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RESEARCH ARTICLE

COMPARATIVE STUDIES ON NUTRITIONAL AND FUNCTIONAL PROPERTIES OF SELECTED TRADITIONAL RICE VARIETIES IN SRI LANKA

Jayadewa CT, Chiranthika NNG and Gunathilake KDPP*

Department of Food Science and Technology, Faculty of Livestock, Fisheries & Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila NWP, Sri Lanka

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ABSTRACT

Non-communicable diseases are chronic metabolic diseases increasing rapidly around the world. Evaluation of natural food sources in terms of controlling or preventing non-communicable diseases is an immerging research area in food science and nutrition schemes. Traditional rice varieties are one of the food commodities currently becoming popular among health-conscious consumers, as they provide health benefits through bioactive compounds or functional food ingredients other than a variety of nutrients. This study aimed to assess the nutritional and functional properties of selected 20 traditional rice varieties in Sri Lanka. Proximate composition was analyzed for raw rice samples. Methanolic extracts of rice were analyzed for total phenolic content (TPC), total flavonoid content (TFC), carotenoid content (TC), antioxidant, anti-inflammatory and anti-diabetic properties. In vitro gastrointestinal digestion was performed to evaluate the bioavailability of bioactive compounds. Rice samples with higher anti-diabetic properties were tested for a postprandial glycemic response using healthy individuals. The results indicated fresh methanolic extracts of Rath Kadha had the significantly ($p<0.05$) highest TPC (9.94±0.01mgGAE/g FW) and TC (2.27±0.06 mg/g FW), Beheth Heenati have the highest DPPH% inhibition (84.93±0.81%), Sudu Heenati had the significantly ($p<0.05$) highest TFC (16.77±0.01mg RE/g FW), anti-diabetic properties (59.54±0.02%) and anti-inflammatory properties (65.85±0.01%). Among the rice varieties subjected to in vitro digestion, Suwadel showed highest significant ($p<0.05$) TPC (0.45±0.02 mg GAE/g FW) and TFC (2.01±0.02 mgRE/g FW), Ma Vee showed the highest TC (0.24±0.015 mg/g FW) and Pachchaperumal showed the highest DPPH% inhibition (61.77±0.02%) in the gastric phase. In the intestinal Phase Sudu Heenati showed the highest TPC (0.99±0.04 mgGAE/g FW), Ma Vee showed the highest TFC (0.8±0.01 mgRE/g FW) and TC (3.24±0.04 mg/g FW), Beheth Heenati showed the highest DPPH% inhibition (63.98±0.07%). Tested all cooked rice varieties exhibited a lower postprandial glycemic response than glucose. Among the cooked rice varieties Maa Vee showed the lowest peak (97mg/dl) concerning glucose. According to glycemic response, values were significantly ($p<0.05$) changed with the rice variety. The results highlighted that traditional rice verities are rich with a wide range of functional properties and useful in the management of non-communicable diseases and their complications.

Keywords: Functional properties, Traditional rice, Gastrointestinal digestion, Glycemic response, Phytochemicals

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's major food crops and it is the staple food for the majority of the world's population. Rice is considered the world's second most important cereal crop with which production slightly below wheat. Rice includes the family Poaceae (Gramineae) and is often considered an annual grass, a semi-aquatic plant. Traditionally, Asian countries have the largest share of rice production in the world. With an output of more than 209 million tons in 2019, China is the world's largest rice producer, followed by India and Indonesia, according to the latest official data (Shahbandeh 2021).

Sri Lanka currently produces 2.7 million tons of rice annually and meets about 95% of

*Corresponding author: kdppgunathilake@yahoo.com

the national demand. Rice is also the most important crop providing 45% of total calories and 40% of total protein needs of an average Sri Lankan and the demand for rice in Sri Lanka is growing at a rate of 1.1% per year (Senanayake and Premaratne 2016). Rice is also providing a variety of vitamins and minerals, dietary fiber, bioactive compounds and phytochemicals. A wide range of rice varieties are grown around the world and their nutrition composition, physio-chemical properties, and functional characteristics vary from one another.

Rice is considered as the staple food in Sri Lanka. Traditional rice varieties have been used by Sri Lankan farmers for over 3000 years. Rice varieties passed down from previous generations are known as "traditional", "indigenous" or "hereditary" rice varieties of Sri Lanka (Rambukwella and Priyankara 2016). Once known as the Granary of the East, Sri Lanka grows more than 2000 types of traditional rice (Dharmasena 2010). In Sri Lanka, most farmers only grow about 400 varieties that are popular with consumers (Abeysekera *et al*. 2008). However, the cultivation of these varieties has increased in recent years. This may be due to the ability of these varieties to withstand harsh climates and respond favorably to organic growing (Dissanayake 2018). And also, these traditional rice varieties have a higher ayurvedic and medicinal value which can be used to address problems such as nutritional deficiencies and non-communicable diseases.

Although there are lots of health benefits from these traditional rice varieties, only a few studies have reported their medicinal properties and quality characteristics (Cruz and Khush 2000; Wickramasinghe and Noda 2008; Premakumara *et al*. 2013). At present global trends in the food industry is also to produce healthy foods worldwide. Therefore, these rice varieties which are rich in physiochemical and nutritional properties can be used in food production on local market as well as foreign markets to solve potential problems related to non-communicable diseases. Therefore, it is necessary to promptly assess the health benefits of traditional Sri Lankan rice varieties and also the data will be useful for further studies as well. Hence this study was conducted to study nutritional and *in vitro* inhibitory activities against non-communicable diseases of selected traditional rice varieties in Sri Lanka.

MATERIALS AND METHODS

Chemicals and Reagents

Amylo glucosidase, phosphor molybdenum reagent, dinitro salicylic acid reagent, Gallic acid, Ascorbic acid, Folin-Ciocalteu reagent, Bovine serum albumin, Porcine pepsin, pig pancreatin, bile salt, Phosphate buffered saline, alpha-amylase used as the main chemicals and all other chemicals used were of analytical grade.

Collection of traditional rice varieties

Twenty whole-grain Sri Lankan traditional rice varieties were collected from Rice Research and Development Centre (RRDC), Bathalegoda, Sri Lanka.

Preparation of Rice Flour

Rice was dehusked, cleaned and washed with tap water. Rice samples were exposed to the sun for 2 - 3 days, for six hours, and in a 55℃ oven for 3 - 4 hours before milling. The dried rice was milled, sieved and stored at 0 - 4 ℃ in an airtight container until used for analysis.

Proximate composition

The moisture, crude protein, crude ash and crude fat contents of selected whole-grain rice varieties were evaluated according to the methods described by the Association of Official Analytical Chemists. Total carbohydrate content was evaluated by subtracting the sum of the values of crude protein, crude fat and ash content (% dry weight) of the sample from 100.

Preparation of methanolic extract

Methanolic extracts of rice varieties were prepared by mixing 10 g of rice with 70 mL of methanol/water (80/20, v/v) and vortexed for thirty minutes followed by centrifugation for 10 min at 792 g. The extracts were then filtered through filter paper, and the prepared extracts were then evaporated in a rotary evaporator at 40°

C under vacuum and stored at -18 °C for further analysis.

Total phenolic content

The total polyphenol content of the methanolic extracts and digested fractions was estimated by Folin-Ciocalteu's method Singleton *et al.* (1999), with some modifications as described by (Gunathilake and Ranaweera 2016). Briefly, 0.5 ml of test sample and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min in the dark. Then, 2.5 ml of 7.5% sodium carbonate was added to the mixture and further incubated for 2 hours in the dark at room temperature, then the absorbance at 760 nm was measured using a UV/ spectrophotometer. The concentration of total phenols was expressed in mmol gallic acid equivalents (GAE) per g fresh weight (FW).

Total Flavonoids content

Total flavonoid content was measured according to the colorimetric method of Zhishen *et al*. (1999), described in Tagliazucchi *et al*. (2010), with some modifications. Briefly, 0.5 ml of the digested or appropriately diluted methanolic extract of the rice standard solution was added to 3 ml of distilled water. Then, 0.3 ml of 5% $NaNO_2$ was added and left to stand for 5 min at room temperature (30 °C). Approximately 0.3 mL of 10% $AlCl_3$ was added 5 min later and allowed to stand for an additional 6 min, then 2 mL of 1 M NaOH was added and the solution was made up to 10 mL with distilled water and it was mixed. Absorbance was determined at 510 nm on a suitable blank using a UV/ VIS spectrophotometer. Total flavonoid content in digested/methanolic rice extract expressed in mmol equivalents per 1 g fresh weight.

Total carotenoid content

The total carotenoid content of rice samples were analyzed according to the method described by Sukran *et al.* (1998). 0.5 ml of test samples were separated and the absorbance was measured at 470, 653 and 666 nm on a UV/VIS spectrophotometer. Total carotenoid content was calculated according to the formula of Kichtenthaler and Wellburn (1983).

Chlorophyll a (C_a) = 11.75(A662) – 2.350 (A645) **……..Eqn 01**

Chlorophyll b (C_b) = 18.61(A645) – 3.960 (A662)**……...Eqn 02**

Carotenoids = 1000(A470) – 2.270(C_a) – 81.4 (C_b)/22**…….Eqn 03**

DPPH radical scavenging activity

The DPPH radical scavenging activity of the methanolic extracts was measured by the method of Gunathilake and Ranaweera (2016). Accordingly, 3.9 ml of freshly prepared DPPH solution was mixed with 0.1 ml of sample and vortexed thoroughly. It was then incubated at room temperature in the dark for 30 min. Sample-free DPPH solution was used as a control. Then, the absorbance of the mixture was measured at 517 nm using a UV/visible spectrometer and calculated the percentage inhibition of the radical scavenging activity.

Antidiabetic properties (Alpha-amylase inhibition assay)

The alpha-amylase inhibition assay was performed on the basis of an approved method Bernfeld (1955), used to evaluate the antidiabetic properties. Accordingly, 100 µL of the sample was mixed with 200 µL of α amylase enzyme and 100 µL of 2 mM phosphate buffer (pH 6.9). After 20 min incubation, 100 µL of 1% starch solution was added. The same was done for controls where 200 µL of the enzyme was replaced with buffer. After incubation for 5 min, 500 µL of 3, 5 dinitrosalicylic acid reagent was added to the control and test solutions. The solutions were kept in a boiling water bath for 5 minutes. Absorbance was measured at 540 nm using a UV/Visible spectrophotometer and the percentage of α amylase inhibition was calculated.

Anti-inflammatory properties (Protein denaturation assay)

A protein denaturation assay described by Gambhire *et al*. (2008), with some modifica-

tions described by Gunathilake, Ranaweera and Rupasinghe (2018, a, b) was performed to assess the Anti-inflammatory properties of rice samples. Accordingly, 0.2 ml of 1% bovine albumin, 4.78 ml of phosphate-buffered saline (PBS, pH 6.4) and 0.02 ml of methanolic extract or digested fractions were mixed and incubated at 37 °C for 15 min in a water bath. After incubation, the reaction mixture was heated at 70 ℃ for 5 min. After cooling, the absorbance of the solution was measured at 660 nm using a UV/ Visible spectrometer. PBS without a sample was used as a control and the percentage inhibition of protein denaturation was calculated.

***In vitro* gastrointestinal digestion**

The rice samples were subjected to gastrointestinal digestion according to the method of Hettiarachchi, Gunathilake and Jayatilake (2021), with slight modifications. 10 g of rice samples were separately mixed with 10 ml of saliva donated by a healthy adult. The mixtures were ground using mortar and pestle for 1 min to imitate chewing. They were incubated at 37 °C for 10 min. Exactly 5 ml of each sample was collected and diluted with 0.9% NaCl followed by filtration through filter paper (Whatman No. 42) And filtered aliquots were stored in amber-colored glass bottles for further analysis. pH of the mixture was adjusted to 2.0 using 6.0 mol/L of HCl solution to inactivate salivary amylase. Then 50 mL 0.9% NaCl and 4.0 mL pepsin solution 940 mg/mL in 0.1 M HCl were added to sample keeping the pH at 2.0. The mixtures were incubated for 1 h in a shaking water bath at 37 °C and 100 rpm. Then, 5 mL aliquots of gastric digestion of each sample were collected, diluted and filtered through filter paper (Whatman No. 42) and stored them for further analysis. Segments of dialysis tubing cellulose membrane (average flat width; 33 mm, MWCO 12,000 Da) were cut into a specified length (15.0 cm) and rinsed on both outer and inner surfaces with 0.9% NaCl solution before starting the intestinal digestion phase with dialysis. Prepared dialysis bags were filled with 5.5 mL NaCl (0.9%) and 5.5 mL NaHCO3 (0.5 M). Then the open end was sealed with clips and immersed into a gastric digestion mixture immediately after digestion. The mixtures were incubated at 37°C and 100 rpm for 45 min in a shaking water bath. Before adding a pancreatin-bile mixture consisting of 2 mg/mL pancreatin and 12 mg/ mL bile extract dissolved in 0.1 M NaHCO3, to each digested mixture, the pH was adjusted to 6.5 with NaHCO3. After adding the pancreatin-bile mixture, each digest was incubated for 2 h at 37 °C. Once the digestion is completed, the pH of the mixtures was between 7-7.5. Aliquots were collected from the intestinal phase, filtered and stored for analysis. The dialysis bags were removed washed with water and dried using a paper cloth before measuring the weight of each. The content in the bags was transferred to measuring cylinders and diluted to a final volume of 14 mL with 0.9% NaCl and then filtered and stored for further analysis. All the aliquots thus collected were analyzed for their TPC, TFC, TC, and antioxidant capacity.

Effect of cooked rice on postprandial blood glucose in human

Each subject was given a cooked rice sample. The start time of the meal will be noted and meals must be finished within 15 minutes. The two-hour limit is counted from the time of starting meal. At the end of two hours, a blood sample will be checked for blood glucose level with a glucometer.

Statistical Analysis

The data were analyzed and expressed as average mean ± SD. Statistical differences between the average values were analyzed using Tukey's HSD comparison test. A 95% confidence level ($p < 0.05$) was employed for all analysis. The statistical analysis was conducted using the software SPSS and MS Excel 2013.

RESULTS AND DISCUSSION

Proximate composition of selected traditional rice varieties in Sri Lanka

The results of the proximate composition of selected 20 traditional rice varieties was given in Table 1. Results showed that moisture, crude ash, crude protein, crude fat, crude fiber, and total carbohydrate contents varied significantly ($P<0.05$) different among studied rice varieties.

Table 1: Proximate composition of selected traditional rice varieties of Sri Lanka

Rice Variety	Moisture(%)	Ash (%)	Fiber (%)	Fat (%)	Protein(%)	Carbohy-drate (%)
Batapola al	12.41± 0.45^{bcd}	1.32± 0.23^{bcd}	0.15± 0.05^{ij}	2.89± 0.06^{e}	11.13± 0.3^{d}	72.12± 0.02^{m}
Beheth Heenati	12.37± 0.6^{abd}	1.35± 0.09^{b}	0.87± 0.07^{de}	3.56± 0.06^{a}	13.39± 0.15^{a}	68.40± 0.01r
Herath Banda	13.20± 0.2^{ab}	1.21± 0.42^{bcde}	0.08± 0.01^{j}	1.84± 0.03^{h}	10.92± 0.29^{fg}	72.74± 0.04^{k}
Kalu Heenati	13.83± 0.35^{a}	1.33± 0.12^{bc}	0.69± 0.01^{f}	1.65± 0.04^{ghi}	11.02± 0.3^{d}	71.48± 0.01^{p}
Kahamaala	13.03± 1.08^{ab}	0.55± 0.29^{efgh}	1.26± 0.17^{bc}	0.65± 0.03^{jk}	8.96± 0.02^{j}	75.55± 0.01^{g}
Kahawanu	13.27± 0.46^{ab}	0.65± 0.14^{efg}	0.07± 0.02^{jk}	0.55± 0.03^{jk}	10.30± 0.5^{fgh}	75.16± 0.04^{k}
Kiri Naran	12.93± 0.75^{abc}	0.52± 0.21^{efgh}	0.04± 0.01^{l}	0.51± 0.02^{jk}	7.85± 0.12^{l}	78.14± 0.02^{c}
Kuruluthuda	10.80± 0.6^{cdefgh}	0.25± 0.34^{ghij}	0.71± 0.02^{def}	3.06± 0.06^{cd}	8.46± 0.15^{k}	76.72± 0.03^{e}
Madathawalu	11.87± 0.87^{cde}	0.73± 0.17^{ef}	0.92± 0.03^{d}	2.05± 0.08^{fg}	11.52± 0.07^{de}	72 92± 0.15^{kl}
Ma Vee	12.03± 0.97^{abcd}	0.33± 0.39^{ef}	5.07± 0.06^{a}	0.7± 0.53^{jk}	11.18± 0.36^{d}	70.69± 0.17^{q}
Pachchaperumal	12.50± 0.3^{bc}	2.38± 0.17^{a}	1.18± 0.01^{b}	3.33± 0.03^{ab}	13.28± 0.31^{a}	67.32± 0.03^{s}
Pokkali	12.07± 0.55^{bcd}	0.91± 0.03^{e}	0.14± 0.01^{g}	0.71± 0.56^{jh}	10.85± 0.19^{fg}	75.3±3 0.05^{h}
Ran Kahawanu	13.13± 0.64^{ab}	0.51± 0.1^{efgh}	0.11± 0.01^{hı}	0.53± 0.02^{jk}	10.42± 0.02^{ı}	75.30± 0.05^{hi}
Rathu Suduru	11.43± 0.45^{cdef}	0.44± 0.03^{gh}	0.03± 0.01^{lm}	1.66± 0.35^{ghi}	8.55± 0.04^{k}	77.88± 0.02^{d}
Rath Kadha	9.70± 0.46^{ı}	0.24± 0.03^{ghı}	0.17± 0.01^{g}	2.21± 0.18^{f}	11.02± 0.17^{def}	76.65 ± 0.01^{f}
Rath Suwadel	13.03± 0.35^{ab}	0.24± 0.03^{ghı}	0.01± 0.01^{m}	0.64± 0.06^{jk}	7.59± 0.01^{m}	78.48± 0.02^{b}
Sudu Suduru	13.23± 0.4^{ab}	0.33± 0.02^{gh}	0.07± 0.01^{jk}	0.63± 0.03^{jk}	12.84± 0.03^{b}	72.90± 0.02^{j}
Sudu Heenati	11.8± 0.87^{cdefg}	0.84± 0.07^{ef}	0.11± 0.03^{hı}	3.42± 0.18^{ab}	12.08± 0.02^{c}	71.76± 0.04^{o}
Sulai	13.30± 0.66^{ab}	0.53± 0.05^{efgh}	0.12± 0.04^{h}	3.19± 0.15^{bc}	10.71± 0.03^{fgh}	72.15 ±0.01^{n}
Suwadel	11.93± 0.7^{cd}	0.64± 0.03^{ef}	0.12± 0.02^{h}	0.33± 0.02^{jk}	6.51± 0.04^{n}	80.47± 0.15^{a}

Values are expressed as mean± SD Values followed by different letters for each assay in the same column are significantly different ($p < 0.05$)

Among rice varieties tested *Kalu Heenati* had significantly (P<0.05) highest moisture content while *Rath Kadha* had the lowest moisture content. Red rice variety *Pachchaperumal* had the significantly (P<0.05) highest crude ash content while the *Rath Kadha and Rath Suwadel* had the lowest crude ash content. Ma Vee had the significantly (P<0.05) highest crude fiber content while the white rice variety *Kiri Naran* had the lowest crude fiber content. Red rice variety *Beheth Heenati* had the significantly (P<0.05) highest crude fat content while the white rice variety *Suwadel* had the lowest crude fat content. *Beheth Heenati* had the significantly (P<0.05) highest protein content while *Suwadel* had the lowest crude protein content. Out of the rice varieties tested *Suwadel* had the significantly (P<0.05) highest carbohydrate content while the red rice variety *Pachchaperumal* had the lowest carbohydrate content.

Main nutrient component of rice is carbohydrate. It varies (80.47 ± 0.15 67.32 ± 0.03) % depending on the variety. In developing countries, rice contributes about 27% of dietary energy. In Southeast Asian countries, where rice is widely consumed, rice contributes more than 50% to dietary energy per capita (Kennedy *et al*. (2017). In Sri Lanka, rice contributes 45% of dietary energy per capita (Senanayake and Premaratne 2016). Foods that are often high in carbohydrates are associated with a high glycemic index (Eleazu 2016). As a prevalence to diabetes-related health problems, it is important to identify rice varieties with a low glycemic index. The presence of high dietary fiber, high amylose content and inhibitors of carbohydrate-digesting enzymes lowers the glycemic index of foods (Eleazu 2016). This study showed that *Maa Vee, Pachhcaperumal* and *Kahamala* had high crude fiber content. Thus, the Consumption of these types of rice may be important in the management of diabetes as well as other chronic non-communicable diseases.

Protein is one of the main nutrients in rice and the rice varieties selected in this study have protein content in the range (13.39 ± 0.15 6.51 ± 0.04) %. Analysis of 2,674 rice varieties by the International Rice Research Institute (IRRI) showed that the protein content of rice varieties ranged from 4.5% to 15.9% Kennedy *et al.* (2003). Therefore, the results of this study are consistent with those of IRRI. According to most studies conducted, protein content of rice varieties worldwide is less than 13% (Juliano 2003; Sompong *et al.* 2011; Oko et al. 2011; Oko *et al.* 2012). There for, rice varieties with protein content greater than 13% can be considered as high protein rice varieties. Rice accounts for 14% of the world's protein supply, and in developing countries it contributes up to 20% of daily protein requirements (FAO 1999). In Sri Lanka, rice's contribution to daily protein requirement is close to 37% (FAO 1999). Therefore, consumption of whole grains, especially the rice varieties *Beheth Heenati, Pachchaperumal* and *Sudu Suuduru* may be beneficial in obtaining a substantial amount of the daily protein requirement.

Rice contributes to about 2.7% of the dietary fat supply in Sri Lanka. In this study *Beheth Heenati, Sudu Heenati* and *Pachchaperumal* showed high fat content. In previous research, it was reported that the outer layers of rice grains are rich in monounsaturated and polyunsaturated fatty acids, which are considered as good quality dietary fats (Juliano 2003). Although rice contributes to the low dietary fat intake in Sri Lanka, consuming these types of rice in its whole grain form helps to obtain good quality dietary fat.

Ash content is an expression of the mineral content of the rice grain. Among selected rice varieties ash content varied from (2.38± 0.17 - 0.24± 0.03) % In this study *Beheth Heenati, Kalu Heenati* and *Pachchaperumal* showed high crude ash content. A study carried out on the micronutrient content of traditional Sri Lankan rice varieties showed that *Kalubala Wee, Pachchaperumal, Dahanala, Rathu Heeneti, Kattamanjal* and *Rathal* had high iron content (2.25–3.73). mg/100 g), while *Kalu Bala Wee, Wanni Dahanala, Rathu Heeneti, Dahanala, Rathal* and *Kalu Heeneti* had high zinc content (2.51–3.91 mg/100 g) Herat *et al.* (2016). Thus, tradition-

al rice varieties of Sri Lanka are staple food to improve the nutritional and health status of the people in the country.

Functional properties of selected traditional rice varieties in Sri Lanka

Rice has been identified as a rich source of several bioactive compounds and in this study total phenolic content, total flavonoid content, total carotenoid content and DPPH radical scavenging activity of studied rice varieties were evaluated. Further, the anti-diabetic property of rice samples was evaluated using alpha-amylase inhibitory assay and the anti-inflammatory property of rice samples was evaluated using protein denaturation assay. The results obtained for the above functional properties are summarized in Table 2.

The total Phenolic content of the methanolic extracts of rice samples were in the range of 9.94± 0.01 - 0.26± 0.01 mg GAE/g FW. Red rice variety *Rath Kadha* had the significantly ($P<0.05$) highest total phenolic content while the white rice variety *Kahawanu* had the lowest total phenolic content. The total flavonoid content of the methanolic extracts of rice samples were in the range of 16.77± 0.01 - 0.09± 0.05 mg RE/g FW Among the studied rice varieties *Sudu Heenati* had the significantly ($P<0.05$) highest total flavonoid content while *Kahamala* had the lowest total flavonoid content. The total carotenoid content of the methanolic extracts of rice samples were in the range of 2.27± 0.06 - 0.01± 0.02 mg/g FW. Red rice variety *Rath Kadha* had the significantly ($P<0.05$) highest total carotenoid content while the white rice variety *Kiri Naran* had the lowest total carotenoid content. DPPH % scavenging activity of methanolic extracts of rice samples were in the range of 84.93± 0.81 - 35.56± 0.05%. *Beheth Heenati* had the significantly ($P<0.05$) highest DPPH inhibition % while *Suwadel* had the lowest DPPH % inhibition. Anti-diabetic property of methanolic extracts of rice samples were in the range of 59.54± 0.02 - 33.63± 0.01%. *Sudu Heenati* had the highest anti-diabetic property while *Herath Banda* had the lowest anti-diabetic property. The anti-Inflammatory property of methanolic extracts of rice samples were in the range of 65.85± 0.01 - 17.07± 0.15%. Among the rice varieties tested *Sudu Heenati* had significantly ($P<0.05$) highest anti-inflammatory properties while *Batapola al* had the lowest anti-inflammatory property.

As described by Zhang *et al.* (2010), Phenolic compounds present in rice mainly include derivatives of hydroxycinnamic acids and benzoic acids. Soluble phenolic compounds located within the cell vacuoles as free or conjugated form and insoluble phenolic compounds esterified with arabinose or galactose residues of hemicellulose or pectic components (Mira *et al.* 2008). The present study indicates total Phenolic content of the methanolic extracts of rice samples was in the range of 9.94± 0.01 - 0.26± 0.01 mg GAE/g FW. The total flavonoid content of the methanolic extracts of rice samples was in the range of 16.77± 0.01 - 0.09± 0.05 mg RE/g FW. Among the tested rice varieties, *Sudu Heenati* had the highest total flavonoid content. The total carotenoid content of the methanolic extracts of rice samples was in the range of 2.27± 0.06 - 0.01± 0.02 mg/g FW. Red rice variety *Rath Kadha* had the highest total carotenoid content while the white rice variety *Kiri Naran* had the lowest total carotenoid content. According to the studies by Prabhu and Jayadeep (2015) generally, non-pigmented rice bran like white rice bran provides only phenolic acids while pigmented rice like red or black rice is rich in pro -anthocyanins and anthocyanin depending on their variety. Hence, red rice bran varieties show higher content of bioactive compounds when compared with white rice bran varieties. Thus, the findings of this study are in agreement with those research findings.

This study has used DPPH radical scavenging assay to evaluate the free radical scavenging ability of the rice extracts. The free radical of DPPH is quenched by phenolic compounds by donating either electron or hydrogen atoms. There a higher percentage of DPPH radical scavenging activity of methanolic extracted samples indicates the higher antioxidant activity of samples in terms of hydrogen donating capacity (Amarowicz *et al.* 2004). The present study indicates that the DPPH radical scavenging activity of rice ranged from 84.93±

Table 2: Content of total phenolic, flavonoid, carotenoids, DPPH, anti-diabetic and anti-inflammatory properties in rice samples

Rice Variety	TPC *(mg GAE/g FW)*	TFC *(mg RE/ g FW)*	TC *(mg/g FW)*	DPPH *(Inhibition %)*	Anti-diabetic property (% inhibition)	Anti-inflammatory property (% inhibition)
Batapola al	1.22± 0.02^{k}	0.15± 0.15^{ij}	0.21± 0.16^{d}	70.71± 0.39^{ef}	44.56± 0.21^{k}	17.07± 0.15^{p}
Beheth Heenati	1.34± 0.01^{i}	0.31± 0.11^{hi}	0.24± 0.21^{d}	84.93± 0.81^{a}	50.00± 0.34^{d}	46.34± 0.01^{gh}
Herath Banda	0.32± 0.01^{n}	0.18± 0.08^{ij}	0.01± 0.03 ^{d}	32.21± 0.24^{r}	33.63± 0.01^{q}	34.14± 0.01^{l}
Kalu Heenati	1.22± 0.01^{k}	0.36± 0.03^{hi}	0.89± 0.15^{c}	76.56± 0.06^{d}	36.81± 0.15^{p}	53.66± 0.25^{de}
Kahamaala	0.26± 0.02^{o}	0.09± 0.05^{ij}	0.12± 0.05^{d}	46.02± 0.15^{i}	40.00± 0.47^{m}	43.90± 1.15^{i}
Kahawanu	0.26± 0.01^{o}	0.12± 0.07^{ij}	0.14± 0.04^{d}	39.33± 0.09^{p}	45.10± 0.03^{hj}	48.78± 2.04^{fg}
Kiri Naran	0.31± 0.01^{n}	0.14± 0.03^{ij}	0.01± 0.02^{d}	54.39± 0.10^{i}	38.63± 0.02^{no}	39.02± 0.03^{jk}
Kuruluthuda	1.87± 0.02^{h}	0.09± 0.51^{ij}	0.01± 0.08^{d}	43.51± 0.32^{m}	45.46± 0.18^{hi}	27.31± 0.01^{n}
Madathawalu	0.65± 0.04^{l}	0.15± 0.05^{ij}	0.15± 0.01^{d}	58.99± 0.15^{h}	38.18± 0.36^{no}	59.66± 1.15^{c}
Ma Vee	0.39± 0.02^{m}	0.14± 0.03^{ij}	0.01± 0.05^{d}	79.28± 0.25^{b}	56.91± 0.51^{b}	63.41± 0.45^{b}
Pachchap-erumal	1.22± 0.15^{i}	8.56± 0.03^{g}	1.40± 0.05^{b}	78.29± 0.13^{c}	52.27± 0.01^{c}	36.59± 0.51^{kl}
Pokkali	1.48± 0.17^{ij}	12.38± 0.21^{d}	0.29± 0.05^{d}	54.81± 0.45^{i}	45.72± 0.28^{h}	34.15± 0.02^{l}
Ran Kaha-wanu	1.80± 0.03^{g}	15.18± 0.01^{c}	0.02± 1.15^{d}	50.20± 0.33^{j}	47.72± 0.01^{g}	34.15± 0.35^{lm}
Rathu Suduru	7.86± 0.02^{b}	0.34± 0.13^{hi}	0.91± 0.03^{c}	44.35± 0.34^{n}	50.56± 0.67^{d}	22.44± 0.01^{o}
Rath Kadha	9.94± 0.01^{a}	10.58± 0.05^{e}	2.27± 0.06^{a}	70.49± 0.47^{e}	48.65± 0.05^{e}	48.78± 0.03^{fg}
Rath Suwadel	3.24± 0.21^{f}	9.76± 0.15^{f}	0.01± 0.02^{d}	69.07± 0.14^{g}	37.27± 1.15^{n}	50.09± 0.03^{f}
Sudu Suduru	4.28± 0.43^{d}	14.48± 0.02^{b}	0.01± 1.04^{d}	49.37± 0.32^{jk}	40.91± 2.05^{l}	52.54± 1.18^{d}
Sudu Heenati	3.72± 0.15^{de}	16.77± 0.01^{a}	0.47± 0.08d^{e}	72.01± 0.08^{e}	59.54± 0.02^{a}	65.85± 0.01^{a}
Sulai	6.00± 0.18^{c}	0.38± 0.07^{hi}	0.01± 0.05^{d}	41.14± 0.15^{o}	48.43± 0.03^{f}	39.02± 2.05^{jk}
Suwadel	9.94± 0.22^{a}	0.54± 0.11^{h}	0.09± 0.05^{d}	35.56± 0.05^{q}	52.27± 0.01^{c}	41.46± 0.45^{j}

TPC = Total Phenolic Content, TFC= Total Flavonoid Content Total Carotenoid, GAE = Gallic Acid Equivalent, RE =Rutin Equivalent, FW = Fresh Weight. Values are expressed as mean ±SD. Values followed by differences in the same column are significantly different ($P < 0.05$).

0.81 - 35.56± 0.05%. In this study *Beheth Heenati, Pachchaperumal* and *Ma Vee* showed the highest DPPH radical scavenging study.

The anti-inflammatory property of the rice samples was evaluated using a protein denaturation assay. Inflammation is one of the defense mechanisms initiated by the invasion of pathogens or tissue injury caused by biological, chemical, or physical damage (Janeway *et al.* 2001). According to the results obtained the anti-Inflammatory property of methanolic extracts of rice samples was in the range of 65.85± 0.01 - 17.07± 0.15%. *Sudu Heenati* and *Madathawalu* showed the highest anti-inflammatory property. In previous research, it is reported that *Sudu heenati* have the highest anti-inflammatory property among selected traditional rice varieties in Sri Lanka (Ginigaddara 2018). Thus, the findings of this study are in agreement with those findings.

Anti-diabetic property of the rice samples was evaluated using an alpha-amylase inhibition assay. Alpha-amylase is a key enzyme that involves in starch digestion. It breaks down polysaccharides into absorbable monosaccharides and disaccharides and then increases the postprandial blood glucose level. Inhibiting the activity of alpha-amylase is one of the important mechanisms to reduce blood glucose levels and control diabetic Mellitus. It is suggested that this inhibitory activity may be due to the phenolic compounds like ferulic acid, ellagic acid, gallic acid, and coumaric acid and the protein fraction of the rice bran (Sangeetha and Vedasree 2012). Results of the present study indicate an anti-diabetic property of methanolic extracts of rice samples was in the range of 59.54± 0.02 - 33.63± 0.01%. In previous research, it is reported that *Ma Vee, Pachchaperumal, sudu heenati, and Suwadel* have the highest anti-diabetic property among selected traditional rice varieties in Sri Lanka (Ginigaddara 2018). Thus, the findings of this study are in agreement with those research findings.

In Vitro digestive studies

The impact of gastrointestinal digestion on the bioactive content of rice also evaluated for rice samples which have the highest anti-diabetic properties and results are indicated in Table 3.

Total Phenolic content in gastric phase was in the range of 0.45± 0.02 - 0.026± 0.011 mg GAE/g FW. *Suwade*l had the significantly ($P<0.05$) highest total phenolic content while *Ma Vee* had the lowest total phenolic content in gastric phase. Total Phenolic content in intestinal phase was in the range of 0.99± 0.04 - 0.35± 0.03 mg GAE/g FW. Out of the rice varieties tested *Sudu Heenati* had the significantly ($P<0.05$) highest total phenolic content in the intestinal phase while *Beheth Heenati* had the lowest total phenolic content in intestinal phase. Among the 5 rice varieties subjected to simulates in vitro gastric and intestinal digestion, all 5 rice varieties had increased the total phenolic content in intestinal phase compared to gastric phase. The change in total Phenolic content of 5 different rice varieties which subjected to simulated in vitro gastric and intestinal digestion is indicated in Figure 1.

Total flavonoid content in the gastric phase was in range of 2.01± 0.02 - 0.02± 0.002 mg RE/g FW. *Suwadel* had the significantly ($P<0.05$) highest total flavonoid content in the gastric phase while *Pachchaperumal* had the lowest total flavonoid content in gastric phase. Total flavonoid content in the intestinal phase was in range of 0.8± 0.01 - 0.09± 0.006 mg RE/g FW. *Ma Vee* had the significantly ($P<0.05$) highest total flavonoid content in intestinal phase while *Beheth Heenati* had the lowest flavonoid content in the intestinal phase. Among rice verities tested *Maa Vee, Pachchaperumal and Sudu Heenati* had increased the total flavonoid content in intestinal phase while *Beheth Heenati and Suwadel* varieties had decreased the total flavonoid content in intestinal phase when compared with gastric phase. The change in total Flavonoid content of 5 different rice varieties which subjected to simulated in vitro gastric and intestinal digestion is indicated in Figure 2.

Total carotenoid content in the gastric phase was in the range of 0.24 ±0.015 - 0.03± 0.015 mg/g FW. *Ma Vee* had the significantly ($P<0.05$) highest total catenoid content while

Table 3: Content of total phenolic, flavonoid, carotenoids and DPPH in rice samples subjected to simulated in vitro gastric and intestinal digestion

Rice Variety	Gastric Phase				Intestinal Phase			
	TPC *(mg GAE/g FW)*	TFC *(mg RE/ g FW)*	TC *(mg/g FW)*	DPPH *(Inhibition %)*	TPC *(mg GAE/g FW)*	TFC *(mg RE/ g FW)*	TC *(mg/ g FW)*	DPPH *(Inhibition %)*
Ma vee	0.026± 0.011^{e}	0.11± 0.023^{d}	0.24 ±0.015^{a}	44.23± 0.22^{c}	0.61± 0.02^{b}	0.8± 0.01^{a}	3.24 ±0.04^{a}	44.00± 0.03^{c}
Pachchap-erumal	0.073± 0.015^{d}	0.02± 0.002^{e}	0.03± 0.015^{de}	61.77± 0.02^{a}	0.37± 0.02^{cd}	0.13± 0.02^{d}	2.19± 0.02^{d}	54.96± 0.07^{b}
Beheth Heenati	0.173± 0.003^{c}	0.43± 0.015^{c}	0.15± 0.02^{bc}	60.69± 0.27^{b}	0.35± 0.03^{c}	0.09± 0.006^{e}	1.57± 0.21^{e}	63.98± 0.07^{a}
Sudu Heenati	0.173± 0.005^{b}	0.59± 0.02^{b}	0.04 ±0.0015^{d}	38.49± 0.19^{d}	0.99± 0.04^{a}	0.64± 0.004^{b}	2.54± 0.02^{c}	36.48± 0.03^{d}
Suwadel	0.45± 0.02^{a}	2.01± 0.02^{a}	0.16 ±0.025^{b}	27.08± 0.03^{e}	0.61± 0.02^{b}	0.22± 0.03^{c}	2.73± 0.15^{b}	34.60± 0.02^{e}

Values are expressed as mean± SD Values followed by different letters for each assay in the same column are significantly different ($p < 0.05$)

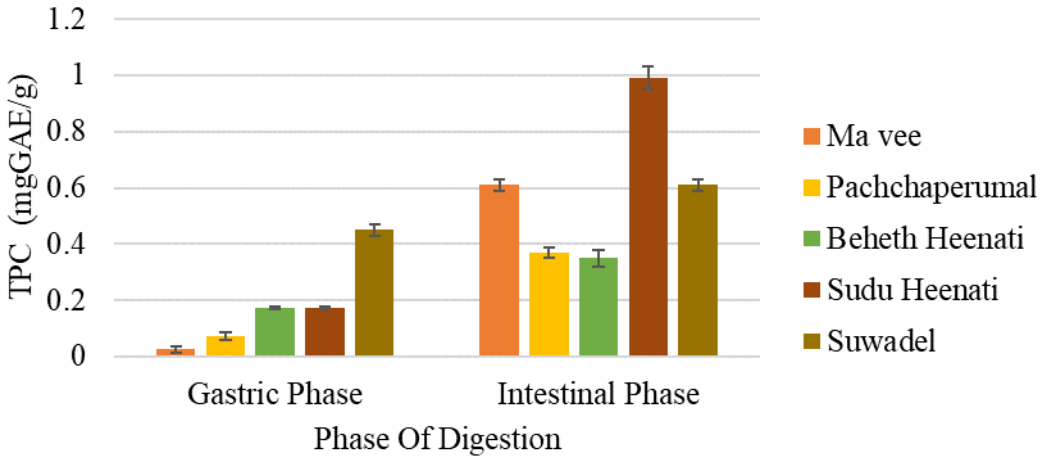

Figure 1: Change in total Phenolic content of 5 different rice samples subjected to simulated in vitro gastric and intestinal digestion. (The data presented in this figure consists of average quantities ± SD of three independent samples.)

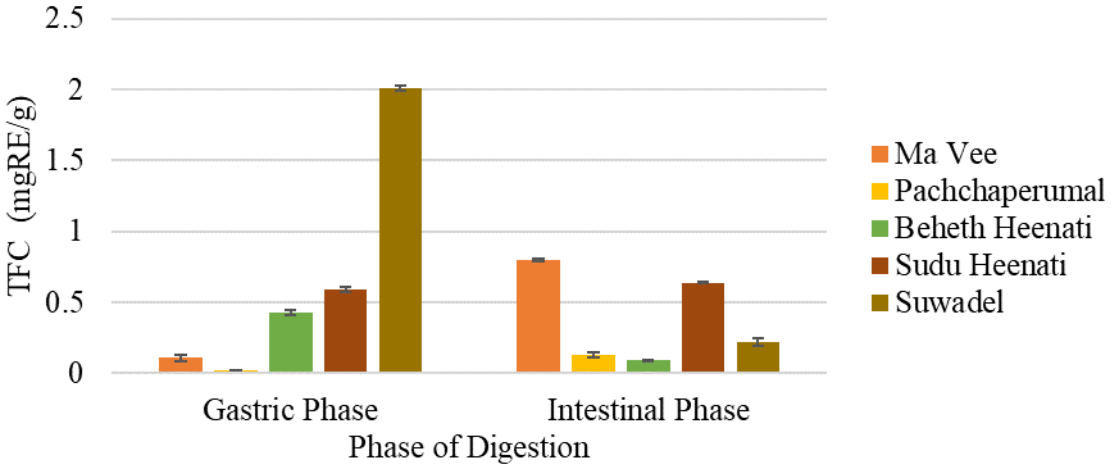

Figure 2: Change in total Flavonoid content of 5 different rice samples subjected to simulated in vitro gastric and intestinal digestion (The data presented in this figure consists of average quantities ± SD of three independent samples.)

Pachchaperumal had the lowest total carotenoid content in gastric phase. Total *carotenoid* content in the intestinal phase was in range of 3.24 ±0.04 - 1.57± 0.21mg/g FW. *Ma Vee* had the significantly ($P<0.05$) highest total carotenoid content in intestinal phase while *Beheth Heenati* had the lowest carotenoid content in the intestinal phase. Among the rice varieties tested, all the rice varieties had increased the total carotenoid content in intestinal phase compared to the gastric phase. The change in total carotenoid content of 5 different rice varieties which subjected to simulated in vitro gastric and intestinal digestion is indicated in Figure 3.

DPPH inhibition % in the gastric phase was in the range of 61.77± 0.02 - 27.08± 0.03%. Red rice variety *Pachchaperumal* had the significantly ($P<0.05$) highest DPPH inhibition % while the white rice variety *Suwadel* had the lowest DPPH inhibition % in the gastric phase. DPPH inhibition % in the intestinal phase was in the range of 63.98± 0.07 - 34.60± 0.02%. *Beheth Heenati* had the highest DPPH inhibition % in the intestinal phase while *Suwadel* had the lowest DPPH inhibition % in the intestinal phase. Among the rice varieties tested *Maa Vee* and Pachchaperumal had decreased the DPPH inhibition % while *Beheth Heenati, Sudu Heenati and Suwadel* had increased the DPPH inhibition % in intestinal phase when compared to gastric phase. The change in DPPH inhibition % of 5 different rice varieties which subjected to simulated in vitro gastric and intestinal digestion is indicated in Figure 4.

The stability as well as the inherent bioactivity of phytochemicals may change after gastrointestinal digestion. The total polyphenol content in all digested fractions of the five rice verities was lower than that of their methanolic extracts, indicating a partial release or breakdown of polyphenols during the gastric and intestinal digestion process. The gastrointestinal tract act as an extractor where both mechanical (mastication) and chemical actions during the digestion contribute to the extraction of bioactive molecules from the food matrix (Tagliazucchi *et al.* 2010). The efficiency of extracting of polyphenols from a food in the gastrointestinal tract can be influenced by many factors as described by Pinelo and colleagues (2006), including extraction temperature and solvent/solid ratio. Recent studies suggest that the presence of certain macromolecules such as dietary fiber (pectin, cellulose) can interact with biomolecules and influence their release into digestive media (Padayachee *et al*. 2012; Mosele *et al*. 2016). The presence of a low pH of around 2.0 during simulated gastric digestion may affect the stability of low molecular weight polyphenols (Mosele *et al.* 2016).

Results of this study summarize that a significant amount of flavonoids which were already

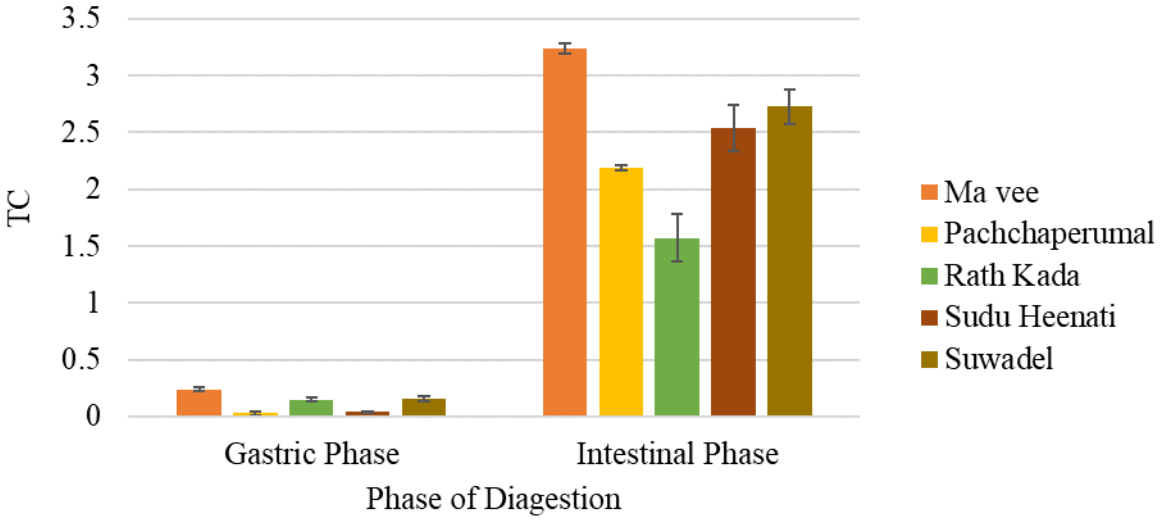

Figure 3: Change in total carotenoid content of 5 different rice samples subjected to simulated in vitro gastric and intestinal digestion (The data presented in this figure consists of average quantities ± SD of three independent samples.)

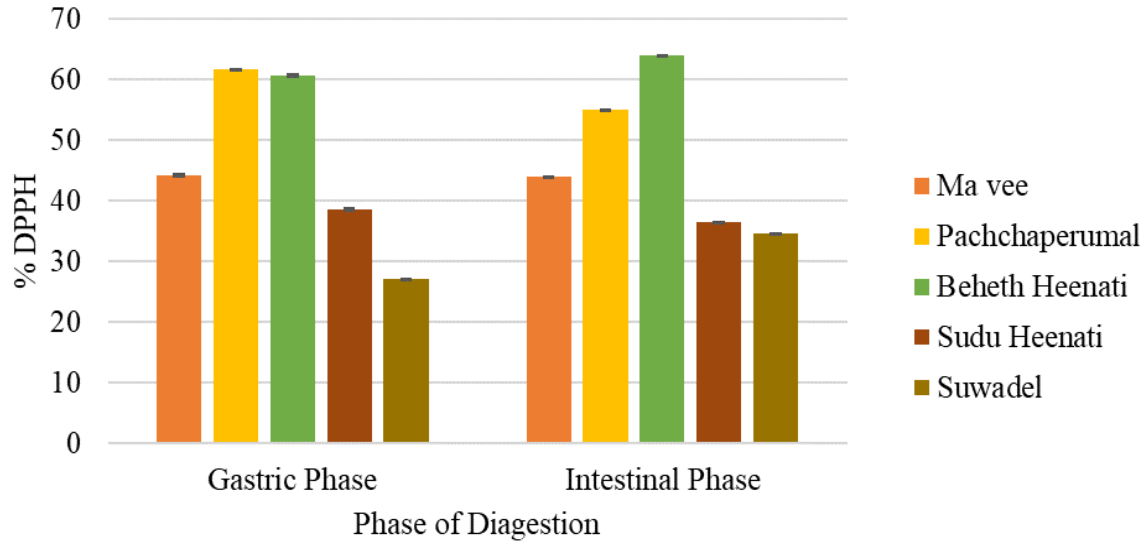

Figure 4: Change in DPPH content of 5 different rice samples subjected to simulated in vitro gastric and intestinal digestion (The data presented in this figure consists of average quantities ± SD of three independent samples.)

available in the gastric phase in *Beheth Heenati and Suwadel*, not all present in the intestinal digesta. This may be due to the brakdown of some polyphenols and flavonoids during the transition from acidic gastric conditions to slightly alkaline intestinal conditions under the influence of bile salts and pancreatin (Bouayed *et al.* 2012). Carotenoids released from the food substrates are an important step in the carotenoid's bioavailability. These carotenoids are readily available in the small intestine are potentially susceptible to becoming available and absorbed across the intestinal barrier Tagliazucchi *et al.* (2010), reported that the radical scavenging activities of polyphenols may be pH dependent. Higher scavenging capacity can observe in the intestine than in the stomach. However, the results obtained for the DPPH scavenging ability of all five rice varieties in the gastric phase (Figure 4) of this in-vitro model do not support the fact since gastric phase extracts have shown considerably higher scavenging activity.

Effect of cooked rice on postprandial blood glucose level

The postprandial blood glucose level after consumption of cooked rice samples is presented in Figure 5. After consuming test meals, blood glucose concentrations increased, peaking at 30 min and then falling to the same values as baseline after 120 min. The peak value of blood glucose response after the ingestion of cooked *Maa Vee* showed at around 30 minutes. The peak value of blood glucose response after the ingestion of cooked *Pachchaperumal, Sudu Heenati, Beheth Heenati, and Suwade*l showed at around 45 minutes. Results highlighted that there were significant ($P<0.05$) differences in the glycemic responses of individuals to different cooked rice varieties. However, the highest postprandial blood glucose levels after ingestion of cooked rice samples had significantly ($p < 0.05$) lower peaks when compared with the pure glucose (control). Among cooked

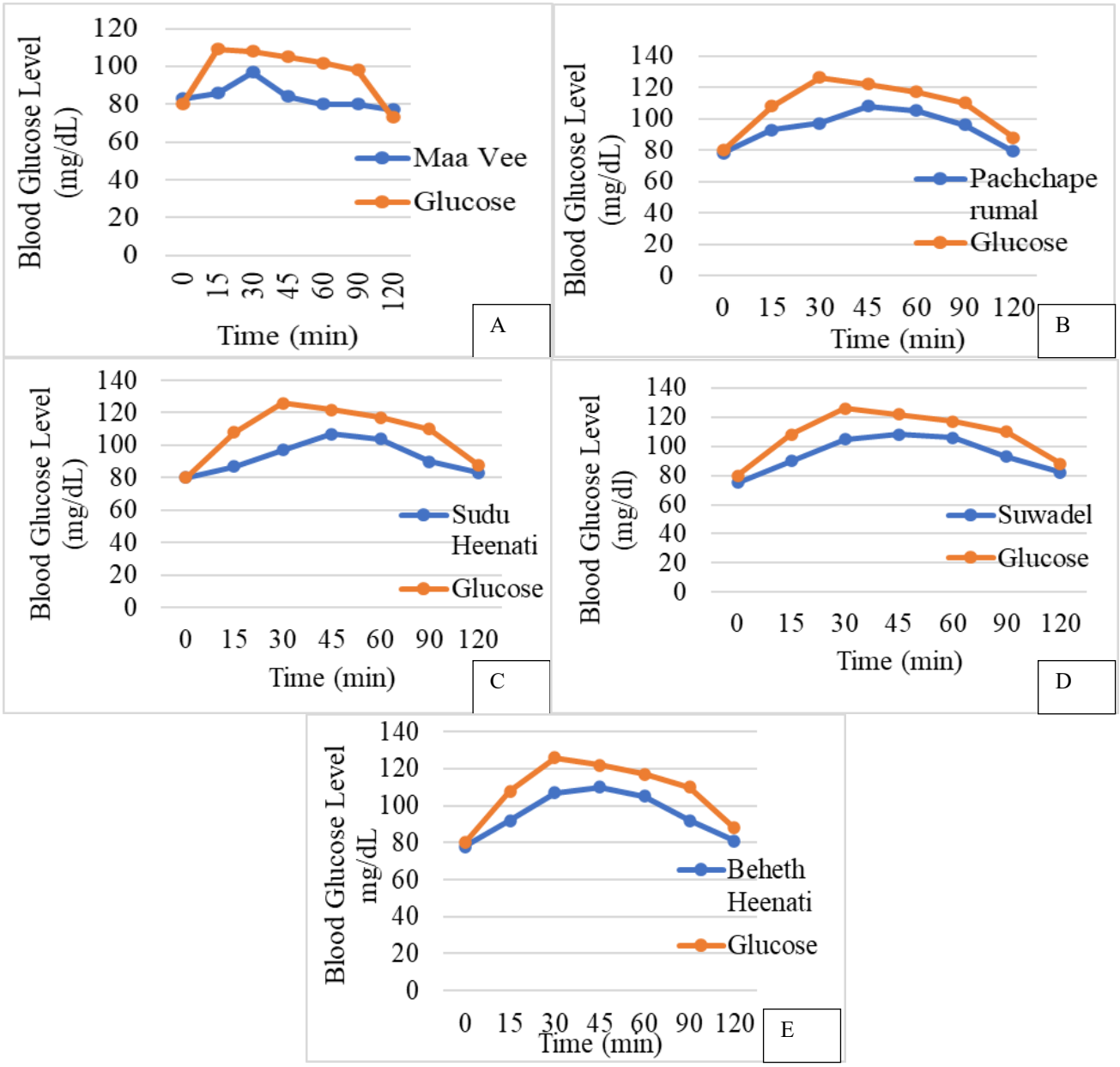

Figure 5: Variation of Postprandial Blood Glucose Level of healthy individuals after consumption of cooked rice samples, *Ma vee* (A), *Pachchaperumal* (B), *Sudu Heenati* (C), *Suwadel* (D), *Beheth Heenati* (E) compared with glucose

rice samples tested *Maa Vee* showed a significantly lower peak while *Beheth Heenati* showed a significantly highest peak in blood glucose response curve. Diets that gradually and slowly raise postprandial blood glucose levels are gaining more demand from consumers because of their potential to reduce the risk of chronic diseases associated with impaired glucose metabolism, such as diabetes (Brigheuti *et al*. 2006). There for tested five cooked rice samples show low GI values which could be helpful to balance blood glucose levels in diabetics when these varieties are incorporated into diet.

CONCLUSIONS

According to the results of the present study, it can be concluded that traditional rice verities such as *Kalu Heenati*, *Pachchaperumal*, *Ma vee*, *Beheth Heenati* and *Suwadel* are rich with bioactive compounds along with nutrients and they are useful in the management of NCDs and their complications. Also, these traditional rice varieties have a wide range of functional properties and they are super staple foods to improve the nutritional status and health of the people. Rice varieties that tested for glycemic response showed low GI values which could be helpful to balance the blood glucose levels in diabetics once incorporated into the diet. These low-GI rice varieties could play an important role in reducing glycemic load of diet.

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AUTHOR CONTRIBUTION

CTJ and KDPPG developed the concept of the study and designed the experiment. CTJ performed the experiment and analyzed the data. KDPPG and NNGC supervised the experiment. CTJ wrote the manuscript. KDPPG AND NNGC critically revised the manuscript.

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