RESEARCH ARTICLE

Comparative Nutritional Analysis of Selected Traditional and TNAU Released Rice Varieties to Identify Nutritionally Potent Landraces for Enhancing Food Security

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ABSTRACT

Widely consumed type of rice in the world with white pericarp and is known as non-pigmented white rice. Recently, pigmented traditional rice varieties have become popular among the consumers due to its dark pigmented pericarp having nutritional value. This type of rice variety is important in determining the digestibility of starch and its Glycemic index (GI). The digestibility of the rice depends on the properties of starch, including its crystallinity and amylose: amylopectin ratio. Hence, the study focuses on the analysis of phyto nutrients in the selected traditional and TNAU released rice varieties to identify the low GI lines. Nutritional analysis of the selected traditional rice genotypes namely Karuppu kavuni, Karun kuruvai (black), Kaattuyanam, Mapillai samba and Kuruvai kalanchiyam and five ruling TNAU released rice varieties (ADT 52, TKM 9,TKM 13, ASD 16 and CO51) has been carried out. Among the selected traditional and TNAU - released rice varieties, Mapillai samba showed the strongest alpha-amylase inhibition (IC50 = 23.15 µg/mL), maximum anthocyanin content (48.37 mg/100g), and phenolic content (45.8 mg/100g), highest total antioxidant activity (51.8% DPPH scavenging activity. Mapillai samba (31) and Kuruvai kalanjiyam (27.5) were identified as low glycemic index lines. Hence, the pigmented traditional rice landraces can be used as a source of functional foods.

INTRODUCTION

The per capita consumption of rice fluctuates around 114 kg per year, depending on the rice and wheat flour price. Rice comprises of 72-80 percent carbohydrates as starch. Besides starch, rice is rich in many nutrients such as proteins, lipid, fibre, minerals, vitamins and antioxidants as well as some bioactive compounds. The rice starch"s digestibility is attributed to the properties of starch, including its crystallinity, granular structure and amylase: amylopectin ratio. The type of rice variety plays an important role in determining the digestibility of starch and its glycemic index (GI). Like most of the other cereals, rice contains large amounts of rapidly digestible carbohydrates that may be detrimental to the health of diabetics as it raises glucose concentration in the blood.

Widely consumed type of rice in the worldwide is having white pericarp, known as non-pigmented white rice. In addition, two other types of pigmented rice consumed in the world known as dark and light- pigmented rice. Anthocyanin pigments are accumulated in different concentrations in pericarp of pigmented rice. Therefore, raw rice kernels appear red or dark red in color. At present, most of the traditional rice cultivars are popular among consumers due to its high nutitional value, dark pigmented pericarp and other factors like unique nutrient content, cooking and eating qualities. Most of the traditional rice varieties have strong pericarp, which cannot be easily removed during the rice milling. Most of the pigmented traditional rice cultivars are being used in the Ayurvedic medical treatments such as diabetes and cancers. The red rice has potential health benefits due to its high dietary fibre content and antioxidant properties, which could help, reduce the GI, thereby reducing the risk of type II diabetes. Dietary fibre helps to delay the gastric emptying and

absorption of carbohydrates and increasing satiety contributes to decreasing the GI.

With this background, the present work is designed to investigate, compare the nutritional content and glycemic index of selected traditional and TNAU released rice varieties to identify the nutritionally potent landraces for enhancing food security.

MATERIAL AND METHODS

Five traditional rice varieties, namely Karuppukavuni, Karunkuruvai (black), Kaattuyanam, Mapillaisamba and Kuruvaikalanchiyam were collected from the farmers of Thanjavur, Pudukkottai, Madurai and Ramnad. Five ruling rice varieties namely ADT-52, TKM -9, TKM-13, ASD-16 and CO-51 were collected from TRRI, Aduthurai and AC&RI, Madurai.

Processing of the collected varieties

The collected rice varieties were manually dehusked and ground to a fine powder for preparation of extracts. Colored rice retains its bran layer (containing vitamins, minerals and fibre), as this has not been polished more to produce white rice. Each cultivar"s whole rice grain (1 g) of was extracted with 25 mL of methanol containing 1% HCl for 24 h at 24°C. The procedure was repeated twice. The methanolic extracts were centrifuged at 4000×g for 15 min and the supernatants were pooled and stored at 4°C.

Karuppukavuni

Kuruvaikalanjiyam   Karunkuruvai (black)   Kattuyanam

Mapillaisamba

Figure.1 Traditional rice genotypes collected from farmers of Thanjavur, Pudukkottai, Madurai and Ramnad

ADT 52

TKM 9   TKM 13   ASD 16

Figure.2 TNAU released rice varieties collected from TRRI, Aduthurai and AC&RI, Madurai

Assessment of non- nutritional high value phytochemicals

The collected traditional and TNAU released rice varieties were analyzed for their anthocyanin, total phenol , total flavonoid contents , alpha amylase inhibition , total antioxidant activity and glycemic index in the methanolic extracts using UV-Vis spectrophotometer as per standard procedures.

Anthocyanins

The anthocyanin content in the rice extracts was estimated by following the modified pH method Two different buffers *viz*., pH 1.0 buffer (KCl buffer) and pH 4.5 buffer (Phosphate buffer) were prepared. 1 mL of each sample was pipetted out in two test tubes and to which 4 mL of pH 1 buffer and pH 4.5 buffer was added separately to the tubes. After 20-30 minutes, the absorbance was measured for both the tubes at 520 nm (A520 nm), and at 700 nm (A700 nm) for the correction of haze.

Total phenolic content

The total phenol content was measured by Folins-–Ciocalteu method. 0.1 mL aliquot was taken in test tube and made up to 6 mL with distilled water, and shaken well. 0.5 mL of Folin-Ciocalteau Reagent (1:1) was added to the tubes. After 3 minutes of incubation, 2 mL of 20% sodium carbonate solution was added and thoroughly mixed. It was then kept in a boiling water bath for 1 minute, cooled and the intensity of blue colour was measured at 650 nm.

Total antioxidant potential

Total antioxidant potential of rice genotypes was measured using DPPH method. Sample and control analysis were performed in separate micro tubes. In the sample tube, 90 µL of ethanol was added, followed by the addition of 1 mL of DPPH. Then, 10 µL of sample was added and mixed well. The control was run in the same way excluding the addition of sample. The tubes are then placed in dark for 20 minutes and the absorbance (Abs) was measured at 517 nm using UV-VIS spectrophotometer.

Total flavonoids

The total flavonoid content was determined by Colorimetric method using quercetin as standard. 0.2 mL of sample was taken in test tube and made up to 3 mL with distilled water. To the tubes, 1.5 mL methanol, 0.1 mL of 1% Aluminium chloride, 0.1 mL of 1M potassium acetate were added and mixed well. The optical density was measured spectrophotometrically at 410 nm.

α – amylase inhibitor

α – amylase inhibitor levels were analysed as suggested by Deshpande *et al.* (1982). In a test tube, 0.1 mL sample aliquot containing the inhibitor was taken to which 0.5 mL of α – amylase enzyme solution was added. Then 1 mL of 1% starch solution was added to determine the – amylase activity. The tubes were incubated for

exactly 2 minutes at room temperature. The reaction was stopped by adding 2mL of DNS (dinitrosalicylic acid) reagent and 1 mL of 40% sodium potassium tartarate. The final volume of the tubes was made up to 5 mL with distilled water. A blank was maintained for all the samples, in which 0.5 mL of phosphate buffer was added instead of α – amylase enzyme. The absorbance of samples and blank were measured at 530 nm using UV-VIS spectrophotometer.

Invitro digestibility and Glycemic index

Procedure described by Korakli et al. (2002) was employed to evaluate invitro digestibility by artificial human gastric juice. FOS was used as a positive control. Artificial human gastric juice was prepared as follows: 8 g of $NaCl$, 0.2 g of KCl, 8.25 g of $Na_2HPO_4.2H_2O$, 14.35 g of $NaHPO_4$, 0.1 g of $CaCl_2.2H_2O$ and

0.18 g of $MgCl_2.6H_2O$ were dissolved in distilled water to make a 1000 mL solution. The pH of the solution was adjusted to 1, 2, 3, 4 and 5 using 6 M HCl, respectively. Subsequently 5 mL of the solution of each pH was added into 5 mL of rice extract (Korakli *et al*. 2002). The reaction mixture was incubated in a water bath at 37 _C for 6 h. 3 mL of the reaction mixture was collected at 0 and 30 min and 1, 2, 5 and 6 h to determine the reducing and total sugar. The degree of hydrolysis and invitro Glycemic index (Goni *et al*. (1997) of samples were calculated as follows:

$$\text{Hydrolysis degree \%} = \text{Reducing sugar released} \times 100$$

Total sugar content- initial reducing sugar content (Equation. 1)

Invitro Glycemic index GI (%) = 39.71 + 0.549 x HI (Equation. 2) (Where GI = Glycemic Index (%); and HI = Hydrolysis Index (%).

Statistical analysis

All the samples were analyzed in triplicates. The values were expressed as means of triplicate analysis of the samples (n = 3) ± SD. The statistical analysis was performed in SPSS software version 22.0. The data obtained were subjected to one way ANOVA and the means were compared using Duncan Multiple Range Test (DMRT) at 5 % level of significance.

RESULTS AND DISCUSSION

Assessment of non-nutritional high value phytochemicals

The non-nutritive plant chemicals with a protective or disease-preventing potential are known as photochemicals. The phytochemical compounds are mainly accumulated in the pericarp and bran of the rice kernel. They prevent oxidative damage in foods and have a wide spectrum of beneficial biological activities. Phytochemicals present in rice can be divided into the following sub-groups namely carotenoids, phenolics, alkaloids, nitrogen and organo-sulphur containing compounds. Phenolic compounds are further sub-grouped as phenolic acids, flavonoids, coumarins and tannins. Anthocyanins, the major pigment responsible for the colour of red and black rice, are a kind of flavonoids. The pigmented cereal grains, such as red and purple/black rice, have phytochemical compounds in higher amounts than non-pigmented varieties. The phytochemicals such as cell wall- bound phenolics and flavonoids are gaining more interest as digestive enzymes and gut microflora can break down these compounds, and as a result, they can be easily absorbed into the body. The coloured rice bran contains anthocyanins that inhibit reductase enzyme and anti-diabetic activities.

Total phenol content

Phenolics are a group of natural antioxidants that has received considerable interest due to their pharmacological functions. Among the phenolic acids, ferulic and ρ-coumaric acids are abundant in grains with light brown pericarp. Between the rice, varieties tested the pigmented varieties showed statistically significant values for total phenolic content compared to that of non-pigmented varieties. Phenolic compounds being potent antioxidants have long been recognized to have protective functions against oxidative damage and are associated with reduced risk of chronic diseases. The total phenolic content of the selected traditional and TNAU released rice varieties were estimated and their graphical representation is given in (Fig.3).

The traditional rice varieties (especially the red rice varieties Mapillai samba has highest phenolic content (45.8+ 0.190 mg/100g) followed by Kattu yanam (41.36+0.075 mg/100g) than the TNAU released rice varieties which were found to contained in the range of (34-40 mg/100g). The higher content of polyphenols in the dark pigmented rice grains (red and black) can be attributed to the difference in pericarp color. In addition to the variations in phenolic compound

concentrations, variations were also observed in the concentration of these compounds in grains with the identical pericarp color. These results are similar to Zhou *et al*., 2004 and Yawadio *et al.,* 2006.
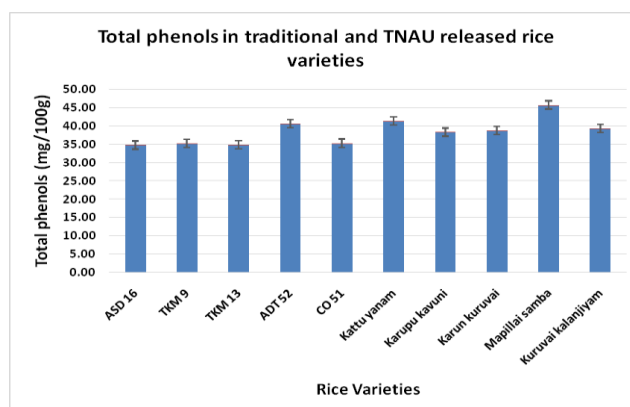

Figure.3 Total phenol content in the selected rice varieties

Total flavonoid content

The phenolic compounds in rice exist in both soluble and insoluble (bound) forms similar to other cereal grains. The pericarp color pigments are derived from polyphenols and the type and concentration of such polyphenols varies among rice genotype. Plant phenolics like isoflavonoids and stilbenes have been found to be beneficial to human health. Flavonoids can counteract cancer cell growth, as well as promote antioxidant, anti-inflammatory activities. The total flavonoid content ranged from 71± 0.29 to 288 ± 0.16 mg/100g rice extract (Fig.4). Accumulation of anthocyanins has a direct relation to the coloration of rice. Thus, it could be presumed that the white rice had flavonoid content lower than those of red and black pigmented varieties of rice. Especially among the pigmented rice varieties, Mappilai samba found to contain highest flavonoid content (288 ± 0.16 mg/100g). The total flavonoid content of the pigmented rice was significantly higher compared to non-pigmented rice varieties. Similar findings were observed by T.Furukawa *et al*. 2007 and Kim *et al*. 2010. No statistical significance was observed among non – pigmented varieties.
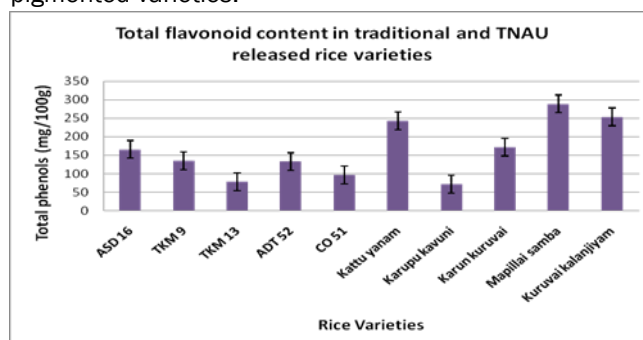

Figure.4 Total flavonoid content in the selected rice varieties

Total anthocyanin content

Anthocyanins, the major pigment responsible for the colour of red and black rice, are a kind of flavonoids. Anthocyanin pigments have been reported to be highly potent in reducing cholesterol levels in the human body. Total anthocyanin content was found to be highest for pigmented varieties (Fig. 5). Among the tested traditional rice varieties, Mapillai Samba has highest anthocyanin content (48.37±0.235 mg/100 g) followed by Kattuyanam (45.02±0.181 mg/100g, compared with the TNAU released varieties having less anthocyanin content in the range of 13 to 25.02 g/100g). The total anthocyanins in the pigmented rice varieties were significantly higher whereas no statistical significance was observed among the non-pigmented varieties tested. The predominant flavonoids in pigmented rice varieties are the anthocyanins and kaempferol, quercetin were identified to be the dominant flavonols, whereas apigenin was the major component of flavones (Sangkitikomol *et al.*2010). The structural properties of anthocyanins make them highly reactive towards reactive oxygen species (ROS). The current study confirmed the findings of a previous study on anthocyanin contents of pigmented rice Mapillai samba, a kind of red rice from Tamil Nadu, has the highest amount of total polyphenolic, flavonoids and anthocyanin content than the varieties from Sri Lanka, China red rice and Manipur black rice (Sompong *et al.*, 2011).
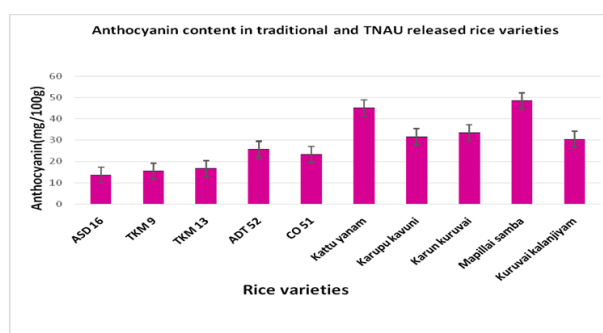

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Determination of alpha-amylase inhibitory potential and total antioxidant potential of the selected rice genotypes

Alpha-amylase inhibitory potential

Diabetes mellitus (DM) is a chronic, endocrine metabolic disorder that occurs due to defective insulin secretion by pancreatic β-cells (type 1 diabetes mellitus, T1DM) or impaired sensitivity to insulin secreted from the pancreas (type 2 diabetes mellitus, T2DM). α-Amylase (α1, 4-glucan 4-glucanohydrolase, EC 3.2.1.1) is a key enzyme produced in human salivary glands and the intestinal lumen; it acts in random locations by hydrolyzing α-1,4-glycosidic bonds in carbohydrates. It breaks down long-chain dietary polymers into maltotriose, maltose, and glucose, which quickly enter the bloodstream (Ao et al., 2007). A sedentary lifestyle and unhealthy food choices can result in increased postprandial glucose levels in the bloodstream, which is associated with an increased risk of T2DM. Alpha-amylase inhibitors are oral antidiabetic drugs known for inhibiting the alpha-amylase enzyme, which carries out starch hydrolysis, enabling its assimilation in the intestine. However, these drugs can cause several adverse side effects.

The present study evaluated the alpha-amylase inhibitory potential of traditional pigmented rice bran crude extracts. The wide spectrum of nutritional metabolites bound to the cell wall in red, purple, and black pigmented rice have gained interest owing to their ability to inhibit reductase enzymes for antidiabetic treatment; these molecules are not found in white rice. Hence, these inhibitors, acquired from consuming traditional pigmented rice, could be used to stabilize the postprandial glucose level. Among the tested traditional and TNAU released rice varieties, Mapillai Samba showed the strongest

alpha-amylase inhibition (IC50 = 23.15 ±0.132 μg/mL followed by Karuppukavuni (IC50 = 20.50±0.190 μg/mL) as given in (Fig.6). Statistically significant amylase inhibitory activity was exhibited in the coloured rice varieties rich in anthocyanins than the non- pigmented rice varieties. This preliminary study will increase the interest in identifying natural and safe antidiabetic compounds from a staple functional food and their use as dietary supplements for maintaining blood glucose levels (Walter *et al.*, 2013) and (Chen *et al.*,2010).
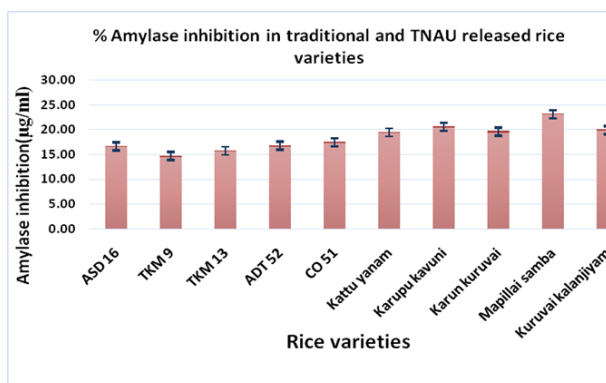

Figure.6 Amylase inhibitor potential of the selected rice varieties

Total anti-oxidant potential

The antioxidant activity was determined with DPPH free radical scavenging for each cultivars of the crude rice extract and the average radical scavenging activity was depicted in Fig. 7. The highest percentage of DPPH radical scavenging inhibition was found in pigmented rice varieties, especially in Mapillai samba (51.8± 0.086% DPPH scavenging activity) and the lowest radical scavenging activity was observed in non-pigmented rice varieties, which could be due to the low content of phytoconstituents. The DPPH scavenging activity of Mapillai samba and Kuruvai kalanjiam were observed to be significantly than the non-pigmented rice extracts. Among the pigmented varieties, there were positive correlations among the total phenol content, anthocyanin and flavonid contents and that of DPPH inhibition confirming the direct relationships between antioxidant activities, active phytoconstituents and density of pigmentation among the rice cultivars (Yodmanee *et al* .2011).

Assessment of Invitro starch digestibility and glycemic index of the selected rice genotypes

Unlike white polished rice, brown rice releases sugars slowly thus helping to stabilise blood sugar in a sustained manner. This trait makes it a better option for people who are suffering from diabetes mellitus. Further, studies in Asia have shown a relationship between the consumption of white rice and risk of type 2 diabetes. Dietary fibres reduce the absorption of carbohydrates by providing an enclosure to the food, hindering the action of hydrolytic enzymes in the small intestine on food, and increasing the viscosity of food in the intestine. This plays a vital role in reducing the GI of food thereby preventing the risk of diabetes type 2. Proanthocyanidins present in red rice provide protection against type 2 diabetes. Similarly, anthocyanins present in black rice is said to have a hypoglycemic effect.

The glycemic index (GI) is a method used to classify dietary carbohydrates based on their impact on the blood glucose response usually 2-hours after meals (Wolever, 2006). A lower diet GI may results in a slower rate of digestion and absorption, hence reducing the rapid elevation of postprandial hyperglycemia and insulin concentration, which will then influence the management of diabetes. GI, is a numerical measure of the extent to which carbohydrates in foods affect postprandial blood glucose levels. Numerous factors influence the GI classification including the amylose content of the rice (Brand-Miller *et al*, 1992). Amylose, a long straight starch molecule that does not gelatinize much during cooking (as it absorbs less water and the swelling of glucose granules) is considered a major determinant of rice appearance and cooking quality. Hence, rice with more amylose content tends to cook fluffy and non-sticky. As previously mentioned, amylose content of the rice influences the GI value where rice that is high in amylose usually has lower GI value .This could be due to the formation of complexes between amylose and lipids upon heating, thus making them less accessible to enzymatic digestion which results in a slower rate of digestion.
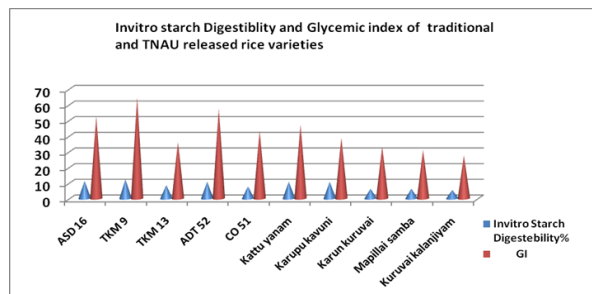

Figure.8 Invitro starch Digestibility and Glycemic index of traditional and TNAU released rice varieties

The *Invitro* starch digestibility and glycemic index of ten different traditional rice landraces is shown in Fig.8. Among the traditional varieties, Karun Kuruvai and Mapillai Samba were found to have lower invitro starch digestibility (6.2%) compared with TNAU released varieties in the range of 10.7 to 12.2%. Similar pattern was observed in case of glycemic

index in the pigmented rice varieties especially Mapillai samba (33.1) and Karun Kuruvai (31.0) which were found to have lower glycemic index compared with TNAU released rice varieties in the range of (43-64). Since there is a strong correlation between the amylose content, total phenol content, anthocyanin , flavonoid, amylase inhibitor potential, antioxidant potential with glycemic index, it was found the rice genotypes with high amylose, total phenol, anthocyanin , flavonoid content ,amylase inhibitor and antioxidant potential has low glycemic index. Among the tested rice varieties Mapillai Samba (33.1) and Karun Kuruvai (31.0) has got lower glycemic index compared with TNAU released rice varieties in the range of (43-64) and they were identified as potential low Glycemic index lines and could be a better alternative for the regular diet of type 2 diabetes patients.

CONCLUSION

It is conspicuous that phenolics, flavonoids content and antioxidant capacity differ from the grain color; nonetheless, these phytoconstituents and antioxidant capacity still vary among that of white color rice grains and pigmented grains. The traditional varieties Mapillai samba and Karun kuruvai were identified as the potential low glycemic index lines and could be a better alternative for the regular diet of type 2 diabetes patients.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research

Originality and plagiarism

This is to certify that Authors should ensure that they have written and submit only entirely original works.

Consent for publication

All the authors agreed to publish the content

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; amirtham@tnau.ac.in

Author contributions

Idea conceptualization-PR, Experiments-DA,PR, Guidance –DA ,Writing original draft – DA, Writing-reviewing &editing – DA.

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