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Research Article Effect of Pre-gelatinization on Physicochemical and Functional Properties of Gayam (*Inocarfus fagifer* Forst.) Flour

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Abstract

Background: Gayam (*Inocarfus fagifer* Forst.), a native plant of Indonesia has a good potential as a source of flour. Currently, gayam flour is made by conventional processing with poor quality. **Objective:** The study was to evaluate the effect of pre-gelatinization treatment on physicochemical and functional properties of gayam flour. **Methodology:** Pre-gelatinized gayam flour was prepared from unpeeled gayam seed by boiling at 100°C for 15, 30 or 45 min, followed by peeling, slicing, drying, grinding and sieving through a 60 mesh. **Results:** The pre-gelatinization treatment decreased the starch and amylose content, while moisture, ash, protein, fat, carbohydrates and dietary fiber content did not differ significantly. The longer pre-gelatinization time led to the higher of ΔE values and whiteness index, whereas pre-gelatinized gayam flour were oval, rough surface, bigger and heterogenous size, while native flour was spherical, smooth surface, smaller and homogeneous size. The pre-gelatinization treatment increased water holding capacity and swelling power but decreased oil holding capacity and solubility. Pasting temperature and setback viscosity of pre-gelatinized gayam flour swith respect to starch, amylose content, color, microstructure, water holding capacity, oil holding capacity and pasting properties. The pre-gelatinization treatment of gayam flours with respect to starch, amylose content, color, microstructure, water holding capacity, oil holding capacity and pasting properties. The pre-gelatinization treatment of gayam flours with respect to starch, anylose content, color, microstructure, water holding capacity, oil holding capacity and pasting properties. The pre-gelatinization treatment of gayam flours with respect to starch, amylose content, color, microstructure, water holding capacity, oil holding capacity and swelling power.

Key words: Gayam flour, pre-gelatinization, physicochemical properties, functional properties

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gayam (*Inocarfus fagifer* Forst.) is a native plant widely grown in Indonesia and it has a number of local names such as gatep (Bali), gayam (Yogyakarta, Central Java), gatet (West Java), bosua and boheng (North Sulawesi), angkaeng (Makassar) and aganyang (Maluku). Globally, gayam has a number of commonly used names, including Tahitian chestnut, Polynesian chestnut and Otaheite chestnut¹. Annual production of gayam seed was 50-150 kg². Gayam seed is rich in carbohydrate (76.74-85.22%) and protein (9.67-11.66%)^{1,3,4}, indicating the potency of gayam seed as a source of a good dietary carbohydrate.

Nowadays, gayam seeds are consumed after conventional processing, such as boiling or being sliced into chips. This conventional processing limits the utilization of gayam seed as a source of carbohydrate, resulting in gayam seeds as a food source being neglected and forgotten⁵. Therefore, the processing of gayam seeds into a flour product is needed to be modified to increase the added value, reduce post-harvest loss and enhance product diversification. Gayam flour can be used for substituting or replacing wheat flour in various baked products such as cakes, cookies or bread⁴.

A major problem of processing gayam seed into gayam flour is the formation of brown color due to the oxidation reaction of polyphenol groups by enzymes. The brown color of gayam flour can affect the nutritional and sensory properties of food products that are substituted or replaced with it. Several methods have been developed to eliminate the enzymatic browning such as pre-gelatinization⁶, blanching⁷ and soaking in sodium bisulphite solution⁸. When compared to blanching or soaking in sodium bisulfite solution, pre-gelatinization of gayam seed could reduce the energy required for processing and production time. Blanching or soaking of gayam seed in sodium bisulfite should be performed on peeled gayam seed, otherwise, pre-gelatinization could be performed on unpeeled gayam seed.

Pre-gelatinization is a hydrothermal process that consists of boiling and drying^{6,9,10}. Pre-gelatinization inactivates polyphenol enzymes⁶. Previous studies have shown that the results of pre-gelatinization are varied and likely depend on the type of the products. Taro flour is pre-gelatinized at 100°C for 20, 40 or 90 min¹¹, cassava flour at 80, 90, 100°C for 10 min¹⁰ and trifoliate yam flour at 100°C for 10 min¹². The hydrothermal treatment such as pre-gelatinization can affect the starch granule with the result that there is increased swelling power, solubility, gelatinization treatment could enhance the physicochemical and functional properties of flour, therefore, it produces a good quality flour which is suitable for developing a new food product.

The previous study of gayam seed has been focused on the utilization of gayam flour for substituting wheat flour in various baked products⁴. These studies revealed the potency of gayam seed as a source of flour alternatively for food diversification. However, there is limited information on the processing of gayam seed into a good quality flour, therefore, the improvement in the processing of gayam flour such as pre-gelatinization of gayam seed is needed. The objective of this study was to evaluate the effect of pre-gelatinization on the physicochemical and functional properties of gayam flour. The result of this study would provide useful scientific information for the production of gayam flour with better properties to meet the standard qualities and current consumer demands.

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⁻¹ (medium-big size). Gayam was washed with tap water and stored at room temperature until further processes. Alpha-amylase (Termamyl 120 L, Sigma-Aldrich Co., St. Louis, MO, USA), pepsin (Merck, Darmstadt, Germany) and pancreatin (BDH laboratory, Poole, England) were used to analyze dietary fiber content. The other chemicals used were an analytical grade.

Preparation of pre-gelatinized gayam flours: Pre-gelatinized gayam flours were prepared by boiling unpeeled gayam seeds at 100°C for 15, 30 or 45 min. Thereafter, the seeds were peeled and sliced (approximately 2-3 mm) and then dried in a cabinet dryer at 50-60°C for 48 h. The dried gayam was ground and sieved through a 60 mesh sieve. As a native gayam flour, unboiled gayam flour was also prepared by peeling, washing, slicing, drying, grinding and then sieving through a 60 mesh sieve. Therefore, four types of flour were prepared: Native Gayam Flour (NGF), 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).

Determination of chemical composition: The chemical analysis of gayam flours was conducted in triplicate according to AOAC¹⁶ procedures, i.e., moisture (thermogravimetri), crude

protein (micro-Kjeldahl), crude fat (Soxhlet extraction) and ash (dry ashing method). Total carbohydrate was calculated by percentage difference. The starch content was determined using the acid hydrolysis method. The amylose content was analyzed according to the method of Juliano¹⁷ and the dietary fiber content using the Asp *et al.*¹⁸ method.

Color measurement: The color of gayam flour was determined using a chromameter Minolta Type CR-400 (Konica Minolta Co., Ltd., Osaka, Japan) and was expressed as the L* (lightness), a* (redness-greeness) and b* (yellowness-blueness). The L* scale ranges from 0 black to 100 white, the a* value ranges from negative (greenness) to positive (redness) and the b* value ranges from negative (blueness) to positive (yellowness). The total color difference (ΔE) to control (NGF) was calculated using the equation according to Popov-Raljic and Lalicic-Petronijevic¹⁹ as follows:

$$\Delta E^* = \sqrt{(L^* - L^{*})^2 + (a^* - a^{'*})^2 + (b^* - b^{'*})^2}$$

where, L'*, a'* and b'* expressed values of control.

Whiteness Index (WI) represents the purity of white color and is considered as an important parameter associated with the browning process. The WI of the sample was calculated using the equation as follows:

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

Scanning electron microscopy: Flour granule morphology was examined by Scanning Electron Microscopy (SEM). A sample was mounted on the aluminum specimen holder with double-sided tape. The specimen holder was loaded in an Emitech SC7620 sputter coater (Emitech, Ashford UK). The sample was coated with gold palladium at a thickness of about 15 nm and viewed under scanning electron microscopy (Inspect S50, FEI Company, Hillsboro Oregon USA) operated at an accelerating voltage of 10 kV.

Water holding capacity and oil holding capacity: Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) were determined according to the Chau *et al.*²⁰ method with slight modifications. Samples (1 g) were suspended in 10 mL of water (for WHC) or vegetable oil (for OHC) in a centrifugal tube. The suspension was centrifuged at 3000 rpm for 30 min. The supernatant was decanted and discarded. The weight of the supernatant was measured. The WHC and OHC were expressed as the number of grams of water or oil held by 1 g of sample (g q^{-1}). **Swelling power and solubility:** Swelling Power (SP) and solubility of flour were determined based on Tester and Morrison²¹ method. A sample (0.2 g) was put into a centrifugal tube and added with 10 mL of distilled water. The sample was equilibrated at 25 °C for 5 min and then put in a water bath at 95 °C for 30 min. Then, it was cooled to 20 °C for 1 min and centrifuged at 3,500 rpm for 15 min to separate gel and supernatant. The gel was weighed to determine swelling power and was expressed in gram per gram. Next, the supernatant was placed in a petri dish and dried at 100 °C for 4 h to determine the dissolved flours. The dried supernatant was weighed to determine the flours solubility. Solubility was calculated as g/100 g of flour on dry weight basis (%).

Pasting properties: The pasting properties of flour were determined using a Rapid Visco Analyzer model RVA-4 (Newport Scientific, Victoria, Australia) equipped with a Thermocline software program for Termoklin for Windows 3 (TCW3). Flour samples (4 g) were weighed into the sample container and then added to 25 g of distilled water. A suspension of flour was held at 50°C for 1 min, heated from 50-95°C at 6°C min⁻¹, held at 95°C for 5 min, cooled to 50°C at 6°C min⁻¹ and held at 50°C for 2 min. The speed was 960 rpm for the first 10 sec, then 160 rpm for the remainder of the experiment.

Statistical analysis: The experimental data were analyzed using one-way analysis of variance (ANOVA) (SPSS 18.0 Statistical Software Program for Windows). Significant differences among experimental mean values were assessed using Duncan's Multiple Range Test (DMRT) (p<0.05).

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of pre-gelatinized gayam flour are presented in Table 1. The moisture content ranged from 8.12-9.12%, which had a similarity with the earlier publication for gayam flour (9.82%)⁴. These values were lower than compared to those reported (11-15%) and in accordance with recommended moisture levels 14% for safety storage²². The lower moisture content were due to the differences of drying method, storage conditions and hygroscopic nature of flour.

The ash and total fat content of gayam flours did not differ significantly. The ash content of gayam flours ranged from 2.79-3.18%, which was higher than the earlier publication for gayam flour (1.95%) and gayam starch (0.04%)^{4,23}. Total fat content of gayam flours ranged from 4.83-5.38%. The protein content of PGF ranged from

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Composition	NGF	PGF-15	PGF-30	PGF-45
Moisture (%)	8.12±0.16 ^c	9.12±0.01ª	8.63±0.02 ^b	8.56±0.04 ^b
Ash (db%)	3.18±0.27ª	3.02±0.27ª	2.79±0.51ª	3.09±0.35ª
Fat (db%)	5.38±0.23ª	4.97±0.22ª	4.87±0.37ª	4.83±0.32ª
Protein (db%)	9.74±0.36ª	9.22±0.20 ^b	9.56±0.34°	9.47±0.56ac
Carbohydrate (db%)	81.69±0.87 ^b	82.41±0.14 ^{ab}	83.41±1.05ª	82.79±0.89ab
Starch (db%)	70.25±0.47 ^{ab}	70.90±1.33ª	68.97±0.80 ^b	66.52±0.45°
Amylose (db%)	28.11±0.16ª	25.80±0.48 ^b	24.75±0.48°	24.33±0.50°
Dietary fiber (db%)	21.00±2.96ª	15.69±2.62 ^b	15.76±0.46 ^b	17.50±0.22ª

Table 1: Chemical composition of pre-gelatinized gayam flours

Values are mean±standard deviation and with different superscript letters in each row are significantly different (p<0.05), db: Dry weight basis, NGF: Native gayam flour, 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

Table 2: Color characteristics of pre-gelatinized gayam flours

Color	NGF	PGF-15	PGF-30	PGF-45
Lightness (L*)	71.38±0.11 ^c	76.51±0.18 ^b	84.13±0.01ª	84.12±0.14ª
Redness (a*)	2.61±0.08ª	1.25±0.42 ^b	0.70±0.01°	0.79±0.15℃
Yellowness (b*)	16.89±0.00ª	14.49±0.04 ^b	13.85±0.30 ^b	14.07±0.62 ^b
Color difference (ΔE)	0.00	5.82±0.03 ^b	13.24±0.04ª	13.18±0.05ª
Whiteness Index (WI)	71.38±0.11°	76.35±0.17 ^b	83.72±0.04ª	83.77±0.10ª

Values are Mean±standard deviation and with different superscript letters in each row are significantly different (p<0.05), NGF: Native gayam flour, PGF-15: Pre-gelatinized gayam flour boiled for 30 min and PGF-45: Pre-gelatinized gayam flour boiled for 45 min

9.22-9.56% and differed significantly with NGF (9.74%). Meanwhile, the carbohydrate content of gayam flours ranged from 81.69-83.41%. All of the pre-gelatinized gayam flours have higher protein and carbohydrate content than earlier reported for gayam flour⁴. These results confirmed the pre-gelatinized gayam flour as a good source of protein and carbohydrate. The starch and amylose content of gayam flours differed significantly and in the range of 66.52-70.90% and 24.33-28.11%, respectively. The PGF-45 has lower starch and amylose content than other gayam flours. It could be concluded that starch and amylose content decreased as the times of pre-gelatinization increased. Decreasing of starch content after pre-gelatinization could be contributed by the degradation of starch, particularly in amylose fraction of starch granules. The starch granules could be damaged since the heating increased and amylose leaching occurs more intensive²⁴. No differences were found in total dietary fiber content of native flour and PGF-45. The total dietary fiber content of PGF ranged from 15.69-21.00%, indicating pre-gelatinized gayam flours as a good source of dietary fiber.

Color: The Hunter color parameter such as L*, a*, b*, ΔE and WI were used to describe the color changes during pre-gelatinization of gayam flours and are presented in Table 2. The lightness (L*) of pre-gelatinized gayam flour ranged from 71.38-84.12. The L* of PGF-30 and PGF-45 had significantly higher than others flour. It means the longer pre-gelatinization time could more retard the enzymatic reactions. Zeuthen *et al.*⁶ have reported pre-gelatinization

was a good color preservative of food, as it retards enzymatic reaction. The a* and b* values of NGF differed significantly and higher than PGF. All of PGF in this study were more yellow as shown in the positive of b* value.

The difference of color of gayam flour could be described from the change in DE values. In this study, the ΔE values of pre-gelatinized gayam flour for PGF-15, PGF-30 and PGF-45 were 5.82±0.03, 13.24±0.04 and 13.18±0.05, respectively. The differences of color can be classified as follows: Different $(1.5 < \Delta E < 3)$ or very different $(\Delta E > 3)^{25}$. Based on these categories, the color of pre-gelatinized gayam flour was categorised as a very different. The WI values of PGF-30 and PGF-45 were 83.72 and 83.77, while PGF-15 and NGF were 76.35 and 71.38, respectively. The increasing of WI could be associated to the inactivation of polyphenol oxidase due to pre-gelatinization treatment. The WI was contributed by the reaction between polyphenol enzyme and depletion of oxygen²⁶. The longer pre-gelatinization time could increase the temperature in gayam seed and more deactivate polyphenol oxidase²⁷.

Microstructure: Figure 1 shows the scanning electron micrographs of gayam flour without and with pre-gelatinization at different times. Starch granules of pre-gelatinized gayam flours were oval, rough surface and heterogeneous sizes, whereas NGF appeared spheric, smooth surface and homogeneous sizes. Heating of starch over the gelatinization temperature could affect the swelling and rupture of starch granules. Sunarti *et al.*²⁸ reported the rupture

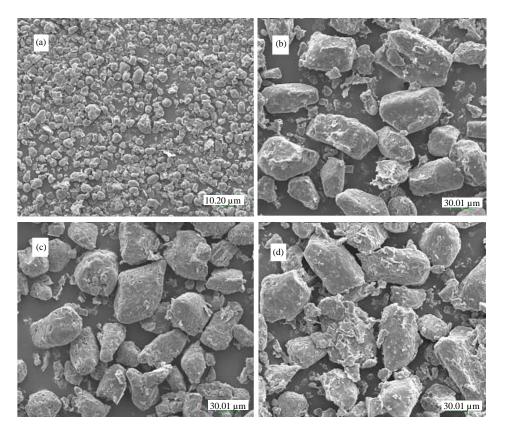


Fig. 1(a-d): Scanning electron microscopic images of (a) NGF, (b) PGF-15, (c) PGF-30 and (d) PGF-45 1000X magnification. NGF: Native gayam flour, PGF-15: Pre-gelatinized gayam flour boiled for 15 min, PGF-30: Pre-gelatinized gayam flour boiled for 30 min and PGF-45: Pre-gelatinized gayam flour boiled for 45 min

of starch granules that have been modified due to the heating process at high temperature. Ambigaipalan *et al.*²⁹ explained probably during granule development, cracking may have resulted in re-distribution of amylose and/or amylopectin reducing ends to the outer regions of the granule. This observation could be attributed to the loss of the amylopectin crystalline region during pre-gelatinization and re-association of the starch chains within the granules.

Water and oil holding capacity: The water and oil holding capacity (WHC and OHC) of pre-gelatinized gayam flour are shown in Table 3. The WHC is defined as the ability to physically hold water against gravity³⁰ and is defined as the amount of water that can be absorbed per gram of sample. The WHC of pre-gelatinized gayam flour ranged from 1.85-2.06 g g⁻¹. The PGFs have higher WHC than NGF. The PGF-45 had higher WHC (2.06 g g⁻¹) and differed significantly with the other flours (p<0.05). The longer pre-gelatinization time could increase the WHC of PGF. This result could be associated to the weakening associative in the starch components during pre-gelatinization with boiling resulting

in high water holding capacity¹². Flour with high WHC can be a substitute for wheat flour for making bakery, since a higher WHC enables bakers to add more water to the dough³¹.

The OHC of pre-gelatinized gayam flour differed significantly and ranged from 0.58-0.71 g g⁻¹. The longer pre-gelatinization time could decrease the OHC of pre-gelatinized gayam flour. Basically, the mechanism of OHC is mainly due to the physical entrapment of oil by capillary attraction³⁰. It was proven that NGF has a higher OHC due to having a higher fat content (5.38%) compared to the other gayam flour. The OHC was affected by the lipophilic nature of the granular surface³². The major chemical affecting OHC is protein, which is consisted of both hydrophilic and hydrophobic chains. Non-polar amino acid side chains can form hydrophobic interactions with hydrocarbon chains of lipid³³. Oil absorption is an important functional property in food formulations since oils improve the mouth feel and retention of flavor³⁰.

Swelling power and solubility: Swelling power and solubility of pre-gelatinization gayam flour were shown in Table 3. The

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Table 3: Functional properties of pre-gelatinized gayam flours

Functional properties	NGF	PGF-15	PGF-30	PGF-45
WHC (g g ⁻¹)	1.85±0.04 ^c	1.94±0.02 ^b	1.98±0.03 ^b	2.06±0.01ª
OHC (g g ⁻¹)	0.71±0.01ª	$0.60 \pm 0.01^{ m b}$	0.58±0.01°	0.61±0.01 ^b
Swelling power (g g^{-1})	1.66±0.464°	3.76±0.35 ^{ab}	4.03 ± 0.06^{ab}	4.16±0.18ª
Solubility (%)	15.53±0.68ª	12.96±0.72 ^b	12.98±1.47 ^b	13.55±1.48 ^{ab}

Values are Mean±standard deviation and with different superscript letters in each row are significantly different (p<0.05), WHC: Water holding capacity, OHC: Oil holding capacity, NGF: Native gayam flour, 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

Table 4: Pasting properties of pre-gelatinized gayam flours

Pasting properties	NGF	PGF-15	PGF-30	PGF-45
Pasting temperature (°C)	81.45±0.28 ^d	87.68±0.25°	89.73±0.04 ^b	90.35±0.01ª
Peak viscosity (cP)	2601.00±48.08ª	891.50±9.19 ^b	930.50±17.68 ^b	853.50±41.72 ^b
Trough viscosity (cP)	2446.50±30.41ª	893.50±9.19 ^b	931.00±18.38 ^b	854.00±41.01 ^b
Breakdown viscosity (cP)	154.50±17.68ª	-2.00±0.01b	-0.50±0.71 ^b	-0.50±0.71 ^b
Final viscosity (cP)	4061.00±65.05ª	1354.50±9.19 ^b	1336.00±0.01 ^b	1404.00±26.87 ^b
Setback viscosity (cP)	1614.50±34.65ª	461.00±0.01°	387.50±6.36 ^d	550.00±14.14 ^b
Peak time (min)	8.93±0.09ª	12.97±0.05 ^b	12.97±0.05 ^b	13.00±0.01 ^b

Values are Mean±standard deviation and with different superscript letters in each row are significantly different (p<0.05), NGF: Native gayam flour, PGF-15: Pre-gelatinized gayam flour boiled for 30 min and PGF-45: Pre-gelatinized gayam flour boiled for 45 min

swelling power of PGF-15, PGF-30 and PGF-45 increased as pre-gelatinization time increased. The PGF-45 had higher values (4.16 g g⁻¹) and differed significantly with other gayam flours (p<0.05). The swelling power of NGF had the lowest value (1.66 g g⁻¹). The shorter pre-gelatinization time could affect the starch contents of the raw flour that were intact and would not swell until it reached the gelatinization temperature. These results indicated starch granules of gayam seed could be more susceptible to water hydration as pre-gelatinization time increased³⁴.

Among the pre-gelatinized gayam flours, solubility value was highest in PGF-45 (13.55%). Solubility values ranged from 12.96-15.53%. The PGF-15 had lower solubility (12.96%) while NGF and PGF-45 have higher solubility. The longer pre-gelatinization time may increase in temperature allowing amylose (water-soluble fraction) molecules located in the bulk amorphous regions to interact with the branched segment of amylopectin (water insoluble fraction) in the crystalline regions. This result implies that the high temperatures weaken the starch granules resulting in increased solubility³⁵.

Pasting properties: The pasting profiles of the gayam flour with the different times of pre-gelatinization are shown in Table 4. The peak (853.50-891.50 cP), through (854.00-893.50 cP), breakdown (-2.00-(-0.50) cP), final (1336.00-1404.00 cP) and setback viscosity (387.50-550.00 cP) of pre-gelatinized gayam flours were lower than the native gayam flour, whereas the pasting temperature (87.68-90.35°C) was higher. These parameters are attributed to the pre-gelatinization process that changed the granule to be much strong and resist swelling at high temperature. Besides the low breakdown value indicated the pre-gelatinized gayam flour was durable against heat and shear³⁶.

The highest pasting temperature was found in PGF-45 and those showed that the longer pre-gelatinization times in gayam flour processing increased the higher pasting temperature. According to Du *et al.*³⁷, the high pasting temperature of gayam flour indicates that its starch is highly resistant to swelling and rupture. The longer times of pre-gelatinized is a hydrothermal process leading to increased starch crystallinity because of the changes in starch granule, so that high temperatures are needed to reach maximum expansion and break down of the starch granule¹⁵.

In general the viscosity of NGF was higher than pre-gelatinized gayam flour but at PGF-15, PGF-30 and PGF-45 there were no significant differences. The setback viscosity of pre-gelatinized gayam flour increased as the pre-gelatinization time increased, however it had no effect on the peak, trough, breakdown and final viscosity. These results indicated the time of boiling affected the pre-gelatinization pasting characteristics. All of the pre-gelatinized gayam flour showed PGF-45 had highest setback viscosity. The setback values indicate the hardness of gel paste upon cooling³⁸, which is used to determine to tendency of retrogradation and syneresis occurrences in the starch^{39,40}. High setback value gave lower retrogradation during cooling and the lower staling rate of the products made from the flour⁴¹.

CONCLUSION

The significant differences in physicochemical and functional properties were observed among the pre-gelatinized gayam flours with respect to starch, amylose content, color, microstructure, WHC, OHC and pasting properties, while moisture, ash, protein, fat, carbohydrate and dietary fiber content did not differ significantly. The pre-gelatinization treatment of gayam seed increased the whiteness index, WHC and swelling power but decreased OHC, solubility, peak viscosity, trough viscosity, breakdown viscosity and final viscosity. The results showed the pre-gelatinized gayam flours have the potency as a source of good dietary flour for substituting or replacing other flour such as wheat flour in a new food product development.

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