

Original Article

Effect of cooling of cooked white rice on resistant starch content and glycemic response

Steffi Sonia MD¹, Fiastuti Witjaksono PhD¹, Rahmawati Ridwan PhD²

¹Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Department of Biochemistry & Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Cooling of cooked starch is known to cause starch retrogradation which increases resistant starch content. This study aimed to determine the effect of cooling of cooked white rice on resistant starch content and glycemic response in healthy subjects. Resistant starch contents were analyzed on freshly cooked white rice (control rice), cooked white rice cooled for 10 hours at room temperature (test rice I), and cooked white rice cooled for 24 hours at 4°C then reheated (test rice II). The results showed that resistant starch contents in control rice, test rice I, and test rice II were 0.64 g/100 g, 1.30 g/100 g, and 1.65 g/100 g, respectively. Test rice II had higher resistant starch content than test rice I, hence used in the clinical study along with control rice to characterize glycemic response in 15 healthy adults. The clinical study was a randomized, single-blind crossover study. In the clinical study, test rice II significantly lowered glycemic response compared with control rice (125±50.1 vs 152±48.3 mmol.min/L, respectively; $p=0.047$). In conclusion, cooling of cooked white rice increased resistant starch content. Cooked white rice cooled for 24 hours at 4°C then reheated lowered glycemic response compared with freshly cooked white rice.

Key Words: cooling, rice, resistant starch, glycemic response, retrogradation

INTRODUCTION

In the past 3 decades, glycemic index (GI) and, later, glycemic load (GL) have been used to quantify postprandial glycemia (glycemic response) induced by various foods.¹ These two concepts (GI and GL) are primarily used to guide patients with diabetes mellitus (DM) in choosing foods. Lower GI/GL foods are considered to benefit diabetic patients because they induce lower glycemic responses, thereby maintaining blood glucose levels as normal as possible. A meta-analysis by Livesey et al² suggested that lower GI diets may reduce fasting blood glucose levels and glycated protein levels. Another meta-analysis by Barclay et al³ suggested that lower GI/GL diets may also be useful in the prevention of type 2 DM, a type of DM characterized by insulin resistance and relative lack of insulin secretion.

White rice is a staple food in many Asian countries. There has been a belief that yesterday's rice (cooked rice which has been stored overnight) is better than freshly cooked rice for diabetic patients. Theoretically, this belief can be explained by the starch retrogradation process that occurs during storage or cooling of cooked rice. This process makes some of the starch in cooked rice resistant to digestion (resistant starch [RS]), hence not absorbed in the small intestine.⁴ Therefore, yesterday's rice may result in lower glycemic response compared with freshly cooked rice.

Retrogradation rate and formation of RS can be increased by higher amylose-amylopectin ratio and storage at 1-25°C.⁵ Retrograded amylose is heat stable up to 117-

125°C before it changes back to being digestible, meanwhile retrograded amylopectin changes back at 40-60°C.⁵

Frei et al⁶ reported that cooling of cooked rice for 24 hours at 4°C reduced starch digestibility *in vitro* and estimated GI. This is supported by Ananda et al⁷ who reported decreased glycemic response *in vivo* after cooling of cooked white rice for 10 hours at 3°C. Conversely, Dewi and Isnawati⁸ reported that cooling of cooked white rice for 24 hours at 4°C followed by reheating had no effect on postprandial blood glucose levels. This difference in study results was probably caused by reheating which changed some RS formed during cooling of cooked rice back into digestible starch. Cooling of cooked rice at low temperature tends to harden the rice and reheating is necessary to soften it. On the other side, there has not been any study using cooked white rice cooled at room temperature.

This study first compared RS contents in freshly cooked white rice, cooked white rice cooled for 10 hours at room temperature, and cooked white rice cooled for 24 hours at 4°C then reheated. One of the two types of

Corresponding Author: Dr Steffi Sonia, Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Salemba Raya 6, Jakarta, 10430 Indonesia.

Tel: +62815-983-1718; Fax: +6221-660-0078

Email: steffisonia@gmail.com; steffishalim@yahoo.com

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cooled rice studied with a higher RS content was then selected for use in the clinical study along with freshly cooked white rice to find a difference on glycemic response. Subjects used were healthy subjects, because glycemic response ratios in healthy subjects and diabetic subjects are similar, and healthy subjects result in better precision.⁹ The objectives of the present study were to determine the effect of cooling method on RS content of white rice and to assess the impact of cooling on glycemic response in healthy subjects.

MATERIALS AND METHODS

Rice content analysis

Variety of rice used was IR-64, grown and harvested in Bandung, Indonesia. The rice was machine milled to remove its husk, bran, and germ, producing white rice. To prepare freshly cooked white rice (control rice), 4 cups of rice (± 600 mg) were washed, combined with about 750 mL water until the 4 cups marker inside the rice cooker bowl (Philips[®] HD-4502), and cooked in the rice cooker (up to $\pm 100^\circ\text{C}$, 22 minutes) until it turned to warm mode automatically. Then, the cooked rice was left in the rice cooker in warm mode for 15 minutes and was mixed evenly before use. Cooked white rice cooled for 10 hours at room temperature (test rice I) was prepared by storing control rice at room temperature ($\pm 27^\circ\text{C}$) for 10 hours. Cooked white rice cooled for 24 hours at 4°C then reheated (test rice II) was prepared by cooling control rice in the refrigerator at 4°C for 24 hours. Reheating of test rice II was conducted by cooking the 24 hours cooled rice combined with 240 mL water in the rice cooker until it turned to warm mode automatically (about 15 minutes). The reheated rice was left in the rice cooker in warm mode for 15 minutes and was mixed evenly before use. Rice contents were analyzed immediately after preparation.

Control rice was analyzed for carbohydrate, protein, fat, ash, total starch, and amylose content. All three types of rice were analyzed for water and RS contents. Carbohydrate content was determined using by difference method.¹⁰ Protein content was analyzed using Kjeldahl method.¹¹ Fat content was analyzed using Soxhlet method.¹¹ Ash content was analyzed using direct/dry method.¹¹ Total starch content was analyzed using phenol sulphate method.¹² Amylose was analyzed using iodometry method.¹⁰ Water contents were determined using oven method.¹¹ RS contents were analyzed using the method by Kim et al.¹³ All analyses, except carbohydrate content, were performed in duplicate. Means of two values obtained from analyses were used as the results. Based on the RS content analysis, the test rice with a higher RS content was selected along with control rice for use in the clinical study.

Clinical study

The clinical study complied with the provision of the Declaration of Helsinki and was approved by the Health Research Ethics Committee of Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo Hospital (ethical approval no. 310/H2.F1/ETIK/2014). Methods of determination of glycemic response were adapted from FAO's methods of determination of glycemic index⁴ with some modifications from Brouns et al.⁹ Fifteen healthy

adults (5 men and 10 women) were recruited from the Department of Nutrition, Faculty of Medicine, Universitas Indonesia in Jakarta and nearby communities. Inclusion criteria included: (1) healthy, (2) age between 20 and 40 years old, (3) able to read and write. Exclusion criteria included: (1) under any medication(s), (2) fasting plasma glucose ≥ 100 mg/dL, (3) body mass index < 18.5 kg/m² or ≥ 25 kg/m², (4) history of DM or impaired glucose tolerance, (5) pregnant or lactating, (6) history of white rice or egg allergy. Written consent was obtained from subjects after a full explanation of objectives, methods, and risks of the study. All subjects finished the study.

The study was a randomized, single-blind crossover study. Two types of rice were used in the study: control rice and one of the test rice with the higher RS content. Each subject attended two breakfast sessions, one with control rice and the other with the high RS test rice. The sessions were set at least two days apart from each other. Subjects were instructed to have dinner between 6 to 10 pm before each session. Subjects were also instructed to have a meal of choice for the dinner before the first session and to repeat that meal for the dinner before the second session. All food and beverages eaten during dinner before each session were recorded by subjects. After 10 pm before each session, subjects were allowed to drink water only. After 6 am before each session, subjects were not allowed to eat or drink anything until breakfast was served. Subjects were also instructed to avoid unusual vigorous physical activity starting one day before each session. Smoking was not allowed on the day of each session.

The type of rice given at the first session was randomized for subjects in blocks of four, and subjects were not informed of which type of rice being served at each session. At each session, subjects consumed 125 g rice, 60 g standard omelette and 240 mL water. Freshly cooked or reheated rice was served warm, immediately after preparation was done. Breakfast started between 8 to 8:30 am and all food and beverage had to be finished in no less than 10 minutes and no more than 15 minutes, with relatively constant rate of consumption.

Blood glucose measurements were conducted using Accu-Chek[®] Active glucometer at time 0 (time of the first bite of food) and 15, 30, 45, 60, 90, and 120 minutes after that. Incremental area under the blood glucose response curve (IAUC) was calculated.

Subject acceptability survey was assessed with a hedonic scale.¹⁴ The subjects answered the following question at each session: "Which statement corresponds with your opinion on the rice served?" 1=Dislike extremely, 2=Dislike very much, 3=Dislike moderately, 4=Dislike slightly, 5=Neutral, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely.

Dietary intake data at dinner before each session was collected and analyzed using the NutriSurvey 2007 software with added Indonesian food database. Total energy, carbohydrate, protein, fat, and dietary fiber were analyzed.

Statistical analysis

Data were analyzed with SPSS (version 20). Results with normal distribution are presented as mean \pm SD. Results with abnormal distribution are presented as median (min-

imum–maximum). Dietary intake at dinner, blood glucose levels, IAUC, and subject acceptability scores were compared using paired *t*-test if normally distributed or Wilcoxon test if abnormally distributed. Significant differences were determined at $p < 0.05$.

RESULTS

Rice content analysis

Results of control rice content analysis can be seen in Table 1. Based on the results, amylose content in the rice studied was 25.6% of dry matter. Water contents in the three types of rice were similar (differences $< 10\%$), meanwhile RS content in each type of rice differed from each other (Table 2). Between the two types of test rice, test rice II had a higher RS content, hence was used in the clinical study along with control rice.

Clinical study

Subject characteristics are shown in Table 3. Intake of energy, macronutrients, and dietary fiber at dinner prior to breakfast sessions did not differ between treatments (Table 4). Based on blood glucose level data, one subject caused several extreme values (> 2 SD above mean) during one breakfast session. The subject admitted unusual vigorous physical activity about 30 minutes before start of the session. Therefore, all of the subject's blood glucose levels and IAUC data was considered invalid and excluded from analysis.

Blood glucose levels at 0 min, 30 min, 90 min, and 120 min did not differ significantly between treatments (Table 5, Figure 1). Blood glucose level at 15 min after test rice

II ingestion was significantly higher than that after control rice ingestion. On the contrary, blood glucose levels

Table 1. Energy, carbohydrate, protein, fat, ash, water, total starch, and amylose content in control rice

	Result
Energy (kcal/100 g) [†]	173
Carbohydrate (g/100 g)	34.0
Protein (g/100 g)	3.9
Fat (g/100 g)	2.3
Ash (g/100 g)	0.09
Water (g/100 g)	59.6
Total starch (g/100 g)	31.6
Amylose (g/100 g)	10.4

[†]Calculated using the formula: energy (kcal/100 g) = carbohydrate (g/100 g) × 4 kcal/g + protein (g/100 g) × 4 kcal/g + fat (g/100 g) × 9 kcal/g

Table 2. Water and resistant starch content in control rice, test rice I, and test rice II

	Control rice	Test rice I	Test rice II
Water (g/100 g)	59.6	58.6	59.9
RS (g/100 g)	0.64	1.30	1.65

Table 3. Subject characteristics (n=15)

	Mean ± SD
Age (year)	30.6 ± 5.2
Body mass index (kg/m ²)	22.2 ± 1.8
Fasting plasma glucose level (mmol/L)	4.97 ± 0.32

Table 4. Intake of energy, carbohydrate, protein, fat, and dietary fiber of subjects at dinner prior to breakfast session (n=15)

	Control rice	Test rice II	<i>p</i>
Energy (kcal)	638 ± 321	663 ± 351	0.511 [†]
Carbohydrate (g)	77.6 ± 39.9	81.8 ± 49.3	0.548 [†]
Protein (g)	20.1 ± 7.2	20.2 ± 7.6	0.915 [†]
Fat (g)	23.4 (0.0–67.0)	28.8 ± 19.6	0.343 [‡]
Dietary fiber (g)	3.3 (0.0–12.1)	4.3 ± 3.3	1.000 [‡]

Values are presented as mean ± SD or median (minimum–maximum)

[†]Result of paired *t*-test

[‡]Result of Wilcoxon test.

Table 5. Blood glucose levels, incremental area under blood glucose response curve (IAUC), and subject acceptability score

	Control rice	Test rice II	<i>p</i>
Blood glucose levels (mmol/L)			
0 min	4.84 ± 0.29	4.84 ± 0.32	0.950 [†]
15 min	5.21 ± 0.56	5.40 ± 0.49	0.039 [†]
30 min	7.18 ± 0.58	7.21 ± 0.60	0.800 [†]
45 min	7.23 ± 0.92	6.72 ± 0.97	0.042 [†]
60 min	6.49 ± 1.03	5.83 (5.33–7.10)	0.037 [‡]
90 min	5.70 ± 0.48	5.44 ± 0.35	0.051 [†]
120 min	5.57 ± 0.41	5.33 (5.16–6.05)	0.238 [‡]
IAUC (mmol.min/L)	152 ± 48.3	125 ± 50.1	0.047 [†]
Subject acceptability score	7 (3–9)	6.3 ± 1.4	0.190 [‡]

n=14 for blood glucose levels and IAUC, n=15 for subject acceptability score; values are presented as mean ± SD or median (minimum–maximum).

[†]Result of paired *t*-test.

[‡]Result of Wilcoxon test.

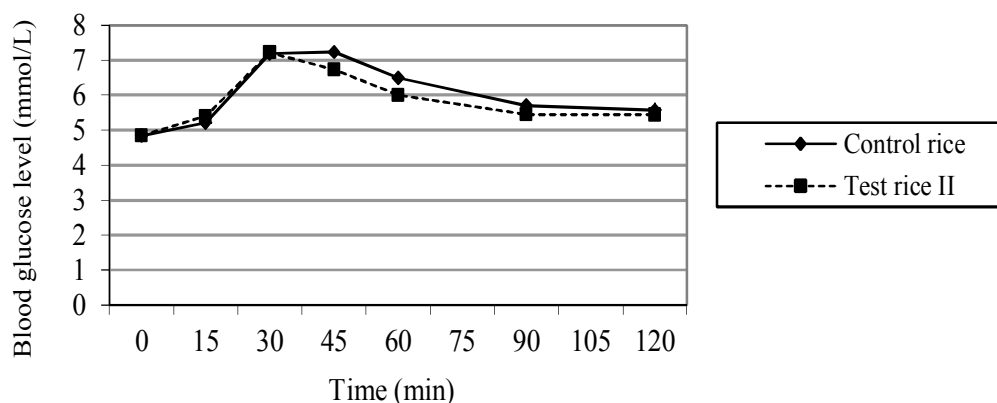


Figure 1. Mean blood glucose levels in response to rice ingestion over time.

at 45 min and 60 min after test rice II ingestion were significantly lower than those after control rice ingestion. Two hours IAUC after test rice II ingestion were also significantly lower than that after control rice ingestion. IAUC difference (mean \pm SD) obtained was 26.3 \pm 44.8 mmol.min/L. Subject acceptability scores did not differ between treatments. No side effect was reported by subjects.

DISCUSSION

RS content is affected by amylose-amylopectin ratio and methods of food processing.¹⁵ Amylose content of the rice used in the present study (25.6% dry matter) was a little higher than amylose content of IR-64 rice in literature (24% dry matter).¹⁶ Higher amylose content may increase amylose-amylopectin ratio and increase starch retrogradation rate.⁵

Water contents in the three types of rice studied were quite similar, thus resulting in little effect on the proportions of other components. Cooling and storage of gelatinized starch allow starch retrogradation which makes some of the starch resistant to digestion (RS type 3).¹⁵ This corresponds to the present study which found higher RS content in both of the test rice compared with control rice. Chung et al¹⁷ also reported that cooling gelatinized waxy rice starch at 4°C increased RS content over time until day-7. Retrogradation is optimal at 1-25°C and longer storage time allows more retrogradation to occur.⁵ This is why test rice II contained more RS than test rice I. Test rice I could not be stored longer than it was because storing rice at room temperature raises the risk of food poisoning from bacterial overgrowth over time.

Test rice II was reheated before served because cold storage of rice makes its texture hard and unpleasant to eat. The reheating method of test rice II was chosen through trial and error to obtain rice with a similar texture to control rice. Retrograded amylopectin melts above 40-60°C,⁵ which is below the reheating temperature. However, retrograded amylose melts above 117-125°C.⁵ Despite the reheating process, test rice II had more RS content than test rice I.

Dietary intake at dinner prior to breakfast sessions was proven to be similar between treatments. This excluded any effect of dinner on blood glucose levels and IAUC at breakfast sessions. Meals at dinner, especially high dietary fiber foods, were known to affect glycemic response

at breakfast.¹⁸

Fasting blood glucose level (0 min) in the remaining 14 subjects didn't differ significantly between treatments. This demonstrated that the results of the remaining blood glucose level measurements and also IAUC obtained were suitable for comparison.

Blood glucose level at 15 min was higher after test rice II ingestion compared with that after control rice ingestion. This was probably due to the different rate of ingestion between treatments. Although instructed to eat at a relatively constant rate and to finish all food and beverage in no less than 10 minutes, some of the subjects ate too fast at first and then slower after being reminded not to finish eating in less than 10 minutes. Heine et al¹⁹ reported that ingestion of 75 g glucose in 1 minute produced earlier glucose response compared with ingestion of the same amount of glucose in 10 minutes. Another alternative reason was that test rice II consisted of smaller fragments of rice due to more mixing in its preparation process, making it faster to digest.

The blood glucose levels at 45 and 60 min after ingestion of test rice II were significantly lower compared with control rice. The blood glucose levels at 90 and 120 min after ingestion of test rice II also tended to be lower compared with control rice. These decreases in blood glucose levels contributed to the decrease in IAUC after ingestion of test rice II compared with control rice. Ananda et al⁷ also reported lower blood glucose levels at 45 through 120 min and significantly lower IAUC after ingestion of cold cooked white rice (cooled for 10 hours at 3°C) compared with warm cooked white rice (freshly cooked). Dewi and Isnawati⁸ found lower postprandial blood glucose levels after ingestion of yesterday's rice (cooled for 24 hours at 4°C and then reheated) compared with freshly cooked rice, although the differences were not statistically significant.

The lower blood glucose levels and IAUC after test rice II compared with control rice found in this study were most probably due to lower available carbohydrate content in test rice II. The higher RS content in test rice II decreased its available carbohydrate content. RS cannot be digested and absorbed in the small intestines, which classifies it as unavailable carbohydrate.⁴

Subjects' opinion about control rice and test rice II did not differ significantly. This allows a long term application of high RS test rice II in everyday diet. Kwak et al²⁰

reported that consumption of 6.51 g RS as a supplement everyday for 4 weeks improved endothelial function, decreased postprandial glucose level, and decreased oxidative stress in prediabetic or newly diagnosed type 2 DM subjects. RS also functions as prebiotic and its consumption may generally improve colonic health.²¹

A limitation of the present study is that there was no rice consumption trial by subjects which caused the subjects to eat at unsteady rates. In addition, blinding of subjects to which type of rice being served might not succeed because some of them were able to differentiate the rice based on experience. Despite the effort to make test rice II similar to control rice, the control rice was relatively stickier than test rice II.

Conclusion

This study demonstrated that cooling of cooked white rice increased its RS content. Cooked white rice cooled at 4°C for 24 hours then reheated had higher RS content than cooked white rice cooled at room temperature for 10 hours. In the clinical study, ingestion of cooked white rice cooled at 4°C for 24 hours then reheated produced lower glycemic response compared with ingestion of freshly cooked white rice at the same portion. Cooked white rice cooled at 4°C for 24 hours then reheated was also accepted nearly as well as freshly cooked white rice. Therefore, changing freshly cooked white rice to cooked white rice cooled at 4°C for 24 hours then reheated can be recommended for diabetic patients in everyday diet.

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AUTHOR DISCLOSURE

The authors declare no conflict of interest.

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Original Article

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Steffi Sonia MD¹, Fiastuti Witjaksono PhD¹, Rahmawati Ridwan PhD²

¹Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Department of Biochemistry & Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

熟白米饭冷却对抗性淀粉含量和血糖应答的影响

煮熟的淀粉冷却会导致淀粉老化，导致抗性淀粉含量增加。该研究旨在确定冷却煮熟的白米饭抗性淀粉含量和对健康人群血糖水平的影响。分析刚煮熟白米饭（对照大米）、白米饭常温冷却 10 小时（试验大米 I）和白米饭在 4°C 中冷却 24 小时后再加热（试验大米 II）3 种状态下抗性淀粉含量。结果表明：对照大米、试验大米 I 和试验大米 II 抗性淀粉含量分别为 0.64 克/100 克、1.30 克/100 克和 1.65 克/100 克。试验大米 II 比试验大米 I 抗性淀粉含量高，因此在临床研究中，用对照大米作对照，研究了 15 名健康成人血糖对试验大米 II 的应答。该临床研究是一项随机、单盲交叉的研究。在临床研究中，试验大米 II 比对照大米能显著降低血糖应答（ 125 ± 50.1 vs 152 ± 48.4 mmol.min/L， $p=0.047$ ）。总之，冷却的熟白米饭增加抗性淀粉含量。与刚煮熟的白米饭相比，煮熟的白米饭在 4°C 中冷却 24 小时然后加热，能降低血糖应答。

关键词：冷却、大米、抗性淀粉、血糖应答、老化