

Physicochemical, functional, pasting and thermal properties of resistant starch type III from banana (*Musa acuminata* X *balbisiana* cv. Awak) pseudostem

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Abstract

In Malaysia, banana is the second most widely cultivated fruit. The banana pseudostem (BP) is cut and dumped as waste after harvesting. Unfortunately, pseudostem is currently underutilised as a functional food ingredient due to its high-quality starch content. Therefore, this study aimed to determine the proximate compositions, physical attributes, and functional characteristics of starches produced from BP, in comparison with commercial starches, such as potato starch (PS) and corn starch (CS). Native starch from banana pseudostem (BPNS) was isolated. Then, resistant starch type III (RS3) was prepared from native starch (RS3BP) by an autoclaving process. The starches were subjected to physicochemical (i.e., proximate composition, water activity, bulk density, and colour), functional (i.e., amylose, amylopectin, water holding, and oil holding capacities), pasting (by using rapid visco analyser), and thermal properties determination (by using differential scanning calorimetry). The results showed that there were significant ($p < 0.05$) increases in the amylose content of RS3BP after the autoclaving process. The water and oil holding capacities of BPNS did not change after autoclaving. The results of pasting and thermal properties indicated that BPNS contained a stronger starch granule and greater thermal stability than commercial starches (PS and CS). In addition, the autoclaving process decreased significantly ($p < 0.05$) the content of moisture, crude protein, crude fat, and ash but increased significantly ($p < 0.05$) the total carbohydrate and energy values in BPNS. The autoclaving process also resulted in a significant ($p < 0.05$) darker and red-yellowish colour of RS3BP than other starches. In conclusion, the findings of this study demonstrated that the isolated BPNS has good starch granule stability and retains its functional properties after autoclaving during the production of RS3BP, even with an increased energy source. Therefore, BP can be considered a viable alternative raw material for producing native starch or resistant starch type III.

1. Introduction

In Malaysia, banana is the second most widely cultivated fruit, whereby banana plantation covers an area of about 23,000 hectares with a total production of 0.3 million metric tons in 2021 (Food and Agriculture Organization of the United Nations (FAO), 2023). However, after harvesting, the pseudostem of the banana is cut as the plant part is useless for the next harvest (Ho *et al.*, 2012). Sokchea *et al.* (2018) reported that approximately 40% of banana plants were considered waste left in the field to decompose. It was reported that about 60–80 tons/ha of banana pseudostem (BP) is generated annually. Therefore, to pursue a better way to resolve the problem of BPs is worthy.

Starch is mainly made up of two polymers of glucose, which are amylose and amylopectin. Amylose is linear or multiple long chains of α -1,4 linked glucose unit, while amylopectin consists of a large number of shorter chains joined together to form branched chains of α -1,4 and α -1,6 branching links of glucose. These two polymers represent approximately 98-99% of starch on a dry weight basis, while the remaining are the presence of small amounts of lipids, protein, phosphorus, and minerals (Horstmann *et al.*, 2017). Moreover, according to Jyothsna and Hymavathi (2017), starch is one of the most important glycemic carbohydrates which contribute up to 70-80% of total carbohydrates in human diets.

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The global production of starch is from corn starch with the production value of approximately 64.6 million tonnes/year, followed by cassava starch (10.2 million tonnes/year), wheat starch (6.0 million tonnes/year), potato starch (3.4 million tonnes/year) and the least, rice starch with the production value of approximately 0.05 million tonnes/year (Waterschoot *et al.*, 2016). Recently, global trends are moving towards the development of 'green products' bio-based, which focus more on reducing waste and maximising resource efficiency (Söderholm, 2020). This circumstance has put pressure on the existing starch production, which mainly relies on the crops grown for human consumption. Therefore, there is an increasing interest in finding and studying new starch resources from less conventional sources. Ho and Wong (2019) reported that BP contained high-quality starch, which is at 44.8%. Moreover, the amylose content of BP starch compares well with that of banana fruit and potato starch (21%) (Shantha and Siddappa, 1970). In addition, Ho *et al.* (2012) reported that BP had a high value of resistant starch (12.81%).

Resistant starch refers to the sum of starch and degraded starch products that resist digestion in the small intestine of healthy people (Sofi *et al.*, 2017; Kwon *et al.*, 2019). There are five general sub-types of resistant starch fraction in food; type I (RS 1), type II (RS 2), type III (RS 3), type IV (RS 4), and type V (RS 5) (Kwon *et al.*, 2019). For the RS 1, it corresponds to the physically inaccessible starches which are entrapped in the cellular matrix. RS 2 is native/raw uncooked granules of some starches. Meanwhile, RS 3 is retrograded starches which cause a reduction in glycemic response (Meenu and Xu, 2018). Chemically modified starches generally belong to RS 4 and their molecular structures are altered chemically in many ways. RS 5 comprises amylose-lipid complexes, which have helical structures with fatty acid tails in the central cavities of the inclusion complex formed by alpha-amylase and polar lipids (Xia *et al.*, 2018). Generally, the native starches of potato, corn, and rice are widely used to prepare RS 3 (Li, Ai and Yuan, 2020; Li *et al.*, 2021; Tandhanskul *et al.*, 2021; Liu *et al.*, 2023). Recently, Liu *et al.* (2023) prepared type III resistant starch from native starch of potatoes by ultrasound-assisted autoclave gelatinization method and investigated its effect on the quality of steamed bread. The authors reported that the incorporation of type III resistant starch from native starch of potato in steamed bread making had significantly shortened the fermentation time of the dough (increased productivity) and improved the network structure and colour of the end product (Liu *et al.*, 2023). An *in vitro* study performed by Tandhanskul *et al.* (2021) showed that the resistant starch type 3 prepared from rice had significantly lower levels of rapidly available glucose

and slowly available glucose as compared to its native starch, indicating that the resistant starch type III of rice is slowly digested to release the glucose into the system. Another study (*in vivo*) conducted by Thompson *et al.* (2023) showed that a diet supplemented with resistant starch from sago (*Metroxylon sagu*) starch can confer health benefits on the fat-induced Sprague Dawley rats.

There are no published reports regarding RS 3 prepared by BP. To fulfil the growing demand, an alternative starch source, such as BP could be utilised in the food industry. The understanding of physicochemical and functional properties is a prerequisite for BP starch. However, there is a lack of knowledge in these areas, in contrast to the commercial starch. This study focused on producing starches (i.e., native and resistant starch type III) from BP and determining its proximate compositions, physical attributes, and functional characteristics. Furthermore, the characteristics of the produced starches were compared to commonly used commercial starches (PS and CS) to determine the potential of native starch of BP as a new ingredient in food preparation.

2. Materials and methods

2.1 Materials

Mature banana (*Musa acuminata* X *balbisiana* cv. Awak) pseudostem was collected from a banana plantation in Pulau Berangan, Besut, Terengganu while the commercial CS and PS were purchased from a local grocery store. All chemicals used in this study were of analytical grade.

2.2 Isolation of banana pseudostem starch

The native starch of BP (BPNS) was isolated by using a simple sample steeping method, modified from the studies by Nakthong *et al.* (2017) and Rahma *et al.* (2019). Before isolation, impurities and dirt were removed, and the pseudostems were cleaned and cut into longitudinal pieces before being mechanically pressed with excess water. The pressed stems were soaked in distilled water at a sample-to-water weight ratio of 1:2 for 24 hrs at 4°C before being filtered by using four layered muslin cloth along with the juice extracted from the BP and left for another 24 hrs to form crude starch sediment. The resultant starch was collected and washed with distilled water several times to distinguish the starch fraction from other water-soluble materials. The precipitated starch was dried in a cabinet drier at 40°C for 8 hrs and subsequently ground in a laboratory mill before being sieved via a 100-mesh screen. The isolated native BP starch was kept in an airtight bottle and stored before further use.

2.3 Preparation of resistant starch type III - retrograded starch

The procedure for producing type III resistant starch from BPNS was conducted by referring to a method adopted by Zi-Ni *et al.* (2015), with minor modifications. In addition, a preliminary study on the optimum condition (i.e., concentration of starch (4%), concentration of enzyme pullulanase (40 %), number of thermal cycles (3%), and drying temperature (40°C) for producing RS 3 from BPNS were conducted by one factor at a time method (unpublished data). BPNS (4% weight per volume on a dry basis) was suspended in sodium acetate buffer (0.1 M, pH5). The mixture was subjected to autoclaving at 121°C for 1 hr. The mixture was then cooled to 60°C before an enzymatic debranching process. Pullulanase (40% concentration) was added to the mixture to debranch the starch. The sample was then heated at 80°C for 15 mins to inactivate the enzyme. Then, the sample was autoclaved at 121°C for 30 mins to completely gelatinise the starch.

The sample underwent three temperature cycles to create and develop high-melting RS 3 crystals. In the first cycle, the sample was autoclaved at 121°C for 1.5 hrs to accelerate the RS 3 crystal formation. Next, the sample was incubated in a water bath at 70°C for 3 hrs to cool the gelatinised starch. In the second cycle, the sample was autoclaved again at 121°C for 1.5 hrs to generate RS 3 crystals after being incubated at 70°C in a water bath for 18 hrs to initiate crystal growth. In the third cycle, the sample was autoclaved once again at 121°C for 1.5 hrs and followed by incubating in the water bath for 3 hrs at 70°C. After cooling, the sample was oven-dried at 40°C until a constant weight of moisture content was achieved. The sample was ground by using a laboratory mill. Then, the sample was sieved by using a mechanical sieve shaker to obtain a fine powder (250 µm). All samples were kept in an airtight container before further analysis.

2.4 Analysis of amylose and amylopectin content

The amylose content of starches was determined by using the colourimetric iodine method as described by Nuwamanya *et al.* (2011). The sample was dispersed into ethanol and then followed with a gelatinisation process using sodium hydroxide (0.1 M). An aliquot of the gelatinised starch was treated with citric acid (0.1 M) before treating with an iodine solution. The resulting solution absorbance was measured by using a UV-vis spectrophotometer (Shimadzu, UV mini-1240, Kyoto, Japan) at 620 nm. For amylopectin content, it was calculated by the differences obtained.

2.5 Determination of proximate composition

2.5.1 Determination of moisture content

The moisture content of the samples was determined according to the oven drying method (Association of Official Analytical Chemists (AOAC) International Official Method 977.11) (AOAC International, 2023). Approximately 5 g of the sample was placed in the pre-dried metal dish, then dried in an oven at 105°C until a constant weight was achieved. The moisture content was calculated by subtracting the dry weight of the samples from the wet weight of the samples and then dividing the result by the wet weight. Results were expressed as a percentage.

2.5.2 Determination of crude protein content

The crude protein content of the samples was determined according to the Kjeldahl method (AOAC International Official Method 955.04) (AOAC International, 2023). Approximately 0.3 g of pre-dried sample was digested with copper sulfate (5 mg) in concentrated sulfuric acid (2 mL) at 350°C until a colourless and clear solution was obtained. The solution was distilled and collected in a flask containing 2% boric acid (10 mL) until all ammonia was distilled, followed by titration with 0.02 M HCl until the boric acid solution changed from green to light purple. The percentage of nitrogen was converted to protein by multiplying by 6.25. Results were expressed as a percentage.

2.5.3 Determination of crude fat content

The crude fat content of the samples was determined according to the Soxhlet method (AOAC International Official Method 960.39) (AOAC International, 2023). The crude fat of the sample (5 g) was extracted with petroleum ether (125 mL, boiling point 40-60°C) using a Soxhlet extractor for 2 hrs until no oil trace remained on the tested filter paper. Then, the extracted fat was then dried in an air oven at 105°C for 1 hr. The extracted crude fat was weighed and the crude fat content was calculated. Results were expressed as a percentage.

2.5.4 Determination of ash content

The ash content of the samples was determined according to the dry ashing method (AOAC International Official Method 923.03) (AOAC International, 2023). Approximately 3 g of the dried sample were placed in the pre-ignited crucible and heated over an open flame until carbonized, then ignited in a muffle furnace at 550°C until the sample became ash. The ash content was calculated as the weight of the ash divided by the initial weight of the sample. Results were expressed as a percentage.

2.6 Computation of total carbohydrate content

The total carbohydrate content of the sample was estimated by subtracting the sum of moisture, crude protein, crude fat, and ash from 100% [% total carbohydrates = 100 - (moisture + ash + crude protein + crude fat)] (Giri and Sakhale, 2019)

2.7 Computation of calorie value

The calorie value of the sample was calculated by multiplying the amounts of crude protein, crude fat, and total carbohydrates with factor values (4, 4 and 9, respectively) and the value was expressed in kcal (Giri and Sakhale, 2019).

2.8 Determination of physical and functional characteristics

Water activity was determined by using a Water Activity Meter (Decagon's AquaLab Series 3, Pullman, USA). For bulk density measurement, approximately 0.5 g of sample was filled into 5-mL graduated cylinder and then gently tapped several times until the sample level had stabilised. Then, the bulk density was computed: weight of sample per volume of sample (g/mL) (Ho *et al.*, 2018). For water holding capacity (WHC) and oil holding capacity (OHC), approximately, 250 mg of sample was added with 10 mL of distilled water or commercial palm oil (for WHC and OHC, respectively), followed by stirring for 30 mins by using a magnetic stir bar. The sample was further centrifuged at a speed of 7,000 rpm for 30 mins after being placed at room temperature for 30 mins. Each centrifuge tube was weighed after decanting the supernatants. Then, WHC and OHC were calculated as g water or g oil per g of dry sample, respectively.

The samples colour was measured according to the Commission Internationale de l'Éclairage (CIE) $L^*a^*b^*$ scale. Colorimeter (Konica Minolta, CR-400/410, Tokyo, Japan) was used to determine the L^* [Lightness ($L^*=0$; black, $L^*=100$; white)], Chroma a^* [green chromaticity (-60) to red (+60)], and Chroma b^* [blue chromaticity (-60) to yellow (+60) space values. The equipment was pre-calibrated with a white standard prior to analysis.

2.9 Determination of pasting properties

The pasting properties of starches were assessed by using the Visco-Analyser (Model RVA 4500 series 4500; Perten Instruments of Australia Pty Limited, Australia). Starch slurries containing 9% w/w starch (dry weight) in a total weight of 3 g were prepared in aluminium canisters. Starch slurries were held at 50°C for 1 min before heating to 95°C, holding at 95°C for 3

mins, and then cooling to 50°C and held at 50°C for 2 mins. The speed of the mixing paddle was set at 960 rpm for the first 10 s, then 160 rpm for the remaining analysis. The starch viscosity parameters measured were pasting temperature (PT), pasting viscosity (PV), breakdown (BD), setback (SB) and final viscosity (FV).

2.10 Determination of thermal properties

The thermal properties of the starches were investigated by using a differential scanning calorimeter (DSC) (Mettler Toledo, model DSC 1, Mettler-Toledo AG, Analytical, Switzerland) according to the procedure described by Espinosa-Solis *et al.* (2021). The starch of 2 mg was weighed in an aluminium pan (ME-26763, Switzerland) and 7 μ L of distilled water was added. The pans were hermetically sealed and allowed to equilibrate in a desiccator for 1 hr before being analysed. The pans were heated from 20°C to 120°C at a heating speed of 10°C/min. The values for the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinisation enthalpy (ΔH_g) of the samples were recorded.

2.11 Statistical analysis

Statistical analyses were performed by using SPSS Version 20.0 software (SPSS Inc., Chicago, IL, USA). The results obtained were represented as mean \pm standard deviation (SD) of triplicate. The significant differences between mean values were analysed by using One-way ANOVA at a significance level of $p < 0.05$.

3. Results and discussion

3.1 Proximate compositions, amylose and amylopectin content of starches

The proximate composition of starches from BP, potato, and corn is shown in Table 1. The moisture content of the starches was in the range of 11.67% to 17.47%. The resistant starch of banana pseudostem (RS3BP) had the lowest moisture content, followed by corn starch (CS), native starch of banana (BPNS), and potato starch (PS). The difference in moisture content of the starches may be attributed to the different processing methods employed, particularly isolation and drying processes. According to Ho *et al.* (2018), flour or powder with moisture content lower than 14% has a good storage quality in terms of lower risk of mould growth, insect infestation, and agglomeration. Therefore, RS3BP exhibited better storage stability with a longer shelf life than PS in this study.

The crude protein content of CS (0.45%) was statistically higher than BPNS, PS, and RS3BP (0.22%, 0.17% and 0.01%, respectively) (Table 1). On the other

Table 1. Proximate compositions, amylose, and amylopectin content of starches.

Composition (%)	BPNS	RS3BP	PS	CS
Moisture	12.71±0.56 ^b	11.14±0.15 ^c	17.47± 0.03 ^a	11.67±0.05 ^c
Crude protein	0.22±0.08 ^b	0.01±0.55 ^c	0.17±0.03 ^b	0.45±0.04 ^a
Crude fat	0.21±0.09 ^a	0.07±0.04 ^b	0.07±0.09 ^b	0.12±0.11 ^{ab}
Ash	0.45±0.06 ^a	0.16±0.02 ^c	0.38±0.01 ^b	0.10±0.00 ^c
Total carbohydrate ¹	86.87±0.69 ^c	88.77±0.26 ^a	82.30±0.08 ^d	87.76±0.10 ^b
Energy ² (kcal/100 g)	350.21±1.96 ^b	355.76±0.27 ^a	330.47±0.54 ^c	353.95±0.16 ^a
Amylose	48.74±0.51 ^b	52.97±0.80 ^a	37.63±0.90 ^c	34.63±0.67 ^d
Amylopectin ¹	51.26±0.51 ^c	47.03±0.80 ^d	62.37±0.90 ^b	65.37±0.67 ^a
Amylose/amylopectin	0.95±0.02 ^b	1.13±0.04 ^a	0.60±0.02 ^c	0.53±0.02 ^d

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different (p<0.05). BPNS: native starch of banana pseudostem, RS3BP: resistant starch of banana pseudostem, PS: potato starch, CS: corn starch.

¹Result obtained by calculation.

hand, BPNS had higher crude protein content than RS3BP (produced by the autoclaving process). It can be explained by the fact that the autoclaving process resulted in the denaturation and solubilisation of proteins, thus reducing the crude protein content after the autoclaving process. Furthermore, as compared to pineapple stem starch, the crude protein content of BPNS was slightly lower by 0.49%, while other authors reported 0.18%, 0.27%, and 0.55% as the crude protein of starches from cush-cush yam, palado seed, and taro, respectively (Lovera *et al.*, 2017; Nakthong *et al.*, 2017; Rahman *et al.*, 2017). However, the crude protein content from the current findings presented a higher value than plantain banana starch (0.1%), as reported by Ssonko and Muranga (2017). Regarding the crude fat content of the starches, BPNS had a crude fat content (0.21%) that was 3, 3, and 1.8 times greater than RS3BP, PS, and CS, respectively. Saeid *et al.* (2015) explained that lower crude fat content indicated a lower tendency to rancidity.

Ash is an inorganic residue that reflects the total amount of minerals, such as calcium, zinc, iron, and phosphorus present in the test sample (Ho *et al.*, 2018; Espinosa-Solis *et al.*, 2021). In this study, BPNS had a statistically higher ash content (0.45%) than RS3BP, PS, and CS (0.16%, 0.38%, and 0.10%, respectively). However, RS3BP and CS showed no significant (p>0.05) differences in ash content. Similar values were also observed in the starches of banana, taro, and palado seed (0.47%, 0.43%, and 0.49%, respectively) (Lovera *et al.*, 2017; Rahman *et al.*, 2017; Ssonko and Muranga, 2017). In addition, it was also observed that RS3BP had significantly (P<0.05) lower ash content as compared to its native starch (BPNS) due to the autoclaving process during resistant starch type III production. According to Espinosa-Solis *et al.* (2021), high pressures and high temperatures applied during the processing of resistant starch type III could cause the breaking of phosphate

groups, particularly the ester bonds, consequently lowering the ash content.

The main component of RS3BP, BPNS, PS, and CS was carbohydrate, expressed as total carbohydrates (Table 1), the majority constituted by starch. The total carbohydrate content of the starches was in a range of 82.3% to 88.77%. The high carbohydrate content translated to the high starch yield acquired from the resources (Michael *et al.*, 2020). As Compared to previous studies, BP starch had higher carbohydrate content than palado seed starch (77.04%) and pearl millet starch (83.4%), however lower than pineapple stem starch (97.52%) (Nakthong *et al.*, 2017; Rahman *et al.*, 2017; Michael *et al.*, 2020). RS3BP (355.76 kcal/100 g) and CS (353.95 kcal/100 g) had significantly (P<0.05) higher energy content than other evaluated starches in the order of BPNS (350.21 kcal/100 g) > PS (330.47 kcal/100 g). The differences in the energy content of the powders could be attributed to the differences in their crude protein, crude fat, and carbohydrate contents.

The results of amylose, amylopectin, and ratio of amylose/amylopectin are presented in Table 1. RS3BP showed higher values of amylose content as compared to other samples (BPNS, PS, and CS). Moreover, the amylose content of BPNS (48.74%), had increased in RS3BP (52.97%), which was the resulting retrograded starch of BPNS after being treated with pullulanase enzyme, subsequently continued with the autoclaving process. An increase in amylose content in RS3BP could be due to the debranching of α-(1-6) linkage of amylopectin and converted amylose molecules which had a smaller linear chain of polysaccharides (Reddy *et al.*, 2015). According to Fitriani *et al.* (2021), amylose can be classified into three categories: low amylose (10% -15%), moderate amylose (>15%), and high amylose (>20%). Therefore, the amylose content of all starches fell in the category of high amylose. High amylose

content in food can decrease blood sugar levels and glycemic response due to its unbranched and compact structure as compared to amylopectin, whereby the straight chain structure of amylose requires a longer time to digest. This resulted in a lower blood sugar level as compared to consuming food with high amylopectin (Zafar, 2018; Fitriani *et al.*, 2021).

For amylopectin, the amylopectin content in the starches varied, ranging from 47.03% to 65.37% (Table 1). Moreover, amylopectin content was the opposite of amylose content. The corn starch contained the significant ($p < 0.05$) highest amylopectin (65.37%), while RS3BP contained the least (47.03%). The amylopectin results of the commercial starch (i.e., PS and CS) were slightly lower than the data revealed by Horstmann *et al.* (2017), whereby PS and CS contain 70%–80% amylopectin. This might be due to the different botanical origins of starch (Zieba *et al.*, 2019).

Statistically, RS3BP presented a significant ($p < 0.05$) higher ratio in amylose/amylopectin (1.13). According to Vaitkeviciene *et al.* (2022), a higher amylose/amylopectin ratio of starch generally reflects higher strength and plasticity of material and influences the gelatinisation process, hence generating a uniform amorphous thermoplastic structure by the presence of heat. Moreover, a high amylose/amylopectin ratio made the food more resistant to being digested by digestive enzymes and produced a higher satiety effect (Zafar, 2018). Overall, RS3BP has a high potential to be used as a functional food ingredient due to its high amylose content and ratio of amylose/amylopectin but low in amylopectin content that may provide health benefits on appetite and body weight management.

3.2 Physical and functional characteristics of starches

The results obtained about the physical and functional characteristics of starches are presented in Table 2. Water activity (a_w) is one of the most critical factors in determining food quality and safety. The a_w of starches produced from BP (i.e., RS3BP and BPNS)

were lower (0.48 and 0.47, respectively) than PS and CS (0.70 and 0.61, respectively). Reducing a_w below 0.6 prevents microbiological spoilage, which subsequently prolongs the product shelf life (Alegbeleye *et al.*, 2022).

Bulk density is one of the basic parameters measured in dried food regarding transport, storage, and packaging. An increase in density resulted in the reduction of product volume (Ding *et al.*, 2020). High bulk density is desirable to reduce shipping and packaging costs as it implies that less packaging material would be required. In this study, starches from BP and PS showed high bulk density values (RS3BP: 0.91 g/mL, BPNS: 0.91 g/mL, and PS: 0.96 g/mL, respectively as compared to CS (0.63 g/mL). These values were also higher than the bulk density of the starches from tapioca (0.58 g/mL), pearl millet (0.67 g/mL), and pineapple stem (0.78 g/mL) (Mohd Noor *et al.*, 2019, Rahma *et al.*, 2019, Michael *et al.*, 2020).

Table 2 also summarises the water and oil holding capacity (WHC and OHC, respectively) of the starches that indicated the ability of the sample to incorporate with water and oil. In this study, WHC between starches produced from BP were not statistically significant ($p > 0.05$), with values of 1.28 water/g dm (RS3BP) and 1.24 water/g dm (BPNS). However, both starches showed a lower WHC than PS (2.44 water/g dm). Previous authors reported low WHC values for starches from sweet potatoes (0.74) (Kale *et al.*, 2017). This could be due to a greater hydrophilic tendency than the hydrophobic tendency of isolated starches.

On the other hand, the OHC of potato starch (2.79 g oil/g dm) was higher than starches produced from BP (RS3BP and BPNS) and CS (1.17 g oil/g dm, 1.21 g oil/g dm, and 1.35 g oil/g dm, respectively). In comparison to other starches, the OHC value of BP was slightly higher than that of arrowhead and cassava starch (1.02, 1.03 and 1.12, respectively) (Astuti *et al.*, 2018; Mohd Noor *et al.*, 2019). The importance of OHC is crucial for

Table 2. Physical and functional characteristics of starches.

Parameter	BPNS	RS3BP	PS	CS
Water activity (a_w)	0.47±0.02 ^c	0.48±0.00 ^c	0.70±0.03 ^a	0.61±0.03 ^b
Bulk density (g/mL)	0.91±0.13 ^a	0.91±0.13 ^a	0.96±0.14 ^a	0.63±0.13 ^b
WHC (g water/g dm)	1.24±0.23 ^b	1.28±0.10 ^b	2.44±0.40 ^a	1.82±0.76 ^{ab}
OHC (g oil/g dm)	1.21±0.18 ^b	1.17±0.08 ^b	2.79±0.18 ^a	1.35±0.15 ^b
L^*	91.27±0.59 ^c	84.20±1.63 ^d	93.69±0.06 ^b	100.35±0.09 ^a
a^*	0.32±0.30 ^b	0.99±0.10 ^a	0.01±0.01 ^c	-0.94±0.02 ^d
b^*	4.28±0.06 ^b	9.59±0.27 ^a	1.10±0.08 ^c	4.17±0.01 ^b

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). BPNS: native starch of banana pseudostem, RS3BP: resistant starch of banana pseudostem, PS: potato starch, CS: corn starch, WHC: water holding capacity, OHC: oil holding capacity.

the use of starch in the cooking or frying process in the food industry.

Results of colour attributes (Table 2) indicated that all samples demonstrated a high luminosity value ($L^* > 90$), except for resistant starch produced from BP (84.20). The L^* value of CS showed the highest significant value among the starches. The L^* value of CS observed in this study was consistent with those values reported by Espinosa-Solis *et al.* (2021) for commercial CS (MaizenaTM, Unilever, Mexico) (100.02). The values of positive a^* ($+a^*$) of PS, BPNS, and RS3BP produced from BP indicated that they presented a reddish colour. However, the reddish colour of native starch of BP (0.32) increased after the autoclave process to obtain resistant starch (0.99).

BPNS presented a yellowish colour ($+b$), which significantly increased after the autoclaved of native starch of BP (RS3BP: 9.59). The variations in L^* , a^* , and b^* values of all starch samples could be associated with pigments, such as xanthophylls and carotenes present in the starch, which impart slightly reddish and yellowish to the RS3BP. In addition, the lowest L^* (lightness) and higher a^* , and b^* values of resistant starch produced from BP could also be attributed to the Maillard reactions (non-enzymatic darkening reaction) that occurred during the autoclaving process; with the presence of heat, the carbonyl groups of reducing sugars and the amino groups of proteins could have interaction (Espinosa-Solis *et al.*, 2021). In addition, according to Barua and Srivastav (2017), a caramelisation reaction also may occur during a heating (autoclaving) process that produces a single unit of sugar by breaking down starch molecules.

3.3 Pasting properties of starches

The pasting properties of the banana pseudostem native starch (BPNS), retrograded resistant starch of banana pseudostem (RS3BP), and commercial starches (PS CS) are tabulated in Table 3. Pasting temperature is the temperature at which the viscosity of the starch pastes starts to rise (Gani *et al.*, 2020). BPNS had significantly ($p < 0.05$) higher pasting temperature (81.07°C) as compared to RS3BP (74.03°C) and commercial starches (PS and CS) (66.97°C and 79.37°C , respectively). The higher pasting temperature represented stronger bonding forces and thus required higher temperature to overcome these forces (Ssonko and Muranga, 2017). This indicated that higher heat or energy inputs were needed during cooking BPNS as compared to cooking RS3BP and commercial starches. Moreover, a significant ($p < 0.05$) decreased in pasting temperature of RS3BP after retrogradation was due to the interruption of starch granules crystallite structure

that occurred during autoclave. Thereby, a lower pasting temperature was required to break the weak crystallite structure before the formation of the paste (Das and Sit, 2021).

Peak viscosity varied significantly ($p < 0.05$) among the tested starches with PS showing the highest value of 10416.67 cP, whereas RS3BP showed the lowest value of 1416.33 cP. The low peak viscosity of RS3BP was attributed to higher values of amylose contents, whereby a high amount of amylose tends to decrease the starch granules melting temperature by disrupting their crystallinity (Gani *et al.*, 2020). In addition, these obtained results indicated that PS had a higher water holding capacity (Table 2). According to Li *et al.* (2020), the longer branch chains of amylopectin in starch may act like amylose in the formation of helical complexes with lipids. This interacted with other branched chains to keep the integrity of starch granules during heating and shearing, then led to a lower peak viscosity as found in wheat starch. The current reported results were in agreement with previous findings by Gani *et al.* (2020) and Li *et al.* (2020) that high amylose starches had lower peak viscosity.

The value of breakdown viscosity represents the resistance of starch paste to shear force (Li *et al.*, 2020). The breakdown value of PS (9111.00 cP) was much higher than the breakdown value recorded in BPNS (480.33 cP), RS3BP (315.67 cP), and CS (894.33 cP). This indicated less resistance of BPNS, RS3BP, and CS to shear during cooking. Meanwhile, setback viscosity showed the extent of recovery viscosity or retrogradation of starch during the cooling period of the heated starch pastes (Li *et al.*, 2020). During this stage, the amylose and amylopectin chains realign themselves to form a more crystalline structure. Through which the increase in setback viscosity during the cooling period indicated the tendency of amylose present in a hot paste to re-associate with a decrease in temperature (cooling) (Balet *et al.*, 2019). BPNS had the highest setback viscosity (2998.33 cP) and the lowest setback viscosity value was found for RS3BP (562.00 cP). This indicated that the starch granules of RS3BP had a lower tendency to retrograde as compared to BPNS.

On the other hand, final viscosity is the determination of the ability of the starch to form a viscous cold starch paste due to the re-association of amylose and amylopectin molecules (Li *et al.*, 2020). BPNS had the highest final viscosity value (6646.33 cP) which was expected as the amylose content of BPNS was higher than PS and CS. However, this was exceptional for RS3BP as the amylose content of BPNS was lower than RS3BP (Table 1). The ability of BPNS to

Table 3. Pasting properties of starches.

Parameter	BPNS	RS3BP	PS	CS
PT (°C)	81.07±0.46 ^a	74.03±0.51 ^c	66.97±0.85 ^d	79.37±0.46 ^b
PV (cP)	3963.67±8.02 ^b	1416.33±31.00 ^d	10416.67±96.46 ^a	2833.67±3.51 ^c
BD (cP)	315.67±23.69 ^d	480.33±69.21 ^c	9111.00±60.89 ^a	894.33±5.51 ^b
SB (cP)	2998.33±40.02 ^a	562.00±19.29 ^d	1994.67±58.76 ^b	1047.00±22.07 ^c
FV (cP)	6646.33±28.54 ^a	1499.00±34.18 ^d	3300.33±24.66 ^b	2986.00±16.65 ^c
a*	0.32±0.30 ^b	0.99±0.10 ^a	0.01±0.01 ^c	-0.94±0.02 ^d
b*	4.28±0.06 ^b	9.59±0.27 ^a	1.10±0.08 ^c	4.17±0.01 ^b

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different (p<0.05). BPNS: native starch of banana pseudostem, RS3BP: resistant starch of banana pseudostem, PS: potato starch, CS: corn starch, PV: peak viscosity, PT: pasting temperature, FV: final viscosity, BD: breakdown, SB: set back.

produce high retrogradation with its lower amylose content (48.74%) as compared to RS3BP (52.97%) could be due to several factors. These included the degree of polymerisation of amylose, size and surface area of granules, crystallinity, moisture content, as well as the presence of other components (i.e., proteins, lipids, salts, sugars, and antinutrients) (Simons *et al.*, 2018).

Furthermore, a reduction in the peak and final viscosities was noted for BPNS after retrogradation. The pasting properties of RS3BP were lower than those of their native starch could be attributed to the degradation of the amylopectin chain into smaller fragments due to the enzymatic hydrolysis. Thereby an increase in the formation of short linear chain molecules and resistant starch content could be substantiated by the much lower values in the pasting viscosity along with the reduced ability of forming gels (Shah *et al.*, 2017; Gani *et al.*, 2020).

3.4 Thermal properties of starches

The results of the thermal properties of the starch are presented in Table 4. BPNS presented higher values for the gelatinisation transition temperatures: onset temperature ($T_o = 71.30^\circ\text{C}$), peak temperature ($T_p = 75.15^\circ\text{C}$), conclusion temperature ($T_c = 80.46^\circ\text{C}$), and enthalpy change ($\Delta H = 13.82 \text{ J/g}$) of gelatinisation as compared to the other samples. The gelatinisation transition temperatures were associated with the internal crystalline structure of starch and reflected in its heat stability (Tarahi *et al.*, 2022). Therefore, these current

findings indicated that BPNS had greater thermal stability than commercial starches (i.e., PS and CS) and its resistant starch counterpart. Moreover, the T_p value of CS was consistent with the values reported by Espinosa-Solis *et al.* (2021) for CS ($T_p = 74.01^\circ\text{C}$). Variations in gelatinisation temperatures of samples could be explained by the fact that the difference in amylose content, starch granule size, dispersion of granular structures, as well as the re-alignment of starch helices within the granule (Barua and Srivastav, 2017).

The preparation of resistant starch (RS3BP) from BP by autoclaving showed significant (p<0.05) differences with their native starch in any of the thermal variables analysed, whereby, after being subjected to the process of autoclaving with high pressure, RS3BP presented lower values of T_o , T_p , T_c , and enthalpy of gelatinisation as compared to native starch. Tarahi *et al.* (2022) explained that the enthalpy of gelatinisation provided information on the degree of crystallinity and the energy required for dissociation of the double helical. It was commonly used as an indicator for the loss of molecular order with the granule that occurred during gelatinisation (Reddy *et al.*, 2015). The melting enthalpy of native starch of BP was found to be significantly (p<0.05) decreased after the autoclaving process. This indicated that less heat was required to break down the crystalline orientation of starch after autoclaving. A similar trend was observed by Espinosa-Solis *et al.* (2021) for Malanga flour and CS. The authors reported that the melting enthalpy value of Malanga flour and CS was

Table 4. Thermal properties of starches.

Sample	Thermal properties			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_g (J/g)
BPNS	71.30±0.27 ^a	75.15±0.59 ^a	80.46±0.29 ^a	13.82±2.34 ^a
RS3BP	56.00±0.67 ^d	65.12±0.33 ^c	73.76±0.94 ^c	4.97±1.31 ^b
PS	58.85±0.64 ^c	62.86±0.62 ^d	67.92±0.78 ^d	12.63±0.47 ^a
CS	64.32±0.30 ^b	70.59±0.09 ^b	75.84±0.37 ^b	11.47±1.59 ^a

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different (p<0.05). BPNS: native starch of banana pseudostem, RS3BP: resistant starch of banana pseudostem, PS: potato starch, CS: corn starch. T_o : onset temperature, T_p : peak temperature, T_c : conclusion temperature, ΔH_g : enthalpy change.

reduced after autoclaving. The disruption of double helices presents in both the crystalline and non-crystalline area of the granules contributed to the reduction of melting enthalpy in RS3BP (Barua and Srivastav, 2017). Furthermore, the formation of resistant starch may result in interference in the double helical structure of starch leading to partial gelatinisation within an amorphous phase of starch granules.

4. Conclusion

The enzymatic treatment with pullulanase and the autoclaving process modified the physicochemical, pasting, and thermal properties of BPNS during the production of resistant starch. The enzymatic and autoclaving treatment had increased the amylose and ratio of amylose/amylopectin but decreased the amylopectin content in RS3BP. The autoclaving process had decreased most of the proximate compositions (i.e., moisture, crude protein, crude fat, and ash) and resulted in a darker and yellowish colour in RS3BP than other starches. BPNS was shown to have a strong starch granule with greater thermal stability than commercial starches (i.e., PS and CS). Furthermore, the water and oil holding capacities of BPNS remain unchanged after autoclave during the production of RS3BP. Therefore, BP can be an attractive raw material for producing native starch or resistant starch Type III.

Conflict of interest

The authors declare no conflict of interest.

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