm resources with high ECQ and low GI.

Materials and methods

Plant materials

Five popular and traditional rice samples (Jaya, Matta, Kuruva, Pokkali and Rakthashali) (Fig. 1) as milled were collected from Rice Research Station, Vyttila and Block Development Office, Angamaly, Kerala. After cleaning and sorting, samples were stored in air tight containers at 4 °C, until use.

Physico-chemical characterization and proximate composition

Grain dimensions, bulk density, thousand kernel weight(TKW) and color

The length, breadth, bulk density, TKW and color of the sample rice grains were evaluated. The length L (mm), and breadth B (mm) of the rice kernels were determined by using a vernier caliper with accuracy of 0.01 mm. Grain shape also was estimated by the ratio of length and breadth (L/B ratio) according to the classification of IRRI (IRRI, 2020). The bulk density (g/cm^3) was calculated according to Akonoret al. (2021) with slight modifications. The 1000 kernel weight (g) was measured as mentioned by Varnamkhasti et al. (2008). Color

Fig. 1 Images of different rice samples used in the study

measurements of the rice kernels were obtained using Lovibond colorimeter (Lovibond LC 100 Spectrocolorimeter, UK). The equipment was calibrated and the color was measured in CIE L^*, a^* and b^*. L is the measure of brightness from black (0) to white (100). The parameter a describes redness (positive) to greenness (negative) and the parameter b describes blueness (positive) to yellowness (negative) (Schoefs et al. 2002).

Gelatinization temperature (GT) by alkali spreading Value (ASV), gel consistency (GC) and amylose content (AC)

Six milled kernels were placed in 10 ml of 1.7% KOH solution in petri dish and were allowed to stand for 23 h at 30˚C to score spreading on 1–7 scale (Chemutai et al. 2016). Based on the ASV, samples were assigned to GT classes (IRRI 1996). The AC was determined by colorimetric method based on the iodine-binding procedure as described by Lauro et al. 1999 by using UV–visible Spectrophotometer (JascoV-730, Japan).

Proximate composition

Moisture, fat, protein and ash content of the samples were determined by standard analytical methods (AOAC 2015). The crude protein content of the rice samples was determined using the Kjeldhal method, which involved protein digestion and distillation; and the factor of 5.65 was used to estimate protein. The dietary fiber was determined by acid–alkali washing method (AOAC, 2005). The total available carbohydrates were estimated by the difference method (FAO 2004).

Available carbohydrate (g%) = 100—[protein + fat + moisture + ash + dietary fiber].

Cooking of rice

Standardization of cooking conditions

The test samples (50g) were taken from each variety and cooking time and temperature were standardized with sufficient quantity of water. All samples were cooked by conventional boiling method under medium flame in lidded pans. Samples were cooked until it reaches a soft consistency with improved texture and a cooked flavor. Excess cooked starch water was decanted. The cooking conditions for all rice samples were standardized after several cooking trials in laboratory settings and demonstrated in Table 3.

Cooking characteristics

The ideal rice to water ratio for an appropriately cooked rice was standardized under laboratory conditions. All cooking trials were done with 50 g of samples. Cooking characteristics of rice samples were estimated according to (Oko et al. 2012), withslight modifications. In brief, the water uptake ratio (WUR) was determined by taking the ratio of cooked rice weight and uncooked rice weight. Solids in cooking water (SCW) was determined by calculating the weight difference of an aliquot of cooked rice water before and after drying. The grain elongation ratio (GER) was determined by measuring the grain length before and after cooking.

Starch digestibility and glycemic index

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS)

- The evaluation of RDS, SDS and RS were done according to the methodology of (Englyst et al. 1992), with slight modifications. About 0.5 g freshly cooked rice sample was ground into fine paste using a pestle and mortar in 10 ml acetate buffer (0.1 M, pH 5.2). This was then incubated on a magnetic stirrer (Kemi, India) at 37˚C with 160 rpm for 10 min, and treated with 2.5 ml of enzyme cocktail (3800 U/ml pancreatin, 188 U/ml amylase, 13 U/ml amyloglucosidase, Sigma–Aldrich, St. Louis, MO, USA). RDS and SDS were determined in the aliquots (0.25 ml) as the glucose released by enzymatic hydrolysis after 20 and 120 min, respectively. The remaining mixture was centrifuged (Eltek, Labspin, TC 450 D) and the supernatant was decanted and the residue was washed twice with 10 ml absolute alcohol. The pellets were treated with 7 M KOH, heated in a boiling water bath, and hydrolyzed further with amyloglucosidase (50 U/ml) to determine RS, which is the starch fraction that remained unhydrolyzed after 120 min. The amount of glucose released for DS and RS estimation was determined with a Glucose assay kit (Sigma–Aldrich, St. Louis, MO, USA). Total Starch (TS) was obtained by the summation of DS and RS.

Glycemic index (GI) and glycemic load (GL)

Estimation of GI involved analysis of available starch and starch hydrolysis index 90 (HI 90) as per the procedure and formula given by (Goñi et al. 1997). The cooked rice samples (250 mg) were homogenized thoroughly with 25 ml distilled water and 200µltermamyl enzyme (Sigma-Aldrich, USA) for 15 min (in boiling water bath-100˚C). The clear supernatant solution (1 ml) was drawn and incubated with sodium phosphate buffer (2 ml, pH 4.75) and amyloglucosidase (Sigma-Aldrich, USA) (1 ml) at 60 °C for 30 min with gentle mixing every 5 min. It was then diluted to 10 ml with distilled water. Glucose was estimated by using Glucose assay kit (Sigma–Aldrich, St.

Louis, MO, USA). The percentage available starch was estimated using the formula:

$$\text{Percent available starch} = \frac{glucose(\mu g) \times 25 \times 0:9}{sampleweight(mg)}$$

The Hydrolysis Index at 90 min (HI 90) was estimated by the methodology suggested by (Holm et al. 1986). Freshly cooked homogenized rice sample (1600 mg) was suspended in 50 ml of 0.1 M phosphate buffer (pH 6.9). Then 5 ml of the same buffer containing 500 mg pancreatin (Sigma-Aldrich, CAS: 8049–47-6), was added and the mixture was incubated at 37 °C with continuous shaking for 90 min. Clear sample solution (0.1 ml) was drawn at 0 min and exactly after 90 min and analyzed for maltose using the Dinitro Salicylic Acid (DNSA) method. Results were expressed as mg maltose released after 90 min of hydrolysis of 1 g sample (dry weight). HI 90 was estimated using the formula:

$$\text{HI90} = 100 \times 0.9 \times \frac{maltose(mg)}{starch(mg)}$$

From value of HI 90, GI was calculated using the following formula:

$$\text{GI} = 39.21 + 0.803 \times \text{HI90}$$

Glycemic load (GL) was estimated by multiplying the amount of carbohydrate contained in a nominal serving size (100 g) of rice with cmerón et al. 1997).

$$GL = \frac{Availablecarbohydratecontentperportion(g) * GI}{100}$$

Genotyping using Wx allele-specific markers

For waxy gene analysis studies, paddy seeds were germinated and genomic DNA was isolated from young leaves of three-week-old plants following the simple CTAB method (Rani et al. 2016) and dissolved in 1X TE buffer. The quality and quantity of extracted DNA samples were determined using spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific, USA). PCR amplification was carried out in a total volume of 20 µl containing a final concentration of 25 ng of genomic DNA and 2XPCR Master Mix (Thermo Fisher Scientific, USA), comprising of 1X Taq Buffer with 4 mM $MgCl_2$, 0.4 mM of each dNTPs and 0.05U/µl Taq polymerase enzyme. The PCR program involved an initial denaturation at 94°C for 5 min followed by 31 cycles of denaturation (94°C for 5 min), annealing at 55–65°C depending on the GC content of the primers for 40 s and extension at 72°C for 40 s. PCR reactions were carried out in Thermocycler (Applied Biosystems, Veriti 96 well thermocycler, U.S).

Table 1 Molecular markers linked to *Waxy* locus used for screening rice varieties

Primer	Sequence
Glu-23F	TGCAGAGATCTTCCACAGCA
Glu-23R	GCTGGTCGTCACGCTGAG
*Waxy-intron1*F	CTTTGTCTATCTCAAGACAC
*Waxy-intron1*R	TTTCCAGCCCAACACCTTAC
wWx^{in} Allele specific primer F	GTTGGAAGCATCACGAGTTT
Wx^{in} Allele specific primer	GAGATCAATTGTAACTCACCAG
Wxb allele specific primer pair F	CCATTCCTTCAGTTCTTTGTCT
Wxb allele specific primer pair R	CACTGACCTGGCAAAGAAGG

Three molecular markers linked to *Waxy* gene were used to screen the rice varieties (Table 1). Glu23 was used to amplify DNA fragment containing the 23-bp duplicate region, which discriminate between non-glutinous varieties with *Wx* and glutinous varieties having *wx* allele. The varieties were also analyzed with Wx^{in} marker to identify intermediate-amylose cultivars. Wx^{b} allele specific primer was used to identify varieties with low amylose content. The amplified products were resolved and visualized in 0.8% polyacrylamide gel electrophoresis.

Restriction digestion of PCR amplified products with Acc1

AGGTATA/AGTTATA polymorphism was detected by using a PCR-CAPS marker Waxy- intron and restriction endonuclease cleavage. The isolated DNA samples were amplified with primer pair Waxy –intron. 10 µL of each amplified product were digested with AccI according to manufacturer's protocol for the AccI enzyme (Thermo Fischer Scientific, USA). The reaction consisted of 10 µL amplified product, 18 µL of PCR graded H_2O, 2 µL of RE 10×TBE Buffer B and 2 µL of Restriction enzyme. The above mixture was incubated for 2 h at 37 °C and a 2% agarose gel used to assess the band size of the digested product.

Statistical analysis

The three-time replicated experimental data were analyzed for Analysis of Variance (ANOVA) at 0.05 level of significance. Ten-time replicated data were considered for the length and breadth determination of uncookedand grain elongation ratio of cooked samples. The Pearson correlation coefficient at 0.05 level of significance was calculated using GRAPES 1.0.0. software (Gopinath et al. 2021). All data are presented as mean ± standard deviation.

Table 2 Physicochemical characteristics and proximate composition of different rice varieties

Parameters	Jaya	Matta	Rakthasali	Kuruva	Pokkali
Physical characteristics					
Length (cm)	0.7 ± 0.0c**	0.6 ± 0.04b**	0.5 ± 0.1d**	0.4 ± 0.1e**	0.7 ± 0.1a**
Breadth (cm)	0.3 ± 0.01a**	0.2 ± 0.02b**	0.2 ± 0.02c**	0.2 ± 0.02b**	0.2 ± 0.01c**
L/B ratio	2.3 ± 0.03d**	3.0 ± 0.03b**	2.7 ± 0.04c**	1.7 ± 0.04e**	3.6 ± 0.4a**
Bulk density (g/cm^3)	0.7 ± 0.02	0.6 ± 0.0	0.6 ± 0.04	0.6 ± 0.01	0.6 ± 0.02
TKW (g)	20.4 ± 0.2c**	21.4 ± 0.2b**	16.3 ± 0.1e**	15.3 ± 0.2d**	24.4 ± 0.4a**
Water uptake ratio	2 ± 0.3a**	1.8 ± 0.9 cd**	1.9 ± 0.8ab**	1.8 ± 0.2d**	1.8 ± 0.5bc**
Solids in cooking water g/100 ml (g)	1.4 ± 0.3d**	2.2 ± 0.9b**	3.5 ± 0.5a**	0.9 ± 0.2e**	1.6 ± 0.7c**
GER (cm)	1.6 ± 0.03bc**	1.7 ± 0.4ab**	1.5 ± 0.2bc**	1.5 ± 0.1c**	1.8 ± 0.6a**
ASV	1	3	6	3	2
Colour characteristics					
L*	59.3 ± 1.0a**	49.3 ± 1.1b**	40.3 ± 0.6c**	57.6 ± 0.4a**	35.9 ± 1.3d**
a*	5.9 ± 0.1d**	9.7 ± 1.4b**	11.6 ± 0.6a**	5.8 ± 0.4d**	7.5 ± 0.8c**
b*	20.5 ± 0.6a**	16.6 ± 0.4c**	10.4 ± 0.3e**	17.6 ± 0.6b**	13.1 ± 0.1d**
Chemical characteristics					
Total starch (%)	78.9 ± 0.6a**	76 ± 0.4c**	72.7 ± 0.8e**	76.7 ± 0.2b**	73.6 ± 0.4d**
AC (%)	25.3 ± 0.4b**	22.9 ± 0.4d**	24.1 ± 0.7c**	25.7 ± 0.2b**	29.1 ± 0.8a**
Proximate composition					
Moisture (%)	11.7 ± 0.4	11.4 ± 0.6	11.1 ± 0.1	11.7 ± 0.8	10.7 ± 0.2
Fat (%)	0.5 ± 0.3c**	0.9 ± 0.1bc**	1.3 ± 0.5b**	0.5 ± 0.1c**	2.0 ± 0.4a**
Protein (%)	7.3 ± 0.3d**	9.4 ± 0.4c**	11.7 ± 0.6a**	9.2 ± 0.2c**	10.7 ± 0.0b**
Ash (%)	0.7 ± 0.2e**	0.9 ± 0.1c**	1.3 ± 0.2a**	0.8 ± 0.1d**	1.1 ± 0.2b**
Crude Fiber (%)	0.9 ± 0.03d**	1.5 ± 0.2b**	1.9 ± 0.3a**	1.2 ± 0.1c**	1.8 ± 0.1a**
Carbohydrates (%)	79.8 ± 0.5a**	77.5 ± 0.4c**	74.6 ± 0.7e**	77.9 ± 0.3b**	75.4 ± 0.6d**

TKW Thousand kernel weight, *GER* Grain elongation ratio, *ASV* Alkali spreading value, *AC* Amylose content. **Treatments with same letters are not significantly different ($p < 0.05$)

Results and Discussion

Grain characteristics

The decorticated grain length (mm) was significantly different ($p^{<}0.05$) among all rice varieties (Table 2). The longest grain length was recorded for Jaya and Pokkali (7 mm), followed byMatta (6 mm), Rakthasali (5 mm) and Kuruva (4 mm). The grain width ranged between 2 and 3 mm, with no statistical significance. Among all the five varieties, the highest L/B ratio was observed for Pokkali. The selection and acceptance of rice varieties often depends on the grain shape and characters (Dias et al. 2022). The B.D showed no statistically significant variation among samples. The TKW (1000 kernel weight) or test weight varied from heaviest 24.4 g in Pokkali to the lightest in 15.3 g in Kuruva (Table 2). TKW is an important parameter to determine grain quality whichindicates the average economic sink size of the plant (Afshari et al. 2011, Zhou et al. 2016).

Alkali spreading value,gelatinization temperature, and amylose content

The results for ASV and AC are furnished in Table 2. The ASV is closely related to GT of raw rice. It measures the degree of spreading using 7-point scale (1- intact, 7- greatly dispersed) and corresponds to the gelatinization temperature: 1–2, high (74.5–80˚C); 3–4, high-intermediate; 5, intermediate (70–74˚C) and 6–7, low (< 70˚C) (Park et al. 2007). The ASV varied between 1 and 6, with Jaya showing intact grain characteristics and Rakthasali with dispersed grain characteristics. Based on AC, the average values ranged from 22.9% in Matta to 29.1% in Pokkali. Amylose content of starches from rice varieties Rakthasali, Jaya and Kuruva were 24.07%, 25.27% and 25.73% respectively. According to the classification crieteria, rice varieties Matta and Rakthasalicould be considered with intermediate amylose starch, whereas Kuruva, Jaya and Pokkali were with high amylose starch respectively.

Proximate composition

The results of the proximate composition (moisture content, protein, fat, fiber, ash content and carbohydrate) of rice varieties under study are shown in Table 2. There were significant differences ($p < 0.05$) in the proximate composition of the rice samples, except moisture. Moisture content is a critical aspect of cereal grains that significantly affect the shelf life. Moisture content above 12% are not recommended as it causes infestations and deterioration (Whitehouse et al. 2018, Dias et al. 2022). The moisture content varied between 10.7% to 11.7% in all the rice samples and were in accordance with earlier reports (Verma et al. 2017, Dias et al. 2022). Nasirahmadi et al., (2014) reported that the moisture content in processed rice sample is influenced by varietal characteristics, initial moisture content and processing conditions. The fat content was highest in Pokkali (2.0%). In general, the fat content showed a significant variation ($p < 0.05$); and varied between 0.5% and 2.0%. Despite the lower quantity, compared to other crops, fat content significantly influences the eating quality of rice (Verma et al. 2017). The possible reason for these variations could be due to the milling practice, degree of aleurone layer retained and the level of oxidation of unsaturated fatty acid (Wang et al. 2006, Verma et al. 2017). The highest percentage of crude fiber, protein and ash content among all rice varieties was exhibited in Rakthasali (1.9%,11.7%, 1.3% respectively).The fiber content plays a significant role in human health including bulking effect, cholesterol lowering, maintaining intestinal pH and preventing colorectal cancer (Fuentes-Zaragoza et al. 2010). The protein content in Pokkali was slightly lesser (10.7%). The protein results were in harmonywith earlier reports. The protein is an essential nutritional component, as it may fulfill the protein deficiency. The variations in the protein content in different rice varieties could be due to genetic makeup, agronomic management practices and environmental factors (Zhou et al. 2016, Amagliani et al. 2017, Verma et al. 2017). The lower ash content was observed in Jaya (0.7%). The ideal carbohydrate content in rice to meet the calorific demand is 80%. All the rice samples were recorded to have carbohydrate content nearer to the ideal range (Verma et al. 2017). The ash content signifies the total amounts of minerals in the sample. The extend of processing during decortication is an important parameter to determine composition of rice.

Cooking characteristics

The evaluation of cooking quality characteristics included water uptake ratio, grain elongation during cooking (mm), solids in cooking water (g) (Table 2) and optimum cooking time (min). The standardized cooking conditions of all samples are presented in Table 3. All samples showed significant differences in cooking conditions and slight difference in cooking water requirement. Samples other than Jaya were cooked in a rice to water ratio of 1:10 whereas the Jaya variety required slightly higher volume of water in the ratio of 1:12. The cooking time was high for Jaya rice (75 min) and lowest for Kuruva (50 min). The requirement of increased volume of cooking water for Jaya rice is due to the elevated cooking time of the kernels. The cooked weight varies according to the water absorption rate and cooking time of each variety. The Jaya variety had the highest cooked weight (200 g) whereas Kuruva rice had the lowest (177 g). The decanted gruel volume was similar for all varieties. The water uptake ratio was observed the highest for Jaya rice (2) followed by 1.9 for Rakthasali, 1.8 for Pokkali,Matta and Kuruva varieties. The solids in cooking water are an index of gruel solid loss during the cooking and decanting process. This is considered as a greater characteristic as the solid loss gives an idea about the cooked grain nature and it is negatively related to the starch percentage and digestibility. The values showed greater differences with a minimum of 0.9 g for Kuruva and 3.5 g for Rakthasali when 100 ml gruel water was taken for the evaluation. Grain elongation after cooking was highest for the Pokkali variety (1.8) and least for the Kuruva variety (1.5) which is positively correlated to the uncooked grain length. The grain length is an important parameter which determines the cooking quality. The long slender rice found the most consumer acceptance. Linear expansion is one of the most appreciable characteristics of cooked rice (Syafutri et al. 2016). The grain length is significantly

Table 3 Standardizedcooking conditions of rice samples

Rice varieties	Rice: water ratio	Cooking time (min)	Initial weight (g)	Solid cooked weight (g)	Gruel volume (ml)
Jaya	1:12	75	50	$200 \pm 8^{a*}$	$240 \pm 15^{b*}$
Matta	1:10	60	50	$178 \pm 5^{c*}$	$179 \pm 18^{c*}$
Rakthasali	1:10	60	50	$191 \pm 12^{b*}$	$150 \pm 16^{d*}$
Kuruva	1:10	50	50	$177 \pm 6^{c*}$	$240 \pm 12^{b*}$
Pokkali	1:10	70	50	$191 \pm 6^{b*}$	$300 \pm 10^{a*}$

* Treatments with same letters are not significantly different ($p < 0.05$)

Table 4 Starch digestibility, Glycemic index and Glycemic load of cooked rice varieties at 75–77% moisture content

Parameters	Jaya	Matta	Rakthasali	Kuruva	Pokkali
RDS*	$9.1 \pm 1.2^{c**}$	$10.8 \pm 1.6^{a**}$	$9.9 \pm 1^{b**}$	$8.4 \pm 0.5^{d**}$	$8.4 \pm 0.8^{d**}$
SDS*	$10.8 \pm 1.3^{a**}$	$9.8 \pm 1.3^{c**}$	$10.1 \pm 1.1^{bc**}$	$10.4 \pm 1.2^{ab**}$	$10.3 \pm 1.2^{b**}$
DS*	$19.9 \pm 2.5^{b**}$	$20.6 \pm 2.8^{a**}$	$20.0 \pm 2.7^{b**}$	$18.8 \pm 2.4^{c**}$	$18.7 \pm 2.1^{c**}$
RS*	$0.6 \pm 0.0^{ab**}$	$0.4 \pm 0.1^{c**}$	$0.5 \pm 0.0^{c**}$	$0.6 \pm 0.0^{ab**}$	$0.8 \pm 0.1^{a**}$
GI	$57.1 \pm 0.6^{c**}$	$58.6 \pm 0.5^{a**}$	$57.8 \pm 0.6^{b**}$	$53.6 \pm 0.4^{d**}$	$51.1 \pm 0.4^{e**}$
GL	$28.6 \pm 0.3^{c**}$	$29.3 \pm 0.2^{a**}$	$28.9 \pm 0.3^{b**}$	$26.8 \pm 0.2^{d**}$	$25.5 \pm 0.2^{e**}$

RDS Rapidly digestible starch, *SDS* Slowly digestible starch, *DS* Digestible starch, *RS* Resistance starch, *GI* Glycemic index, *GL* Glycemic load, **Treatments with same letters are not significantly different ($p < 0.05$)

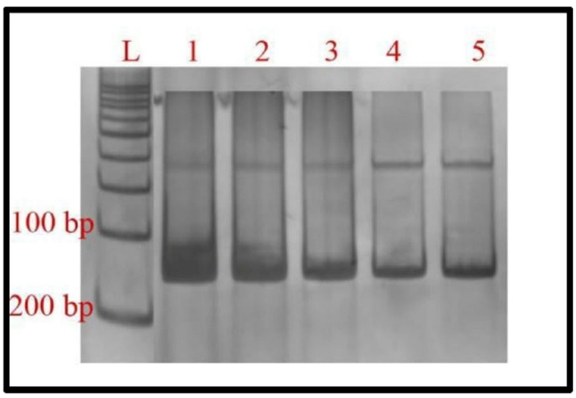


Fig. 2 Genotypic screening of rice varieties with GLU 23. L: 100 bp ladder, 1: Rakthashali, 2: Pokkali, 3: Jaya, 4: Kuruva, 5: Matta

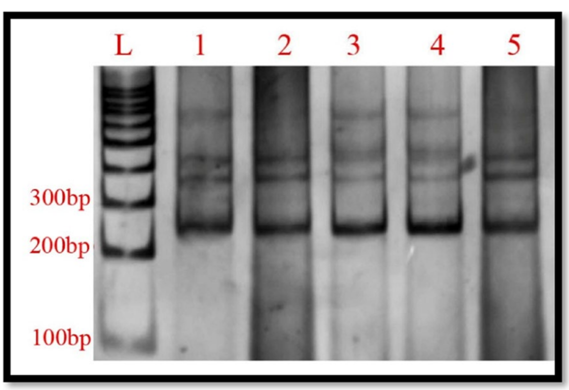


Fig. 3 Genotypic screening of rice varieties with Waxy- intron. L: 100 bp ladder, 1: Rakthashali, 2: Pokkali, 3: Jaya, 4: Kuruva, 5: Matta

altered and elongated upon cooking. The most extended grain length after cooking was recorded for Pokkali (13 mm), followed by Jaya (11 mm).

Starch digestibility and glycemic index

Measurement of different starch fractions provide a means for predicting the rate and extent of starch digestion in the human small intestine. The total starch and its fractions, RDS, SDS and RS, in the cooked rice samples are shown in Table 4. The starch fraction profile showed slight variations with different rice varieties. Pokkali rice showed lower DS (18.7%) followed by Kuruva (18.8%) and Jaya (19.9%). The RDS fraction was low in Pokkali and Kuruva (8.4%) varieties. Both RDS and DS were high in the Matta variety with values of RDS (10.8%) and DS (20.6%) respectively. The SDS value was low for Rakthasali rice (10.1%) and high for Jaya rice (10.8%). The RS content was high in Pokkali rice variety (0.8%) followed by Jaya and Kuruva(0.6%).

All samples showed significant lower GI than reference food. The GI was statistically significantbetween Jaya (57.49), Rakthasali (57.02) and Matta (58.62). On the basis of classification of carbohydrate foods described by (Wolever et al. 1985), these varieties came under intermediate GI food (56 to 60). GI of remaining two varieties was found to be lower again with values; Kuruva 53.57 and Pokkali 51.76 and both came under low GI rice (˂55). GL indicates the extend of raise in blood glucose levels by each gram of carbohydrate present in food. Among the five rice samples, the GL of Jaya, Matta and Rakthasali were comparatively high with values 28.6,

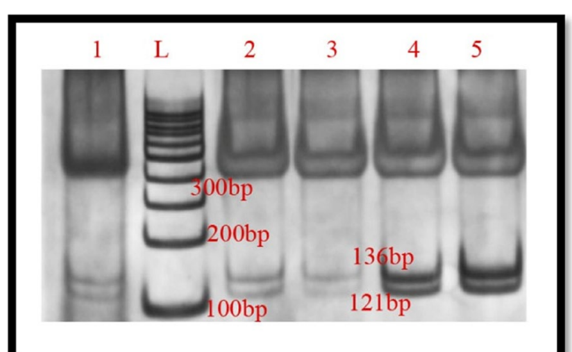


Fig. 4 Restriction enzyme digestion of Waxy intron amplified PCR products. L: 100 bp ladder, 1: Rakthashali, 2: Pokkali, 3: Jaya, 4: Kuruva, 5: Matta

29.3 and 28.9 respectively. The GL of Kuruva and Pokkali were 26.8 and 25.5 respectively. All five samples showed high GL.Several studies have demonstrated the impact of lower GI and GL meals in reducing blood glucose levels (Willett et al. 2002, Brand-Miller et al. 2003, Zafar et al. 2019). Low GI diets have also been proven to help prevent the occurrence of cardiovascular diseases (CVD) (Aston, 2006, Maki et al. 2007).

Molecular profiling of rice varieties for amylose content

GLU 23 marker was used to identify the waxy and non- waxytypes. All the rice varieties screened showed amplification of 173 bp allele which corresponds to *Wx* allele classifying them as non-waxy rice varieties (Fig. 2). Screening with marker specific to Wx^b produced no amplification. Wx^b allele is expressed in rice varieties with amylose content in the range 15–18%. As all the rice varieties under study belong to intermediate and high amylose content group, there will be no Wx^b

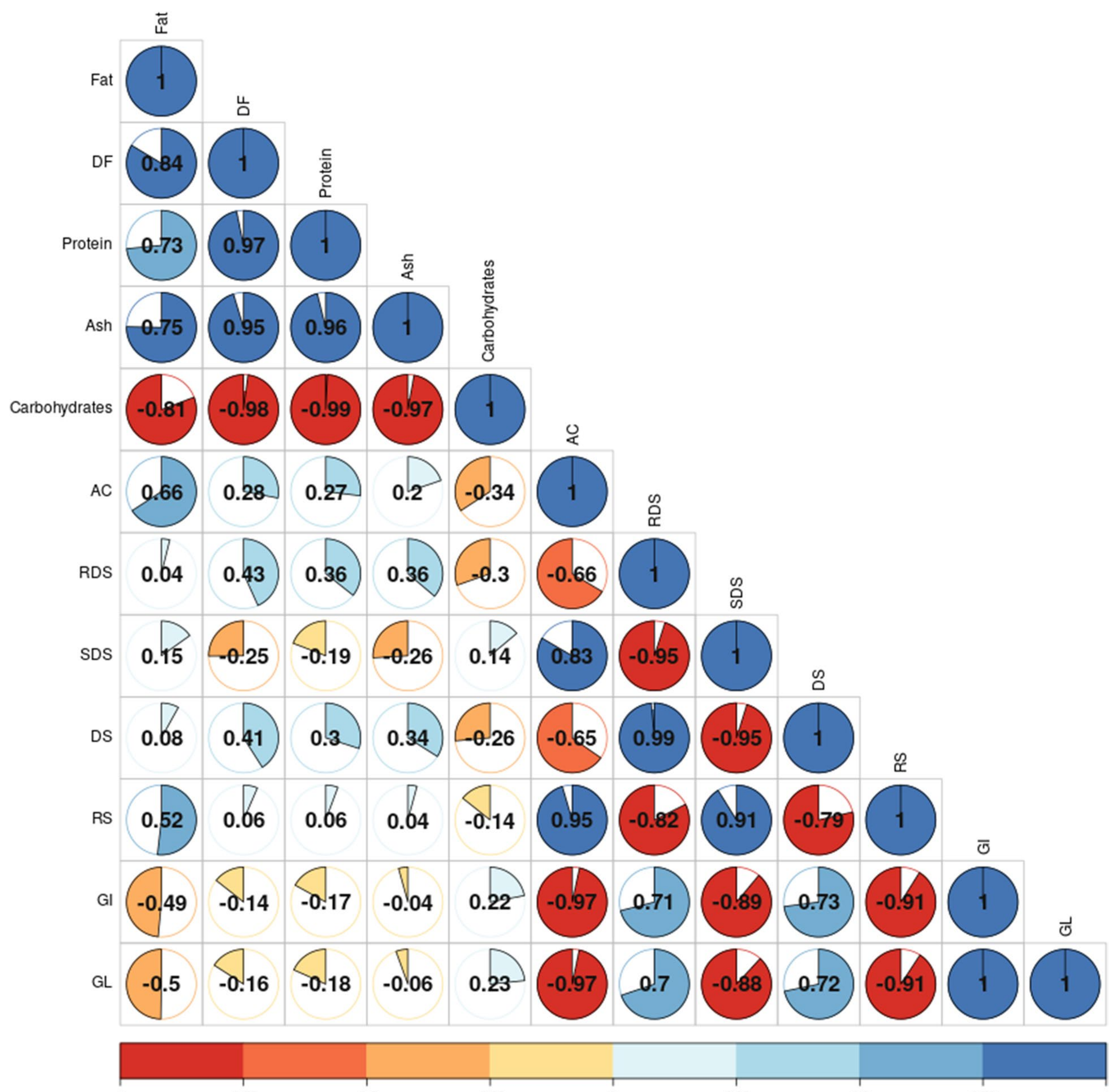


Fig. 5 Correlogramof different characteristics of rice samples. *DF* Dietary fiber, *GC* Gel Consistency, *AC* Amylose content, *GI* Glycemic index, *RDS* Rapidly digestible starch, *SDS* Slowly digestible starch, *DS* Digestible starch, *RS* Resistant starch, *GL* Glycemic load. Correlation is significant at the level 0.05

alleles present in the DNA samples. G/T polymorphism in intron 1 of the *Waxy* gene was identified by restriction digest of the PCR fragment of the region amplified by *Waxy-intron1*F. The samples were screened with Waxy-intron primer which amplified a 257 bp product (Fig. 3) followed by restriction digestion of the PCR fragment of the region amplified using restriction endonuclease AccI.After analysis of the resulting banding pattern, 129 and 128 bp bands were observed indicating restriction digestion which confirms the presence of Wx^{a}allele (Fig. 4). The varieties were also analyzed with $\mathrm{Wx}^{\mathrm{in}}$ marker to identify cultivars with amylose content in the range of 18–22%. However, there was no amplification observed with all the test samples indicating that these varieties contain amylose content higher than 22%. Amylose content of all the rice varieties under study were found to be above 22% as estimate by biochemical estimation which can be associated with the type of allele present in the respective variety. As waxy locus is correlated with amylose content in rice, the allelic forms: Wx^{a} and Wx^{in}corresponding high to intermediate amylose content respectively, can be used for breeding rice varieties with lower GI and also to introgress these alleles into popularly consumed rice varieties and make them diabetic friendly.

Relationship between GI and starch content, physicochemical characteristics, ECQ

Significant correlations were observed among the starch content, physico-chemical characteristics, cooking quality traits and GI (Fig. 5). The RDS and DS showed a significant strong positive correlation with GI. Whereas, the AC, SDS, and RS showed strong negative correlation with GI. These observations corroborate the results of previous studies (Naseer et al. 2021, Hu et al. 2004, Ritudomphol and Luangsakul, 2019). Furthermore, a significant negative correlation values were observed between AC with GI and RDS. This could be explained as rice with low amylose content tends to hydrolyze faster during digestion leading to an increased production of RDS. Among the rice varieties screened, GL showed highly significant positive correlation with GI and RDS and a significant strong negative correlation with AC. Previous studies have reported similar correlation trends (Thiranusornkij et al. 2019). GI and GL showed non-significant positive correlation with total starch content of uncooked rice. Therefore, it can be concluded that amylose to amylopectin ratio is the major factor to determine GI of rice ratherthan its total starch content. Pokkali rice variety had the highest fat content, highest amylose content and lowest.

Conclusion

A total of five commonly cultivated and consumed rice varieties were analyzed for starch content, rate of hydrolysis of starch, cooking and eating quality parameters and their influence on glycemic index. The rice varieties were classified according to amylose content using molecular markers. The standardized cooking conditions help consumers to gather an awareness towards ECQ. It was inferred, from the current study, that the popular Kerala rice varieties generally exhibit medium to low GI and amylose content is the major criterion which determines the digestibility properties. The RDS, SDS and RS also influenced GI in these rice varieties as inferred from correlation study. Pokkali rice variety was found to have lowest GI and can be effectively recommended for diabetic management in Kerala, where rice is a staple diet.

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