



Glycemic Index, Antioxidant Activity and Starch Composition of Various Varieties of *Oryza sativa L* (Rice) Available in Pakistan

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Abstract

The 10 samples of different rice were collected from Ashraf road firdos bazaar Peshawar and were tested for glycemic indices, total starch content, free radical scavenging activity, and amylose to amylopectin ratio. The glycemic indices were in the range of 72.88 ± 1.70 to 89.08 ± 6.19 , and total starch content from 7 to 35.1%. The free radical scavenging activity of Basmati Chawal 18.46 ± 0.33 (lowest) to Mota Chawal 44.98 ± 2.02 (highest). The Amylose to Amylopectin ratio was from 0.12 to 1.822. The highest Amylose to Amylopectin ratio was of Seela Lazat (1.822) and the lowest one was of Seela Dakra Dobar (0.12). The sample of lowest glycemic index was Seela Dakra Dobar (72.88 ± 1.70) and the highest was Mota Chawal (89.08 ± 6.19). The DPPH method was followed for the measurement of antioxidant activity. The %inhibition of DPPH activity by an antioxidant was in the range of 18.46 ± 0.33 to 44.98 ± 2.02 for $100 \mu\text{g/ml}$.

Conclusion: The research performed was to determine radical scavenging activity, Glycemic Index Starch content and Amylose to Amylopectin ratio for different rice varieties. A variety which has high radical scavenging activity, low Glycemic index and high Amylose Content will be a good variety to use to be healthier. That variety should be preferred for diabetic patient.

Keywords: Starch; Glycemic; Diabetes Mellitus; Amylopectin; Amylose

Abbreviations: GI: Glycemic Index; AOA: Antioxidant Activity; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; AUC: Area Under Curve; HFWR: High Fibre White Rice.

Introduction

Plants are major sources of food contributing around 93% of the food the people of the world, among which two-third is contributed by cereals (Rice, wheat, maize, barley, sorghum, and millet). Around 80% of the global cereals production is shared by Rice, wheat and maize [1]. Rice is the second most cereal crop that is widely grown and staple food for more than half of the world's population. More than 3000 million people eat greater than 100kg of rice per

year. Rice is cultivated on 155.5 million hector area with an average growth rate of 0.39% a year [2]. Rice is the most important food crop and primary sources of food for more than one third of the world's population. More the 90% of rice is produce and consumed in Asia where 60% of Earth's population live. The largest rice producer countries are China, India, Indonesia, Bangladesh, Vietnam, Thailand and Pakistan [3].

Pakistan is the world's 11th largest country that produce rice. Pakistan's export 8% to total world's rice trade, Pakistan annual rice export stands at about 2.5 million tons. In Pakistan rice occupies 10% of the total cultivated area, accounts for 6.1% of value added in agriculture and 1.3% in gross domestic

products. There are more than 20 different varieties of rice which are cultivated in Pakistan [4]. In Khyber Pakhtunkhwa about 8% rice is produce while remaining 92% is produce in other provinces of Pakistan. In Khyber Pakhtunkhwa about 128 tons rice is produce per year [5]. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and is caused by defect in insulin secretion or insulin action. Insulin is the major hormone secreted by islets of Langerhans which is responsible for glucose homeostasis through a negative feedback mechanism [6]. Diabetes mellitus is a disease affecting 200 billion people in the whole world [7]. Diabetes mellitus is a complex syndrome involving abnormalities in carbohydrates fat and protein metabolism due to defect in action of insulin on target tissue cause by inadequate insulin secretion or diminished tissue response to insulin [8].

Diabetes mellitus is divided into two major subgroups. Insulin dependent diabetes mellitus is mostly found in young's due to lack of insulin secretion because of autoimmune destruction of insulin producing cells [9]. IDDM is also called 'juvenile-onset diabetes or type 1 diabetes mellitus in which there is total loss or near total loss of insulin secretion from islets of Langerhans in pancreas resulting in dis inhibition of gluconeogenesis leading to high blood glucose level and gross loss of muscles and fats [10]. In IDDM insulin secretion is loss due to destruction of beta cells as a result of viral infection, heredity or autoimmune disorders [11]. Non-insulin dependent diabetes mellitus is mostly found in aged people due to tardy or low secretion of insulin so that blood glucose level rise into hyperglycemic range because of resistance of target tissue to insulin [10]. Non-insulin dependent diabetes mellitus is also called onset-diabetes' or Type II diabetes in which there is low secretion of insulin from beta cells of pancreas because of diminished sensitivity of target tissue to metabolic activity of insulin resulting gradually in increased weight, obesity and elevated blood glucose levels [12].

Elevated levels of reactive oxygen species in the body is called oxidative stress which play an important role in the etiology of diabetic complications. Many biochemical pathways are strictly associated with hyperglycemia (glucose autoxidation, polyol pathway, prostanoid protein glycation) and can increase the production of free radicals [13]. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complication of diabetes mellitus

[14]. Insulin has an important effect on lipid and glucose metabolism. Hyperlipidemia in diabetes mellitus results from altered lipoprotein metabolism due to increased production, decrease clearance or change in lipoprotein composition cause by the change in insulin, glucose, and free fatty acid level associated with diabetes mellitus. Although diabetes mellitus appears to be an independent cardiovascular risk factor, abnormalities in lipid and lipoprotein metabolism in diabetes also contributes to the observed excess in cardiovascular risk [15].

The term diabetes is associated with both macro vascular complications (coronary, cerebrovascular and peripheral vascular disease) and so called 'micro vascular complications' (retinopathy (eye disease), nephropathy (kidney disease) and neuropathy (peripheral nerve damage). The individuals with diabetes have a 25-fold increase in the risk of blindness, a 20- fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischaemic brain damage [15]. The clinical manifestations of neuropathy in diabetic patients are protean. Various neuropathic disorders are associated with diabetes [12]. Retinopathy occurs in all forms of diabetes and depends on the duration of disease. After seven years, 50% of patients with IDDM have some degree of retinopathy. The prevalence of any retinopathy reaches more than 90% after 20 years [16]. It is diabetes specific complication associated with great mortality. Clinically significant nephropathy is identified with the development of micro albuminuria. After 5 to 10 years of diabetes, overt proteinuria develops in patients. In next stage glomerular filtration rate falls resulting in end-stage renal disease. It develops in only 35 to 45% patients with IDDM, less than 20% patients with NIDDM suffer from nephropathy. It does not rise continuously with increasing duration of diabetes [14].

Glycemic index is the area under the curve of the glucose responses to a carbohydrate-containing food compared to either a specific glucose dose or a specific amount of rice [17,18]. Food having low glycemic responses has been shown to improve overall blood glucose control in patients with diabetes mellitus and to reduce total serum cholesterol and triglyceride levels in hyperlipidemia subjects. Recently it has been reported in type 2 diabetic patients, that a low glycemic index (GI) diet causes important reductions in plasminogen activator inhibitor, leading to a normalization of the fibrinolytic activity in these patients [19].

Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury [19]. Many constituents in plant foods

exert protective properties by acting independently or synergistically as anticancer or cardio protective agents by a variety of mechanisms. One such protective mechanism attributed to dietary bioactive components is antioxidant activity which is a fundamental property important to life. It has been demonstrated that many of the biological functions including anti-mutagenicity, anti-carcinogenicity and anti-aging are related to oxidation/anti-oxidation balance. In the human body endogenous antioxidant systems may not be sufficient to scavenge ROS completely and hence may cause oxidative damage to lipids, proteins and nucleic acids, among others. Therefore, dietary antioxidants may be particularly important in protecting against numerous chronic diseases resulting from oxidative stress. In general, food plants such as cereals, fruits, vegetables, nuts and spices form the primary source of naturally occurring antioxidants in the human diet [20].

Antioxidants are classified, at least, into two categories, depending on the action sites in an oxidative sequence: preventive antioxidant and chain-breaking antioxidant. The former prevents the initiation of radical chain reaction by scavenging reactive oxygen species, or, more importantly in serum, by binding transitional metals in forms that will not generate reactive species. Chain-breaking antioxidant interrupts the oxidative process by reacting with chain propagating radicals, thereby forming a stable product. In clinical settings, the preventive antioxidant activity (AOA) in serum can be measured as the ability to inhibit lipid autoxidation in brain homogenates, and the AOA, measured by this method, is known to be largely determined by the metal-binding capacity of ceruloplasmin, transferrin, and albumin [21].

Materials and Methods

Chemicals

Hydrochloric Acid (Analar (BDH), Sodium Hydroxide (Merck KGaA), Ethanol (SIGMA ALDRIC Methanol (DAEJUNG 99.5%), DPPH (Wako), Acetic acid(99%), Iodine, Potassium Iodide (Merck Schuchardt OHG).

Equipment's

Centrifuge (Model-800, China), Thermostatic water bath (China), Oven (Model U-10, Germany), Spectrophotometer (Sp-300 Optima, Japan), Glucometer (Code free).

Samples Collection

Samples were collected from Ashraf Road Firdos bazaar Peshawar. Rice Sample varieties

S.N	Samples	Sources
1	Basmati	Ashraf Road Pesh
2	Basmati Kacha	Ashraf Road Pesh
3	Seela Lazzat	Ashraf Road Pesh
4	Seela Purana kainat	Ashraf Road Pesh
5	Seela Kahjor Marka	Ashraf Road Pesh
6	Mota Chawal	Ashraf Road Pesh
7	Begamai Chawal	Ashraf Road Pesh
8	Seela Charg Marka	Ashraf Road Pesh
9	Seela Dakra Dobar	Ashraf Road Pesh
10	Seela Mahal Kianat	Ashraf Road Pesh

Table 1: Rice sample varieties.

Determination of in Vivo Digestibility of Starch (Glycemic Index)

After 10-12 hours of fasting, the normal healthy volunteers were selected for the test. Before taking breakfast the venous blood were collected from the volunteers, then the test food (50 gram of boiled rice samples) were provided to the volunteers. After 15, 30,45,60,90, and 120 minutes of taking the test food the venous blood samples were collected. One-day gap was provided between two tests and the standard was D-Glucose solution (50g/250mL).

Data Analysis

GI = Incremental area under glucose curve test food × 100
Incremental area under glucose curve for standard food

Determination of Antioxidant Activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was used as a free radical to determine the antioxidant activity. The 0.1mM solution of DPPH was prepared by dissolving 3.8mg of DPPH in small amount of methanol and then diluted up to 100mL and incubated in the dark for 24 hours. 2mL of 0.1mM DPPH solution was added to every 0.5ml of extract. By taking 0.5mL of distilled water and 2mL of 0.1mM DPPH solution, the control was prepared and at room temperature incubated for 30 minutes. The absorbance was determined at 517 nm in contrast to blank solution which is 0.5 of distilled water mixed with 2mL methanol.

Determination of Total Starch

25mL of 0.1 molar NaOH solutions was taken and 5g of Grinded rice samples were added to the solution, then stirred on magnetic stirrer for 30 minutes. The mixture was centrifuge for 20 minutes, the supernatant was discarded and, to these diment, the 20mL of distilled water were

added. The samples were riddled with a special cloth, the filtrate was neutralized with 1M HCl solution and the debris was discarded and the filtrate was centrifuged again for 20 minutes. The tailing starch was removed and the samples were kept in oven for drying at 37°C, after complete drying weighted and total starch were determined.

Amylose to Amylopectin Ratio Determination

The determination of Amylose to Amylopectin Ratio was done by following Aziz et al method. The NaOH solution of 1M was prepared and 0.1g was taken from starch, 9mL from 1M NaOH solution and 1mL of ethanol 99% were added to the 0.1g of taken starch. After mixing, for the gelatinization of starch the sample were kept in boiling water for 10 minutes. The hundred mL volumetric flask was filled with 5mL of starch along with 1mL of 1M acetic acid. The sample solution was followed by the addition of 2mL of iodine solution and to make the solution to the mark point of volumetric flask distilled water was added. Spectrophotometer was used

to measure the absorbance of the solutions at 620 nm. To obtain the amylose and amylopectin content the equation used are; Amylose content (%) = 3.06 x absorbance x 20 and Amylopectin content (%) = 100 - % Amylose content.

Results

Glycemic Index of Rice Varieties

In order to find out GI, the area under the curve (AUC) for samples and standard (glucose) were calculated using Ms Excel. The areas under the curve for various samples are given in Table 1. GI of all samples was calculated using glucose as a standard. The values were expressed as mean \pm SD (Table 2). The values ranged between 89.08 \pm 6.19 and 72.88 \pm 1.70. In GI of most of the samples, the significant difference was present. Mota Chawal sample was found to have highest glycemic index while lowest glycemic index was found in Seela Dakra chawal sample.

S.No	Variety	Subject1	Subject2	Mean
1	Seela Lazat	74.69	71.58	73.13 \pm 2.20
2	Seela Charg Marka	80.29	91.46	85.87 \pm 7.89
3	Seela Purana Kainaat	81.9	94.72	88.31 \pm 9.06
4	Seela TajMahal Kainat	84.92	86.53	85.72 \pm 1.13
5	Basmati Kacchha Chawal	82.29	76.4	79.34 \pm 4.16
6	Basmati Chawal	82.76	84.69	83.72 \pm 1.36
7	Mota Chawal	84.7	93.46	89.08 \pm 6.19
8	Seela Dakra Dobar	74.09	71.68	72.88 \pm 1.70
9	Seela Kahjoor Marka	77.65	77.64	77.64 \pm 0.007
10	Baigmi Chawal	83.36	81.02	82.19 \pm 1.65
11	Glucose	100	100	100

Table 2: Glycemic index of rice samples.

Samples	Area under curve sub 1	Area under curve sub 2	Mean AUC
Seela Lazzat	10403	10808	10605
Seela Charg Marka	11183	11970	11576.3
Seela Purana Kainat	11408	12398	11902
Seela Tajmahal	11828	11325	11575.3
Basmati Kacha	11475	11535	11505
Basmati Chawal	11528	11085	11306
Mota Chawal	11798	12233	12015.3
Seela Dakra Dobar	10320	10823	10571.3
Seela Kahjoor Marka	10815	11723	11268.8
Baigmi Chawal	11610	12233	11966.3
Glucose	13928	13088	13507.5

Table 3: Area under the curves for various samples.

Determination of Total Starch Content

The starch content of various rice varieties was determined and shown in table. The values were expressed as mean and values ranged between 7 and 35.1. The highest amount of total starch was found in Baigmi Rice and the lowest amount was present in seela dakra rice.

S.NO	Sample	% Starch
1	Baigmi Chawal	35.1
2	Seela purana Kainaat	22.4
3	Seela TajMahal Kainaat	10
4	Mota Chawal	10
5	Seela Basmati	33.4
6	Basmati Kachha Chawal	32.8
7	Seela Dakra	7
8	Seela Lazat	15.6
9	Seela Charg Marka	11.2
10	Seela Kahjoor Marka	19.08

Table 4: Total starch content of Rice samples.

Antioxidant Activity

The Percent inhibition of DPPH by the antioxidants present in various rice varieties was determined and most of the varieties differ significantly in terms of their antioxidant

S.N	Sample	Amylose content (%)	Amylo pectin Content (%)	Amylose to Amylopectin Ratio
1	Seela Basmatti Chawal	41.30±8.22	58.69±8.22	0.7
2	Seela Puraana Kainaat	47.73±2.59	52.29±2.55	0.91
3	Mota Chawal	42.22±6.05	57.77±6.05	0.73
4	Baigmi Chawal	26.00±0.43	73.99±0.43	0.35
5	Seela Charg Marka	31.21±0.86	68.79±0.86	0.45
6	Seela Kahjoor Marka	11.32±1.30	90.52±1.30	0.12
7	Seela TajMahal Kainaat	52.93±8.22	47.06±8.22	1.12
8	Basmati KacchaChawal	47.73±0.86	52.27±0.86	0.91
9	Seela Lazat	64.56±9.08	35.43±9.08	1.822
10	Seela Dakra Dobaar	11.32±1.30	90.52±1.30	0.12

Table 6: Amylose to amylopectin ratio of rice samples.

Discussion

The Diabetes Mellitus and the Carbohydrates containing foods are closely related to each other. The different varieties of rice differ in terms of their glycemic index. Most of the rice samples showed significant difference in GI. The variation in

potential status. The values were expressed as mean ± SD. The antioxidant potential of samples ranged between 44.98±2.02 and 18.46±0.33. Mota Chawal showed highest DPPH scavenging activity while Basmati Chawal had lowest antioxidant potential among all the rice varieties.

S.No	Samples	Mean
1	Baigmi Chawal	31.79±1.32
2	Seela purana Kainaat	44.98±2.02
3	Seela TajMahal Kainaat	21.67±2.79
4	Mota Chawal	44.98±2.02
5	Seela Basmati	18.46±0.33
6	Basmati Kachha Chawal	22.60±0.23
7	Seela Dakra	26.35±0.06
8	Seela Lazat	20.07±1.27
9	Seela Charg Marka	28.95±0.70
10	Seela Kahjoor Marka	31.79±1.32

Table 5: Antioxidant activity of rice samples.

Amylose to Amylopectin Ratio

% Amylose and Amylopectin were calculated for all the rice varieties. The Amylose to Amylopectin ratio was in the range of 0.12 to 1.822. The Amylose content, amylopectin content and amylose to amylopectin ratio of various rice samples are shown in table 6.

GI and other parameters with in different varieties of same cereals is also reported previously. For example Mohan, et al. [22] compared the various parameters including glycemic index, fibre content and amylose content of commercial white rice and high fibre white rice (HFWR). The study concluded that the white rice possessed five times lower

dietary fibre content than HFWR and white rice also had 6.5 times lower resistant starch than HWFR while HFWR contained pointedly more amylose content than white rice. The white rice was found to have 23 times higher GI than HFWR. In short, the white rice were concluded to be unhealthier than HFWR because of having high GI [23]. Prasad, et al. [23] worked on the GI of different Indian rice varieties. The different chemical, biochemical, agronomical, and morphological characteristics of Indian standard rice varieties, Lalat, Jaya, Savithri, Sasyasree, Salivahana, PR-113, NDR-97, Tellahamsa, Varalu, Triguna, and DRRH-3 were studied. The GI was negatively linked with resistant starch. For the dietary regulation of diabetes, the Lalat was reported to be best because of having low GI [24].

The difference in GI of various varieties of same cereal can be attributed to a number of factors. The difference in genetic makeup is one factor which can affect the glycemic index of different varieties. Furthermore the carbohydrate quantity and quality can also affect GI. The type of starch present along with amylose content can also affect the glycemic index. Further studies regarding the determination of resistant and slowly digestible starch in these varieties can account for more clear reasons in difference in GI of rice samples [25-29].

A low glycemic index food has a number of health benefits for normal people as well as people suffering from glucose associated problems including diabetes. Furthermore low GI foods are also recommended for people with heart diseases and for obese people. Among the tested varieties of Rice, the Mota chawal showed highest Glycemic Index. The higher GI value of Mota Chawal makes this variety unsuitable to be consumed by diabetic individuals. Seela Dakra Chawal showed the lowest glycemic index among all the analyzed samples, which make this variety as a better variety on comparative basis to be consumed by diabetic individuals.

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing. Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract and cancer. Antioxidants are the compounds which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. These antioxidants are also produced by biological system and occur naturally in many foods and the balance between oxidants and antioxidants

decide the health and vigor. The Percent scavenging activity of DPPH free radical of each rice variety was determined. A variety which scavenges more percentage of free radical is good for health. In the varieties that we tested for radical scavenging activity, Mota chawal had highest radical scavenging activity while Basmati Chawal was of lowest Antioxidant activity. So Mota chawal is considered to be used [30-34].

Rice starch is a finely textured, flour like, gluten free powder that comes from endosperm (seed) of rice. Rice is an excellent source of starch, which is normally hydrolyzed by enzymes in the digestive tract to be converted into glucose that cells use to produce energy for their metabolic functions. Digestibility of cooked rice starch is usually determined by the amount of amylose in the grain. The more amylose in the grain, the slower is the digestion of rice, and the lower is the Glycemic index. The Starch content in Percent was determined for all varieties. Highest amount of starch was found in Baigmi chawal and was low for Seela Dakra Chawal. The range was from 7% to 35.1%.

Amylose is a polysaccharide made of Glucose units bonded to each other through glycosidic linkage. Because of its tightly packed helical structure, amylose is more resistant to digestion than other starch molecules and therefore an important form of resistant starch. The Amylose/ Amylopectin ratio was also determined for each variety. The highest Amylose

Amylopectin ratio was found for Seela Lazat Chawal which mean that it also contain high Amylose content. The lowest ratio was found for Seela Kahjoor Marka and Seela Dakra Dobaar. A variety which has highest amylose content is preferred over other. So seela lazat would be preferred to be use by the diabetic patient.

Conclusion

The research performed was to determine radical scavenging activity, Glycemic Index Starch content and Amylose to Amylopectin ratio for different rice varieties. A variety which has high radical scavenging activity, low Glycemic index and high Amylose Content will be a good variety to use to be healthier. That variety should be preferred for diabetic patient. Here 10 different rice varieties were tested for these properties. In all of the varieties Mota Chawal has highest Antioxidant Activity while the Seela Dakra has the lowest Glycemic Index. The Amylose to Amylopectin ratio was highest for Seela lazat variety .So it is recommended to use Seela Dakra for its low Glycemic Index. A Diabetic Patient can use this Variety to keep his/her glucose level normal.

Author Contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and have approved its submission.

Conflict of Interest

This study has no conflict of interest to be declared by any author.

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