International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(4): 2329-2335

Received: 16-05-2019
Accepted: 20-06-2019

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Grain quality attributes of different rice (*Oryza sativa* L.) Genotypes cultivated in Tamil Nadu, India

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Abstract
A study was conducted to evaluate the Brown Rice grain quality attributes of different Genotypes at Agricultural College & Research Institute, Killikulam-628 252, India during the period from April 2015 to March 2017. In the present study, twenty different rice varieties inclusive of two traditional cultivars cultivated in and around Thoothukudi district of Tamil Nadu as the test rice grains in the form of Brown rice in completely randomized design with three replications were tried. Most of the physico-chemical characteristics such as amylose content, gel consistency, alkali spreading value and gelatinization temperature were significantly correlated (positively or negatively) with some of the quality traits. Amylose content was highest (31%) in CO 43 rice with very lowest recorded for Purple puttu variety (9.167%). Even though highest Gel Consistency was recorded in variety, Purple puttu (92mm) and lowest in CO 43 (72mm), all twenty rice varieties are categorised as soft gel consistency(>60 mm) taken for analysis. The highest score (5) was given for CO (R) 49 and lowest score (2.5) for ASD 16 and ASD 18 subjected for Alkali degradation using 1.7% potassium hydroxide solution. Among the various rice varieties investigated, thousand grain weight varied between 13.7 and 29.7 g, length/breadth (l/b) ratio was between 2 and 3.5. Significant variation (P<0.05) was detected among the 20 rice varieties for the traits, Amylose content and Gel Consistency evaluated. Results of this study provide vital informations for consumers to identify 'superior quality of rice' cultivated and marketed in Tamil Nadu.

Keywords: Alkali spreading value, amylose content, gel consistency, *Oryza sativa*, quality traits

Introduction

Rice (*Oryza sativa* L.) is a monocotyledonous plant belonging to the grass family (Gramineae) and the genus *Oryza.* Rice is the essential crop for half of the world population and considered as one of the major carbohydrate sources for human consumption**.** Starch molecule belong to the category of carbohydrates is the major constitute of rice grain. Protein is the second vital component of rice. Rice starch is a polymer of glucose composed of two polysaccharides: amylose and amylopectin.

Amylose is principally composed of a linear polymer of α (1→ 4) linked glucose, whereas amylopectin is a more complex mixture of both α (1→ 4) linked glucose branched by α (1→ 6) linkages. In its native state, rice starch has a semicrystalline structure which is disrupted by cooking, transforming the starch into a softer, edible, gel-like material. Because it is associated with the cooking time and texture of cooked rice and cool cooked rice, the temperature at which rice starch gelatinizes is an important component of rice eating quality (Maningat and Juliano, 1978) [22].

Low-amylose rice starch results from the relative inactivity of granule-bound starch synthase (GBSS) (Sano *et al*., 1986; Hirano and Sano, 2000; Mikami *et al*., 2000) [32, 10, 24], whereas rice starch with a low GT results from the presence of a relatively inactive starch synthase (SSIIa**)** (Umemoto *et al*., 2002, 2004) [41, 42].

Grain quality of rice is determined by the factors such as grain appearance, nutritional value, cooking and eating quality (Juliano *et al*. 1990) [15].

The physical traits include grain length (L), grain breadth (B) and L/B ratio. The Biochemical and Cooking qualities are Amylose Content, Alkali spreading value and Gelatinisation Temperature.

Different cultivars of waxy and non-waxy rice are usually classified according to their grain dimensions, amylose content, amylograph consistency, gelatinisation properties of the extracted starches and the texture of cooked rice (Juliano, 1985) [14].

Amylose Content, Amylopectin structure and protein composition explained the difference in cooking quality of rice (Lisle *et al*. 2000) [20].

Amylose content as well as gelatinization temperature and gel consistency can highly influence cooking and eating qualities of rice, which can vary based on the varieties (Juliano, 1972) [12].

Cooked rice is composite food consist of different biopolymers, including starch and proteins along with moisture as plasticizer (Ahmed *et al*. 2007) [1].

Rice varieties with amylose content of more than 25% absorb more water and have a fluffy texture after cooking (Frei and Becker, 2003) [8]. Within an individual rice particle, various processes occur during cooking. The heating, water uptake and swelling of the rice particle all involve diffusive processes. When water is present at high temperatures, the starch undergoes a gelatinization reaction (Oko *et al.* 2012) [25].

In general rice grains has to be soaked in water at 25℃ for one hour before cooking at temperatures above 70℃ for 20 minutes. As water is taken up by a rice particle, the starch granules undergo a gelatinization reaction, the term generally used to describe the swelling and hydration of the granular starch (Whistler, 1984) [44].

The aim of this work is to evaluate the specific biochemical parameters to distinguish the quality of rice among the different varieties.

Materials and Methods

Materials

This research was conducted at the Agricultural College & Research Institute, Killikulam-628 252, India during the period from April 2015 to March 2017. The materials used in this research were 20 rice cultivars being cultivated in Thoothukudi, Tirunelveli and Kanyakumari districts of Tamil Nadu.

50g sample of each rice variety were procured from five different sampling stations. Damaged kernels and debris were not considered for observation. Many varieties are unique in their morphological characters of shape, size and colour.

Biometric observations

Length and breadth measurement

Ten unbroken brown rice in three sets were measured using vernier calipers and the mean length, breadth was expressed in millimeter (mm).

Length / breadth ratio

The Length / breadth ratio was determined by dividing cumulative length of ten grains by the cumulative breadth of ten grains. The average of three replications was reported.

Thousand grain weight

The thousand grain weight was determined by means of a digital electronic balance having an accuracy of 0.000g. One thousand rice kernels were randomly selected from the bulk sample by taking different lot for 3 times and weighed separately.

Estimation of amylose content (Juliano 1979) [13]

The simplified procedure of Juliano (1979) [13] was used for estimating the amylose content. Three samples of Brown rice flour [50mg] were taken in 50ml volumetric flask. To this, 0.5ml of 95% ethanol was added to wash the sample adhering to the flask followed by 5ml. of 1N Sodium hydroxide. The material was left undisturbed overnight to gelatinize the starch. The solution was made up to 50ml. Sample extract of 2.5ml was pipetted out into another 50ml. volumetric flask. To this, 20ml. of distilled water was added followed by three drops of phenolphthalein to develop pink colour. Then 0.1N HCL was added drop by drop until the colour disappeared. The volume was made up to 50ml after the addition of 1ml. iodine reagent. The Absorbance was measured by the UV-VIS-spectrophotometer at a wavelength of 590nm based on the intensity of the blue colour developed. The standard curve was designed based on standard amylose concentrations and their absorbance. Amylose concentration for analyte was obtained by plotting the absorbance in the standard curve. Amylose content of each genotype was expressed as percentage of total quantity of sample taken for analysis. Experiments were performed in triplicate.

Gel consistency (Cagampang *et al.* 1973) [4]

Rice flour prepared with pestle & mortar from Brown rice [100mg] was placed in 13×150mm culture tubes and wetted with 0.2ml 95% ethanol containing 0.03% thymol blue, according to the method of *Cagampang et al.* (1973) [4]. 2ml of 0.2N KOH was added and mixed sufficiently with a vortex Genie mixer set at speed six. Tubes were covered with glass marbles and placed in a boiling water bath for 8 min, making certain that the contents reached two thirds the height of the tube. The tubes were removed from the water bath and kept at room temperature for 5 min, and finally transferred to an ice-water bath for 15min. After this treatment, the tubes were laid flat on a laboratory table over ruled graph paper for 1h. The height or total length of the blue-colored gel was measured from the bottom of the tube to the end of the gel was measured in millimeters.

Alkali spreading value (Juliano *et al*. 1993) [17]

Six numbers of brown rice grains were placed in a petri dish containing 10ml of 1.7% potassium hydroxide solution which is kept on a black plastic sheet. The kernels were arranged randomly with a spatula in such a way to provide space between kernels for spreading. The petri dish plates were covered and left overnight, undisturbed at room temperature for 23 hrs. Next day, the grains were observed carefully and the extent of degradation was determined. The appearance and disintegration of kernels were rated visually.

Gelatinization temperature (Little *et al*. 1958) [21]

The Gelatinization temperature was indicated based on Alkali spreading value.

Statistical analysis

The experiment was carried out in a completely randomized design. All results were expressed as the mean value. The data obtained were subjected to statistical scrutiny for the parameter under study. The level of significance was considered at $P<0.05$.

Results and Discussion

Results

The results so obtained in both years were analyzed separately and are presented for the period of April 2015 to March 2017 since values obtained for the period of April 2015 to March 2017 followed a similar pattern. Most of the physico-chemical characteristics such as amylose content, gel consistency,

alkali spreading value and gelatinization temperature in 2016 followed a trend very similar to that of 2015.
Among the various rice varieties investigated inclusive of two traditional, thousand grain weight varied between 13.7 g (ADT 49) and 29.7 g (Mapillai samba), length/breadth (l/b) ratio was between 2 and 3.5 as depicted in Table 1.

Table 1: Biometric observations of 20 different rice varieties / hybrids with their duration.

S. No.	Variety/Hybrid	Duration [Days]	1000 grain weight [g]	L mm	B mm	L/B ratio
1	White ponni	135	17.3	6.0	2.0	3.0
2	TRY-1	140	24.5	6.0	2.5	2.4
3	MDU 5	95-100	23.0	7.0	2.5	2.8
4	ASD 16	110-115	21.9	5.5	2.5	2.2
5	ASD 18	105-110	25.2	7.5	2.5	3.0
6	ADT 36	110	21.4	6.0	2.5	2.4
7	ADT 39	120-125	18.3	7.0	2.3	3.04
8	ADT 43	110	18.5	5.0	2.0	2.5
9	ADT 45	110	20.4	6.0	2.0	3.0
10	ADT 47	118	13.9	6.0	2.0	3.0
11	ADT 49	132	13.7	6.0	2.0	3.0
12	CO 43	135-140	19.7	6.0	2.0	3.0
13	CO 47	110-115	17.8	6.0	2.0	3.0
14	CO (R) 48	130-135	19.3	6.5	1.9	3.42
15	CO (R) 49	135	18.8	6.0	2.0	3.0
16	CO (R) 50	135-140	21.5	6.0	2.0	3.0
17	CO RH3	115	23.8	7.0	2.0	3.5
18	CO RH4	130-135	18.3	6.0	2.0	3.0
19	Purple puttu	125-150	18.6	6.0	3.0	2.0
20	Mapillai samba	160	29.7	6.0	2.5	2.4
Statistical analysis		SEm	0.0955			
		SEd	0.1351			
		CD 1%	0.3596			
		CD 5%	0.2703			

The experimental material comprised of twenty rice genotypes. Based on average grain length and L/B ratio before cooking, size and shape of Brown rice grains were determined by classification method of International Organisation for Standardisation (ISO) for paddy (IRRI, 2013) as depicted in Table 2 and 3.

Table 2: Brown rice classification based on Length

Scale	Length (mm)	Size category	Rice varieties
1	>7.50	Extra long	ASD 18.
2	6.61 to 7.5	Long	MDU 5, ADT 39 & CO RH3,
3	5.51 to 6.6	Medium	White ponni, TRY-1, ADT 36, ADT 45, ADT 47, ADT 49, CO 43, CO 47, CO (R) 48, CO (R) 49, CO (R) 50, CO RH4, Purple puttu, Mapillai samba.
4	5.5 or less	Short	ASD 16 & ADT 43,

Table 3: Shape classification of Brown rice grains

Scale	L/B ratio	Shape category	Rice varieties
1	>3.0	Slender	CO (R) 48 and CO RH3
2	2.1 to 3.0	Medium	White ponni, TRY-1, MDU 5, ASD 16, ASD 18, ADT 36, ADT 39, ADT 43, ADT 45, ADT 47, ADT 49, CO 43, CO 47, CO (R) 49, CO (R) 50, CO RH4, Mapillai samba.
3	1.1 to 2.0	Bold	Purple puttu
4	<1.1	Round	Nil

The values of the physico-chemical characteristics such as amylose content, gel consistency, Alkali spreading value and gelatinization temperature of the grain among the 20 cultivars used in this study is shown in Table 4&5.

Table 4: Amylose content and Gel Consistency property of Brown rice grain in 20 different rice varieties / hybrids

S. No.	Variety/Hybrid	Mean values	Category	Gel consistency (mm)	Category
1	White ponni	23.433	Intermediate	79	Soft
2	TRY-1	23.000	Intermediate	80	Soft
3	MDU 5	21.400	Intermediate	81	Soft
4	ASD 16	24.700	Intermediate	78	Soft
5	ASD 18	22.400	Intermediate	88	Soft
6	ADT 36	28.167	High	75	Soft
7	ADT 39	24.500	Intermediate	79	Soft
8	ADT 43	24.600	Intermediate	78	Soft
9	ADT 45	23.600	Intermediate	79	Soft
10	ADT 47	20.300	Intermediate	83	Soft

11	ADT 49	21.600	Intermediate	81	Soft
12	CO 43	31.000	High	72	Soft
13	CO 47	21.000	Intermediate	82	Soft
14	CO (R) 48	24.003	Intermediate	79	Soft
15	CO (R) 49	15.000	Low	88	Soft
16	CO (R) 50	23.667	Intermediate	80	Soft
17	CO RH3	20.333	Intermediate	83	Soft
18	CO RH4	23.953	Intermediate	79	Soft
19	Purple puttu	9.167	Very Low	92	Soft
20	Mapillai samba	20.667	Intermediate	88	Soft
Statistical analysis	SEm	0.3951		0.2236	
	SEd	0.5587		0.3162	
	CD 1%	1.4872		0.8417	
	CD 5%	1.1180		0.6328	

Table 5: Alkali digestion value and Gelatinization temperature of Brown rice grain in 20 different rice varieties / hybrids

Alkali digestion value			**Gelatinization Value**		**Rice Varieties / Hybrids**
Scale	**Features**	**Inference**	**GT(°C)**	**Inference**	
1	Kernel not affected	Low	75-79	High	Nil
2	Kernel swollen	Low	75-79	High	CO 47, CO (R) 50, CO RH4, Mapillai samba.
2.5	Kernel swollen, collar incomplete or narrow	Low to Intermediate	70-79	Intermediate to High	ASD 16 & ASD 18
3	Kernel swollen, collar incomplete and narrow	Low to Intermediate	70-79	Intermediate to High	ADT 36, ADT 39, ADT 43, ADT 45, ADT 47, ADT 49, CO 43, CO (R) 48, CO RH3, TRY-1, White ponni.
4	Kernel swollen, collar complete or wide	Intermediate	70-74	Intermediate	MDU 5 & Purple puttu
5	Kernel split or segmented collar complete and narrow	Intermediate	70-74	Intermediate	CO (R) 49
6	Kernel dispersed, merged with collar	High	55-69	Low	Nil
7	Kernel completely dispersed	High	55-69	Low	Nil

Discussion

In respect of short duration paddy varieties, MDU 5 and ASD 18 are found to be the best choice for the farmers.

Biometric observations

Grain size and shape largely determine the market value and consumer acceptance of rice, while cooking quality is influenced by the properties of starch. Some varieties expand more in size than others upon cooking. Length -wise expansion without a corresponding increase in girth is considered a highly desirable rice grain quality trait (Sood *et al*. 1979) [19].

Among the varieties studied, rice varieties such as CO (R) 48 and ADT 39 showed good physical characteristics (maximum L/B ratio).

In respect of Long duration paddy varieties, ‘Mapillai samba’, the higher grain weight is found to be maximum [29.7g] per 1000 seeds. In respect of short duration variety, ASD 18 found to have higher grain weight of 25 grams per 1000 seeds. When we compare the same, ASD 18 found to generate higher grain weight within 108 days will be of much appreciable one to the farmer’s point of view.

Amylose content

The variation in amylose classification among samples between very low to high agrees with literature that amylose content in rice is influenced by variety (Vlachos *et al*, 2008, Frei *et al*. 2003) [8, 43].

Suggested classification of amylose content identified classes as waxy (0–5%), very low (5– 12%), low (12–20%), intermediate (20–25%), and high (25–33%), even considering that commercially rice is classified by amylose content as either low (less than 20% amylose), medium (21–25%) and high (26–33%) (Juliano 1992; Suwannaporn *et al*. 2007) [16, 37].

In this study, Purple puttu variety recorded very low amylose content followed by CO (R) 49, sixteen varieties inclusive of two hybrid and one traditional recorded Intermediate category. CO 43 rice variety has recorded high amylose followed by ADT 36. Rice varieties with amylose content of more than 25% absorb more water and have a fluffy rice texture, hard, and dry after cooking. Most consumers prefer rice with intermediate AC ranged between 20- 25% (Rachmat *et al*. 2006) [27].

Park *et al.* postulated that the crystalline regions of non-waxy (high amylose) rice starch restricted the hydration of amorphous regions whereas waxy (high amylopectin) rice starch consisted mostly of crystalline regions and thus could begin gelatinization at a lower temperature. Park *et al.* also stated that gelatinization temperatures increase with higher amylose content in rice starches.

Increase in soil nitrogen decreased the amylose content (Gu *et al*. 2015, Zhu *et al*. 2017) [9, 46].

Cold weather fluctuations caused higher amylose content in the same variety even though amylose content is cultivar dependent (Asaoka *et al*. 1985; Umemoto *et al*. 1995) [2, 40].

Differences in amylose content of rice are influenced by genotypes and nitrogen levels in the soil. The study by Juliano (1972) [12] showed that the rice with the same variety but planted in the regions which have different nitrogen content in the soil and the air temperature of different planting locations will produce the rice with different amylose content.

Amylose content showed significantly negative correlating with gel consistency suggesting that those varieties with high amylose content will result in hard and shorter length of gel than varieties with low amylose content due to retrogradation behaviour of amylose during the cooling of gel (Rani *et al*. 2006) [29]. Negative correlation between amylose content and gel consistency was also reported earlier (Khatun *et al*. 2003) [18].

Elongation of rice can be influenced by both the l/b ratio and the amylose contents (Singh *et al*., 2005; Danbana *et al*., 2011) [33, 5].

Rice varieties with higher amylose content are more prone to leaching out into the cooking water as starch grains expand during cooking (Juliano, 1971) [11].

Rice cultivars with high amylose content will have less amylopectin content and gel consistency.

Gel consistency

The gel consistency value can be used as the index of the softness of the Brown rice. The conducted research showed that several rice varieties in Tamil Nadu, India had a gel length that is more than 61mm. This trait was observed in most of the rice genotypes under study. The rice gel consistency was ranged from 72mm (CO 43) to 92mm (Purple puttu) which shows the index of high softness category of all the rice varieties taken for analysis. Amylose levels had influence on gel consistency.

Table 7: Classification of Gel Consistency (Tang *et al.* 1991) [39]

Classification	Length of Gel (mm)
Hard	26-35
Medium Hard	36-40
Medium	41-60
Soft	61-100

Gel consistency test was developed as an indirect method used in screening cooked rice for its hardness especially in rice with high amylose content (Juliano 1985) [14]. Rice differ in gel consistency from soft to hard (Cagampang *et al.* 1973; Juliano 1979) [4, 13]. Cooked rice with hard gel consistency hardens faster than those with a soft one. Rice with soft gel consistency cook tender, and remain soft even upon cooling (Juliano 1979) [13]. Rice with soft gel consistency is preferred by most rice consumers. Breeders are therefore trying to develop high-yielding varieties with soft gel consistency (Khush *et al.* 1979) [19].

Alkali digestion value and Gelatinization temperature

Alkali digestion value and Gelatinization temperature are major traits, which are directly related to cooking quality.

The alkali spreading value and gelatinization temperature were calculated for all the rice varieties examined (Table 5). The alkali spreading value was calculated as low, intermediate and high. The rice grains that were highly affected by alkali solution had a high Alkali Digestion Value. Alkali spreading value indirectly correlates with the gelatinization temperature.

Rice with low GT disintegrates completely in 1.7 % KOH solution, whereas rice with intermediate GT showed partial disintegration. Rice with high GT remains largely unaffected in alkali solution. If the alkali spreading value is low, the GT is high. If the alkali spreading is intermediate, the GT is intermediate. If the alkali spreading value is low intermediate then the GT is high intermediate. The intermediate alkali digestion group consisted of grains that were either swollen or segmented with complete and wide collars.

The intermediate alkali digestion genotypes are the most preferred worldwide given their good cooking qualities such as water absorption, moistness, volume expansion and softness upon cooling (Sthapit *et al.* 2004) [36].

Consumer preference of the low GT rice is minimal due to the negative outcome on the linear kernel elongation, water absorption and volume expansion of the rice genotypes (Tuano *et al.* 2011) [38].

The cooking quality of rice is associated with the starch gelatinization temperature (GT). Rice genotypes with low GT have probably been selected for their cooking quality by humans during domestication (Daniel 2006) [6].

Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water (Sobkowska, 2001) [34].

GT is positively correlated with the amount of time required to cook rice. Rice varieties with high GT require more water and cooking time than those possessing low or intermediate GT. A low or intermediate GT is desired for a high-quality rice variety (Juliano *et al*, 1993) [17].

Rahman (1995) [28] reported that starch gelatinization is affected by various electrolytes, and the effects depend on the concentration and type of anions and cations.

Fan *et al.* reported that non-starch components in rice flour such as protein, ash, fiber and lipids reduce enthalpy for gelatinization. Saif *et al.* stated that starch concentration and varied level of water / starch ratio have significant influence on gelatinization properties of rice flour.

Protein and fat molecules also has little influence on Gelatinization temperature. High protein content of rice makes more time to cook since protein fills the spaces between starch granules in the endosperm (Masniawati *et al* 2018) [23].

GT is responsible for cooking time, water absorption and the temperature at which starch irreversibly loses its crystalline order during cooking. The GC is responsible for softness and the AC for texture of cooked rice (Sabouri 2009) [30].

Some of the most popular rice varieties were of intermediate GT, grown and consumed in Tamilnadu. Cooking quality of grains directly correlates with the Gelatinization Temperature; a low GT favours fuel conservation.

Conclusion

Rice available in thousands of cultivars that vary greatly in their attributes including cooking, eating and product making quality. Every year breeders release many rice varieties with higher yield potentials, better resistance to biotic and tolerance to abiotic stresses. However, quality aspects of rice grown up to the consumer’s expectation are ignored. Quality aspects include both the physical and chemical traits. In this research, physicochemical properties are used as parameters in identifying the cooking quality of Brown rice. It is concluded by analysing amylose content, gel consistency, alkali spreading value and gelatinization temperature of twenty rice genotypes that genotype MDU 5 have good grain quality for consumer preferences as well as rice breeding research for further crop improvement.

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