In vivo screening for low glycemic index (GI) rice varieties in Bangladesh and evaluate the effect of differently processed rice and rice products on GI

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Abstract

In quest of screening for low glycemic index (GI) rice varieties in Bangladesh and evaluate the effect of differently processed rice and rice products on GI, a total of 72 Bangladesh Rice Research Institute (BRRI) released high yielding varieties (HYV) were subjected to estimate the glycemic index (GI), in an in-vivo experimental rat model using glucose as standard (control). Our data revealed that BR16, BRRI dhan46 and BRRI dhan69 are categorized as low GI rice varieties in addition to 50 varieties are intermediate GI and 19 varieties are high GI in vivo experimental rat model. In order to assess the effect of differently processed rice such as un-parboiled, parboiled, pressure parboiled, double parboiled milled rice and brown rice on glycemic response in an in vivo experimental rat model, we had selected three rice varieties from three scales of GI group namely BR16 (Low GI), BRRI dhan29 (Intermediate GI) and BRRI dhan28 (High GI). We found that GI value reduces at parboiled mill rice than unparboiled one. It further reduces towards pressure parboiled mill rice, double parboiled milled rice and the most reduced at the brown rice condition. Due to fiber content brown rice showed the lowest GI among all rice processing methods. High GI value grouped BRRI dhan28 rice (GI 70.96 at unparboiled milled rice condition) become intermediate GI grouped rice (GI 65.01 at parboiled mill rice condition) due to temperature effect (forming a kind of resistant starch). In addition our research also reveals that GI of rice based products i.e. flattened, popped and puffed rice are higher GI than rice itself. We concluded that thermal and pressuring process lowering GI value than original because of possible retrogradative changes in starch composition. Rice based rice products might possess higher GI than rice itself because of possibly lesser amount of solid matters.

Keywords: Glycemic index, Bangladesh Rice Research Institute, High yielding variety
INTRODUCTION
The glycemic index (GI) is a numerical classification based on human in vivo chemical trails designed to quantify the relative blood glucose response to foods drinks, nutraceuticals, pharmaceuticals and any edible agents (Freeman, 2008). The glycemic index ranks foods on how they affect blood glucose levels (Foster-Powell, 2002). The development of diabetes mellitus, obesity, cancer and cardiovascular disease (CVD) has been reported to be linked to high GI foods, with regards to the treatment of these diseases (Ludwig, 2002). Rice in the human diet serves underprivileged populations in Asia as a means of nutritional replenishment for energy and protein as well serving as a vehicle for micronutrient fortification. About 85% of rice consumption is mainly white rice. A possible relationship between white rice consumption and health risk exists. The threat is real enough for the scientific community to promote wholegrain consumption in place of refined grains. In the transitioning food environment, white rice is categorized as a refined grain and is thus implicated in the development of non-communicable diseases (NCDs). There is considerable interest in exploring glycaemic index (GI) in relation to the consumption of different rice varieties. The variable glycaemic response to rice types is better appreciated from the viewpoint of factors that moderate this response. Genetic make-up, physicochemical properties, amylase and dietary fibre content, post-harvesting processing as well as cooking methods are influential factors in determining GI variability (SE, 2015). Rice breeding and improvement programs play a major role in safeguarding the food environment, by taking into account traits that will improve rice quality in terms of GI (SE, 2015). Glycaemic index (GI) is a metrological approach to quantifying the human postprandial glucose response immediately after consuming fixed amounts of carbohydrate-rich foods (Jenkins, 1981). As rice is a major global staple, there is considerable interest in exploring postprandial glycaemia in relation to the consumption of different rice varieties. The GI values derived from many studies have been published in The International Tables of GI and GL Values (Foster-Powell, 2002; Atkinson, 2008). Epidemiological studies describing the carbohydrate quality in diets consumed by different populations have utilized the GI tables to quantify daily dietary GI and GL to elucidate the diet-disease relationship (Murakami, 2006). Most of the carbohydrates specially rice that we commonly consume are complex carbohydrate essentially made up of starches belonging to the different scales of amylose categories (Montignac method, 2017). The amount of amylose in proportion to amylopectin, basically determines the physical-chemical nature of rice and their nutritional impact on human. The proportion of amylose to amylopectin can vary from one rice variety to other. In order for starches to be absorbed and enter our bloodstream, they have to be broken down into glucose. This decomposing process is the work of our digestive enzymes. Digestion of starch normally begins in the mouth where an enzyme, salivary amylase, is secreted, catalyzing the break up of the starch by hydrolysis. After a quick passage through our stomachs, additional breakdown of starch occurs in the small intestine with pancreatic amylase secreted from the pancreas. Glycemic Index (GI) corresponds to carbohydrates potential to raise blood sugar levels. In other words, GI tell us the degree to which certain carbohydrates make our bodies secrete insulin. Insulin is a hormone secreted by the pancreas that regulates glucose levels in the blood. Without insulin, cells cannot use the energy from glucose to carry out functions within the body. In this experiment, we were aimed to screen low glycemic index (GI) rice varieties in Bangladesh and evaluate the effect of differently processed rice and rice products on GI. Diabetes mellitus is a metabolic chronic disorder 3rd leading cause of death worldwide. Nearly 80% people of diabetes live in low and middle-income countries. In Bangladesh, we had 165 million people according to recent data of CIA (2016). A total of 8.5 million people in our country suffering from diabetes but
only 30 per cent of them were availing treatments. Of the total diabetic patients, only 5 per cent suffer from type-1 diabetes and the remaining 95 per cent suffer from type-2 diabetes. Since our country is an agricultural country and our main food is rice, which is the major energy source (carbohydrate). We have done this experiment in vivo to identify low glycemic rice varieties for diabetic patient in Bangladesh. Since rice is the staple food of Bangladeshi people and we consume approximately 416 g rice a day per person on an average, so it is high time to focus our scientific attention on rice in terms of different aspects of health related issue especially in case of diabetes. Usually we consume milled rice so the outer layers of bran and germ are removed during milling that significantly reduce the vitamin mineral and fiber content of rice. GI is a measure of the glycemic effect of carbohydrate in a particular food, compared to an equivalent amount of carbohydrate in a standard amount of glucose. In the in vivo experiment we use rat because these are small, easily housed and maintained, adapted well in the environment. Their physiology and anatomy well understood by the researcher. Their genetic, biological and behavioral characteristics resemble to those of human being and many human symptoms can be replicate in rat (Abdullah, 2010; Xia, 2006; Iannaccone, 2009; Wang, 2010; King, 2012). Since human trial is not ethically sound for any research activities, we use an experimental rat model in this regard to identify low glycemic indexed rice varieties in Bangladesh.

METHODS AND MATERIALS
The animals used for this experiment were Long evan rats obtained from department of Physiology of Bangladesh University of Health Science (BUHS), Mirpur, Dhaka and experiment took place at rat room facilities (controlled condition) in Grain Quality and Nutrition division, BRRI, Gazipur. The rats were divided into seventy three groups of three rats each. The rats weighed between 150 and 160 grams. Approximately three months old (~12 weeks), weighing 150 ± 2g, rats were used in this study. Animals were housed individually in cages in a room maintained at 22–24°C with a controlled 12 hrs light–dark cycle, and had free access to tap water and cooked rice feed. Generally, rat is not accustomed with eating cooked rice so, we used to accustom rat providing cooked rice before starting experiment. We systematically withdrawal commercial rat diet gradually and replace cooked rice instead. We weighted rat before and after experiment but did not get any significant weight loss in this regards (data not shown). In case of rice, we get carbohydrate, fat, protein and vitamin content. So, rice is an ideal source of energy for maintaining healthy life. We used cooked rice instead of powder or grinding rice because if we provide powdery rice then it would digest very quickly and it will not reflect our pattern of consuming rice. Since we are consuming cooked rice, the experiment was subjected to reflect our pattern of consuming. The rats were fasted for 12-15 hours before starting GI experiment. Blood samples were collected from the tip of rats' tails at fasting (0 min) and also at 30, 60, 90 and 120 minutes post feed respectively. Blood glucose level was determined using Accu-Chek glucometer and test strips at mmole/L unit. Blood glucose level of all 72 groups was monitored with Accu-Chek glucometer (mmole/L unit) at time intervals of 30 minutes, 60 minutes, 90 minutes and 120 minutes after diet (50g equivalent carbohydrate or glucose; 50 g rice or glucose /70 kg x 0.15 g = 0.11 g rice per rat) withdrawal. GI value was measured by AUC (Area under curve) method and following formula (Haffner, 1986; Psyrogiannis, 2003, Keh, 2004)

AUC = 0.25×(fasting value) + 0.5×(half-hour value) + 0.75×(1-hour value) + 0.5×(2-hour value)

A total of 72 HYV including 68 inbred and 4 hybrid rice like BR1, BR2, BR3, BR4, BR5, BR6, BR7, BR8, BR9, BR10, BR11, BR12, BR14, BR15, BR16, BR17, BR18, BR19, BR20, BR21, BR22, BR23, BR24, BR25, BR26, BRRI dhan27, BRRI dhan28, BRRI dhan29, BRRI dhan30, BRRI dhan31, BRRI dhan32, BRRI dhan33, BRRI
dhan34, BRRI dhan35, BRRI dhan36, BRRI dhan37, BRRI dhan38, BRRI dhan39, BRRI dhan40, BRRI dhan41, BRRI dhan42, BRRI dhan43, BRRI dhan44, BRRI dhan45, BRRI dhan46, BRRI dhan47, BRRI dhan48, BRRI dhan49, BRRI dhan50, BRRI dhan51, BRRI dhan52, BRRI dhan53, BRRI dhan54, BRRI dhan55, BRRI dhan56, BRRI dhan57, BRRI dhan58, BRRI dhan59, BRRI dhan60, BRRI dhan61, BRRI dhan62, BRRI dhan63, BRRI dhan64, BRRI dhan65, BRRI dhan66, BRRI dhan67, BRRI dhan68, BRRI dhan69, BRRI Hybrid 1, BRRI Hybrid 2, BRRI Hybrid 3 and BRRI Hybrid 4 were surveyed for glucose response in this experiment. In order to account the effect of differently processed rice such as unparboiled, parboiled, pressure parboiled and double parboiled milled rice on glycemic response in an in vivo experimental rat model. We had selected three rice varieties from three GI group namely BR16 (Low GI), BRRI dhan29 (Intermediate GI) and BRRI dhan28 (High GI) and processed all three varieties in different methods i.e. parboiled mill rice (soaking, steaming and drying), pressure parboiled mill rice (Paddy moisture 12% and under 3 kg/cm$^2$ gauge pressure for 20 mins) and double parboiled mill rice (Two times pressure parboiled). Later on we have compared these methods with unparboiled milled rice (Atap rice; Just dehusking and removal of bran from paddy) regarding GI value changes. GI value was measured accordingly to discussion above. Flattened rice (also called beaten rice) was prepared by dehusked rice which is flattened into flat light dry flakes. Puffed rice was made by heating rice kernels under high pressure in the presence of steam. Amylose in starch is released by treatment with dilute alkali. By the addition of Tri-iodide ion, amylose produces blue color. Then the absorbance of blue color produced in aqueous solution was measured (Juliano, 1971). Resistant Starch (RS) was determined using the enzymatic method of Goni et al. (1996). In this study, CRD design was followed and Duncan’s multiple range test (DMRT) was applied on GI value at differently rice processing conditions, resistant starch (RS%) and rice based products (Table 2, Table 3 and Table 4) for statistical analysis using SPSS, version 20.0.

RESULTS AND DISCUSSION

Rice (*Oryza sativa* L.) provide carbohydrates, proteins, fat, fibre, minerals, vitamins etc. and considered as one of the major sources of nutrients due to its daily consumption. Our research aim was to focus on genetic variability of rice on glucose response at unparboiled milled rice condition. A total of 72 (Seventy two) HYV varieties including 68 BRRI Inbred and 4 BRRI Hybrid rice were studies for in vivo glycemic response at unparboiled milled rice condition. Our data reveals that three (3) out of seventy two (72) rice varieties were found low GI(<55) values ranges from 52.4 to 54.9 which resemble 4.2% of total tested samples (n=72). Varieties are named as BR16, BRRI dhan46 and BRRI dhan69. A total of fifty (50) varieties were categorized as Intermediate GI (56-69) which ranges from 56.4 to 69.5. Approximately 69.44% rice samples were classified as Intermediate GI group. A total of 19 rice varieties were categorized as High GI (≥70) value ranges from 70.7 to 87.8 which resemble 26.4% of total tested samples. Means of all three categories were summarized at tabular format (Table 1). All details glucose response data of 72 varieties at four time points (0 mins, 30 mins, 90 mins and 120 mins) were kept in supplementary document (SD).

**Table 1: Means of different GI categories.**

<table>
<thead>
<tr>
<th>GI Categories</th>
<th>No. of sample</th>
<th>Value (Mean ± SE)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GI group</td>
<td>3</td>
<td>53.46±0.75</td>
<td>4.2</td>
</tr>
<tr>
<td>Intermediate GI group</td>
<td>50</td>
<td>62.67±0.52</td>
<td>69.4</td>
</tr>
<tr>
<td>High GI group</td>
<td>19</td>
<td>76.78±1.45</td>
<td>26.4</td>
</tr>
</tbody>
</table>

We have estimated GI value of 72 HYV rice varieties by AUC (increment Area under curve) method (Supplementary data) (Keh, 2004). Since
we did not have any postbaseline measures which were lower than the baseline value in this study so we adopt this increment AUC method for GI estimation. But if we got post baseline glucose response lower than baseline then we would prefer total AUC method instead to minimize the effect of negative area (Potteiger, 2002).

Later part of this experiment we had focused on whether different rice processing procedures have effects on glucose response. In order to account the effect of differently processed rice such as unparboiled, parboiled, pressure parboiled and double parboiled milled rice on glycemic response in an in vivo experimental rat model, we had selected three rice varieties from three GI group namely BR16 from Low GI, BRRI dhan29 from Intermediate GI and BRRI dhan28 from High GI group and processed all three varieties in different methods i.e. parboiled mill rice (soaking, steaming and drying), pressure parboiled mill rice (Paddy moisture 12% and under 3 kg/cm² gauge pressure for 20 mins) and double parboiled mill rice (Two times pressure parboiled). Our data reveals that brown rice possess the lowest GI value for all rice varieties and GI categories. Parboiled milled rice including pressure parboiled and double parboiled milled rice showed lower GI value than unparboiled milled rice condition (Table 2). It is quite interesting that high GI grouped, unparboiled milled rice BRRI dhan28 (70.96) become reduced significantly to 65.01, 61.04, 60.65 and 59.33 at parboiled milled rice, pressure parboiled milled rice, double parboiled milled rice and brown rice respectively. Our data suggested that thermal and pressuring process lowering GI value than original because of possible retrogradative changes in starch composition and due to fiber content brown rice showed the lowest GI among all rice processing methods irrespectively to all rice varieties (Table 2). Since, we are consuming

Table 2: GI value of rice at different processed condition.

<table>
<thead>
<tr>
<th>Methods</th>
<th>BRRI dhan28</th>
<th>BRRI dhan29</th>
<th>BR16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Rice (BR)</td>
<td>59.33±0.21c</td>
<td>57.54±0.33cd</td>
<td>46.54±0.58c</td>
</tr>
<tr>
<td>Pressure Parboiled Milled Rice (PPMR)</td>
<td>61.04±0.2c</td>
<td>59.28±0.41cd</td>
<td>48.66±0.69bc</td>
</tr>
<tr>
<td>Doubled Parboiled Milled Rice (DMPR)</td>
<td>60.65±0.11c</td>
<td>60.65±0.31bc</td>
<td>47.82±0.19bc</td>
</tr>
<tr>
<td>Parboiled Milled Rice (PMR)</td>
<td>65.01±0.41b</td>
<td>61.02±0.25b</td>
<td>48.23±0.21b</td>
</tr>
<tr>
<td>Un-Parboiled Milled Rice (UMR)</td>
<td>70.96±0.34a</td>
<td>62.36±0.11a</td>
<td>52.41±0.27a</td>
</tr>
</tbody>
</table>

Any two means having common letter (s) are not statistically different at P< 0.05, as measured by the Duncan Multiple Range Test (DMRT).

Table 3: Resistant Starch (RS%) of rice at different processed condition.

<table>
<thead>
<tr>
<th>Methods</th>
<th>BRRI dhan28</th>
<th>BRRI dhan29</th>
<th>BR16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Rice (BR)</td>
<td>2.48±0.10a</td>
<td>2.80±0.19a</td>
<td>5.15±0.20a</td>
</tr>
<tr>
<td>Pressure Parboiled Milled Rice (PPMR)</td>
<td>2.35±0.20cd</td>
<td>2.58±0.11i</td>
<td>4.56±0.11d</td>
</tr>
<tr>
<td>Doubled Parboiled Milled Rice (DMPR)</td>
<td>2.20±0.11bc</td>
<td>2.40±0.15i</td>
<td>4.30±0.13c</td>
</tr>
<tr>
<td>Parboiled Milled Rice (PMR)</td>
<td>2.06±0.13b</td>
<td>2.00±0.11b</td>
<td>3.11±0.14b</td>
</tr>
<tr>
<td>Un-Parboiled Milled Rice (UMR)</td>
<td>1.51±0.21a</td>
<td>1.75±0.14a</td>
<td>2.54±0.11a</td>
</tr>
</tbody>
</table>

Any two means having common letter (s) are not statistically different at P< 0.05, as measured by the Duncan Multiple Range Test (DMRT).
parboiled milled rice in our Bangladeshi population, so almost all rice may vary considerably in the postprandial blood glucose (PPG) response to either low or intermediate level of GI at parboiled milled rice condition. Moreover, the post-harvest treatment of rice and the method of consumer preparation can also play a significant role in this variation. Starch comprises two glucose polymers: amylose and amylopectin. Amylose is a linear and relatively short polymer of glucose units linked by a (1 - 4) bonds. Amylopectin is a branched and longer polymer where glucose units are arranged linearly through a(1- 4), with branches emerging via a(1- 6) bonds occurring every twenty-four to thirty glucose units. It is well known that starches with a higher amount of amylose are more resistant to digestion. In addition to the variation in amylose content, cooking (and cooling) processes can influence starch digestibility via the degree of gelatinization and retrogradation of rice starch. Gelatinization is the collapse (disruption) of molecular order (breaking of H bonds) within the starch granule, manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence and starch solubilization during hydrothermal treatment. This leads to the dissociation of crystalline regions in starch with associated hydration and swelling of starch granules, leading to higher starch availability to human digestive enzymes. Retrogradation is the re-crystallization of amorphous phases created by gelatinization and, in the case of amylose, results in the formation of type 3 resistant starch (RS3). RS3 is resistant to digestion, because it is heat stable and melts above 120°C. In contrast, retrograded amylopectin is thought to melt upon reheating (cooking) due to the low melting point (46–65°C) of these crystallites, and therefore it is digestible upon cooking. We have analyzed resistant starch (RS %) (Table 3) content of three rice varieties such BRRI dhan28 (High GI), BRRI dhan29 (Intermediate GI) and BRRI dhan16 (Low GI) at different processed conditions such as brown rice, parboiled rice, double parboiled rice, pressure parboiled rice and un parboiled or clean rice condition. Our data reveals that RS content is the highest at brown rice condition and the lowest at clean or un-parboiled rice of all three varieties. RS content at pressure parboiled condition is the highest among parboiled condition for all three varieties considering low, intermediate and high level of AAC (Table 3) and correlation between GI and RS data found negative (significant at the 0.01 level; Pearson correlation value -0.949***). We have estimated AAC for BRRI dhan28 (27%), BRRI dhan29 (27.4) and BRRI dhan16 (27%) at cleaned or un-parboiled rice condition. Since, RS content varied over rice processing methods so it is likely that AAC might be changed during parboiling process. In further investigation we will try to reveal the mechanism behind the issue including possible involvement of α-Amylase inhibitory activities in rice and differently processed rice. We also investigated GI of rice based products such as fattened rice, popped rice, puffed rice and found rice products posses higher GI than rice itself because. For instance, BR16 is a low GI rice (GI 52.41) even in unparboiled milled rice condition but it increased its’ GI value at fattened, puffed and popped rice to 56.35, 58.42 and 60.76 respectively (Table 4). Similar fashion was also observed in other varieties like BRRI dhan28 and BRRI dhan29.
Table 4: GI value of rice based products.

<table>
<thead>
<tr>
<th>Rice varieties</th>
<th>BRRI dhan29</th>
<th>BRRI dhan28</th>
<th>BR16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flattened rice</td>
<td>72.47±0.46a</td>
<td>69.98±0.31a</td>
<td>56.35±0.19a</td>
</tr>
<tr>
<td>Puffed rice</td>
<td>78.64±0.34b</td>
<td>76.21±0.22b</td>
<td>58.42±0.45b</td>
</tr>
<tr>
<td>Popped rice</td>
<td>81.44±0.51c</td>
<td>80.09±0.41c</td>
<td>60.76±0.35c</td>
</tr>
</tbody>
</table>

Any two means having common letter (s) are not statistically different at a P< 0.05, as measured by the Duncan Multiple Range Test (DMRT).

Flattened rice is nothing more than rice which has becomes rich in probiotic elements because of getting dried. However, since it is basically rice, it is composed primarily of starch and carbohydrates. It is as good as eating rice and is not necessarily good for diabetics. Since both popped and puffed rice posses lesser mount solid materials, these are easily digestible compare to rice itself which ultimately increase glucose level in blood.

CONCLUSIONS

Rice is high in carbohydrates which are needed for energy. People with high physical activity such as sportspersons, daily laborer, farmers need carbohydrates. But if a person leads a sedentary lifestyle without much physical movement, high energy isn't required and hence rice consumption should be reduced and should be chosen for Low GI (≤55) rice varieties for consumption such as BR16, BRRI dhan46 and BRRI dhan69 in Bangladeshi population. These low GI indexed rice varieties can also be used as potential parent in low GI rice breeding program in Bangladesh. Compare to unparboiled milled rice, GI value of brown rice reduces the most and parboiled milled rice is better than unparboiled milled rice consumption in term of slower glucose responses in blood stream. RS content showed negatively correlated with GI data at different processed condition which resembled that parboiling process including heating, cooling, streaming and pressure treatment might have direct effect on retrogerative changes in starch composition. In addition fiber content of brown rice might possibly cause of possessing higher RS content. Rice product such as flattened, popped and puffed rice are of the ready-to-eat type in which the rice starch provides texture-modifying properties due to thermal modification. Lesser amount of solid materials might fasten their digestivity over rice itself which ultimately showed higher GI value than rice. Rice consumption should be rational according to individuals lifestyle and energy requirement to lead a healthier life.

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