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Contributed Paper

Effects of Storage Temperature and Time on the Glycemic Response of White Rice

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ABSTRACT

This study examined the glycemic response of white rice to determine the effects of storage temperature (4°C, 0°C, and -20°C) and storage duration (1, 3, and 5 days). Results were compared with cooked white rice (control, 25°C). Measurements were performed on Days 1, 3, and 5 to test the glycemic index (GI), incremental area under curve (IAUC), and blood glucose response. Ten healthy adults aged 18-26 years were fed white rice or a reference food containing 50 g of available carbohydrates. Venous blood samples were collected before the meal and at 15, 30, 45, 60, 90, and 120 min after the meal. The results showed that the mean GI was highest for the control (93.5 ± 3.6), followed by those for the -20°C (87.4 ± 3.1), 4°C (84.1 ± 2.8), and 0°C (84.1 ± 2.7) test foods (Day 1). The mean GI of the 0°C test food decreased from 84.1 ± 2.7 on Day 1 to 77.9 ± 1.8 on Day 3 and then 74.8 ± 1.5 on Day 5. The GI values differed significantly with the storage duration ($p < 0.05$). In conclusion, white rice stored at 0°C for 5 days has the lowest glycemic response. Moreover, control group (25°C) exhibited the highest GI.

Keywords: glycemic response, white rice, storage temperature, storage duration

1. INTRODUCTION

Rice is one of the main crops in most countries worldwide and a staple food in Asia [1]. The main constituent of rice is starch, which is made of α glucans comprising polymerized glucose molecules. The two components of starch are amylose (20%-30%) and amylopectin (70%-80%), the properties of which have been widely studied [1-3]. The structure and form of starch determine its sensitivity to pancreatic enzymes. Amylose have few enzyme active sites and are therefore digested slowly and incompletely. By contrast, amylopectin is digested and absorbed rapidly, resulting in a high glucose response and high glycemic index (GI). Other physicochemical properties of starch that determine the GI include retrogradation [4], the amylose–amylopectin ratio [5], and sensitivity of amylase [6]. The processing and cooking methods [7] as well as the particle size [8] also affect the GI [9]. Long-term intake of high-GI foods correlates with an increased

risk of diabetes, coronary heart disease, and obesity [10-13]. In clinical trials, a low-GI diet has been shown to improve insulin sensitivity, resulting in reduction of blood lipids and blood sugar content [13-15]. Therefore, determining the postprandial blood glucose response and GI of white rice is imperative.

Englyst et al. [16] found that some starches cannot be hydrolyzed by enzymes; hence, these starches are named resistant starches (RSs). RS escapes digestion by resisting hydrolysis from enzymes such as α-amylases and glucoamylases. The reasons for this include the physical and chemical structure of starch as well as its resistance to gelatinization, staling, and modification. Studies have classified RS into four types according to its physical characteristics (RS1, RS2, RS3, and RS4). RS3 refers to staling starch which is formed when starch-containing foods are cooked and cooled. Examples of RS3 include rice, processed grains, processed flour, and bakery products. Rice is classified into three types according to the grain quality; in descending order of amylose content, they are indica rice, japonica rice, and waxy rice. The amylose content of rice is a crucial determinant of its physicochemical properties and a key indicator of starch gel hardness [17-19]. Rice that is high in amylose forms RS3 because it is prone to retrogradation [16]. In addition to the level of amylose [20], the effects of RS3 on glycemic response are determined by factors such as storage time and temperature [16] and the degree of gelatinization [19].

Cooked rice is often stored in a refrigerator to extend its shelf life. However, the storage temperature and time tend to cause changes in the physicochemical properties of rice (e.g., retrogradation and staling of the starch). This reduces the GI of cooked rice because such changes make it more difficult for the small intestine to digest the rice. According to this principle and modern food processing technology, the present study was aimed at developing nutritious and healthy grain products, including instant rice (congee), puffed rice cereal, rice pasta, rice noodle, rice bread, and puffed rice cake. Some studies have examined the effects of storage temperature and duration for starch and starch-based foods. For example, the effects of storage temperature and duration on staling and retrogradation have been investigated for potatoes [21], chapati [22], and wheat flour tortillas [23]. However, few studies have examined white rice to determine the effects of these factors on postprandial blood glucose response and GI. Therefore, the present investigated the physicochemical properties of rice by observing the effects of storage temperature and duration on postprandial blood glucose response, incremental area under curve (IAUC), and GI. The findings of this study may serve as a reference to people concerned about glycemic response and food processing information with which to manage their diets and developing rice products.

2. MATERIALS AND METHODS

2.1 Subjects

Ten healthy adults of East Asian descent (5 women and 5 men) were recruited from National Pingtung University of Science and Technology. The participants were weight-stable and not on any medication during the study period. All participants had normal fasting blood glucose concentrations and were asked to restrict their intake of alcohol, beans/legumes, and fried foods, and to refrain from unusual eating habits and physical activities before each test day [25,26]. The mean age (±SEM) of the

participants was 20 ± 1 years (range, 18-26). The mean body mass index was 22.0 ± 0.4 kg/m^2 (range, 20.0-24.0), the mean weight was 67.8 ± 1.1kg (range, 49.0-75.0), and the mean height was 174.4 ± 1.8 cm (range, 147.0-183.0). The average fasting glucose was 74.0 ± 0.6 mg/dL (range, 69.5-76.5). This research project was conducted in accordance with the Declaration of Helsinki. Prior to conducting the study, we obtained informed consent from the study subjects and submitted this information to the Research Ethics Committee of Pingtung Christian Hospital (International Review Board) for review and evaluation. The committee provided guidance and approved this research project.

2.2 Test Foods

The test foods were made from white rice and the reference food using a 50 g glucose that closest to the available carbohydrate content, a value of 100 represents the standard, an equivalent amount of pure glucose. The white rice variety used in this study is the most common edible rice (japonica rice) in Taiwan (Kaohsiung 145, Pingtung Sin Fang, Taiwan). The composition of the rice is as follows: total milled rice (82.68 %), head rice (73.8%), white center (0.17), white back (0.00), white belly (0.15), crude protein (6.02%), amylose (19.8%), overall palatability (0.159), calories (354 kcal/100 g), crude fat (0.7 g/100 g), crude protein (7.0 g/100 g), total carbohydrates (80.0 g/100 g), and sodium (10 mg/100 g). The chemical composition of the test food was analyzed using the standard method described in AOAC 46-11 (2000).

The test foods were prepared on the morning of the test days. White rice (62.5 g of raw rice containing 50 g of available carbohydrates) was first soaked in water (rice:water ratio, 1:1) and then cooked using a rice cooker for 40 min (TAC-10A-G, Tatung Co., Ltd., Tatung, Taiwan). After it was cooked, the rice was allowed to stand for 10 min with the rice cooker in warming mode. It was then immediately moved to a sterile tray for rapid precooling to -20°C. The purpose of precooling was to minimize bacterial contamination and water activity in the rice). After the rice returned to room temperature, the middle layer of the rice was removed from the tray and placed in sterile food-grade plastic bags. Each bag contained 107 g of rice with 50 g of available carbohydrates. Subsequently, the bags were stored at 0°C, 4°C, and -20°C for 1, 3, and 5 days. Prior to the rice being served to the subjects, the bags were returned to room temperature for 2 h and reheated in the rice cooker for 10 min. Each subject was asked to consume glucose three times at the beginning, middle, and end of study to observe the effect of daily variation in glucose tolerance [25]. This procedure was applied in strict compliance with the protocol recommendations described in the ISO guidelines (ISO/FDIS 26642:2010).

2.3 Experimental Procedures

The subjects fasted 10-12 hours overnight and performed the test morning at 8 a.m. in a laboratory. Each subject was randomly fed the test food or reference food (each containing the equivalent of 50 g of available carbohydrates). Blood samples were taken before the subjects ate (at 0 min) and at 15, 30, 45, 60, 90, and 120 min after the meal [24-26]. The blood samples collected were processed as follows. A researcher withdrew intravenous blood (1.5 mL) by inserting a scalp needle into each subject's forearm. When the blood samples were taken, the outer tube was filled with normal saline (0.9%) to prevent the scalp needle from clotting. The collected blood samples were placed

in heparinized tubes and centrifuged further for 3 min at 12,500 ×g at 4°C to separate the plasma. The plasma was subjected to a principle analysis (glucose oxidase and peroxidase) with a biochemical analyzer (YSI 7100 Multiparameter Bioanalytical System, YSI, Yellow Springs, USA) to determine the blood glucose levels. Peak rise was the maximum glucose concentration achieved minus fasting glucose. To calculate the GI values, the trapezoidal rule and incremental area under the 2-hour blood glucose response curve method were used to determine the blood glucose response curve (ignoring the area beneath the fasting concentration). The GI of the test food was the ratio of test food IAUC to the standard glucose IAUC, multiplied by 100 [27].

2.4 Statistical Analysis

Data were first processed using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA) and are presented as the mean ± Standard Error of Mean (SEM). All figures were produced using SigmaPlot Version 12.5 (Systat Software Inc., San Jose, CA, USA). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used for each time point for each sample to observe the difference in the test foods versus the control (white rice). One-way ANOVA with Tukey's post hoc test was performed to observe the between-groups differences. All ANOVA tests were performed using SPSS Version 21.0 (IBM Corp., Armonk, NY, USA).

3. RESULTS

3.1 Postprandial Glucose Responses

Figure 1 shows the glucose responses for the 4°C, 0°C, and -20°C test foods and the control (25°C) on Days 1, 3, and 5. The highest responses curve for the mean glucose concentration was observed at every time point for the control group (25°C), followed by those for the test foods stored at 4°C, -20°C, and then 0°C. The figure also shows the postprandial blood glucose responses of the research subjects after ingesting glucose on Days 1, 3, and 5. The blood glucose concentrations for the 4°C, 0°C, and -20°C test foods peaked at all three time points 45 min (Figure 1). Notably, the postprandial blood glucose concentration for the 0°C test food was significantly lower than those for the 4°C and -20°C test foods at all three time points ($p < 0.05$). Moreover, the postprandial blood glucose concentration for the 0°C test food fluctuated less than those for the 4°C and -20°C test foods, and no significant difference was observed in this concentration between the 4°C and -20°C test foods ($p > 0.05$).

Figure 2 shows scatter plots of all the results for three storage temperatures and durations. Regarding the correlation analysis, the ANOVA results in Figure 2 depict the differences in the glucose concentrations. The largest correlation (R^2) was between the mean storage temperature and glucose concentration on Day 1 ($R^2 = 0.969$), followed by that on Day 3 ($R^2 = 0.899$), and that on Day 5 ($R^2 = 0.814$). All correlations were significant ($p < 0.01$), as determined through a two-tailed Pearson's correlation test. Comparing the glucose concentration difference among the three storage temperatures (Table 1) revealed that the mean ± SEM glucose concentrations of the test and control foods, the control (25°C) had the highest glucose concentration, followed by the -20°C and 4°C test food and finally the 0°C test food (Table 1). The largest mean differences were between the 0°C test food (9.8%) at 45 min and 0°C test food (7.5%) at 60 min. The smallest mean differences were observed between the 4°C (0.6) and -20°C (0.7%) at 0 min.

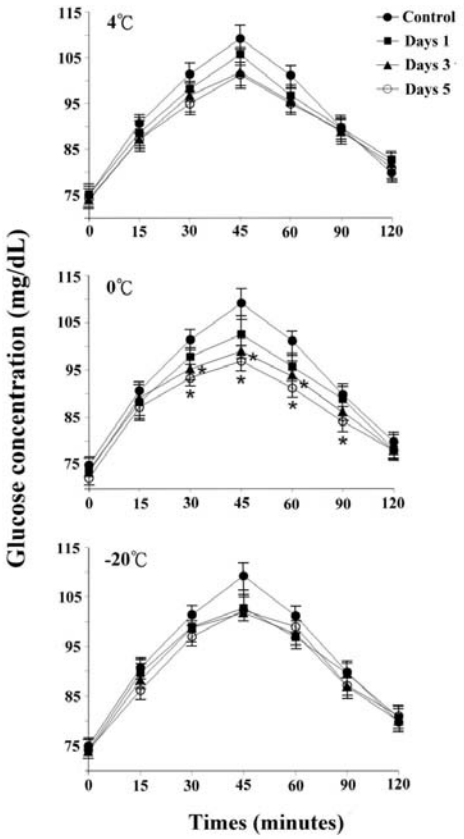

Figure 1. shows the glucose responses for the 4°C, 0°C, and -20°C test foods and the control (25°C) on Days 1, 3, and 5. The asterisks indicate that the values (mean ± SEM, n = 10) differ significantly from the control group values at specific time points (one-way ANOVA, Tukey's post hoc), * Data indicates $p < 0.05$.

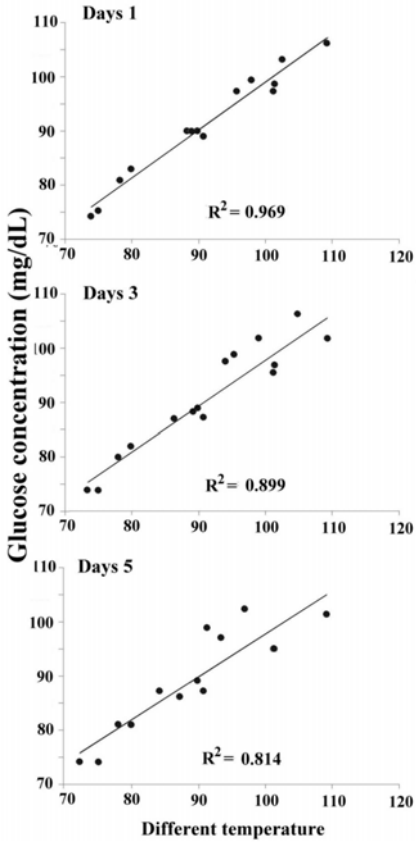

Figure 2. Comparison of the glucose concentrations for three storage temperatures and durations. on Day 1 ($R^2 = 0.969$, $p < 0.01$), on Day 3 ($R^2 = 0.899$, $p < 0.01$), and on Day 5 ($R^2 = 0.814$, $p < 0.01$).

Table 1. Comparing the glucose concentration difference among the three storage and control temperatures.

Time	0	15	30	45	60	90	120
control (25°C)	74.9±3.9	90.6±3.1	101.4±5.1	109.2±4.3	101.1±5.2	89.8±4.8	79.8±1.9
(4°C)	74.3±2.1	87.3±4.1	96.6±5.2	102.9±2.2	95.7±7.9	89.2±1.1	81.9±1.6
(0°C)	74.1±2.2	88.1±3.2	95.5±4.9	99.4±6.4	93.6±4.2	86.4±4.3	78.1±1.8
(-20°C)	74.2±2.3	88.0±2.7	98.2±4.7	102.3±2.3	97.7±5.4	87.9±1.1	80.5±1.6
different with white rice (4°C)	0.6^{ab}	3.3^{a}	4.8^{ab}	6.3^{ab}	5.4^{ab}	0.7^{ab}	-2.1^{ab}
different with white rice (0°C)	0.8^{a}	2.5^{ab}	5.9^{a}	9.8^{a}	7.5^{a}	3.4^{a}	1.7^{a}
different with white rice (-20°C)	0.7^{ab}	2.6^{ab}	3.2^{ab}	6.9^{ab}	3.4^{ab}	1.9^{ab}	-0.7^{ab}

[a-b]Value (mean±SEM) with different letter indicate significant differences as determined by one-way ANOVA with Tukey's post hoc ($p<0.05$).

3.2 IAUC

Table 2 shows the IAUC of all the test foods, as determined by the measurements conducted on Days 1, 3, and 5; specifically, the table shows the mean IAUC for the 4°C, 0°C, and -20°C test foods and the 25°C control. Results with $P < 0.05$ were considered significant. However, the Day 5 measurements for the 0°C test food were significantly lower than those for the 4°C and -20°C test foods on Days 1 and 3.

Table 2. The IAUC differences in storage temperature and storage duration.

	Incremental area under the curve							
	25°C	CV(%)	4°C	CV(%)	0°C	CV(%)	-20°C	CV(%)
Control	2258±191	35.3	2258±191[a]	35.3	2258±191[a]	35.3	2258±191[a]	35.3
Day 1			2032±193[ab]	36.4	2032±191[ab]	36.5	2111±182[ab]	36.2
Day 3			2025±193[ab]	36.9	1881±184[b]	35.9	2025±187[ab]	35.7
Day 5			1935±194[b]	37.1	1806±182[b]	36.1	1995±192[b]	36.7

[a-b]Value (mean±SEM) with different letter indicate significant differences as determined by one-way ANOVA with Tukey's post hoc (p<0.05).

3.3 Glycemic Index

Figure 3 shows the mean GI for the three storage temperatures (4°C, 0°C, and -20°C), control temperature (25°C), and three storage durations (Days 1, 3, and 5). On average, the mean GI of the control food (25°C) was the highest (93.5 ± 3.6), followed by those of the -20°C (87.4 ± 3.1), 4°C (84.1 ± 2.8), and 0°C (84.1 ± 2.7) test foods on Day 1. The mean GI value of the 0°C test food declined progressively from Day 1 (84.1 ± 2.7) to Day 3 (77.9 ± 1.8) to Day 5 (74.8 ± 1.5). The mean GI differed significantly among the three storage temperatures ($p < 0.05$).

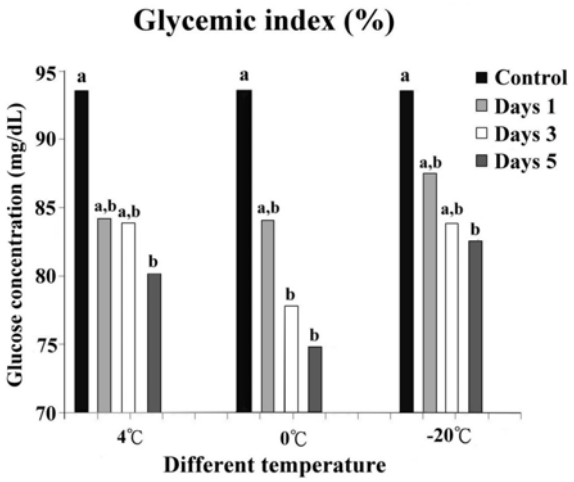

Figure 3. Glycemic index of three storage temperatures (4°C, 0°C, and -20°C), control temperature (25°C), and three storage durations (Days 1, 3, and 5). Values (mean±SEM, n = 10) with different letters are significantly different (one-way ANOVA, Tukey's post hoc, p<0.05). Closed square, Control; light gray square, Days 1; open square, Days 3; gray square, Days 5.

4. DISCUSSION

This primary purpose of this study was to examine the effects of storage temperature (4°C, 0°C, and -20°C) relative to a control group of cooked white rice (25°C) on GI, IAUC, and postprandial blood glucose responses at three time points (Days 1, 3, and 5). Our results show that, among the control and 4°C, 0°C, and -20°C test foods, the 0°C test food exhibited the lowest glucose concentrations, IAUCs, and GIs. Compared with the foods tested on Days 1 and 3, the Day 5 samples had the lowest glucose concentrations, IAUCs, and GIs. However, the control had the highest glucose concentrations, IAUCs, and GIs. Moreover, the largest correlation was between the storage temperature and glucose concentration on Day 1 (R^2 = 0.969), followed by that on Day 3 (R^2 = 0.899), and that on Day 5 (R^2 = 0.814). All correlations were significant ($p < 0.01$). The GI of the test foods compared with that of the control was in the order of -20°C > 4°C > 0°C and rice consumed on Day 1 > Day 3 > Day 5. Under the fasting condition, we observed no significant difference between the blood glucose concentrations at different temperatures and storage durations. However, 2-hour postprandial tests revealed significant differences in the largest mean difference with the 0°C test food (9.8%) at 45 min, and the smallest mean difference with the 4°C test food (0.7%) at 90 min.

Numerous reports have revealed that the retrogradation of starch at low temperatures mainly affects the postprandial glycemic response and GI. For example, Lu et al. [21] used amylose solutions of potatoes to investigate the effects of temperature on the retrogradation of amylose. The results revealed that the retrogradation rate decreased from 58.8% to 7.1% (in 24 h) when the temperature was increased from 5°C to 45°C. In addition, the retrogradation rate varied according to the molecule size. For example, when the amylose solutions were stored at 5°C for 100 days, retrogradation occurred to most amyloses, whereas only amyloses with small molecules exhibited retrogradation for those stored at 45°C. Kelecki et al. [23] evaluated the effects of various temperatures (-60°C, -12°C, 0°C, 4°C, 22°C, and 35°C) on wheat flour tortillas. The results revealed that tortillas stored at ≥12°C retained their fresh attributes for +25 days, and the optimal temperatures for retrogradation were between 4°C and 35°C. However, Shaikh et al. [22], who investigated how to prevent staling in chapatti (an unleavened Indian flat bread made from whole wheat flour, usually consumed immediately after preparation as it becomes hard when stored because of staling), reported dissimilar results. Specifically, to delay staling and retrogradation, enzymes, such as α-amylase, xylanase, and maltodextrin, were added to chapati dough, and the chapatis were stored at 29 ± 1°C and 4 ± 1°C for 10 days. The results revealed that although retrogradation and staling occurred at both storage temperatures, the 4 ± 1°C chapatis exhibited less retrogradation than did the 29 ± 1°C chapatis. The findings of Shaikh et al. [22] indicated that low temperature does not cause retrogradation or staling, which contradicts with the experimental results obtained in the present study. In addition, Ji et al. [28] examined the staling process of Chinese rice cake (*mi gao*). Hardness began to set in on Day 1 of storage; the water content decreased on Day 2, and the hardness increased slightly on Day 3. On Day 5, the rice cake was significantly stale. The findings of Ji et al. [28] are similar to the results of the present study, in which the glycemic response and GI of test foods were reduced significantly on Day 5 of storage.

Storage temperature and duration are crucial factors affecting the retrogradation of starch and quality of starch-based foods. Storing such foods at the appropriate temperature can effectively inhibit retrogradation, thus maintaining food quality. However, the effects of storage duration and temperature vary substantially according to the type of starch food. Studies have revealed that the size, concentration, and temperature of amylose molecules determine the retrogradation rate [21]. in the present study the type of white rice was japonica rice, which has an amylose content of 19.8%. In addition to knowing that heating with water increase the GI of rice starch, however, refrigerating cooked rice alters the molecular structure of the starch, causing rearrangement of the molecules. Moreover, polymerization and recrystallization would also occur between starch chains, and the released amylose molecules link with each other and form an ordered structure that renders the starch more difficult to dissolve, digest, and absorb [29-30]. Because of starch retrogradation from reheating refrigerated rice that produce RS3 Therefore, affecting the glycemic response [31]. In the present study, the 0°C test food exhibited the lowest postprandial blood sugar, IAUC, and GI relative to the control group (cooked white rice at 25°C), and the lowest glucose concentrations, IAUCs, and GIs were observed in the test foods on Day 5. One limitation of the present study is that the longest storage duration for the test foods was only 5 days because of concerns over food safety (e.g., possible *Bacillus cereus* infection).

Studies on the retrogradation of rice have mostly focused on the retrogradation of starch paste. However, the composition of rice grains is complex [32]. Rice is an unevenly distributed complex composed of constituents such as starch, proteins, lipids, vitamins, water, and ash. The center of a rice grain is mostly starch, whereas the outer layer is made of copolymers with multiple constituents with an extremely low ratio of starch [32]. Amylose retrogradation is a short-term process consisting of three stages [33]. At Stage 1, chain extension depends on the fracture of internal supermolecules that maintain the structure of amylose; at Stage 2, the molecules are repositioned because of the loss of bound water; and at Stage 3, hydrogen bonds form between adjacent molecules. The first two stages are exothermic, whereas the final stage is endothermic. The entire process of the retrogradation of amylose paste is endothermic [33].

The glycemic response of rice varies with the storage temperature and duration. In the present study, the 0°C test food exhibited the lowest glucose concentration, IAUC, and GI among the test foods stored at different temperatures, and the test foods stored for 5 days had the lowest glucose concentrations, IAUCs, and GIs (relative to the test foods stored for 1 or 3 days). In addition, the control group (freshly cooked white rice) exhibited the highest glucose concentration, IAUC, and GI. The experimental results reveal that white rice stored at 0°C for 5 days has the lowest glucose concentration, IAUC, and GI. These findings may serve as a reference for developing rice products and relevant practical applications.

5. CONCLUSIONS

The glycemic response of rice varies with the storage temperature and duration. In the present study, the experimental results reveal that white rice stored at 0°C for 5 days has the lowest glycemic response. Eating foods with a lower glycemic index may confer health advantages. Not only can it help you to live healthy, it may reduce the risk of developing diabetes and cardiovascular

disease. In recent years, food choice and eating habits have changed dramatically. In addition to rice products, other instant and convenient rice products prepared for ever-increasing. When more and more people emphasize dietary health, the GI value of rice should be further investigated and known to consumers. Furthermore, it is necessary to develop the strains of rice and consequent processed rice products with low-GI values in a highly technical society. Our findings may serve as a reference for developing fast-food or low-GI emerge and conform to nutrition and health concepts applied to production of rice products, for example, instant rice (rice porridge), puffed breakfast rice grain, rice bread, rice cake and diversified, high-quality and convenient rice products under development.

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