PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 15 (7): 649-654, 2016
ISSN 1680-5194

Changes in Resistant Starch Content and Glycemic Index of Pre-Gelatinized Gayam (*Inocarfus fagifer* Forst.) Flour

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Abstract: Gayam seed (*Inocarfus fagifer* Forst.) has a high amylose content and is a good source of resistant starch (RS). Pre-gelatinization during the preparation of gayam flour can increase the RS content, which is associated with a lower glycemic index (GI). The objective of this research was to study the changes in both the RS content and GI of gayam flour with pre-gelatinization treatment. Pre-gelatinized gayam flour was prepared from unpeeled gayam seed that was boiled at 100°C for 15, 30, or 45 min. The results showed that the RS contents of pre-gelatinized gayam flour (by dry weight analysis) boiled for 0, 15, 30 and 45 min were 15.78±0.99, 16.95±0.54, 17.06±1.10 and 17.92±0.39%, respectively. The RS content of pre-gelatinized gayam flour boiled for 15 and 30 min did not differ significantly compared with the control, whereas pre-gelatinization for 45 min produced the highest RS content. Using glucose as the reference food, the GI values of pre-gelatinized gayam flour boiled for 0, 15, 30 and 45 min were 74, 75, 61 and 57, respectively. Thus, a longer pre-gelatinization time can produce a lower GI value for gayam flour, which is associated with an increased RS content.

Key words: Gayam flour, pre-gelatinization, resistant starch, glycemic index

INTRODUCTION

Gayam (*Inocarfus fagifer* Forst.), a native plant of Indonesia, is grown in the swamps or riverside areas in Java, Sumatera, Kalimantan and the Malay Peninsula. Gayam has a number of commonly used names, including Tahitian chestnut, Polynesian chestnut, Otaheite chestnut, *aila* and *bosua* (Pauku, 2006). Gayam seeds contain high amounts of amylose. As reported by Epriliati (2002), the amylose content of gayam starch is 52.7% of the total starch content in the seed. This value is equivalent to the amylose content of corn starch and is higher than that in arrowroot starch (Faridah *et al*., 2013). Gayam seeds are consumed after conventional processing, such as boiling, roasting in hot ash, or being sliced into chips (Heyne, 1987). This conventional processing limits the utilization of gayam seed as a source of carbohydrate, resulting in gayam seeds commonly being forgotten as a food source. Therefore, the processing of gayam seeds into a flour product is necessary to increase the added value, reduce postharvest loss and enhance product diversification.

Pre-gelatinization is a hydrothermal process in the preparation of gayam flour that consists of boiling and drying (Zeuthen *et al*., 1984; Padmaja *et al*., 1996; Palupi *et al*., 2011). Pre-gelatinization inactivates polyphenol enzymes (Zeuthen *et al*., 1984) and retrogrades the starch contained in gayam seeds (Englyst and Cummings, 1987). Previous studies have shown that the results of pre-gelatinization are varied and likely depend on the type of the products. Taro flour was pre-gelatinized at 100°C for 20, 40 or 90 min (Njintang and Mbofung, 2006), cassava flour at 100°C for 20, 30, 40, or 50 min (Khasanah, 2009) and mixed flour of sorghum and pigeon pea at 60-100°C for 55 min (Mbaeyi-Nwaoha and Onweluzo, 2013).

Pre-gelatinization led to the retrogradation of the starch granules (Englyst and Cummings, 1987; Chung *et al*., 2009), altered starch structure, increased gel hardness (Jacobasch *et al*., 2006) and increased starch crystallinity (Jacobasch *et al*., 2006; Dupuis *et al*., 2014), thus, pre-gelatinization increases the resistant starch (RS) type-III fraction (Guha *et al*., 2011; Dupuis *et al*., 2014). RS is the total amount of starch and its degradation products that escape digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon (Englyst *et al*., 1992). RS has some physiological benefits resembling those of soluble dietary fiber. The physiological importance of RS ingestion has been investigated in several studies and the benefits include the prevention of gastrointestinal diseases and cardiovascular disease, a reduction of the risk of ulcerative colitis and colon cancer and the promotion of bacterial

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growth and mineral absorption (Zhang *et al*., 2014). Agama-Acevedo *et al*. (2012) reported that RS intake can control diabetes mellitus by altering the glycemic impact of ingested carbohydrates.

Because high RS foods result in a lower glucose response when consumed, these foods have a lower glycemic index (GI). GI describes the rate of blood glucose absorption after food consumption (Wolever *et al*., 1991). Several reports have associated high GI food with metabolic disorders such as diabetes mellitus (Jenkins *et al*., 2002; Sun *et al*., 2010). The British Nutrition Foundation (BNF) (1990) explained that RS has a lower glycemic response and can prevent the increase of blood glucose and insulin after meals. Marsono (2002) showed that processing tubers can affect the starch gelatinization and thereby impact both the RS content and GI value.

Previous studies of gayam seeds were focused on the chemical composition and functional properties of gayam starch (Epriliati, 2002). However, little information is available on gayam flour processing, especially on the effect of pre-gelatinization in flour preparation on RS content and GI. Therefore, studying whether the processing gayam seeds into flour products results in a potential source of high RS food is needed to provide scientific information for increasing the utilization of gayam seeds in food product development. In the present study, the changes in the RS content and GI of pre-gelatinized gayam flour after various pre-gelatinization times were evaluated.

MATERIALS AND METHODS

Materials: Gayam seeds were obtained from Bantul, Yogyakarta, Indonesia and had the following characteristics: russet color, ripe on the tree, age 3-4 months and weight 75-110 g/seed (medium-big size). Pepsin was obtained from Merck (Darmstadt, Germany) and alpha-amylase and amyloglucosidase were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Measurement of the blood glucose response in a volunteer was conducted using pure glucose (anhydrous glucose) as the reference food. Blood sample and blood glucose were analyzed using an Accu-chek glucometer (Roche-Diagnostics Germany) as previously described (Hettiaratchi *et al*., 2012; Ihediohanma *et al*., 2012).

Preparation of pre-gelatinized gayam flour: Pre-gelatinized gayam flours were prepared by boiling unpeeled gayam seeds at 100°C for 15, 30 and 45 min. Thereafter, the seeds were peeled and sliced (approximately 2-3 mm) and then dried using a cabinet dryer at 50-60°C for 48 h. The dried gayam was ground and sieved through a 60 mesh sieve. As a control, unboiled gayam flour was also prepared by peeling, washing, slicing, drying and then sieving through a 60 mesh sieve. Therefore, four types of flour were prepared: gayam flour without pre-gelatinization (W-PGF), pre-gelatinized gayam flour boiled for 15 min (PGF-15), pre-gelatinized gayam flour boiled for 30 min (PGF-30) and pre-gelatinized gayam flour boiled for 45 min (PGF-45). The flour was packed in polyethylene bags until further analysis.

Determination of RS: The RS content was determined using the method of Goni *et al*. (1996) with slight modifications. To digest the protein, the flour sample (100 mg) was treated with pepsin (0.2 ml, 1 g pepsin/10 ml of KCl-HCl buffer, pH 1.5) and hydrolyzed with pancreatic α-amylase (1 ml, 40 mg/ml of Tris-maleate buffer) and the samples were then incubated at 37°C for 16 h. The pellet (which contained RS) obtained after centrifugation (15 min, 3000 g) was washed with ethanol followed by distilled water and dispersed with 4 M KOH followed by stirring for 30 min at room temperature. The aliquots (which contained the alkali-solubilized starch equivalent to the amount of RS) were treated with 80 µl of amyloglucosidase (5 mg/ml of acetate buffer pH 4.75) and kept in a water bath at 60°C for 45 min with constant shaking. The aliquots were centrifuged (15 min, 3000 g) and the supernatant (containing glucose obtained from hydrolysis of alkali-solubilized RS) was collected in a 25 ml volumetric flask. The glucose content was determined using a glucose oxidase-peroxidase assay GOD-POD kit (DiaSys, Diagnostic Systems GmbH, Alte Strasse 9, Holzheim, Germany). One milliliter of GOD-POD reagent was added to aliquots (10 µl) of the sample and the mixture was incubated at 37°C for 10 min. Absorbance was measured using a spectrophotometer at wavelength 510 nm. RS was calculated as the amount of glucose X 0.9.

Determination of GI of pre-gelatinized gayam flour: The GI of pre-gelatinized gayam flour was determined according to the protocol described by Marsono (2002). Ten healthy volunteers aged 19-55 years old with body mass indices of 18.5-22.9 and normal blood glucose profiles (fasting and postprandial) were asked to participate in the experiment by signing the informed consent form. None of the volunteers were smoking, drinking alcohol, under medication or consuming any dietary supplements. The volunteers fasted 10-12 h before consuming samples equivalent to 50 g glucose. The blood glucose level of each volunteer was measured every 30 min over a period of 2 h after consuming the samples by using a commercial kit consisting of an Accu-chek Glucometer (Roche-Diagnostics Germany). The same procedures were conducted for the other samples from the same volunteers daily. Glucose was used as the reference food. Data were plotted as time versus blood glucose level, with time as the X-axis and the blood glucose content as the Y-axis. The area under the curve (AUC) was determined for every blood glucose level of each sample (Wolever *et.al*., 1991). The GI was calculated as follows:

$$GI = \frac{AUC\ of\ the\ sample}{AUC\ of\ the\ reference\ food} \times 100$$

Statistical analysis: RS content was measured in triplicate. Data were analyzed using one-way analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using the SPSS 18.0 Statistical Software Program.

RESULTS AND DISCUSSION

RS content of pre-gelatinized gayam flour: The RS content (by dry weight analysis) of gayam flour without pre-gelatinization (W-PGF) and pre-gelatinized gayam flour boiled for 15 min (PGF-15), 30 min (PGF-30) and 45 min (PGF-45) were 15.78±0.99, 16.95±0.54, 17.06±1.10 and 17.92±0.39%, respectively and significantly differed (Fig. 1). The lowest RS content was found in gayam flour without pre-gelatinization, whereas the highest was found in the flour with pre-gelatinization for 45 min.

The results showed that pre-gelatinization of gayam seed by boiling and then drying affects starch gelatinization and retrogradation. Retrogradation due to heating and drying (Hoover, 1995; Sajilata *et al.*, 2006) can lead to the formation of indigestible starch. This starch is called RS type-III (Englyst *et al.*, 1992). Retrograded starch has a different crystal structure compared to the native granule starch and is more indigestible by amylase enzyme (Marsono, 1998). Englyst and Cummings (1987) and Asp (1992) stated that the formation of RS during food processing might vary depending on the temperature and time of heating and drying.

Hydrothermal treatments (e.g., steaming, autoclaving and boiling) of flour preparation induce retrogradation (Zeuthen *et al.*, 1984; Padmaja *et al.*, 1996; Palupi *et al.*, 2011), including new crystallization or recrystallization. This process changes the crystalline regions of the starch granule (Englyst and Cummings, 1987; Chung *et al.*, 2009), alters the starch structure and increases the gel hardness (Jacobasch *et al.*, 2006). Retrogradation also increases starch crystallinity (Jacobasch *et al.*, 2006; Dupuis *et al.*, 2014). Moreover, Guha *et al.* (2011) and Dupuis *et al.* (2014) reported that hydrothermal treatments increase slowly-digestible starch and RS type-III. Goni *et al.* (1996) categorized food based on RS content (based on dry weight analysis) as follows: very low (<1%), low (1-2.5%), medium (2.5-5%), high (5-15%) and very high (>15%). Based on these categories, the gayam flours are categorized as a very high RS food.

GI of pre-gelatinized gayam flour: Weight of pre-gelatinized gayam flour to be consumed by volunteers: In this study, the amount of steamed gayam flour given to the volunteers was equivalent to 50 g available carbohydrate. The amount of gayam flour sample equivalent to 50 g of total available carbohydrate ranged from 79.91-90.40 g (Table 1). In the preparation of samples for GI determination, pre-gelatinized gayam flour was steamed for 45 min and the amount of sample consumed was 208-217 g. The weight of steamed gayam flour administered was varied to be equivalent to 50 g total available carbohydrate.

Blood glucose response of pre-gelatinized gayam flour: The blood glucose response of the volunteers after consuming gayam flour samples and reference food is presented in Table 2. The glucose response was determined using the blood glucose level after the volunteers consumed the experimental food. In this study, anhydrous glucose was used as the reference food. Brouns *et al.* (2005) recommended the use of glucose as the reference food for the determination of GI value because it results in more stable values than do other reference foods. The results revealed that all of gayam flour samples have a lower blood glucose response than the reference food. Longer pre-gelatinization times in gayam flour processing produced the lower glucose response, which is associated with an increased RS content. RS is the starch fraction that escapes enzymatic digestion in the small intestine. The unavailability of RS for digestion in the small intestine reduces the postprandial response. Bahado-Singh *et al.* (2011) reported the beneficial influence of RS in starch digestion and its consequent ability to decrease glycemic responses. The rate of gastric emptying and the digestibility of starch affect the glucose response (Nugent, 2005; Chung *et al.*, 2008). Previous studies have reported that RS is an indigestible starch and could delay gastric emptying (Vonk *et al.*, 2000; Lin *et al.*, 2010).

Figure 2 shows the changes in the volunteers' glucose responses after they consumed glucose and pre-

Table 1: Carbohydrate, total fiber, available carbohydrate and weight of pre-gelatinized gayam flour equivalent to 50 g glucose to be consumed by volunteers

Samples	Carbohydrate (by difference, % wb)	Total fibre (% wb)	Available carbohydrate (% wb)	Weight of sample equivalent to 50 g glucose (g)
W-PGF	74.59	19.28	55.31	90.40
PGF-15	74.83	12.26	62.57	79.91
PGF-30	75.59	14.40	61.19	81.71
PGF-45	75.23	16.01	59.22	84.43

W-PGF: Gayam flour without pre-gelatinization, PGF-15: Pre-gelatinized gayam flour boiled for 15 min, PGF-30: Pre-gelatinized gayam flour boiled for 30 min, PGF-45: Pre-gelatinized gayam flour boiled for 45 min

Table 2: Fasting and postprandial blood glucose concentration (mg/dl) after consuming glucose (reference food) and pre-gelatinized gayam flour

	Time (min)				
Samples	0	30	60	90	120
Glucose	79.2	173.8	182.2	115.2	107.9
W-PGF	81.3	128.7	113.7	105.1	96.4
PGF-15	84.2	140.2	116.2	106.0	89.8
PGF-30	83.8	132.8	110.9	95.6	92.8
PGF-45	83.7	124.3	106.2	102.0	91.7

W-PGF: Gayam flour without pre-gelatinization, PGF-15: Pre-gelatinized gayam flour boiled for 15 min, PGF-30: Pre-gelatinized gayam flour boiled for 30 min, PGF-45: Pre-gelatinized gayam flour boiled for 45 min

Table 3: Incremental area under curve (AUC) and glycemic index (GI) of pre-gelatinized gayam flour

Samples	Area under curve (AUC) (mg/dl × min)	Glycemic index (GI)
Glucose	4514	100
W-PGF	3335	74
PGF-15	3378	75
PGF-30	2772	61
PGF-45	2562	57

W-PGF: Gayam flour without pre-gelatinization, PGF-15: Pre-gelatinized gayam flour boiled for 15 min, PGF-30: Pre-gelatinized gayam flour boiled for 30 min, PGF-45: Pre-gelatinized gayam flour boiled for 45 min. Glucose was used as a reference food

gelatinized gayam flour. The increase in blood glucose level was calculated from the fasting blood glucose level, which was used to determine the GI (Marsono, 2002). All of the pre-gelatinized gayam flour samples led to a lower increase in the blood glucose level than did the reference food. The reference food gave the highest peak blood glucose concentration and the largest overall blood glucose responses, followed by the PGF-15, whereas PGF-45 gave the lowest blood glucose responses. Based on a calculation using the formula developed by Wolever *et al.* (1991), the incremental areas under the curve (mg/dl x min) were 4514 (glucose); 3335 (W-PGF); 3378 (PGF-15); 2772 (PGF-30) and 2562 (PGF-45) (Table 3). These values were used to calculate the GI value.

GI of pre-gelatinized gayam flour: The GI of W-PGF, PGF-15, PGF-30 and PGF-45 were 74, 75, 61 and 57, respectively (Table 4). Jenkins *et al.* (2002) and Wolever (2006) classified the GI value of food using pure glucose as the reference food as follows: low (<56), medium (55-69) and high (>69). Based on this classification, the W-PGF and PGF-15 have a high GI, whereas PGF-30 and PGF-45 have a medium GI. PGF-45 has a lower GI than do the other samples. The results revealed that the pre-gelatinization treatment in the preparation of gayam flour processing can affect the GI value. The longer pre-gelatinization times can lead to a greater decrease in the GI.

Presumably, this phenomenon of GI reduction is due to the starch retrogradation of the gayam seed resulting from pre-gelatinization, including boiling followed by drying. Starch retrogradation leads to an increased RS content of gayam flour. Increasing RS content resulted in lower starch digestibility and thus decreased the GI value, as shown in Fig. 1, which indicates that increasing

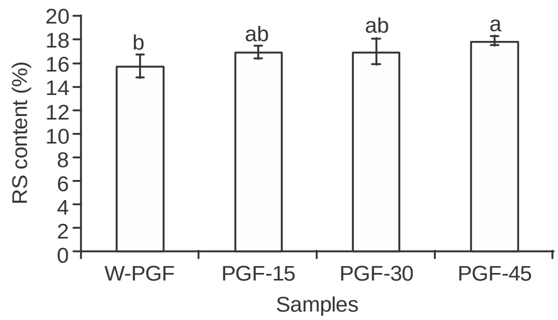

Fig. 1: Resistant starch (RS) content of pre-gelatinized gayam flour. Values represent Means±standard deviations (n = 3). Bars with same letters do not differ significantly (p<0.05). W-PGF: gayam flour without pre-gelatinization, PGF-15: pre-gelatinized gayam boiled flour for 15 min, PGF-30: pre-gelatinized gayam flour boiled for 30 min, PGF-45: pre-gelatinized gayam flour boiled for 45 min

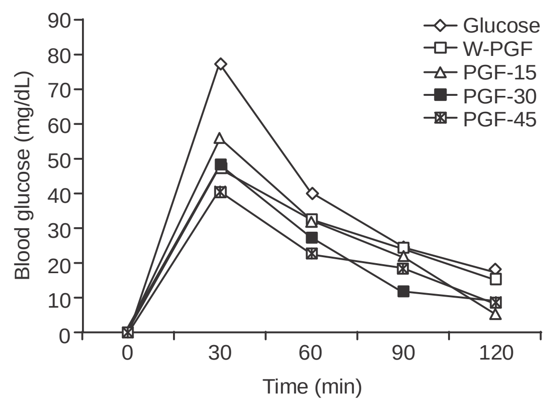

Fig. 2: Changes in the blood glucose level of volunteers after consuming glucose (reference food), W-PGF: gayam flour without pre-gelatinization, PGF-15: pre-gelatinized gayam flour boiled for 15 min, PGF-30: pre-gelatinized gayam flour boiled for 30 min and PGF-45: pre-gelatinized gayam flour boiled for 45 min

pre-gelatinization time increases the RS content. The British Nutrition Foundation/BNF (1990) explained that the GI value can be affected by several factors, e.g., the RS content or the starch availability, the amylose and amylopectin ratio, viscous fiber content and anti-nutrition substances, such as amylase inhibitors and phytates. In this study, a lower GI in the pre-gelatinized gayam flour correlated with the RS content. These results revealed that pre-gelatinization treatment in gayam flour processing can increase the RS content and decrease the GI value. Several studies suggested that the RS content positively influences the GI (Bjorck and Elmstahl, 2003; Fuentes-Zaragoza *et al*., 2010; Srikaeo and Sangkhiaw, 2014). Pre-gelatinized gayam flour can be used as a functional food for the prevention of diabetes mellitus, obesity and dietary management, as suggested in previous studies (Nugent, 2005; Sajilata *et al*., 2006; Agama-Acevedo *et al*., 2012; Srikaeo and Sangkhiaw 2014).

Conclusion: Longer pre-gelatinization times increase the RS content and decrease the GI value to a greater extent. Pre-gelatinization for 45 min significantly increased the RS content of gayam flour, whereas pre-gelatinization for 15 and 30 min did not significantly affect RS content compared to flour without pre-gelatinization. Pre-gelatinization for 45 min led to the highest RS content of 17.92±0.39% and the lowest GI value of 57; therefore, gayam flour with this level of pre-gelatinization can be used as a functional food for preventing diabetes mellitus and obesity. It is necessary to conduct the further research to evaluate the hypoglycemic effect of pre-gelatinized gayam flour in animal and/or human tests.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan/LPDP), Ministry of Finance, Republic of Indonesia for financial support through the program of Dissertation Scholarships in 2015. The authors would like to thank the students and the staff of Nutrition Department, Yogyakarta Health Polytechnic, for the participation of the volunteers in this research.

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