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Pandan Wangi rice as reference food and the use of 25g portion in glycemic index test

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ABSTRACT

The notion of the Glycemic Index (GI) can be utilized to aid in the choosing of foods for a healthy diet, leading to low GI claims that have been commonly found in various food products. The convenience of the test subject could affect GI results, such as the type of reference food and how much of it to be consumed. Therefore, the goals of this research were to see if white rice (Pandan Wangi) could be used as a reference food and to compare GI result obtained from a different portion of available carbohydrate. The recruited subjects ranged in age from 21-36 years old with BMI of 18.5-24.9 kg/m^2). Glucose measurement and sample testing (white rice and cookies) were conducted in the experiment. Overnight fasting was required of volunteers, and blood samples were obtained using One Touch Ultra Lifescan. There were seven points of blood sampling with triplication of glucose testing GI values were calculated with Incremental Area Under Curves (IAUC). The result means of GI were 74±16 (white rice), 60±24 (wheat cookies), 38±14 (NS-cookies), and 39±16 (HMT-cookies). The correlation between IAUC glucose and rice was significant with r=0.834 (n=10, p<0,01). GI of white rice based on 25g available carbohydrates (AVCHO) was significantly different from GI based on 50g AVHCO (n=10, p<0.05). Pandan Wangi white rice can be used to substitute glucose or white bread in GI tests with a conversion factor of 0.74. It needed further study regarding the use of 25g AVCHO as the basic portion in GI test.

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Keywords: glycemic index, Pandan Wangi, reference food, test portion

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INTRODUCTION

Sc et al. (1981) familiarized the Glycemic Index (GI) concept in measuring the role of carbohydrates in food in its effect on postprandial blood glucose response. GI was defined by the Food and Agriculture Organization (FAO) (1998) as the incremental area under the curve (the blood glucose response) of a 50 g carbohydrate of a test food, expressed as a percent of the response to the same amount of carbohydrate from a standard ingested by the same person. The GI concept can be utilized to help with the food selection in a healthy diet. Low-GI food has been known to positively affect human health, such as improvement in blood glucose control (Bouché et al., 2002; Gilbertson et al., 2001; Liu et al., 2000), and reduce coronary heart disease (Barakatun et al., 2009). The calculation of GI value should become a concern because of low GI claims that are regularly encountered in various food products.

White bread or glucose, according to the FAO, can be used as a reference food. Other reference foods could be utilized. However, the new reference food's GI relative to standardized glucose or white bread should be investigated. In Western cultures, white bread is their staple food, though, for Eastern cultures, predominantly Asian, the staple food is rice. It showed a possibility of white rice as a reference food in GI determination. Both FAO (1998) and Badan Pengawas Obat dan Makanan (BPOM) (2011) recommend that the food tested in GI determination should contain 50g of available carbohydrate (AVCHO). However, using 50 g of AVCHO in low density product like rice could result in enormous amounts of AVCHO being consumed. Therefore, the goals of this research were to see if white rice (Pandan Wangi) could be used as a reference food and to compare GI result obtained from a different portion of AVCHO. Pandan Wangi type of rice was used for the reason that the main component that forms the aroma of pandan in Pandan Wangi rice is a component that is identical to that of the aroma producer in pandan leaves, namely 2-acetyl-1-pyrolline (Nurjaya & Maulida, 2018) and findings that Pandan Wangi leaf water extract has the potential to reduce blood glucose levels in diabetic rats (Prameswari & Widjanarko, 2014).

MATERIALS AND METHODS

Materials

Pandan Wangi rice (Upper Right Cianjur, PT Midi Utama Indonesia, Tbk), wheat flour (Bogasari, Indonesia), native arrowroot starch (NS) (Kelompok Wanita Tani Yogyakarta, Indonesia), and HMT-modified arrowroot starch were employed in this study. The reference food as glucose/dextrose monohydrate (Qinhuangdao Lihua Starch CO, China). Blood was drawn using glucometer, lancets, and strips from One Touch Ultra Lifescan (Johnson & Johnson Company, USA).

Subjects

The researchers looked for people who were healthy, did not have diabetes mellitus, were not pregnant, did not smoke, were between the ages of 21 and 36, and had normal BMI (Body Mass Index). BMI was computed by dividing weight (kg) by height (m^2). Ten people had given their informed consent to take part in the study. The Ethics Committee of the Indonesian Ministry of Health granted approval (LB.02.01/5.2/KE.142/2014).

Methods

Modification of arrowroot starch

By spraying water on native arrowroot starch, the moisture content was increased to 20%. The following equilibrium wet mass calculated the amount of water. Stirred starch with a moisture level of 20% wb was saved in a HDPE (high density polyethylene) plastic pouch. To homogenize moisture content, the starch was left at room temperature for one night. After that, wet starch was treated with HMT in an autoclave at 121^0C for 15 minutes. In addition, HMT-Modified Arrowroot Starch was dried for 2 hours at 50°C in a tray dryer.

Production of rice and cookies

The type of rice used in this research was Pandan Wangi. Rice was boiled with water to rice ratio of 3: 1 in a rice cooker. Rice was prepared on the same day as the test. Wheat, native arrowroot starch, and HMT-modified arrowroot starch were used to make cookies. The cookies were made with 57.04% flour/starch/modified starch, 5.13% fine sugar, 5.13% palm sugar, 23.30% margarine, 4.24% skim milk powder, 4.97% yolk, 0.14% salt, 0.02% baking soda, and 0.02% vanilla. The process of making cookies was started with mixing margarine, fine sugar, and palm sugar. Palm sugar was used because it has been proven that GI of the mixture of bread using palm sugar as the sweetener was lower than using sugarcane (Srikaeo & Thongta, 2015). The yolk was added to the mixture and mixed for 2 minutes. Flour or starch, salt, baking soda, vanilla essence and skim milk powder were added into the mixture and mixed in 8 minutes. The dough then was sheeted in flat-round shape and baked in oven at 170°C in ±15 minutes. Cookies were placed in the sealed plastic jar until used.

Analysis

We performed proximate dietary fiber, resistant starch, and glycemic index (in vivo test) analysis in this research. Proximate analysis was measured by (AOAC, 2012), including water content (925.10), protein (960.52), fat (920.39), ash (923.03), and carbohydrate (by difference).

An in vitro method based on the procedure of (AOAC, 2012) was adopted to determine dietary fiber (DF). To measure any contribution from reagents to residue, a blank was conducted through the entire procedure alongside test portions. Blank was processed through the entire procedure along with test portions to detect any contribution from reagents to the residue. Samples containing >10% fat were extracted using soxhlet extraction with hexane as the solvent. Duplicate fat-free test portions were weighed 1 g and inserted into a glass beaker, then 50 mL of phosphate buffer pH 6 (0.1 M) was added. Termamyl (Sigma A3403, Sigma-Aldrich, USA) 100 mL was added and closed with aluminum foil, and incubated at a temperature of 100ºC for 15 minutes. Solutions were cooled to room temperature, then prepared to pH 7.5 by adding 10 mL 0.275M NaOH and 100 mL of protease solution (Sigma P4630, Sigma-Aldrich, USA), incubated at 60°C for 30 minutes in a water bath shaker. The solution was made from pH 4.0 to 4.6 by adding 10 ml of HCl and 300 mL amyloglucosidase (Sigma A9913, Sigma-Aldrich, USA) at 0.325M, incubation at 60 ° C for 30 minutes in a water bath shaker. 280 mL Ethanol 95%, which had previously been heated at a temperature of 60°C, was added into the solution and settled at room temperature for 60 minutes. The solution was filtered using a vacuum filter and washed three times with 20 ml ethanol 78%, two times with 10 mL ethanol 95%, and twice with 10 mL acetone. The residue was then dried overnight at a temperature of 105ºC. One duplicate was analyzed for protein residue, and the other was analyzed for ash residue. The total DF of the samples were calculated using the following formula.

$$\text{TDF (\%)} = \frac{\text{WR}-\text{P}-\text{A}-\text{B}}{\text{WT}}\ x\ 100$$

WR : average of weight of residue from duplicate samples (mg)
P : protein (mg)
A : ash (mg)
B : weight of blank – weight of blank protein – weight of blank ash
WT : weight of test portion (before tested)

Resistant starch analysis was conducted with an *in vitro* method based on the procedure of Goñi et al. (1996). This was adopted to determine Resistant Starch (RS) with slight modification in buffer and glucose calculation. Samples containing fat ≥5% were defatted using Soxhlet extraction with hexane as the solvent. Fifty mg of food portion were passed through an 80-mesh filter and were placed into a centrifuge tube. Ten mL HCI-KCI buffer (pH l.5) was added. Then 200 µL pepsin solution (4000U/10mL HCI-KCI buffer, Sigma P7000, Sigma-Aldrich, USA) was added to each sample. After

that, the solutions were incubated at 40°C for 1 hour in a water bath shaker. Phosphate buffer (pH 6.9) was added to reach pH 6-7.

One mL α-amylase solution (400U α-amylase per mL buffer phosphate, Sigma 10065, Sigma-Aldrich, USA) was added to each centrifuge tube and incubated for 16 h in a water bath at 37°C with constant shaking. Samples were then centrifuged (3000 g) for 15 minutes, and the supernatant was discarded. Samples' residues were washed with 10 mL distilled water twice, centrifuged again, and discarded. Residues were added with 1.5 mL distilled water and 1.5 mL 4M KOH, mixed, and left for 30 min at room temperature with constant shaking. Aliquots were added with 2.75 mL 2M HCl and 1.5 mL of 0.4 M sodium acetate buffer (pH=4.75). Then 80µL of amyloglucosidase was added to the aliquots and incubated in a water bath for 45min at 60°C with constant shaking. Aliquots were then centrifuged (3000 g) for 15 minutes, and supernatants were collected and saved in a volumetric flask. Residues then were washed with 10 mL distilled water at least once, centrifuged again, and combined supernatants with that obtained previously. Volume was adjusted to 10-l00 mL with distilled water. Then, 0.5 mL of the solution was tested with phenol sulphuric acid to get the glucose content. The glucose was converted into starch by multiplying for 0.9.

Lastly, the glycemic index was done. Glucose (dextrose monohydrate) was used as the reference food. The portion consumed by subjects is AVCHO portion (total carbohydrate minus DF). In the examination of the feasibility of white rice as a reference food, experiments were carried out involving three times (triplication) for glucose testing and once for each sample (white rice and cookies). Meanwhile, in the comparison of different AVCHO portions, experiments were carried out involving single glucose testing and triplication of sample testing (Pandan Wangi rice). Ten subjects were instructed to fast overnight for 10-12 hours. Within 12 minutes, glucose or samples containing 50 g or 25 g AVCHO were consumed with 200 mL of water. Using the One Touch Ultra Lifescan (Johnson & Johnson Company, USA), blood samples were taken at 15, 30, 45, 60, 90, and 120 minutes after the samples were consumed. The tests were carried out over a period of at least three days. The GI values were determined using Incremental Area Under Curves (IAUC) of the blood glucose curve of samples and compared to the GI values of reference food, ignoring the area below the fasting concentration.

$$\mathrm{GI} = \frac{\text{IAUC of test sample } \times 100}{\text{IAUC of reference food}}$$

Data Analysis

To calculate the GI, one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test (p<0.05). Pearson test (p<0.05) was used to examine the correlation between IAUC from both reference foods. SPSS® version 20 was utilized as the statistical software.

RESULT AND DISCUSSION

As stated in FAO/WHO (1998) and BPOM (2011), 50 g of AVCHO should be present in the portion of food tested. Each subject had to consume 50 g AVCHO or equal to one portion of 146 g white rice / 87 g wheat cookies / 74 g NS-cookies / 78 g HMT-cookies (Table 1). Glycemic index for white rice, wheat cookies, NS-cookies, and HMT-cookies are 74±16, 60±24, 38±14, and 39±16, respectively. The Glycemic index of HMT-cookies and NS-cookies were not significantly different (n=10, p>0,05). It can be caused by resistant starch content on cookies obtained from HMT modification was measured as DF. Thus, the effect of resistant starch did not reflect in GI since the method used AVCHO as the basis portion, which was calculated as total carbohydrate minus DF.

Table 1. Composition of samples

	Cooked Pandan Wangi Rice	Wheat Cookies	NS Cookies	HMT Cookies
Moisture Content (%)	59.31	5.26	4.76	4.15
Ash (%)	0.11	0.85	0.85	1.64
Protein (%)	3.95	6.98	3.44	1.53
Fat (%)	0.98	22.85	22.02	23.29
Carbohydrate (*by diff*) (%)	35.65	64.06	68.93	69.39
Dietary Fiber (%)	1.33	6.51	1.60	5.20
AVCHO (g/portion)	50	50	50	50
Resistant Starch (g/portion)	0.22	2.22	1.17	2.71
Portion (g)	146	87	74	78

NS: Native Starch. HMT: Heat moisture Treatment, AVCHO: Available Carbohydrates

Cooked Pandan Wangi rice contains 1.33% dietary fiber (DF), higher than white-polished rice stated in ASEAN Food Composition Database, which includes 0.61% DF (Institute of Nutrition, 2014). The difference observed in the results might be due to the different types of rice. Low DF in white rice can be caused by de-husking and milling, in which vitamins and fiber were removed. Dietary fiber in wheat cookies was higher than NS-cookies and HMT-cookies might be caused by the higher DF in wheat flour compared to arrowroot starch. In starch processing, there is an extraction process that may decrease the DF value of starch (Mustafa, 2016).

The aims of classical GI concept is to control the glycemic response without reducing AVCHO intake (Sc et al., 1981). AVCHO is carbohydrates that can be digested, absorbed, and metabolized. With this concept and AVCHO definition, resistant starch (RS) obtained from starch modification should be excluded from the basis portion since the increase of RS content reduce the AVCHO in starch. For products rich in RS or DF, it can be questioned whether to use AVCHO portion or whole meal portion. For example, to test a rich DF jelly products using AVCHO portion, it can be imagined how much jelly should be consumed by subject to achieve 50 g AVCHO. Therefore, GI testing should be performed on AVCHO rich product, and there should be a consideration of the food characteristics. To know the functional effect of the addition of the DF or the increase in RS, the test needs to be conducted separately and claimed separately as well. Comprehensive research and discussion about the classical concept of GI and the problems raised in the GI method are needed.

It can be seen in Table 2, IAUC glucose and rice had a significant correlation with r=0.834 (n=10, p<0,01). Because of that high correlation, white rice (Pandan Wangi) could be used as a reference food in GI test. Sugiyama et al. (2003) tested Satou rice, local Japanese rice, as a reference food and found a similar correlation between Satou rice and glucose (r=0.853, n=10, p<0,002). It is shown in Table 3 that the mean GI of Pandan Wangi white rice based on triplicate 50g glucose trials was 74±16, while Satou rice in (Sugiyama et al. (2003) was 80.

Rice has become the staple food in Eastern culture. FAO increased its prediction for global paddy production in 2017 by 2.9 million tonnes to 759.6 million tonnes (503.9 million tonnes, milled basis). At that level, worldwide paddy output would be 0.6 percent higher than the previous high of 2016. Much of the expected growth would be the reflection of improved yields and would be concentrated in Asia (FAO, 2018). It shows that in Asian culture, rice is more available than white bread. In GI test, the subject is asked to consume 50g AVCHO of glucose or white bread. In an attempt to make the subjects feel more comfortable consuming certain grams of reference food, then the reference food should be palatable. Some parameters that are often considered in selecting rice are the aroma and fluffiness. (Darmasetiawan, 2003) reported that rice from Pandan Wangi varieties has an intensity of pandan aroma,

cereal, buttery, and cream higher than IR64. For the fluffiness, Pandan Wangi varieties have the highest intensity among Peutey, Hawara Batu, and Beureum Seungit (Waluya, 2008). Pandan Wangi (*Oryza sativa* L.) paddy has been certified as Cianjur local varieties released by the decree of the Minister of Agriculture No. 163 / kepts / LB.240 / 3/2004 by the name Pandan Wangi.

Table 2. Correlation of incremental area under curve (IAUC) glucose and rice

		IAUC of Glucose	IAUC of Rice
IAUC of Glucose	Pearson Correlation	1	.834*
	Sig. (2-tailed)		.003
	N	10	10
IAUC of Rice	Pearson Correlation	.834*	1
	Sig. (2-tailed)	.003	
	N	10	10

**Correlation is significant at the 0.01 level (2-tailed)*

The difference in starch composition between local Japanese rice and local Indonesian rice could possibly affect, especially in the ratio of amylose and amylopectin. High amylose foods tend to be difficult to digest due to the unbranched structure of amylose, making it more tightly bound, so it is difficult to gelatinize, while amylopectin has a branched structure and larger molecular size, so it is easy to gelatinize (Afandi et al., 2019). The primary type of rice grown in Japan is japonica, while in Indonesia are indica and javanica. Japonica rice tends to have lower amylose (10-22%), while Indian rice tends to have higher amylose (18-32%) (Lang & Buu, 2004). The amylose content of Pandan Wangi, which is included in javanica rice, was 24.66% (db) for its brown rice and 30.06% (db) for its white rice. For comparison, the amylose content of other Indonesian local rice was varied, including Cisokan (28.5%), Anak-daro (27.4%), Randah-kuniang (27.2%), Kuriak-kusuik (27.4%), and Saratuih-hari (27.6%), Cibeber (22.11%), Campaka (22.46%), Warungkondang (22.45%), Cianjur (20.43%), Gekbrong (21.88%), Cugenang (23.03%), and Mande (26.56%) (Anhar, 2016; Syamsiah & Masliah, 2019). It also affected the conversion factor. In Pandan Wangi white rice based-GI, the conversion factor was 0.74 taken from glucose-based-GI of rice per 100 (GI of glucose). Before the GI was multiplied by the conversion factor, all of the samples' rice-based-GI were higher than glucose-based-GI. The conversion factor of Pandan Wangi white rice was similar to wheat bread at 0.7 (Atkinson et al., 2008).

Table 3. Glycemic Index (GI) value based on glucose and white rice as reference foods

Reference Food	GI values							
	CPWR		Wheat cookies		NS-Cookies		HMT-cookies	
	Mean ±s.d	%CV	Mean ±s.d	%CV	Mean ±s.d	%CV	Mean ±s.d	%CV
Glucose	74±16	22	60±25[a]	42	38±14[a]	37	39±16[a]	41
CPWR	-	-	82±33[a]	40	51±13[b]	25	52±17[a]	33
CPWR (factor=0,74)	-	-	61±24[a]	40	38±10[a]	25	39±12[a]	33

CPWR: Cooked Pandan Wangi Rice, NS: Native Starch, HMT: Heat Moisture Treatment. Values in the same column with the same letter superscripts did not differ significantly (P >0.05). Means for all subjects (n=10). ANOVA. CV= (s.d./mean)x100

Rice-based-GI of NS cookies and HMT-cookies showed a lower CV than using glucose as a reference food. The use of cooked Pandan Wangi rice as a reference food was preferred because it was more delectable than glucose. GI testing for food intended for people with diabetes involves diabetics as the subject, and it is more appropriate to use rice as a reference food than glucose. Consumption of fairly concentrated glucose solution can cause the subject to experience nausea.

Figure 1 showed that concentrations of the blood glucose subject after consuming 25 g of glucose decreased sharply after reaching the peak. Meanwhile, blood glucose concentrations of the subjects after consuming 25 g of rice decreased slowly after reaching the peak. This is probably due to differences in the composition of glucose standard and white rice. Glucose standard only consists of pure glucose, while white rice consists of not only starch but also protein and fat (Zhou et al., 2002). So, it takes longer to break down the sugar.

It can also be seen that IAUC based on consumption of 25 g glucose was narrower than IAUC based on consumption of 50g glucose, with mean of IAUC 3133 and 4933 for glucose 25 g and glucose 50 g, respectively. The mean peak of the glucose 25 g curve was in 30 minutes, while for glucose 50 g curve was in 45 minutes. The curve of blood glucose concentration of rice 25 g did not follow the blood glucose curve of glucose 25 g, but followed the blood glucose curve of glucose 50 g.

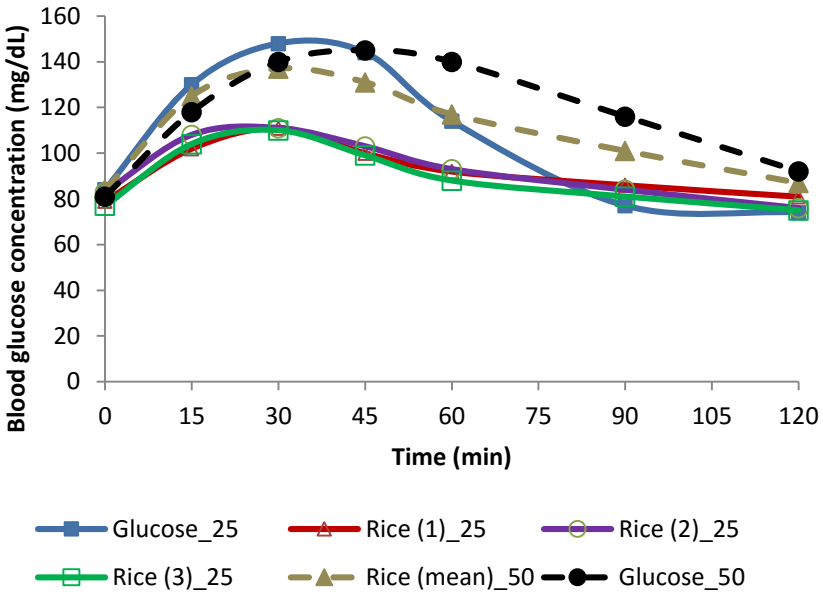

Figure 1. Blood glucose curves of glucose and rice-based on 25 g and 50 g available carbohydrate; values are mean, n=10

Using 50g of AVCHO in low carbohydrate density such as rice might require the ingestion of enormous quantities. Therefore, 25g portion of AVCHO has also been studied. If previously, to determine GI value there been used three times reference food tests and once sample tests, this time were used once reference food and triplicate sample tests. This was done to know whether it was necessary to test the sample more than once. Thus, white rice was tested three times on different days. The mean GI of white rice in triplicate sample tests was not statistically different, as indicated in Table 4 (n=10, $p>0,05$). For the comparison with glucose 50 g, was used the GI based on the mean of triplicate glucose trials. It was shown that GI of white rice based on 25 g AVCHO was significantly different from GI based on 50 g AVHCO (n=10, $p<0.05$).

Table 4. Mean glycemic index (GI) of white rice

AVCHO	White Rice Curve		Mean GI of White rice	%CV
25g	Single	1	56±12[a]	22
		2	50±18[a]	35
		3	53±14[a]	26
		x̄	53±15	28
	Mean of duplicate sample tests	1&2	52±14[a]	27
		1&3	54±11[a]	21
		2&3	50±14[a]	27
		x̄	52±13	25
	Mean of triplicate sample tests	1,2&3	52±12[a]	24
50g	Mean of triplicate glucose tests	1,2&3	74±16[b]	22

Means for all subjects (n=10). ANOVA. Values in the same column with different letter superscripts did not differ significantly (p>0.05). CV= (s.d./mean) x 100

GI based on 25 g AVCHO was lower than GI based on 50g AVCHO. Even though CV based on mean triplicate 25g sample tests and mean of triplicate 50g glucose test was not significantly different (n=4, p>0.05), testing reference food more than once is more necessary than testing sample more than once because it is used as the basis of calculation in every test food in the series.

GI based on 25 g AVCHO has lower GI value compared to GI based on 50 g AVCHO in the same product. This trend indicates the possibility of grouping GI by the basic portion of 25 g AVCHO. GI value of rice which was 53, was classified as medium GI on the general GI grouping, but it was possibly classified as high GI by 25 g AVHCO-based GI grouping. Further studies regarding this issue are needed. The use of 25 g AVCHO as the basic portion will help in the implementation of the GI test because the subject does not need to consume large quantities of samples.

CONCLUSION

We concluded that with a conversion factor of 0.74, white rice (Pandan Wangi) could be used in GI tests as a reference food. Further research into the usage of 25 g AVCHO as the basis portion and the possibility of GI-grouping is required.

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