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Research Article

Evaluation of Glycemic Index of Selected Black Rice Cultivars in Bangladesh

Md. Hanjala Pipil, Md. Mahadi Hasan, Arifa Setu, Shuma Rani Ray, Shayla Sharmin and Muhammad Javidul Haque Bhuiyan✉

Department of Biochemistry and Molecular Biology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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Correspondence
Muhammad Javidul Haque Bhuiyan
✉: abir062003@gmail.com

ABSTRACT

The Glycemic Index (GI) helps consumers select foods with a low GI, which can reduce the risk of diabetes. An *in vivo* investigation was conducted to screen for optimal glycemic index (GI) content rice varieties suitable for regular diets, particularly for individuals managing diabetes mellitus. Ten healthy volunteers with no diabetes participated in the study, consuming glucose as a reference food and various rice cultivars after overnight fasting. The rice cultivars included black rice from Indonesia, the Philippines, and Vietnam, along with ACI rice serving as the control. Proximate analysis assessed moisture, fat, fiber, protein, ash, and carbohydrate content in all rice samples. Amylose content (%) was determined using spectrophotometric analysis following alcoholic-alkaline gelatinization, acidification, and iodine mixing. The results indicated GI values of 67.23, 54.19, 52.64, and 53.50, and amylose content (%) of 19.42, 21.77, 28.50, and 27.52 for Indonesia line, Philippines line, Vietnam line, and ACI rice, respectively. An inverse relationship between GI levels and amylose content was observed, suggesting a potential mechanism for regulating postprandial blood glucose levels. Based on the findings, the Philippines line and Vietnam line were classified as low-GI rice varieties, indicating their suitability for regular consumption by individuals with diabetes mellitus or without the condition. These results underscore the importance of selecting appropriate rice varieties to manage glycemic response effectively in dietary management strategies for diabetes mellitus. Further research is warranted to explore the underlying mechanisms and to validate these findings in larger cohorts.

Introduction

Rice (*Oryza sativa*) is one of the most significant and staple food which feeds the most of the world's population. Half of the world's population and almost 100 countries use rice as their main meal. Consuming rice helps people to meet their needs for several nutritional macromolecules, the most common of which are carbohydrates, which are followed by protein, fat, vitamins, and minerals. The average amount of carbohydrate and protein in rice are 80% and 7%, respectively (Carcea, 2021). Diabetic persons must be careful during intake of carbohydrates in order to maintain healthy blood glucose levels, as carbohydrate is directly responsible for rising postprandial blood glucose levels (Marsh *et al*. 2011).

Black rice is rich in iron, vitamin E, antioxidants, anthocyanins, and coloring agents such as cyanidin-3-glucoside, malvidin-3-glucoside, and peonidin-3-glucoside (Zhang *et al*. 2006). In addition, black rice is devoid of cholesterol, low in sugar and fat, in contrary, it has significantly higher phenolic compounds, dietary fiber, and antioxidant activity than regular white rice (Ito and Lacerda, 2019; Shen *et al*. 2009). Much more vitamins and minerals are present in this rice. It is a source of fiber and contains plant-based proteins. Phenolic substances found in black rice include tannins, phenolic acids, and flavonoids (Prasad *et al*. 2019). Black rice is related with lower GI and higher health benefits. Black rice starch's thermal properties are greatly enhanced by the amylose-lipid complex during gelatinization which is mostly mediated by increased resistant starch (RS) and slowly digestible starch (SDS) with decreased rapidly digestive starch (RDS). The SDS, RS, and GI of black rice were higher than those of white rice (Zhang *et al.* 2020).

GI is a widely accepted indicator of the tendency for a diet high in carbohydrates to elevate blood glucose levels. Numerous studies have shown that consuming

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high-GI foods frequently increases the risk of type 2 diabetes (Salmerón, *et al*. 1997). Due to their prolonged digestion and absorption, meals with a low GI gradually raise blood sugar and insulin release. Eating meals low on the GI has been shown to help persons with diabetes mellitus reduce their insulin resistance and blood sugar, fat, and cholesterol levels. Low-glycemic foods also aid in controlling appetite, postponing the beginning of hunger, and supporting patients in maintaining a healthy weight. Low-GI diets appear to lower the risk of diabetes, (Björck *et al*. 1994), hyperlipidemia, (Jenkins *et al*. 1987) and cardiovascular disease based on available data (Ludwig *et al*. 1999).

Jenkins and Wolever (1981) introduced the idea of GI which can quantify carbohydrates' performance in elevating blood glucose. A glycemic index is a number on a scale of 0 to 100 that represents the various rates at which a diet high in carbohydrates is absorbed by the human digestive system. A food's GI value and carbohydrate content are critical variables in regulating blood sugar and insulin response (Pinhero *et al*., 2016). Thus, by reducing blood glucose and insulin level fluctuations, utilizing carbs with a low GI may have positive consequences (Ludwig, 2002).

Although it is a genetically inherited characteristic, the glycemic index of different types of rice might vary for a number of reasons. Howlader and Biswas, (2009) conducted research to check low to medium GI in black rice and local cultivars. In the present study, our objectives were to assess the GI values of some selected black rice cultivars in Bangladesh and to categorize the rice varieties based on their assessed GI (values).

Methodology

Design, location, and time

The experiment was conducted in the Prof. Muhammed Hossain Post-Graduate Laboratory of the Department of Biochemistry and Molecular Biology at Bangladesh Agricultural University from February to June 2023.

Sampling

For this study, ten healthy (male and female ratio as 1:1), non-diabetic volunteers between the ages of 24 and 26 were selected. Their body weights were in normal range (BMIs between 18.5 and 24.9 kg/m^2, according to WHO guidelines) with no medical conditions, food allergies, or medication use. They were suggested to maintain a regular life style and food schedule for the duration of the experiment. The study protocol was followed as per the Declaration of Helsinki's founding principles. Prior to participation, all individuals were well informed, and gave written consent about the study protocol.

Data collection

Test foods: In order to determine the GI values, three distinct black rice cultivars from (Indonesia, Philippines, and Vietnam) were employed in this experiment, along with ACI as a control (established low GI). The local or brand names of Indonesia line are Indonesian black rice, Purple rice. In the case of Philippines line the local or brand names are Philippines black rice, Heirloom balatinaw black rice, and for Vietnam line, Vietnamese black rice, Black glutinous are the local or brand names. These black cultivars were all bought from the local market of Shibganj, Bogura city, Bangladesh. In an electric rice cooker, rice (containing about 50 grams of carbohydrate) of each type was cooked for a duration of 17 to 20 minutes (rice to water ratio as 1:3). Before being fed to each subject, the cooked rice was allowed to keep outside for half an hour to achieve room temperature. From the first bite, the test food took about ten minutes to be ingested. Every test food's performance was measured just once. Subjects were maintained with minimal physical exertion throughout the testing period.

Proximate analysis of test foods

Standard protocols guided by AOAC (2000) were followed, and calculations were performed accordingly to determine the proximate parameters of the rice varieties. Moisture content was calculated by the following formula:

$$\% \text{ Moisture} = \left(\frac{\text{Initial weight of crucible} - \text{Final weight of crucible}}{\text{Weight of sample}}\right) \times 100$$

The Soxhlet apparatus involved inserting the thimble into the extraction chamber, which was connected to a round-bottom flask holding 200 ml of petroleum ether. The following formula was used to determine the fat content: $\% \text{ Fat} = (W_1\text{-}W_2)/W$

Here, W_1=Weight of evaporated flask with sample, W_2=Weight of empty flask, W=Weight of sample

Ash content was determined using the standard non-enzymatical approach. Using the following formula, the ash content was determined:

$$\% \text{ Ash} = \left(\frac{\text{Initial weight of crucible} - \text{Final weight of crucible}}{\text{Weight of sample}}\right) \times 100$$

Dietary fiber content (%) was estimated by the AOAC (2000) method, and the percentage was calculated as follows:

$$\frac{\text{Loss of weight}}{\text{Weight of sample}} \times 100.$$

Micro Kjeldahl apparatus was used to determine the percent N followed by protein content (Saez-Plaza *et al*.2013) using the following formula:

%Nitrogen =

$$\left[\frac{(TS-TB)\times \text{Normality of acid} \times meq\ of\ N}{\text{Weight of sample}}\right]\times 100$$

Where T_S = titer value of sample, T_B = titer value of blank, and meq of N = 0.014, and thus, finally: % Protein = (Burette reading × Normality of H_2SO_4 × mL equivalent of N_2 × protein factor ×100)/Weight of the sample

Here, Normality of H_2SO_4=0.2N, Protein factor = 5.5 mL, Equivalent of N_2=0.014 mL, Weight of the sample=0.1 gm.

Total available carbohydrate content (%) was measured using a formula according to FAO (2004) as follows: 100 – (%Moisture + %Ash + %Fat + %Fiber + %Protein).

Reference food

As the reference food, 50 grams of glucose (Glucomax-D, Uniliver Company Ltd.) were dissolved in 200 milliliters of water. To prevent the carry-over effect, blood glucose levels were tested every two days to evaluate the reference food's performance (Brouns et al., 2005). Conversely, a washout interval of at least 4 to 5 days was observed in between the trials of two test foods.

Blood glucose measurements

Due to obtain the high degree of accuracy, blood glucose levels were measured six times for reference food, and on separate days for each rice cultivar. The subjects were prepared to be included in the testing procedure in the morning after an overnight fast of 10–12 hours. Before consuming the food (either reference food or test food), fasting blood glucose levels (mmol/L) were tested at -20 and 0 minutes in advance, with the mean values serving as the baseline. Blood glucose levels were taken again at 15, 30, 45, 60, 90, and 120 minutes after having the meal. Using a glucometer (Bioland, Model: G-423E, China), the blood glucose level was measured by pricking a finger.

Glycemic index calculation

Using the trapezoid rule, the MS-Excel-2016 application computed the geometric incremental area under curves (IAUC) for the blood glucose level fluctuation in reference food (glucose) and test food (rice types), ignoring the area below the reference food's baseline value (FAO, 1998). It was usually assumed that the reference food's IAUC was 100. When comparing the test foods to the reference food, the corresponding glycemic index (GI) conversion value was determined using the subsequent formula: Once every GI result was obtained, the GI was classified appropriately followed by Jenkins *et al*. (1981).

Glycemic Index =

$$\frac{\text{Blood glucose IAUC value for test food}}{\text{Mean IAUC value for reference food (glucose)}}\times 100$$

Determination of amylose content (%)

Amylose content was estimated according to the procedure of Robyt & French (1969). A 10 mg finely powdered sample, measured exactly twice, was put into an Erlenmeyer flask. After adding 1 milliliter of 95% ethanol and 9 milliliters of 1N NaOH, the starch was heated in a water bath for five minutes to cause gelatinization. After that, the substance was quantitatively transferred into a 100 ml volumetric flask, which was subsequently cooled and filled with water. Prior to performing optical density measurements, a five-milliliter aliquot of this solution was placed in a 100-milliliter volumetric flask, filled to the brim with water, stirred, and left for 20 minutes. 1ml of 1N acetic acid and 2ml of iodine solution were then added to the mixture. For the preparation of the standard curve, 100 mg of anhydrous rice amylase (J.T. Baker Chemical Co., Phillips-Burge, N.J.) was dissolved in 100 ml of alcoholic NaOH (10 ml ethyl alcohol and 90 ml 1 N NaOH). Eight hundred milliliter flasks were filled with portions of amylose having 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2 mg. To each of the foregoing solutions, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4 ml of acetic acid were added, correspondingly. A Spectrophotometer (Coleman Junior, USA) was used to measure optical density at 590 mµ. Lastly, using the standard curve, the amylose content of each sample was determined.

Data Analysis

Using IBM SPSS software (version 24), a one-way analysis of variance (ANOVA) was performed on all the data, which were derived from duplicate observations. The information was displayed as mean, standard error of mean (SEM), and standard deviation (SD). The study employed the Duncan Multiple Range Test (DMRT) to assess treatment differences at the 5% ($p \le 0.05$) significance level.

Results and Discussion

Observation of study subjects

Various demographic characteristics were recorded at fasting condition (shown in Table 1). No statiscal differences were found among the observed demographic properties of the studied subjects. Such homogenous subjects with proper BMI, fasting blood glucose level and blood pressure were included to minimize the residual effect from them.

Table 1. Anthropometric Measurements of the Study Subjects (N=10)

Parameter	Mean ± SD
Age (years)	25±1
Height (m)	1.64±0.08
Weight (kg)	59.29±8.18
BMI (kg/m^2)	22.02±2.76
Fasting blood glucose (mmol/L)	5.37±0.43
Blood Pressure	
Systolic (mm/hg)	121±5.16
Diastolic (mm/hg)	77±6.32

Values are shown as mean with standard deviation, N= Number of volunteers used in the research work, BMI: Body Mass Index.

Nutrient composition

Nutrient composition followed by subsequent serving amounts of rice and cooking times are shown in Table 2. According to the present finding, the black rice varieties showing a range of moisture contents (10.35–13.00%), protein contents (7.15–8.89%), fat contents (0.07–3.41%), fiber contents (3.00–8.47%), ash contents (1.0–1.8%), and carbohydrate contents (73.0-76.20%). The amount of fat, protein, ash, and carbohydrates were in consistent with the previous research work conducted by *Ito et al*., (2018); Sompong *et al*., (2011); Thomas *et al*., (2013) and Ferdous *et al*., (2018).

Table 2. Nutritional Composition of the different rice cultivars

Amylose content (%) variation

	Rice Variety			
Nutritional Composition	Indonesia line	Philippines line	Vietnam line	ACI rice
Moisture (%)	10.64±0.3	11.07±0.2	11.96±0.45	12.5±0.25
Fat%	3.2±0.3	2.15±0.6	1.57±0.2	1.03±0.07
Fiber%	4.32±0.2	4.7±0.2	4.83±0.4	1.21±0.06
Protein%	7.61±0.2	8.89±0.3	7.15±0.2	7.3±0.55
Ash%	1.41±0.02	1.33±0.04	1.55±0.03	1.18±0.04
Carbohydrate%	72.82±0.33	71.86±0.46	72.94±0.6	76.78±0.61
Rice cooked (g) per serving	68.66	69.57	68.55	65.12
Cooking timme	20±1	17±1	19±1	18±1

Amylose content (%)

The amylose content (%) are shown as mean± SD in Table 3. The amylose content of the black rice cultivars and the control ranged from 19.42% to 28.50%. Similar to this, Howlader and Biswas (2009) revealed that milled rice samples from several cultivars in Bangladesh ranged in amylose content from 21.3% to 29.1%. According to DMRT based on the amylose content, the rice cultivars were subsequently categorized as low level (Indonesia line), intermediate level (Philippines line) and high level (Vietnam line and ACI) following Juliano *et al*., (1993) proposed amylose content (%) range chart.

Table 3. Categorization of amylose content (%) among the selected black rice cultivars

Name of cultivars	Observed Amylose content%	Suggested amylose content (%) range*	Classified groups based on observed amylose content (%)
Indonesia line	19.42±0.65%^{c}	12-20%	Low level
Philippines line	21.77±1.67%^{b}	20-25%	Intermediate level
Vietnam line	28.50±2.97%^{a}	25-33%	High level
ACI	27.52±2.09%^{a}		

Data (mean±SD) are grouped into their classes(a-c) by Duncan's multiple range test. * Suggested amylose content (%) ranged by Juliano (1993).

Blood glucose level (mmol/L) variation in glucose and rice cultivars

After giving glucose (50 g) and rice (containing carbohydrate equivalent to 50 g) with accessible carbs at a 4 to 5-day interval, the subjects' blood glucose levels were assessed in both scenarios. Figures 1A–D, Glucose levels were recorded at 0,15,30,45,60,90, and 120 minutes, of consuming glucose or rice. All of the results except the ones at 0 and 120 minutes had demonstrated a considerable deference. From the graph it was observed that the amount of glucose increased after glucose or rice intake.

In Fig. 1(A-D), the test food rice and the reference meal (glucose) are categorized together with their mean glycemic response and mean incremental area under the curve (IAUC), respectively. When it came to fasting blood glucose levels, all test items and reference foods had similar impacts. Between 30 and 45 minutes after ingestion, the greatest blood glucose levels were frequently observed for both the rice test dish and the reference meal (glucose). When compared to the test food (rice) and reference meal (glucose), the blood glucose response trends of the Indonesian line and the reference meal (glucose) showed slower blood glucose level reductions (Fig. 1A) after 45 to 120 minutes. As a result, 45 minutes was the maximum blood glucose level recorded, and between 15 and 120 minutes, the test food's blood glucose level differed significantly (p≤0.05) from that of the Indonesia line reference food.

Similarly, Zafar et al. (2015), found that after 30 minutes of consumption, the glucose disposal stage had started, as evidenced by the blood glucose response to the reference meal. After ingesting the Philippines line, Vietnam line, and ACI (control) Fig. 1(B-D), a delayed phase of blood glucose elimination has also been observed. After 45 minutes, there was a progressive decline in glucose levels, which continued until 120 minutes. The blood glucose levels of the test food, as indicated by Fig. 1(B-D), differ considerably (p≤0.05) at different time points from the reference diet, which consists of the Vietnam line, the Philippines line, and ACI rice (control).

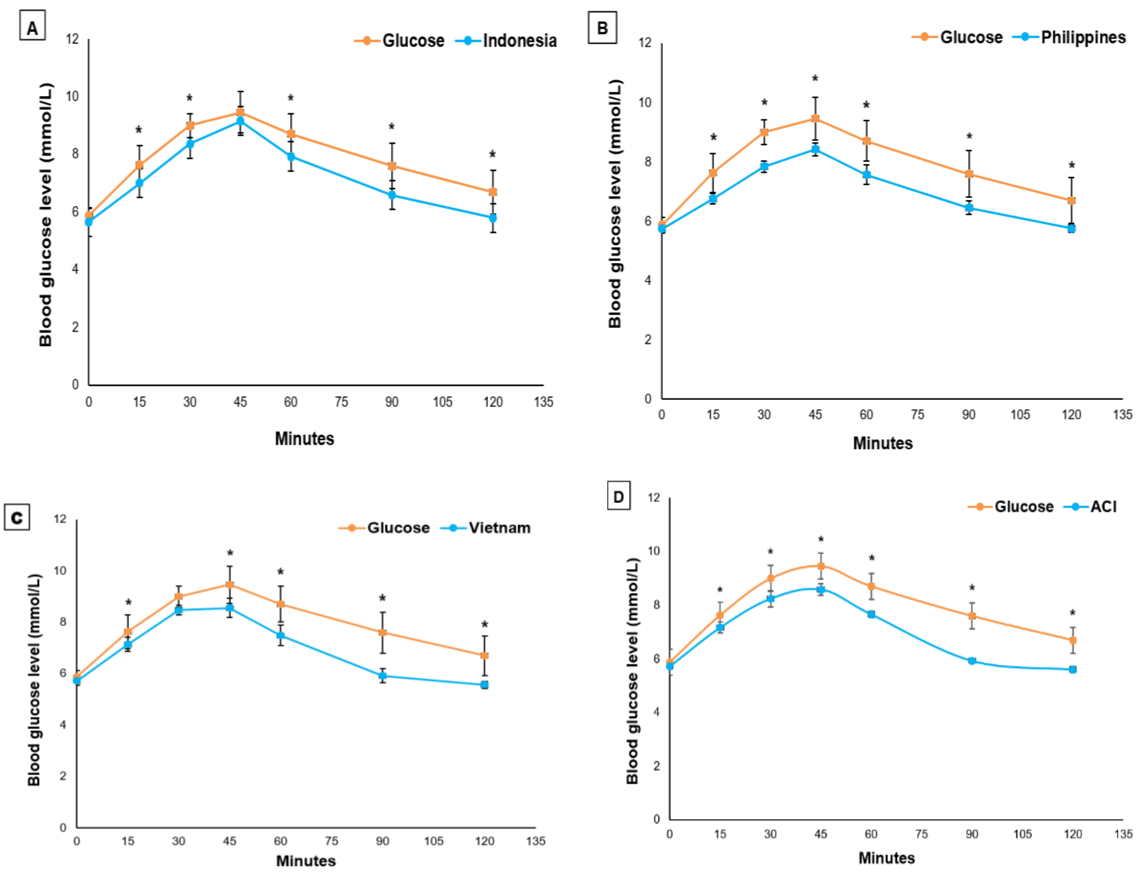

Figure 1A-D. Variation in blood glucose levels (mmol/L) at different times following two hours of consumption of glucose (reference food), rice from Indonesia, the Philippines, Vietnam, and Indonesia, and ACI (test food): When two foods (the reference food and the test food) are compared at various times, an asterisk (*) denotes a significant difference (p≤0.05).

Calculation of Incremental Area Under Curve (IAUC) mmol/L min and glycemic index (GI)

Table 4 shows the calculated values of incremental area under the curve (IAUC) mmol/L min, glycemic index values (GI), as well as their subsequent classification. The IAUC values of test foods decreased as expected compared to those of reference foods. IAUC ranges from 138.86 (mmol/L min) to 177.32 (mmol/L min) in various test foods. Calculated GI values from the IAUC of reference and test foods were categorized accordingly (Brand-Miller *et al.*, 2009). A 120-minute time span was used to measure the blood glucose levels and incremental area under the curve (IAUC) (Table 4). It was expected that the reference food glucose's IAUC would have the highest value.

Table 4. IAUC and calculated GI of reference food and test food.

Reference & Test food	IAUC mmol/L min	GI	GI class (Brand-Miller *et al.*, 2009)
Glucose	263.75	100 [a]	-
Indonesia line	177.32	67.23[b]	Medium (56-69)
Philippines line	142.93	54.19[c]	Low (≤ 55)
Vietnam line	138.86	52.64[c]	Low (≤ 55)
ACI	141.11	53.50[c]	Low (≤ 55)

Values indicate mean±SEM with the subscript [a-c] as ranking by DMRT at $p \leq 0.05$.

GI variation, categorization and relationship with amylose content

The Indonesia line of test items (rice) has moderately higher GI levels than the Vietnam line, the Philippines line, and the ACI rice (control). Table 4 shows that, out of all the black rice varieties, the Indonesia line had a relatively middle GI value, while the Vietnam and Philippines lines had the lowest values. Another study discovered that a low level of amylose (20%) was associated with a high glycemic index (GI) value (Bhupathiraju *et al.*, 2014). Our research revealed a consistent inverse correlation between the amylose content and glycemic index (GI) values of the rice cultivars (Figure 3). This observation matches the findings of a study conducted by Meera *et al.* (2019). According to Li *et al*. (2020), a high amylose content in the starch structure is thought to slow down the rate of digestion.

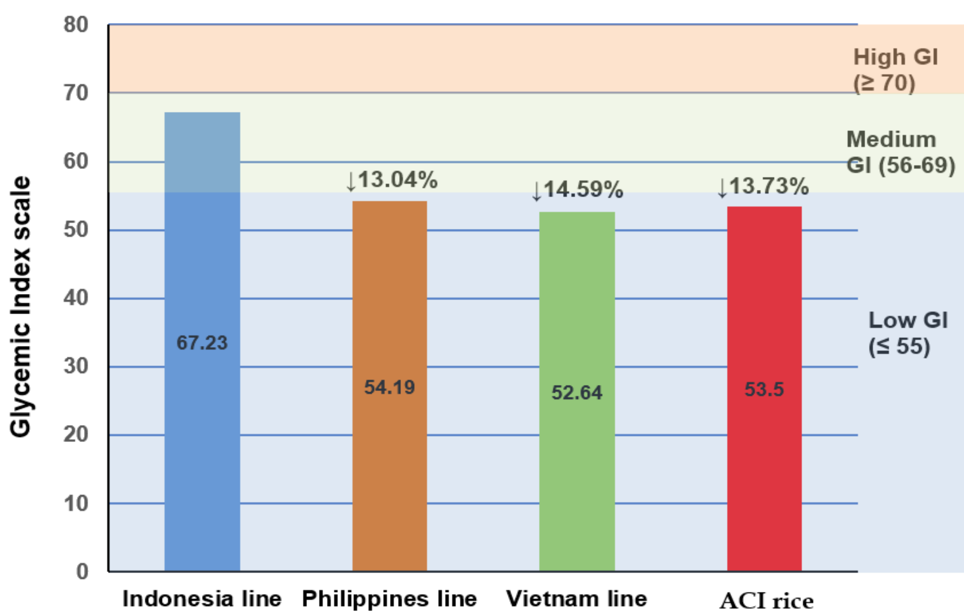

Figure 2. GI response, along with categorization of different test foods and GI reduction rate, are mentioned at the top.

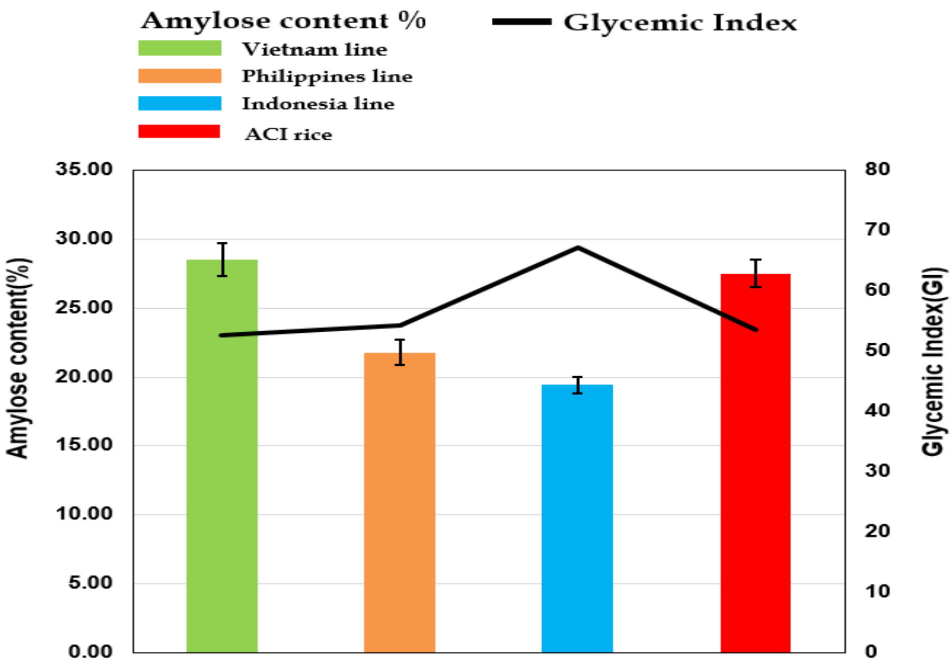

Figure 3. Comparison between glycemic index and amylose content.

Conclusion

Our investigation revealed glycemic index (GI) and amylose content for black rice varieties from Indonesia, Philippines, and Vietnam, alongside a recognized low-GI rice variety (ACI). The estimated GI values were 67.23, 54.19, 52.64, and 53.50 for the Indonesia line, Philippines line, Vietnam line, and ACI rice, respectively, while amylose content (%) measured at 19.42, 21.27, 28.50, and 27.52. We observed an inverse relationship between GI levels and amylose content, indicating a potential mechanism for regulating postprandial blood glucose levels. Categorizing rice based on GI, the Philippines line and Vietnam line emerged as low-GI rice varieties, suitable for regular consumption by individuals managing diabetes mellitus or without the condition. These findings highlight the significance of selecting appropriate rice varieties for effective glycemic control in dietary management strategies for diabetes mellitus. Further investigation is essential to delve into the underlying mechanisms and corroborate these findings in larger study populations.

Ethics approval

The study protocol was approved by the Diabetic Association of Bangladesh (DAB- HT73/2018) following the guideline described in the declaration of Helsinki.

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