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Resistant Starch Content and Glycaemic Index of Sago (*Metroxylon spp.*) Starch and Red Bean (*Phaseolus vulgaris*) Based Analogue Rice

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Abstract: Foods with low glycaemic index (GI) are part of the functional aliments recommended for patients with diabetes mellitus. These foods can be produced from materials with high amylose and/or high dietary fibre content by using specific processing technologies that increase their resistant starch content. Here, analogue rice formulations were prepared using sago and red beans as main materials, employing extrusion technology. We determined their resistant starch (RS) content and GI to evaluate their potential as functional foods for diabetics. Six formulations were tested: pure sago analogue rice (BS100) and five sago analogue rices with the addition of 5, 10, 15, 20 and 25% red bean flour (BSKM5, 10, 15, 20 and 25, respectively). The GIs were determined for 20 healthy volunteers. RS contents of BS100, BSKM5, 10, 15, 20 and 25 analogue rices were 12.25, 11.80, 11.18, 10.97, 10.24 and 9.28%, respectively. The lowest GI value was obtained with BS100 (40.7), while BSKM5 and BSKM10 had GIs of 48.3 and 50.4, respectively. Analogue rice BSKM15 and 20 had medium GIs, 68.8 and 69.5, respectively and BSKM25 was included in the high GI category (76.5). Pure sago analogue rice (BS100) and analogue sago rice with red bean flour added up to 10% (BSKM5 and 10) were included within low GI category, making them potential functional foods for type 2 diabetics.

Key words: Analogue rice, glycaemic index, extrusion, sago starch, red bean flour

INTRODUCTION

Diabetes mellitus is one of the four non-communicable diseases (NCDs) causing death. In 2015, there were around 415 million adults aged 20-79 with diabetes worldwide (IDF Diabetes Atlas-7th Edition 2015). The number of diabetics is predicted to increase more than 45% within the next 18 years, affecting 551 million people (10% of the global population), if no actions and serious prevention measures are undertaken. The WHO (World Health Organization) predicts that the number of people with diabetes mellitus type 2 will increase in Indonesia from 8.2 million people in 2000 to 21.3 million in 2030 (Sidartawan *et al.*, 2006).

The recommended intake to prevent diabetes and help affected people is 50% energy from low glycaemic index (GI) foods, a maximum of 35% energy from fat intake and low saturated fat, as well as a dietary fibre intake of 10-13 g/1000 kcal or 30-40 g/day (The British Diabetic

Association, 1982). Low GI foods can improve insulin sensitivity and decrease the rate of glucose absorption, which are useful properties to control glucose levels in the blood of diabetic patients (Riccardi *et al.*, 2008). Rice is the major source of carbohydrates for Indonesian people, with a high GI of 80 (Marsono, 2001). However, the intake needs to be restrained in order to control blood glucose levels. According to SUSENAS (BPS, 2014), in 2014, rice consumption within the Indonesian population reached 114 kg/cap/year, far above the average consumption amongst Asian countries. Rice consumption in Indonesia needs to be reduced gradually through food diversification, replacing rice by other products with similar properties and appearance, for example analogue rice. Analogue rice is made of non-rice material, for example local flour or non-rice flour, including sago starch. Research by Hariyanto *et al.* (2013) revealed that sago had various GI values

depending on the types of processed products. For example, macaroni obtained from sago has a low GI of 28. However, to date, conversion of sago starch into analogue rice has never been studied. Sago starch has low protein content and therefore, an additional protein source, such as red bean flour, needs to be introduced when preparing a substitute product for rice. In addition to being a good source of protein, red beans (*Phaseolus vulgaris*) also have a high dietary fibre content and a low glycaemic index of 26 (Marsono *et al.*, 2002). Analogue rice is prepared using an extrusion process following a pre-gelatinization step. Extrusion technology is used because it can generate a shape similar to that of a grain of rice. Extrusion combines material moisture, high pressure, thermal and mechanical friction, with the power to cut unprocessed or partially gelatinized materials in a short time, causing both physical and chemical changes. The resulting product of extrusion will then be dried so that starch retrogradation can occur, increasing the RS content (Gomes *et al.*, 2015; Mishra *et al.*, 2012; Riaz, 2000).

Sago starch contains high amylose, red bean flour holds a high protein content and extrusion enables the increase of RS levels. Consequently, the produced analogue rice has properties similar to those of rice, with high protein content and low GI. The purpose of this study was to determine the levels of RS and glycaemic index values of analogue rice obtained from various mixtures of sago starch and red bean flour.

MATERIALS AND METHODS

The main components of this research are sago starch (*Metroxylon* spp.), a variety of meranti and a variety of local red bean (*Phaseolus vulgaris*). Sago starch was obtained from Selat Panjang, Riau, while the red beans were obtained from farmers of the Parakan district, Temanggung Regency. Red bean flour was prepared in accordance with the procedures described by Wahjuningsih and Kunarto (2013). The enzymes used for analysis, such as thermamyl, pepsin and pancreatin, as well as the glucose detection Kit (GOD) were obtained from Sigma Co., USA.

The chemical analysis units used for this study, such as the Soxhlet tube, micro-Kjeldahl unit, spectrophotometer and centrifuges, Blood sampling and blood sugar analysis equipment (Accu-check glucometer) was obtained from Roche Diagnostics GmbH, Germany.

For formulation process for analogue rice, six formulations of analogue rice were made from composite flour consisting of sago starch and red bean flour: one analogue rice of pure sago starch (BS100) and 5 rice analogues of sago starch with the addition of 5% (BSKM5), 10% (BSKM10), 15% (BSKM15), 20% (BSKM20) and 25% (BSKM25) red bean flour, as shown as in Table 1.

Table 1: Formulations of analogue rice composite flours with sago starch and red bean flour

Formulation	Sago starch (%)	Red bean flour (%)
BS100	100	0
BSKM5	95	5
BSKM10	90	10
BSKM15	85	15
BSKM20	80	20
BSKM25	75	25

In addition to composite flour, other ingredients for the manufacture of analogue rices consisted of GMS, carrageenan, coconut oil and salt (Wahjuningsih *et al.*, 2015). Before the manufacture of analogue rice, we added the additional ingredients to the composite flours, supplemented this mixture with water and homogenized it for 5 min, before carrying out a steaming process for 15 min. The steamed mixture was then directly inserted into the extruder and a homogenization was carried out for 2 min, before starting the printing process to convert it into analogue rice. After the granules were formed, they were dried into a dryer cabinet at 50°C for 12 h.

For chemical analysis, all the analogue rice formulations were analyzed to evaluate the levels of carbohydrates using a different method (proximate analysis data are not shown) (AOAC, 2000), the dietary fibre content using enzymatic methods (Asp *et al.*, 1983), the amylose content by spectrophotometric analysis (Englyst *et al.*, 1992) and the resistant starch content *in vitro* following the method described by Goni *et al.* (1996), with slight modifications.

For determination of the glycaemic index, determination of the rice analogue GI was conducted in accordance with the method reported by Marsono (2002). Twenty volunteers were selected who fulfilled the following inclusion criteria: normal blood sugar levels; a glucose response of normal standard food; normal body mass index (BMI) and provision of written informed consent to follow the specified study protocol. This study has received ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, University of Gadjah Mada in 2015 (No. KF/FK/480/EC). The selected volunteers were randomly divided into 2 groups of 10 people. Each group consumed a standard meal (glucose solution) and 3 test meals. The volunteers were asked to fast for one night (10 h) and in the morning, blood was collected and sugar levels analyzed (fasting glucose). The volunteers were then given a standard diet (50 g of glucose) and their blood sugar levels were monitored every half hour for the next 2 h. From these results, we obtained standard meal glucose responses. For the tested food preparations, the following procedure was applied before carrying out the glucose response test: Firstly, the water, carbohydrates and dietary fibre content of all the analogue rices was determined. Based on the data obtained for

carbohydrates and dietary fibre content, we calculated the amount that the volunteers were required to eat in order to reach the equivalent of 50 g of glucose.

The determination of the glucose response was conducted as for the standard food testing. Using the fasting blood sugar levels measured after eating, the glucose response of analogue rice could be graphed. The GI represents the ratio between the tested food glucose response curve and the standard meal glucose response curve (standard glucose). Blood glucose levels were determined using the Accu-Check Active Glucometer (Roche Diagnostics GmbH, Germany). This method had been used previously by other researchers (Marsono, 2014; Hettiaratchi *et al.*, 2012; Ihediohanma *et al.*, 2012; Lukaczer and Barbara, 2000).

For statistical analysis, we evaluated the difference between rice analogue formulations using variance analysis (ANOVA) with the statistical analysis system ver.9.2. The significance of the differences between analogue rice formulations was determined by the Duncan test, where $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Data regarding amylose, resistant starch and dietary fibre content are presented in Table 2.

When the amount of red bean flour is increased, the analogue rice has higher dietary fibre content but lower resistant starch (RS). The source of dietary fibre in this formulation is red bean flour; therefore, with increase in the proportion of red bean, the amount of dietary fibre also increases. During the process, amylose retrogradation generates retrograded starch, which is one of the RS3 (Marsono, 1998). In this formulation, sago is the biggest provider of amylose, thus contributing significantly to the formation of RS. Therefore, the higher the proportion of sago, the greater the content in RS.

Analysis of carbohydrate and dietary fibre content was required to determine the amount of analogue rice, equivalent to 50 g of glucose, to be consumed by the volunteers in order to test the GIs. Based on the data obtained from the carbohydrates and dietary fibre content measurements, the amounts of analogue rice (equivalent to 50 g of glucose) to be given are shown in Table 3.

The cooking conversion factors applied are 2.01 (BS100), 1.92 (BSKM5), 2.09 (BSKM10), 2.06 (BSKM15), 2.06 (BSKM20) and 2.06 (BSKM25).

The average responses of blood glucose 2 h after a meal are shown in Fig. 1 and 2. The GIs ranged from 40.7 (BS100) to 76.5 (BSKM25) as shown in Table 4. From Table 4 it can be seen that the greater the proportion of sago starch, the lower the value of the GI. Increasing addition of red bean flour yielded analogue rices with higher dietary fibre content but lower resistant

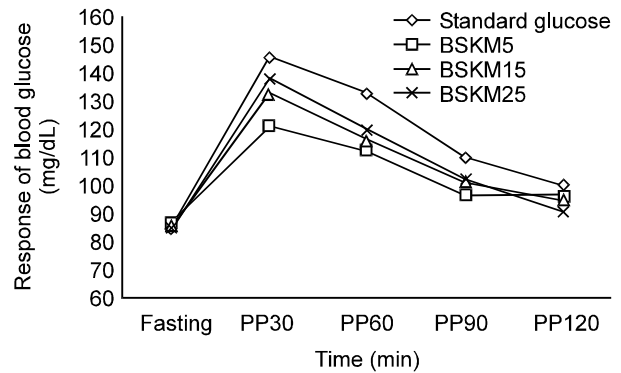


Fig. 1: Volunteers' glucose response after consuming standard glucose, BSKM5, 15 and 25 meals

starch (RS) (Table 2) and increased GIs (Table 4). These results show that the dietary fibre content does not contribute in decreasing the GI. This can be explained by the fact that most of the dietary fibres in the analogue rice formulation are insoluble (IDF) and non-viscous and thus, do not have any effect on the glucose absorption resistance (Marsono, 2001). Several previous studies indicated that the role of soluble fibres in decreasing blood glucose is greater than that of insoluble fibres (Wood *et al.*, 1990; Jenkis *et al.*, 2002; Parastouei *et al.*, 2011; Wordu and Banigo, 2013; Nader *et al.*, 2014).

In this study, the effect of resistant starch (RS) and amylose in lowering the GI is significant. With greater proportions of sago starch, the higher are the levels of RS and amylose and the lower are the values of GI. RS is affected by the amylose content and the processing method, causing retrogradation. Increased amylose content in starchy material will cause slower digestion, because amylose is a polymer of glucose that has an unbranched structure, with strong hydrogen bonding (Behall and Hallfrisch, 2002), forming a compact crystal limiting accessibility for the amylase enzyme (Marsono, 1998). Amylose plays an important role in the process of gelatinization. Reheating and cooling of starch that has undergone gelatinization modulate its structure and facilitate the formation of retrograded starch, causing a decrease in the value of GI (Hazila *et al.*, 2006). The analogue rice prepared in this study contained sago flour with high amylose content. The extrusion treatment used to prepare the analogue rice is a combination of heat treatment and limited provision of water. The main changes made to this process compared with the method described by Camire *et al.* (2007) and Larrea *et al.* (2005) concern the starch gelatinization, the protein denaturation, the formation of amylose-lipid complexes and the degradation of the components sensitive to heat. The gelatinization process allows the retrogradation of sago starch with resistant characteristics, thereby decreasing the digestion level.

Table 2: Amylose, resistant starch (RS) and dietary fibre (TDF) content

Formulation	Amylose	RS (%)	TDF (%)	SDF (%)	IDF (%)
BS100	31.61±0.13 ^a	12.25±0.15 ^a	2.73±0.36 ^e	1.76±0.32 ^c	0.97±0.05 ^e
BSKM5	30.62±0.26 ^b	11.80±0.29 ^a	4.18±0.48 ^d	2.16±0.35 ^b	2.02±0.18 ^d
BSKM10	29.48±0.48 ^c	11.18±0.61 ^b	5.35±0.56 ^c	2.43±0.34 ^b	2.92±0.24 ^c
BSKM15	28.43±0.82 ^d	10.97±0.15 ^b	7.29±0.17 ^b	2.59±0.34 ^a	4.70±0.28 ^b
BSKM20	27.39±0.61 ^e	10.24±0.19 ^c	8.90±0.27 ^a	3.04±0.14 ^a	5.87±0.35 ^a
BSKM25	26.94±0.62 ^e	9.28±0.16 ^d	9.08±0.41 ^a	2.98±0.40 ^a	6.10±0.20 ^a

Description: superscripts a, b, c, d, e with different letters in the same column show significant differences (p<0.05)

Table 3: Quantity of analogue rice equivalent to 50 g of glucose

Sample	TKH (g/100 g)	TDF (g/100 g)	AKH (g/100 g)	Analogue rice (g)	Cooked analogue rice (g)
BS100	93.92	2.73±0.36 ^e	91.19	54.83	110
BSKM5	90.80	4.18±0.48 ^d	86.62	57.72	111
BSKM10	89.13	5.35±0.56 ^c	83.78	59.68	125
BSKM15	87.90	7.29±0.17 ^b	80.61	62.13	128
BSKM20	86.75	8.90±0.27 ^a	77.85	64.23	132
BSKM25	85.82	9.08±0.41 ^a	76.74	65.16	133

TKH = Total carbohydrates (carbohydrates by difference)

AKH = available carbohydrates (TKH-TDF)

TDF = Total dietary fibre content

Cooked analogue rice = quantity of rice x cooking conversion factor

Table 4: Glycaemic index values of sago starch and red bean flour analogue rices

Sample	Glycaemic index value
Standard (glucose)	100
Analogue rice BS100	40.7
Analogue rice BSKM5	48.3
Analogue rice BSKM10	50.4
Analogue rice BSKM15	68.8
Analogue rice BSKM20	69.5
Analogue rice BSKM25	76.5

The process can increase the RS content of analogue rice. Moreover, the formation of amylose-starch complex will also increase the RS (Marsono, 1998). Marsono (2015) stated that there are four possible roles played by RS in the decrease of blood glucose, that is (i) because RS is not digested, the availability of glucose is low; (ii) the viscous nature of RS inhibits the absorption of glucose; (iii) RS improves insulin sensitivity and (iv) the fermentation of RS produces butyric acid that increases the level of transporter glut 4. The role played by RS in lowering the GI was also investigated by Lehmann *et al.* (2003), as well as by Srikaeo and Janya (2014). The restoration of the glycaemic response after consuming high amylose foods or RS has also been reported by Behall and Hallfrisch (2002), Behall *et al.* (2006). Behall and Hallfrisch (2002) reported a significant reduction in glucose and insulin responses after consuming bread containing resistant starch (8-13.4 g).

Another potential explanation of the low glycaemic response seen with analogue rice containing more sago might reside in the role played by sago starch structure. According to Govindasamy *et al.* (1992), sago starch is more resistant to hydrolytic enzymes attacks, due to their crystalline structure and the polyphenol compounds coating the sago starch granules. Polyphenols are also believed to act as inhibitors of the starch digestion enzyme.

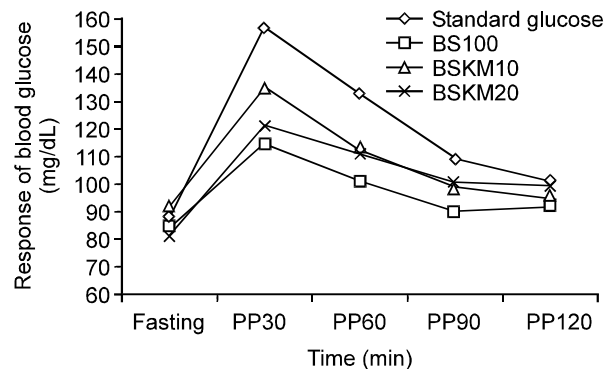


Fig. 2: Volunteers' glucose response after consuming standard glucose, BS100, BSKM10 and BSKM20 meals

Sago analogue rices with higher sago proportions have higher RS content and lower GI values. The RS content ranges from 12.25% (BS100) to 9.28% (BSKM25). The analogue rices BS100, BSKM5 and BSKM10 were within the low GI food category with GI values of 40.7, 48.3 and 50.4, respectively. The sago analogue rices BSKM15 and 20 are part of the medium category, with GI values of 68.8 and 69.5, respectively. The sago rice BSKM25 falls in the high GI category (76.5). Sago analogue rice represents a potential functional food for type 2 diabetics.

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