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Resistant Starch Content of Thirty Eight Selected Rice (*Oryza sativa L.*) Varieties of Sri Lanka

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Abstract

Background: Rice is the staple food of half the world's population including Sri Lanka. Resistant starch (RS) is the starch and starch degradation products that escape digestion in the small intestine by human digestive enzymes and reported to have wide range of health benefits. Rice consists of varying amounts of RS depending on the rice variety. However, RS content of Sri Lankan rice varieties has very limited studied. Objective: To evaluate the RS content of thirty eight selected rice varieties of Sri Lanka. Methods: Twenty seven new improved (NI) [Bg: Bathalagoda and At: Ambalanthota], two old improved (OI) and nine traditional rice varieties of Sri Lanka were used in this study. RS content of whole grain rice varieties were determined according to the Megazyme assay protocol with some modifications. Results: RS content varied significantly ($P < 0.05$) among the tested rice varieties and it ranged from 0.30 ± 0.02 to 4.65 ± 0.19%. RS content of NI, OI and traditional rice varieties ranged from 0.30 ± 0.02 to 3.11 ± 0.06, 0.55 ± 0.05 to 1.92 ± 0.40 and 0.44 ± 0.14 to 4.65 ± 0.19% respectively. Interestingly, mean RS content was significantly ($P < 0.05$) high in traditional rice varieties compared to NI and OI rice varieties. Further, rice varieties which showed > 3% RS contents were all traditional rices (except Bg 3-5, a new improved white rice variety). Among the rice varieties studied, a traditional white pericarp rice variety Suduru Samba (4.65 ± 0.19%) had the highest RS content, while NI white pericarp rice variety Bg 305 (0.30 ± 0.02%) had the lowest RS content. Conclusion: It is concluded that RS content varied among the tested rice varieties and especially Sri Lankan traditional rice varieties had the highest RS content. Thus, such rices may have the potential to utilize in developing value added novel functional foods and neutraceuticals for prevention and dietary management chronic diseases.

Keywords: Rice; Sri Lankan rice; Traditional rice; Old improved rice; New improved rice; Resistant starch.

1. Introduction

RS is starch and starch degradation products that escape digestion in the small intestine by human digestive enzymes. RS is classified into five subtypes as RS1: physically inaccessible starch granules which is present in whole grains and seeds; RS2: native granular starch which is found in potato and banana; RS3: retrograded starch produce during cooking/cooling processes, RS4: chemically modified starch and RS5: type of RS formed due to formation of amylose-lipid complexes [1-4]. RS is considered as type of dietary fiber [1-3] and increase consumption of RS is associated with variety of health benefits [1-3]. It involves in improving blood glucose and insulin responses and promotes body fat utilization rather than its deposition [1, 2, 5]. Thus, RS plays a vital role in managing diabetes and obesity [1, 2, 5]. It is also essential in prevention and dietary management of cancers, hemorrhoids, diverticulosis, constipation and cardiovascular diseases [1, 2, 6]. Therefore, foods having high RS content are of great importance in the food industry for addressing such health issues [1, 2, 5, 6].

Rice is the staple food of half the world's population and widely consumed in Asian countries [7]. It is the staple food in Sri Lanka and popularly consume by the people in the country [8]. Recent health statistics of the Sri Lankan population has shown rising incidences of non-communicable diseases [9, 10] and this has been explained due to variety of risk factors including in proper dietary habits [9]. Thus, foods having additional health benefits beyond its basic nutrition are really important in alleviating such health problems [11, 12] in the country. Rice as the staple food

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in Sri Lanka it can be easily used as a vehicle to address such health issues if the rice varieties consume in the country are nutritionally and functionally sound. Research conducted in past 8-10 years on health benefits of Sri Lankan rice varieties was able to discover wide range of nutritional and health benefits of Sri Lankan rice varieties [8, 13-19][8, 13-19]. Further, these studies clearly showed some rice varieties in the country are more nutritious and have enhanced health benefits compared to the other tested rice varieties [8, 13-19]. However, research on starch properties including RS content of Sri Lankan rice varieties had been very limitedly studied. This study evaluated RS content of thirty eight NI, OI and traditional rice varieties of Sri Lanka.

2. Materials and Methods

2.1. Materials

Twenty seven NI [Bg: Bathalagoda (22 rice varieties) and At: Ambalanthota (5 rice varieties)], two OI and nine traditional rice varieties were obtained from Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka. These varieties were cultivated and harvested in experimental field conditions at RRDI, Bathalagoda.

2.2. Sample Preparation

Rice seeds were dehulled using a laboratory dehuller (THU 35B, Satake, Hiroshima, Japan) and ground to fine flour using a laboratory miller (0.5mm sieve mesh) and used for the RS content analysis.

2.3. Resistant Starch Content of Selected Rice Varieties

RS contents of selected rice varieties were determined according to the method described in Megazyme Assay KIT, K-RSTAR 08/2011, Ireland [20]. Briefly, 100mg rice flour from each rice variety was weighed into screw cap falcon tubes and added with 4ml of pancreatic α-amylase (10mg/ml) containing amyloglucosidase (3300 Units/ml). Samples were then digested for 16hrs at 37ºC in a shaking water bath at 200rpm. After the digestion period, 4ml of ethanol was added to each tube, vortexed for 2 minutes, centrifuged at 3000rpm for 10 minutes and the supernatants were discarded. The resulting pellets were re-suspended in 2ml of 50% ethanol and stirred vigorously on a vortex mixer. To each sample tube 6ml of 50% ethanol was added and centrifuged at 3000rpm for 10 minutes. Then, supernatants were decanted and falcon tubes were inverted on absorbent paper to drain excess liquid. Samples were then added with 2ml of KOH and the pellets were re-suspended by stirring for 20 minutes in an ice/water bath over a magnetic stirrer. After the hydrolysis, 8ml of sodium acetate buffer (1.2M, pH 3.8) and 0.1ml amyloglucosidase (3300 U/ml) were added and incubated at 50°C in a water bath for 30 minutes. Then, samples were centrifuged at 3000rpm for 10 minutes and the glucose concentration was determined by glucose oxidase peroxidase kit. RS content of each rice sample was calculated according to the calculation procedure given in the Megazyme Assay KIT, K-RSTAR 08/2011, Ireland [20].

2.4. Statistical Analysis

Data were analyzed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences among treatment means. $P < 0.05$ was regarded as significant.

3. Results

RS content (%) of selected Sri Lankan rice varieties and variation in RS content among the selected Sri Lankan rice varieties is given in Table 1 and Table 2 respectively.

Significant differences ($P < 0.05$) were observed for the RS content among the tested rice varieties of Sri Lanka. RS content of the rice varieties in this study ranged from 0.30 ± 0.02 to 4.65 ± 0.19%. NI, OI and traditional rice varieties had RS contents in the range of 0.30 ± 0.02 to 3.11 ± 0.06, 0.55 ± 0.05 to 1.92 ± 0.40 and 0.44 ± 0.14 to 4.65 ± 0.19% respectively. Interestingly, mean RS content of traditional rice varieties were significantly higher ($P < 0.05$) than mean RS content of NI and OI rice varieties. A traditional white pericarp rice variety Suduru Samba (4.65 ± 0.19%) had the highest RS content, while NI white pericarp rice variety Bg 305 (0.30 ± 0.02%) had the lowest RS content.

4. Discussion

Range of selected NI, OI and traditional rice varieties of Sri Lanka were studied for RS content using *in vitro* assay protocol which is widely used in measurement of RS content of various substrates [20]. The NI rice varieties tested in this study covered the rice varieties developed from Bathalagoda (Bg varieties) and Ambalantota (At varieties) Rice Research Institutes. Most of these varieties are popular, widely cultivating and consuming rice varieties in the country [21, 22]. The selection of OI (H varieties) and traditional rice varieties for the present study was based on our previous research findings [8, 13, 14, 16-19, 22-24].

Wide variation in RS content (0.30 ± 0.02 - 4.65 ± 0.19%) was observed among the selected rice varieties. Mean RS content of traditional rice (0.44 ± 0.14 - 4.65 ± 0.19%) was greater than the mean RS content of NI (0.30 ± 0.02 - 3.11 ± 0.06%) and OI (0.55 ± 0.05 - 1.92 ± 0.40%) rice varieties. Interestingly, the rice varieties which showed > 3% RS contents were all traditional rices (except Bg 3-5, a new improved white rice variety). Suduru Samba, the traditional rice variety which showed the highest RS content in this study exhibited 4.4, 4 and 1.9 fold respectively high RS contents compared to the most popular NI rice varieties Bg 300, Bg 352 and Bg 94/1 which account ~ 45%

extent of the paddy cultivation in Sri Lanka [21, 25]. In general, the rice varieties developed from Bathalagoda (Bg varieties) Rice Research Institute had greater RS content than rice varieties developed from Ambalantota (At varieties) Rice Research Institute. The observed variations in RS content among the selected rice varieties may be due to the varietal differences. Previous workers have also shown wide variation in RS content of different rice varieties and it has ranged from 0.5 - 6.1% [26-28]. The Sri Lankan rice varieties (0.30 ± 0.02 - 4.65 ± 0.19%) tested in the present study showed low to moderate RS content in comparison to the findings of the previous workers [26-28]. However, RS content of most of the Sri Lankan traditional rice varieties were higher than medically important traditional rice varieties (0.68 ± 0.08 - 0.83 ± 0.01%) from India [28] and high to moderate in comparison to some traditional rice varieties (3.06 - 6.13%) from Thailand [26].

Rice as the principle staple food among half of the world's population provides significant carbohydrate content to the rice consumers [29]. However, foods having high carbohydrate content along with quickly digestible carbohydrates are reported to have high glycemic index [30]. Such foods are associated with high blood glucose and insulin responses and long term consumption of such foods leads to diabetes, obesity and other chronic diseases [30, 31]. Thus, low glycemic index foods having slowly digestible carbohydrates are used in current food and nutrition guidelines for the dietary management of chronic diseases [31, 32]. Rice varieties to have low glycemic indices increase amounts of RS, dietary fiber, amylose content and inhibitors of carbohydrate digestion enzymes are of great importance [17, 33]. In our previous research studies we have shown that most of the rice varieties (except Suduru samba) tested in the present study were high amylose rices and especially Sudu Heeneti rice variety had high dietary fiber (6.1%) content [23, 34]. Further, brans of Sudu Heeneti had potent anti-amylase activity and showed low starch digestion rate *in vitro* [17, 18]. Considering the findings from the present study and the findings from our previous studies it can be hypothesized that flour from whole grains of Sudu Heeneti rice variety may be superior to Suduru samba rice variety. Thus, consumption of especially whole grains of Sudu Heeneti may play and important role in the prevention and dietary management of diabetes and other chronic diseases.

RS has wide variety of applications in the food industry [35, 36]. It is used as an ingredient in foods for lowering caloric value, improving organoleptic characteristics, textural properties and gut microbial population (prebiotic agent) and also as a wall material for microencapsulation [35, 36]. Commercial preparations of RS such as CrystaLean®, Novelose® and Amylomaize VII are also available to increase the dietary fiber content and to provide other functional properties in foods [36]. The high RS containing rice varieties especially traditional rice varieties of Sri Lanka also have the potential for use in such applications in the functional food industry for addressing ever increasing diabetes, obesity and other chronic diseases in the country. Further, this is the 1st first study to evaluate RS content of large set of Sri Lankan rice varieties.

5. Conclusion

It is concluded that RS content varied among the selected rice varieties of Sri Lanka. Mean RS content of traditional rice varieties were higher than the RS mean content of OI and NI rice varieties. Suduru Samba, a traditional rice variety had the highest RS content among the varieties studied. The high RS containing Sri Lankan rice varieties may have the potential to utilize in developing value added novel functional foods and neutraceuticals for prevention and dietary management chronic diseases.

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Declaration of Conflicting Interests

There is no conflict of interests.

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Table-1. Resistant starch content of selected rice varieties of Sri Lanka

No	Rice Variety	Rice Type	Pericarp Color	Resistant Starch Content %
1	Suduru samba	Traditional	White	4.65 ± 0.19^{a}
2	Sudu Heeneti	Traditional	Red	3.75 ± 0.15^{b}
3	Thatu Wee	Traditional	Red	3.37 ± 0.28^{c}
4	Bg 3-5	New Improved	White	3.11 ± 0.06^{cd}
5	Kurulu Thuda	Traditional	Red	3.02 ± 0.52^{d}
6	Dhosthara Heeneti	Traditional	Red	2.50 ± 0.05^{e}
7	Bg 94/1	New Improved	White	2.39 ± 0.03^{e}
8	Bg 360	New Improved	White	2.28 ± 0.05^{ef}
9	Kalu Heeneti	Traditional	Red	2.01 ± 0.06^{fg}
10	Bg 450	New Improved	White	1.99 ± 0.09^{fg}
11	H 7	Old Improved	White	1.92 ± 0.40^{g}
12	Bg 366	New Improved	White	1.35 ± 0.40^{h}
13	Bg 369	New Improved	White	1.34 ± 0.11^{h}
14	Bg 352	New Improved	White	1.17 ± 0.04^{hi}
15	Pachchaperumal	Traditional	Red	1.14 ± 0.09^{hij}
16	Bg 250	New Improved	White	1.09 ± 0.05^{hijk}
17	Bg 400	New Improved	White	1.09 ± 0.21^{hijk}
18	Bg 300	New Improved	White	1.05 ± 0.05^{hijk}
19	At 307	New Improved	White	0.99 ± 0.08^{ijklm}
20	At 306	New Improved	White	0.93 ± 0.04^{ijklmn}
21	At 353	New Improved	Red	0.85 ± 0.10^{jklmno}
22	Bg 454	New Improved	White	0.84 ± 0.03^{jklmno}
23	At 405	New Improved	Red	0.78 ± 0.08^{klmnop}
24	Bg 379/2	New Improved	White	0.73 ± 0.19^{lmnopq}
25	Bg 304	New Improved	White	0.73 ± 0.40^{lmnopq}
26	Rathdal	Traditional	White	0.71 ± 0.08^{almnopq}
27	Bg 38	New Improved	White	0.69 ± 0.05^{mnopq}
28	At 362	New Improved	Red	0.67 ± 0.08^{mnopq}
29	Bg 406	New Improved	Red	0.66 ± 0.07^{mnopq}
30	Bg 407	New Improved	White	0.62 ± 0.05^{nopqr}
31	H 4	Old Improved	Red	0.55 ± 0.05^{opqr}
32	Bg 357	New Improved	White	0.46 ± 0.05^{pqr}
33	Bg 359	New Improved	White	0.45 ± 0.10^{pqr}
34	Suwadal	Traditional	White	0.44 ± 0.14^{qr}
35	Bg 358	New Improved	White	0.42 ± 0.11^{qr}
36	Bg 745	New Improved	White	0.41 ± 0.06^{qr}
37	Bg 403	New Improved	White	0.32 ± 0.04^{r}
38	Bg 305	New Improved	White	0.30 ± 0.02^{r}

Results were given in dry weight basis and represented as mean ± SD (n=3).Varieties with the same letter are not significantly different at $P < 0.05$.

Table-2. Variation in resistant starch content among the selected rice varieties of Sri Lanka

Rice type/rice variety		Resistant starch content (%)
New Improved varieties	All varieties	0.30 ± 0.02 - 3.11 ± 0.06
	Red varieties	0.66 ± 0.07 - 0.85 ± 0.10
	White varieties	0.30 ± 0.02 - 3.11 ± 0.06
Old Improved varieties	All varieties	0.55 ± 0.05 - 1.92 ± 0.40
	Red varieties	0.55 ± 0.05
	White varieties	1.92 ± 0.40
Traditional varieties	All varieties	0.44 ± 0.14 - 4.65 ± 0.19
	Red varieties	1.14 ± 0.09 - 3.75 ± 0.15
	White varieties	0.44 ± 0.14 - 4.65 ± 0.19
Red varieties		0.55 ± 0.05 - 3.75 ± 0.15
White varieties		0.30 ± 0.02 - 4.65 ± 0.19
Bg varieties		0.30 ± 0.02 - 3.11 ± 0.06
At varieties		0.67 ± 0.08 - 0.99 ± 0.08

Results were given in dry weight basis and represented as mean ± SD (n=3).