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# Exploring differences in the physicochemical, functional, structural, and pasting properties of banana starches from dessert, cooking, and plantain cultivars (*Musa* spp.)

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#### ABSTRACT

Banana starch, with its nutritional and functional properties, opens up new opportunities for the food industry, which is seeking new starch sources to fulfil rising demand. Herein, physico-chemical, and functional properties of banana starches isolated from dessert, plantain, and cooking cultivars were investigated. Starch yield was higher in Popoulu (30.58%) and Monthan (27.82%). Starch granules registered irregular forms with granule sizes ranging from 8.9 to 55.09  $\mu$ m. Among the cultivars, the amylose content was ranged between 25.05 and 31.86%. Total starch (95.86 and 95.60%,) and resistant starch (65.56 and 59.20%) were higher in Saba and Monthan respectively. Flour colour index (86.2–90.6) was higher in banana starches. Differential scanning calorimetry and rapid viscosity studies confirmed that starches from Saba (87.67 and 85.71 °C) Monthan (85.36 and 81.65 °C) have a higher gelatinization property. Banana starches were B and C-type with varying crystallinity levels (21.19–52.01%). The *in-vitro* starch digestibility revealed that Saba starch has a lower hydrolysis rate with lesser glycemic index. PCA showed the greater impact of amylose and resistant starch content on the grouping of varieties. These findings would be useful for food and non-food industries in terms of using banana starch in various food compositions and other industrial applications.

#### 1. Introduction

Starch is the major component of green banana and the diet source for humans. Also, starch is used as a basic ingredient in many food and non-food industries [1,2]. With global yearly production of around 70 million tons [3], starch is employed for thickening, gelling, providing stability and other diverse purposes [4]. Food industries (snacks, bakery, sausages, ice cream, etc.) utilizes 60% of starch, whereas other sectors (textiles, pharmaceuticals, paper industry) consume 40% of starch [5]. On dry weight basis, starch content varies greatly among crops: cereals (65–80%), legumes (25–50%), and tubers such as potato, cassava, elephant foot yam, and arrowroot (60–90%) [6].

With rising urbanization, the food industry requires more starch,

instigating researchers to explore a novel plant sources for starch extraction. Banana is a tropical fruit, consumed every day owing to their higher nutritional properties. They are grown in more than 115 countries (tropical and subtropical areas), yielding 155 million tons from 8.02 million ha [3]. Banana contains higher level of starch which ranges from 80–90% (dwb) [7]. India leads with the production of 30.0 million tons of bananas from 0.8 million hectares, accounting for one-fifth of global production. In recent years, resistance starch (RS), present in significant amount in banana (30–50% dwb), attracts attention due to slow release in calories [8], positive impact on human colon, making it an important component of nutrition. Furthermore, RS is a prebiotic, promotes intestinal microbiota, promotes excellent mineral absorption (Iron and calcium), and helps to regulate blood sugar and cholesterol

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level. Therefore, with its higher concentration of starch and resistant starch, banana becomes a promising source of starch other than traditional sources such as maize, corn, and cassava. With its higher productivity, biomass and starch content, banana provides opportunity to be a complementary source for the extraction of starch and to meet the ever-growing demand.

Banana starch and its functional properties need to be extensively studied before its utilization. Cooking bananas like Monthan and Saba (Bluggoe, ABB) are underutilized in India and other regions in spite of their high starch and resistant starch [7]. Plantain (Nendran, AAB) varieties are considerably grown in sub-Saharan Africa and India. They have different functionalities than other dessert bananas. During the handling of Cavendish (Grand Naine, AAA) banana, the most traded, a multibillion-dollar loss occurs due to massive wastage (15-20%) during on-farm, packhouse handling, shipping, and other marketing channels. In the competitive global starch market, the use of discarded green bananas and cooking banana varieties for the extraction of starch could yield a higher monetary advantage [9]. Different banana varieties have various structural components which therefore influences their physical and functional properties. Hence, it is necessary to evaluate the physicochemical (e.g., amylose, RS) and functional (e.g. swelling power, water holding capacity, freeze-thaw stability, and rheological behavior) features for the comprehensive utilization of banana starch in food and nonfood industries [5]. Based on the demand and importance of starch production from new raw materials, this study was aimed to isolate banana starches by simple sieving and washing and investigate their physicochemical, structural, pasting, and textural properties from commercial dessert cavendish variety, Grand Naine (AAA); cooking varieties, Monthan (ABB), Saba (ABB); plantain variety Nendran (AAB); and an unique variety, Popoulu (AAB) in comparison to commercial corn starch for their possible use in various industrial applications.

#### 2. Materials and extraction of starch from banana

# 2.1. Materials

Potassium metabisulfite (KMS) and potassium hydroxide (KOH) were purchased from HIMEDIA, India. Glycerol, Amyloglucosidase (AMG) from Aspergillus niger,  $\alpha$ -Amylase from porcine pancreas (PPA), and Glucose (GO) Assay Kit were purchased from Sigma-Aldrich, India. Resistant starch measurement kit was purchased from Megazyme International, Wicklow, Ireland.

# 2.2. Starch isolation

All the banana cultivars were grown in the experimental block of ICAR-National Research Centre for Banana, Tiruchirappalli, Tamil Nadu, India. Fully matured green bananas were harvested, the peel was removed manually, and the pulp was immediately dipped in distilled water containing KMS (0.1%, w/v) for 5 min to prevent the oxidation process. The pulp was sliced, macerated for 4-5 min in a commercial blender at low speed (100-125 rpm). The slurry was kept at room temperature (13  $\pm$  2 °C) for 2 h before being sieved through a 100  $\mu m$ mesh screen. After washing for 2–3 times, the slurry was filtered using a 60 and 100 µm mesh screen to remove non-starch particles. For improved starch sedimentation, the starch slurry was kept at 4 °C for 2 h and rinsed repeatedly until a clear supernatant was obtained. The white starch cake settled at the bottom was dehydrated at 50 °C, ground into powder and stored at 5  $^{\circ}\text{C}$  for further use. The starch yield in percentage from various bananas was calculated using Eq. (1). The basic parameters of the banana such as bunch weight (kg), number of hands, number of fingers, fruit length (cm), girth (cm), and hardness (N) were measured before the starch extraction process. Fig. S1 depicts the stepwise starch extraction procedure.

Starch yield 
$$(g) = \frac{\text{Weight of the starch obtained } (g)}{\text{weight of the pulp used } (g)}$$
 (1)

#### 2.3. Colour profile characteristics

The colour properties of pure starch were evaluated using a Minolta CR-400 hand-held chroma meter (MINOLTA Co., Ltd., Japan). Whiteness index (WI), yellowness index (YI), and flour colour index (FCI) were derived by measuring the L (lightness), a\* (degree of redness (+a) to greenness (-a)), and b\* (yellowness (+b) to blueness (-b)), respectively, using Eqs. (2), (3), and (4) [10].

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

$$YI = \frac{142.86(b^*)}{L} \tag{3}$$

$$FCI = L^* - b^* \tag{4}$$

#### 2.4. Proximate content of banana starches

AOAC official methods were carried out to determine proximate content such as moisture (925.10), protein (N 6.25) (991.20), fat (920.35), ash (923.03) and pH [11].

#### 2.5. Measurement of starch fractions

Amylose content was estimated by Williams et al. [12]. A 20 mg of starch was suspended in 10 mL of 0.5 N KOH and then diluted to 100 mL using DDW. Next, an aliquot (10 mL) was pipetted into 50 mL volumetric flask together with 5 mL of 0.1 N HCl and 0.5 mL of iodine reagent, and the total volume was adjusted to 50 mL. The absorbance of reaction solution at 625 nm (UV-3200 spectrophotometer, Lab India, New Delhi) was measured. At the same time, amylose from potato was used as a standard. The amylopectin was calculated as a percentage by subtracting the amylose content from the starch. Total starch was determined according to the previous method [13]. A 50mg of starch was dispersed in 6 mL of 2 M KOH and incubated at room temperature for 30 min. The solubilized starch was subsequently hydrolyzed with AMG (60  $\mu$ L) and incubated in a shaking water bath at 60 °C for 45 min. The glucose content in the supernatant was tested using the GO kit and the total starch content was estimated as mg of glucose  $\times$  0.9.

Megazyme resistant starch Assay Kit was used to analyze the Digestible starch (DS) and Resistant starch (RS) of the samples. DS and RS were estimated according to the procedure of Megazyme resistant starch Assay Kit. Sample was digested with  $\alpha$ -amylase, AMG incubated in the water bath for 16 h at 37  $^{\circ}\text{C}$  for the hydrolyzation of digestible starch. 4 mL absolute ethanol was then mixed with the suspension to arrest the enzyme activity. With centrifugation (5000 g) for 10 mins, resistant starch was formed as a pellet. It was then washed with ethanol to eliminate the DS. 2 mL of 2 M KOH was used to dissolve the sediment in an ice bath by stirring for 20 min. AMG (0.1 mL, 3300 U/mL) was added to the solution, and 8 mL sodium acetate buffer was used to neutralize the solution. 3 mL of GOPOD reagent was added with an aliquot (0.1 mL) and incubated (50  $^{\circ}\text{C}$ , 20 min). Absorbance was noted at 510 nm using the spectrophotometer. A factor of glucose  $\times$  0.9 was used to measure the RS and DS and expressed as a percentage.

#### 2.6. In-vitro kinetic study of starch hydrolysis

A method by Goni et al. [13] was used to measure the rate of starch hydrolysis. Starch sample (50 mg) was transferred into a tube contains 10 mL of HCL-KCL buffer (pH 1.5) and 0.2 mL (20 mg) of pepsin in HCL-KCL buffer. Then, the tubes were incubated at 40  $^{\circ}$ C for 1 h in shaking

water bath. The incubated solution was made up to 25 mL with Tris-Maleate buffer (pH 6.9) and added 5 mL of PPA (3.3 IU) in Tris-Maleate buffer (pH 6.9). The samples were then placed in shaking water bath and incubated at 37 °C for 3 h. An aliquot of 1 mL sample was taken at various period (0.5, 1.0, 1.5, 2.0, 2.5, and 3 h) and placed in 100 °C to deactivate the enzyme. To this, 3 mL of sodium acetate buffer (0.4 M, pH 4.75) and 60  $\mu$ L of AMG was added to each tube to hydrolyze the residual starch at 60 °C for 45 min. After centrifugation (4500g, 15 min), the amount of glucose was measured using Glucose assay kit and the digestible starch was calculated using equation.

Starch hydrolysis (%) = 
$$\frac{\text{Gt} \times 30 \times 0.9}{50} \times 100$$
 (5)

where, Gt is the glucose content produced at t (h). A conversion factor 0.9 was used to convert molar mass from glucose to starch monomer unit.

The rate constant of starch hydrolysis was estimated based on the approach depicted by Butterworth et al. [14]. In general, the digestibility curve of starch follows the first-order equation:

$$Ct = C\alpha \left(1 - e^{-Kt}\right) \tag{6}$$

where, "Ct" is the fraction of starch digested at time "t", "Ca" is the degree of starch hydrolyzed at the end of the reaction, "K" is a first order rate constant for in vitro starch hydrolysis ( $h^{-1}$ ), and "t" is the time (h). The slope of a linear-least-squares fit of a plot of LN (1-C) against t [15] can be used to calculate the value of k. The integral of the kinetic equation was used to calculate the area under the hydrolysis curve (AUC). By dividing the AUC of starch samples by the AUC of a reference sample (white bread), the hydrolysis index (HI) was calculated. The estimated glycemic index (eGI) was calculated using an equation given by Goni et al. [13].

$$eGI = 39.71 + 0.549HI \tag{7}$$

# 3. Gelatinization characteristics of banana starches

# 3.1. Swelling power (SP), water holding capacity (WHC), and solubility (S)

SP, WHC, and S of the starch were determined following our earlier adopted method [7]. Briefly, 40 mL of starch suspension (2.5%, w/v) was heated at various temperatures (55, 65, 75, 85, and 95 °C) in constant agitation. The cooled samples were then centrifuged (2500 g, 20 min). The weight of wet sediment, dried sediment, and dried supernatant were noted. The SP, WHC, and S were calculated as following equations (Eq. (8), (9), and (10));

$$SP\left(g/g\right) = \frac{\text{Weight of the wet sample}}{\text{Weight of the sample - weight of the dried supernatant}}$$
 (8)

$$WHC (g/g) = \frac{\text{Weight of the wet - weight of the dried sediment}}{\text{weight of the sample}}$$
(9)

Solubility (%) = 
$$\frac{\text{Weight of the dried supernatant}}{\text{Weight of the sample}} \times 100$$
 (10)

#### 3.2. Paste clarity and syneresis (%)

A method developed by Kaur et al. [16] was adopted to measure the paste clarity at 640 nm in UV-Spectrphotometrically. Similarly, a method was given by Reddy et al. [17] used to determine the syneresis of starch suspensions. The values for paste clarity and syneresis were noted for five days by storing the samples at 4 °C. Syneresis was calculated using the following formula (Eq. (11)).

$$Synersis (\%) = \frac{\text{Weight of the water released}}{\text{Weight of the gel}} \times 100$$
 (11)

#### 3.3. Thermal analysis by light microscopy (LM)

Method by Pelissari et al. [18] was employed to measure the thermal effect on starch granules. Starch suspension in DDW (0.25%, w/v) was heated with a varying constant temperature range from 55 to 95 °C at an interval of 10 °C. The suspension was then centrifuged (3500 g, 10 min) and a small amount of precipitate stained with iodine solution and glycerol (50%, v/v), mounted on a microscopic slide. The morphological changes were observed under the digital camera (OLYMPUS, U-TV1X-2, T7, Tokyo, Japan) attached light microscope (Olympus BX 50, Tokyo, Japan) at 40× magnification and 100  $\mu$ m size.

#### 3.4. Differential scanning calorimetry (DSC)

The thermal behaviors of starches were analyzed by differential scanning calorimetry (DSC-8000, Perkin Elmer, USA). In brief, 5.0–5.5 mg of banana starch was directly weighed into the aluminum pan and mixed with 12  $\mu L$  deionized water. Then, the pan was closed and left to attain equilibrium for 1 h before the measurement. Samples were subjected to heating range of 10.0 °C/min was employed for scanning and 35 to 150 °C was used as the temperature range for the study. An empty aluminum pan was maintained as a reference without a sample. Pyris 1 software (Perkin Elmer, USA) software was used to obtain transition temperatures like onset temperature  $(T_{\rm o})$ , peak temperature  $(T_{\rm p})$ , conclusion temperature  $(T_{\rm c})$ , gelatinization temperature range (GELR), and enthalpy ( $\Delta H_{\rm gel}$ ) were calculated.

## 3.5. Pasting properties by rapid visco analyzer (RVA)

The pasting properties of banana starches were determined using a rapid visco analyzer (RVA starch master 2, Newport Scientific, Warriewood, NSW, Australia). Banana starch (3.5 g) in 25 mL of distilled water, kept in the canister was used to record the pasting profile. In brief, the slurry was heated at 50 °C with continuous stirring for 10 s and held at the same temperature for 1 min. The heating temperature was then increased to 95 °C for 7.3 min and held for 5 min and finally cooled at 50 °C. From the graph, the following parameters were evaluated: pasting temperature (PT), peak viscosity (PV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV).

## 4. Morphological and structural characterizations

# 4.1. Scanning electron microscopy (SEM) and light microscopy (LM)

SEM images were taken by scanning electron microscope (SEM) (FEI Magellan 400 L microscope, Hillsboro, OR, USA) to observe the surface morphology of starch samples. The samples were fixed on double-sided tape and coated with a layer of gold plating. For the light microscopic (LM) study, starch suspension (50 mg) was dissolved in 1 mL of 50% water-glycerol ( $\nu/\nu$ ) solution. The starch suspension (10  $\mu$ L) was kept on a microscopic slide and was observed under a bright field light microscope (Instruments details mentioned in Section 3.3).

#### 4.2. Particle size distribution

Particle distribution of starch granules was analyzed using Laser Scattering Particle Size Distribution Analyzer (HORIBA, LA-960). A paste with a concentration of 0.001% was made to obtain the refraction index of water and that of starch 1.335 with the absorption of 0.1 [19].

#### 4.3. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra of banana starches were analyzed by an Attenuated Total Reflectance (ATR) incorporated FTIR spectrophotometer (Perkin Elmer: Spectrum RXI, Ohio, USA) using the the range of 400–4000 cm<sup>-1</sup>. Starch pellets were prepared with potassium bromide (KBr) for the analysis. A neat KBr pellet was employed as a standard.

#### 4.4. X-ray diffraction pattern (XRD) and relative crystallinity (RC)

X-ray diffraction pattern was performed using Bruker AXS D8 Advance diffractometer (Bruker Inc., Germany) with K- $\beta$  filtered Cu KR radiation (154.2 pm) at a voltage of 35 kV and current supply of 32 mA. The 2 $\theta$  was measured by a scanning range of 5–80°. The Relative crystallinity (%) was obtained from the diffractograms, using the following equation (Eq. (12)).

$$RC (\%) = \frac{\text{Crystalline peaks}}{(\text{Crystalline peaks} + \text{Amorphous peak})}$$
 (12)

#### 4.5. Texture profile analysis (TPA)

Textural properties of retrograded gel for the starch suspension (8%, w/v) were evaluated using TA-TX Plus Texture analyzer (Stable Micro Systems, Surrey, UK) as per our earlier adopted protocol [7]. The program settings for TPA were adopted from Shittu et al. [20]. Then the gel (2 cm length) was analyzed, using a 10 kg load cell with a setup of pretest speed 5.0 mm/s, test speed 1.0 mm/s, and post-test speed 8.0 mm/s; penetration distance 5 mm and a rest period of 5 s between 2 cycles. The values for primary textural properties like hardness, cohesiveness, springiness, and secondary properties like gumminess and chewiness were obtained from exponent connect software.

#### 4.6. Statistical analysis

All the experiments were performed in triplicate and the values are

expressed as mean value  $\pm$  standard deviation (SD). One way ANOVA analysis and Duncan's Multiple Range Test (DMRT) were used for comparing the mean values (p < 0.05) using SPSS software (SPSS, version. 21, IBM, USA). The predicted maximum level of starch hydrolysis (C $\alpha$ ) was obtained from fitting of first-order equation in Origin software (Version 9, MA, USA). Principal component analysis (PCA) was performed using XL stat to correlate and discriminate the varieties and to identify the relationship among the different components.

#### 5. Results and discussion

#### 5.1. Physical characteristics of banana fruits and starch yield

The physical properties of banana such as bunch weight, fruit weight, and the number of fruits per hand, length, diameter, pulp and peel weight, pulp firmness, and pulp: peel ratio were significantly varied (Table S1). The varieties Popoulu and Nendran recorded higher fruit and pulp weight. In our study, the starch yield was higher with banana varieties like Popoulu and Monthan (641.87 and 559.7 g for 2.99 and 2.12 kg of pulp), indicating its feasibility for potential starch extraction. In contrast, Grand Naine has produced low amount of starch in spite of higher pulp weight. Higher moisture content in Grand Naine than other cultivars could be a reason for their low starch production [7]. The percentage of starch yield was calculated and showed that Popoulu (30.58%) and Monthan (27.82%) recorded higher starch recovery than other banana varieties. The variation in the starch extraction is also related to the intramolecular association of starch with water molecules, crude fibers (non-starch polysaccharides), proteins, lipids, and other macromolecules. Besides, genomic types also play a vital role in economical production of starch. Though the starch yield from Grand Naine is low, the rejected banana could be used as a candidate to produce a large quantity of starch as a non-conventional source. Generally, starch yield in this study was higher than those reported previously (Agbagba-plantain (14.5%) and Bobo-cooking banana (21.5%), Dominico Hartón (12.7%), White and Yellow plantain (4.51% and 6.82%, respectively), Macho (43.8%), and Criollo (11.8%)) [21-23,1].

**Table 1**Colour properties of banana and corn starches.

Colour properties	Varieties							
	Grand Naine	Monthan	Saba	Nendran	Popoulu	Corn		
L a* b* WI YI FCI	$92.55 \pm 0.24^{\rm f}$ $-11.55 \pm 0.01^{\rm b}$ $6.26 \pm 0.04^{\rm b}$ $86.58 \pm 0.02^{\rm b}$ $9.67 \pm 0.09^{\rm b}$ $86.28 \pm 0.28^{\rm cd}$	$93.30 \pm 0.12^{e}$ $-10.75 \pm 0.64^{a}$ $5.47 \pm 0.38^{d}$ $87.66 \pm 0.70^{a}$ $8.39 \pm 0.57^{d}$ $87.83 + 0.37^{bc}$	$94.00 \pm 0.18^{d}$ $-11.78 \pm 0.4^{b}$ $5.82 \pm 0.02^{c}$ $86.64 \pm 0.03^{b}$ $8.84 \pm 0.02^{c}$ $88.19 \pm 0.17^{b}$	$94.55 \pm 0.44^{c} \\ -11.79 \pm 0.03^{b} \\ 6.24 \pm 0.09^{b} \\ 86.45 \pm 0.03^{b} \\ 9.43 \pm 0.18^{b} \\ 88.31 \pm 0.53^{b}$	$95.71 \pm 0.14^{b} \\ -11.89 \pm 0.03^{b} \\ 5.42 \pm 0.06^{d} \\ 86.77 \pm 0.03^{b} \\ 8.09 \pm 0.10^{d} \\ 90.29 + 0.19^{a}$	$98.02 \pm 0.12^{a}$ $-13.04 \pm 0.06^{c}$ $7.33 \pm 0.04^{a}$ $84.97 \pm 0.03^{c}$ $20.41 \pm 0.07^{a}$ $90.69 \pm 0.15^{a}$		

WI: whiteness index, YI: yellowness index, FCI: flour colour index. Data presented are mean value  $\pm$  standard deviation (n = 5). Different superscripts in the same row are significantly different (p < 0.05) by Duncan's test.

 Table 2

 Chemical composition of banana and corn starches.

Chemical compositions	Grand Naine	Monthan	Saba	Nendran	Popoulu	Corn
Moisture (%, db)	$3.45\pm0.07^{b}$	$2.70\pm0.08^{d}$	$2.91\pm0.17^{\rm c}$	$3.47\pm0.31^{\text{b}}$	$3.15\pm0.12^{\rm b}$	$6.85\pm0.33^{\mathrm{a}}$
pН	$5.99\pm0.14^{ab}$	$5.89\pm0.23^{ab}$	$5.98\pm0.11^{ab}$	$5.85\pm0.22^{ab}$	$6.22\pm0.36^a$	$5.54\pm0.20^{\rm c}$
Fat (%, db)	$0.13\pm0.05^{\rm c}$	$0.10\pm0.05^{\rm d}$	$0.09\pm0.06^{\mathrm{de}}$	$0.10\pm0.02^{\rm d}$	$0.17\pm0.02^{\mathrm{b}}$	$0.23\pm0.06^a$
Protein (%, db)	$0.52\pm0.02^{\mathrm{b}}$	$0.37\pm0.02^{\rm d}$	$0.23\pm0.04^{\rm f}$	$0.39\pm0.03^{\rm c}$	$0.30 \pm 0.04^{e}$	$0.94\pm0.01^a$
Ash (%, db)	$0.40 \pm 0.05^{\mathrm{b}}$	$0.53\pm0.04^{\mathrm{b}}$	$0.47\pm0.08^{\mathrm{b}}$	$0.53 \pm 0.06^{\mathrm{b}}$	$0.83\pm0.03^a$	$0.23\pm0.08^{\rm c}$
TS (%, db)	$93.19 \pm 0.80^{\mathrm{bc}}$	$95.60 \pm 0.97^a$	$95.86 \pm 0.86^{a}$	$93.77 \pm 1.01^{bc}$	$94.48 \pm 0.93^{ab}$	$89.40 \pm 0.67^{c}$
RS (%, db)	$49.80 \pm 0.61^{d}$	$59.20 \pm 0.51^{\mathrm{b}}$	$65.56 \pm 0.50^{a}$	$57.62 \pm 0.89^{c}$	$57.30 \pm 0.78^{c}$	$38.43 \pm 0.94^{e}$
DS (%, db)	$42.39 \pm 1.13^{\mathrm{b}}$	$36.40 \pm 0.86^{d}$	$30.30 \pm 0.66^{e}$	$36.15 \pm 1.28^{ m d}$	$37.19 \pm 0.71^{c}$	$50.96 \pm 0.85^{a}$
AM (%, db)	$25.05 \pm 1.00^{\rm e}$	$31.86 \pm 0.97^{b}$	$29.03 \pm 0.90^{c}$	$27.38\pm0.95^{\mathrm{d}}$	$29.52 \pm 0.94^{c}$	$34.83 \pm 0.13^{a}$
AMP (%, db)	$68.14 \pm 1.01^{a}$	$63.74\pm0.92^{d}$	$66.83\pm0.86^{\mathrm{b}}$	$66.38 \pm 1.02^{\rm b}$	$64.96\pm0.98^{c}$	$54.56 \pm 0.72^{e}$

Dry basis (db); TS (Total starch); RS (Resistant starch); DS (Digestible starch); AM (Amylose), and AMP (Amylopectin). Data presented are mean value  $\pm$  standard deviation (n = 3). Different superscripts in the same row are significantly different (p < 0.05) by Duncan's test.

#### 5.2. Colour properties and chemical composition of Banana starches

Consumer acceptance and quality of the starch are primarily assessed with colour of the products. The results (Table 1) showed that L, which reflects the relative lightness or darkness of the products varied significantly (p < 0.05) from 92.55 to 95.71. The commercial corn starch recorded a higher value (98.02) than banana starches. The high lightness of starch (>90) of all the banana varieties implies its use in various food applications. The yellowness index values significantly (p < 0.05) ranged from 8.09 to 9.67 which were lower than that of corn starch (20.41). A lower value of YI indicated the superiority of banana starch over corn starch. Flour colour index (FCI) of banana starches significantly varied from 86.28 to 90.29. Generally, the extraction procedure and the drying condition significantly influenced the colour of the starch. Both higher WI and lower YI strongly indicated that the banana starch could be supplemented with corn starch to meet the ever-growing demand for starch [20].

#### 5.3. Chemical composition of native banana starches

The chemical composition of isolated starch is shown in Table 2. The moisture content of all banana starches was ranged from 2.70 to 3.47%. As evidenced by Reddy et al. [17], the product moisture content was affected by the innate, inherent characteristics, drying and storage condition along with environmental humidity. The pH of the starches significantly varied from 5.85 to 6.22. The purity of obtained starches were evidenced by the lower value for the fat (0.09–0.17%, db), protein (0.23–0.52%, db), and ash (0.40–0.83%, db) content, indicating that the starch isolation method adopted could render high purity of starches from the banana. The commercial corn starch recorded higher moisture (6.85%) and protein (0.94%) content than banana starch. Generally, the variation in the proximate composition and starch content are due to innate genetic nature and environment factors. In this study, the banana varieties were grown in similar environmental conditions but showed significant variations in starch and proximate composition, indicating that the variations resulted from their different genotype background [9]. Moisture, fat, and ash content in the starch of our results were lower than previously reported for various bananas including plantain and cooking varieties [21,18,24].

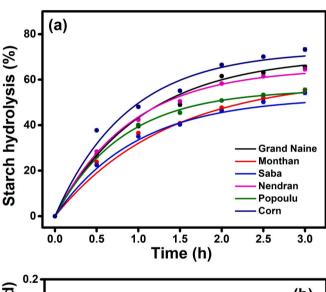
The difference in the carbohydrate value of starches is given in Table 2. The total starch was significantly varied between 93.19 and 95.86% Plantains (95.60-95.86%) and cooking (93.77-94.48%) starches recorded more starch content than Grand Naine (93.19%). The starch results of our study were slightly more than those reported by De Barros Mesquita et al. [25] and Reddy et al. [18]. Resistant starch (RS) rich products are gaining popularity in the commercial food market due to their resistance to enzymatic hydrolysis during digestion in small intestine [26]. In our study, the amount of RS (II) from various green banana starch significantly varied from 49.80 to 65.56%. The cooking bananas recorded a higher RS (65.56%) than plantain and dessert varieties. However, banana cultivars recorded higher RS, in comparison with commercial corn starch (38.43%). The higher RS content reduced the digestion of starch into glucose units over a period of time [9]. Digestible starch (DS) values were inversely proportionate to RS (II) values. It was found that Grand Naine (42.39%) showed the highest DS content. In the previous reports, RS content observed in common banana cultivars ranged from 50.7 to 68.1% [27], 13.34 (Gros Michel) to 71.71% (FHIA 20) [22], 27.4 (Morado) to 67.6% (Macho) [24].

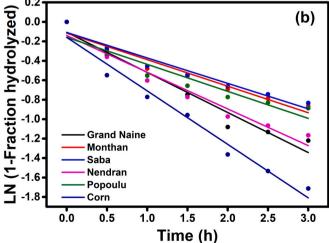
The amylose content plays a main role in starch since the ratio of the amorphous region and crystallinity differs with the arrangement pattern of amylose and amylopectin in the starch molecules [2]. Nonetheless of its smaller fraction, amylose in the starch greatly influences various functional properties such as crystalline buildup, gelatinization, retrogradation, and susceptibility to enzymatic digestion. More dense and stronger gels could be made with starches that have higher amylose.

These starches could be used in edible packaging, emulsions, sausages, and other industrial applications [25,17]. The Monthan starch had higher amylose content (31.86%), differing from other banana starches. The starches with high amylose content resulted higher resistance to hydrolytic enzymes. The amylose level of starches in this study are in line with amylose content reported from different banana varieties, ranging from 19.32% (Valery) to 26.35% (Macho) [24]. The variation in the carbohydrate content is dependent on the length and degree of glucan polymerization of amylose, amylopectin, enzymes responsible in the starch synthesis, genetic variations, environmental and nutritional conditions during plant growth development, and extraction procedures [9,28].

# 5.4. In vitro digestion of starch and first order kinetics fit to the degree of hydrolysis

Fig. 1a depicts the percentage of starch hydrolysis by PPA and AMG. During a 0–3 h incubation period, Saba and Monthan starches recorded the lowest digestibility in response to digestive enzymes. However, varieties Grand Naine and Nendran possessed a higher degree of starch hydrolysis than other banana starches at the end of incubation period. The lowest degree of digestibility in Saba and Monthan may attributed to the resistant starch content (Table 2), amylopectin arrangement, and





**Fig. 1.** (a) In vitro starch hydrolysis rate of native banana starches at different time incubation periods. The dots indicate the degree of hydrolysis and SD (n=3), and the lines indicates non-linear fits. (b) The fit of first-order kinetics to the degree of hydrolysis of starch from 0 to 3 h.

the breakdown of starch over the period. Also, the starches whose granular crystalline organization corresponds to a B-type pattern are more resistant to enzymatic digestion [8,9]. The digestion rate constant (K) and the estimated glycemic index (eGI) of each banana starch were obtained by fitting first-order kinetics (Eq. (6)) and shown in Table 3. The resulted  $C\alpha$  of all banana starches ranged between 52.35 to 70.42%. The largest Cα value was obtained for Grand Naine. The digestion rate, which reflects the reaction rate of the first-order kinetic model of starch hydrolysis, was different among the starch samples. The K value obtained from slope of a linear-least-squares fit of a plot of LN (1-C) was plotted against t (Fig. 1b). Saba, Monthan, and Popoulu resulted the lowest K ( $h^{-1}$ ) value among the samples (Table 3), indicating that these starches were digested at the slowest rate. The eGI of the starches were ranged from 72.54 to 80.72. The starch from Saba had the least eGI among the starch samples. However, the commercial corn starch showed the highest HI and eGI indicating that banana starches could be an alternative source for making food products with low glycemic properties.

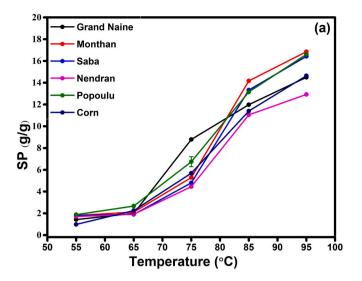
### 6. Gelatinization properties

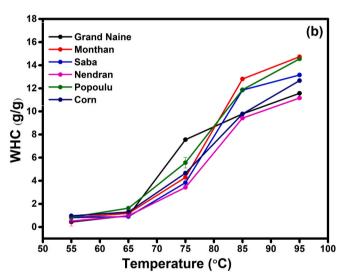
# 6.1. Swelling power (SP), water holding capacity (WHC), and solubility (S)

In this study, SP, WHC, and S were directly associated with increasing temperature of 55-95 °C. Fig. 2(a) indicated that the SP of all the varieties was minimal (<3 g/g starch) between 55 and 65 °C which suggested that the starches were resilient to swelling and strong forces acted between the molecules which maintained the structural integrity of the starch. However, the SP increased exponentially with the temperature above 75 °C. The overall SP value (g/g starch) ranged from 12.93 (Nendran) to 16.85 (Monthan) at 95 °C. The higher SP of Monthan starch is directly related to the amylose-amylopectin bonding level as water diffusion is more crucial in the starch granules to lose structure and swell. In this study, we obtained lower SP for all the starches at 95  $^{\circ}\text{C}$ (12.93-16.85) than that of starch from different banana varieties; 31.1 g/g (Macho) [1], ~17 g/g (yellow and white plantain) [23], and other crops; 16.8 g/g (Corn), 16.6 g/g (cassava) [5]. At temperatures above 75 °C, starch WHC (g/g starch) increased gradually (Fig. 2(b)). The WHC, as exhibited was found to be in order as follows; Monthan (14.73) > Popoulu (14.55) > Saba (13.15) > Grand Naine (11.57) > Nendran (11.16) at 95 °C. The WHC of commercial Corn starch was (12.67) lower than Popoulu (14.55) and Monthan (14.73) at 95 °C. The Though the native banana starches are resistant to solubility in normal water, the rise in temperature in the aqueous starch, led to the weakening of binding forces in the crystalline area of the starch granules [1]. The hydroxyl position of amylose and hydrogen bonds of double helix amylopectin are hydrated with water and allowing progressive and irreversible water absorption resulting in increased swelling and water holding by the starch molecules [29]. The nature of the arrangement of starch molecules (amylose and amylopectin), granule size, and its

Varieties	Cα	K (h <sup>-1</sup> )	HI	eGI
Grand Naine	$70.40 \pm 1.01^{b}$	$0.412\pm0.03^{b}$	$72.57 \pm 1.27^{\mathrm{b}}$	$79.55 \pm 1.10^{\rm b}$
Monthan	$61.12 \pm 0.95^{d}$	$0.272\pm0.02^{\rm d}$	$66.90 \pm 0.99^{d}$	$77.43\pm0.96^{\mathrm{c}}$
Saba	$52.35 \pm 0.86^{\rm b}$	$0.261 \pm 0.02^{e}$	$56.17 \pm 0.95^{\rm f}$	$72.54 \pm 0.65^{d}$
Nendran	$65.04 \pm 1.05^{c}$	$0.377\pm0.03^{c}$	$69.25\pm1.01^{c}$	$80.72 \pm 1.02^{\rm b}$
Popoulu	$55.67\pm0.85^{\mathrm{f}}$	$0.277 \pm 0.06^{\rm d}$	$61.76\pm1.12^{\text{e}}$	$77.61\pm0.99^{c}$
Corn	$72.72\pm0.97^a$	$0.549\pm0.04^a$	$79.61\pm1.00^a$	$88.42 \pm 1.05^{a}$

<sup>&</sup>lt;sup>A</sup> Values are mean  $\pm$  standard deviation (n=3). Different superscripts in the same column are significantly different (p<0.05) by Duncan's test. C $\alpha$ , equilibrium constant; k, kinetic rate constant; HI, hydrolysis index; eGI, estimated glycemic index.





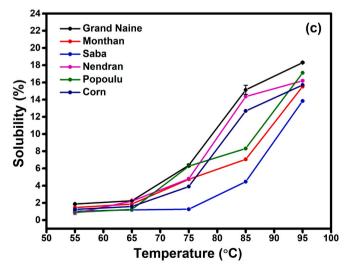


Fig. 2. Swelling power (a), water holding capacity (b), and solubility (c) of banana and corn starch. The data presented are mean value  $\pm$  standard deviation (n = 3).

distribution also affects the SP and WHC [16,21,25]. In both SP and WHC, Monthan and Popoulu starches resulted maximum value which might be related to its granule size and crystallinity level. Starches with superior SP and WHC could cook readily [30]. Starches with large granules and higher mass of amylopectin led to higher swelling because of their less molecular assembly while smaller sized granules were resistant to swelling [21] as seen in Grand Naine and Nendran.

Solubility is the degree of soluble compounds which are leaked out from starch granules while heating with water [29]. The solubility behavior of starches is shown in Fig. 2(c). Until the temperature range of 55–75 °C, the solubility slightly increased, whereas, at a temperature above 85 °C, starches showed higher solubility. However, Saba and Monthan recorded lower solubility even at 95 °C whereas, with the existence of smaller starch granules, Grand Naine showed higher solubility. Corn starch exhibited lower solubility 15.69% at 95 °C when compared to that of Grand Naine, Nendran, and Popoulu at 95 °C.

## 6.2. Paste clarity and syneresis (%)

Paste clarity is one of the most essential functional characteristics of starch in certain starch-based food products [31] is presented in Fig. 3 (a). At zero hour, the light transmittance (%) was higher but as the amylose leached into the solution, it lowered the transmittance due to

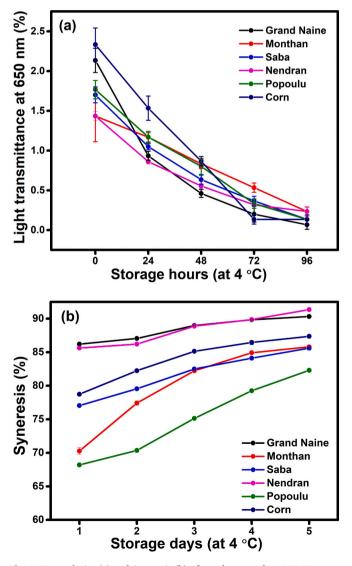


Fig. 3. Paste clarity (a) and Syneresis (b) of starches stored at 4  $^{\circ}$ C. Data presented are mean value  $\pm$  standard deviation (n = 3).

the higher absorbance of light. Among the varieties, Grand Naine recorded a higher light transmittance value (0.07%) as it had a loosely packed structure, lesser granule size and thus the amylose leached easily. After 48 h of storage in 4 °C, starch from Grand Naine and Nendran exhibited a lesser amount of transmittance indicates the high paste clarity. The starch paste with clear clarity is preferred to be used as thickening agents in soups and pie fillings [29].

Retrogradation of starch at low temperatures (4 °C) is a desired property for the foods which are inclined towards freeze-thaw stability [9]. Generally, the low temperature stored products, loose their quality when the water bound with the food matrix, started moving out from the gel. In this study, over the period of storage at 4 °C, the syneresis of starches amplified gradually as presented in Fig. 3(b). At first day of storage, Monthan, Saba, and Popoulu recorded the lowest syneresis (70.26, 77.05, and 68.19%, respectively) while Grand Naine and Nendran recorded the highest syneresis (86.21 and 85.62% respectively). The high degree of paste clarity and syneresis of Nendran, Grand Naine, and commercial Corn starch represents that they have smaller granule sizes which contributed to the rapid recrystallization and reaggregation than cooking banana starch. At fifth day of storage, the syneresis for all starches varied from 82.30 g to 90.32% gel. It noted that cold storage induces retrogradation and reaggregation of amylose and amylopectin which were broken down during gelatinization, thus the formed crystals enhance the high transparency [23]. Differences in both paste clarity and syneresis among starches could be due to several factors such as amylose, amylopectin, lipid and protein contents, granules size and distribution, crystallinity, and glucan chain intermolecular distance significantly affect the strength of the gel [16].

#### 6.3. Thermal analysis by light microscopy (LM)

From Fig. 4, it is evident that the granules at the temperature range of  $55-65\,^{\circ}\text{C}$  were stable and intact. Grand Naine, Nendran, Popoulu and

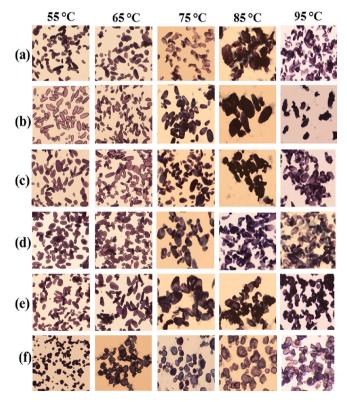


Fig. 4. Light microscopic images (40× magnification) of gelatinized starch granules (100  $\mu m)$  at various temperatures.

Grand Naine (a), Monthan (b), Saba (c), Nendran (d), Popoulu (e) and Corn (f).

corn starches were partially gelatinized at 75 °C and entirely disintegrated at 85 °C whereas the starch from Saba and Monthan had intact granules till 85 °C and started to lose its structure after 95 °C. The integrity of starch granules was wrecked and starch from Grand Naine, Nendran, and Corn dispersed their soluble components especially amylose into the solution. The results revealed that Monthan and Saba starches maintained good structural integrity with absorbing water at higher temperatures (<90 °C) along with little birefringence. This can be linked with the higher swelling powder and water holding capacity of the varieties as discussed earlier. Agama-Acevedo et al. [32] and Pelissari et al. [18] analyzed the thermal behavior of isolated starch and confirmed that in most of the cases, the gelatination took place between 70 and 90 °C.

#### 6.4. Thermal behavior of starches by DSC

The gelatinization properties of banana starches were measured by DSC and presented in Table 4. The endothermic transition of all starch samples is dependent on the association with amylose-amylopectin and amylose-lipid complex, granule shape, chain length, and different alignment of hydrogen bonds in crystalline regions [33]. The endothermic peak (T<sub>D</sub>) value of all banana starches significantly varied from 70.61 to 87.67 °C. Saba starch required more temperature (87.67 °C) to break the internal chain arrangements in the starch granules. From T<sub>D</sub>, it is concluded that plantains and cooking bananas required higher heat energy to loosen the internal arrangements of starch. This is evidenced even with the studies related to thermal analysis of starches, swelling powder and water holding capacity. Chávez-Salazar et al. [22] found that the T<sub>p</sub> of plantain starches ranged between 73.55 and 74.33 °C and stated that higher amylopectin content in the starch necessitated greater energy to break the double helices. Besides, Saba and Monthan with larger granule sizes and higher crystallinity levels attributed to their higher gelatinization temperature (87.67 and 85.36  $^{\circ}\text{C})$  whereas Grand Naine and commercial corn starch registered lower gelatinization temperature (70.61 and 73.43 °C). The gelatinization range (GEL<sub>R</sub>) was employed to determine the heterogeneous crystalline nature of banana starches. The gelatinization range for the banana starches ranged from 12.32 to 27.21 °C. Both plantain and cooking bananas recorded wider GEL<sub>R</sub> than the dessert Grand Naine, reveals greater heterogeneity in the granule size distribution and atypical arrangements of starch components in the granule.  $T_c$  reflects the melting of a high crystallinity area with high stability [34], and it was more in Saba (99.35 °C) and Monthan (96.81 °C). The larger particle size with higher  $T_c$  implies that crystallites of starches are more stable and absorb more water than starches with lower particle size and  $T_c$  [34].

Saba starch recorded the highest gelatinization enthalpy ( $\Delta H_{gel}$ ) (24.40 J/g) while Grand Naine (12.36 J/g) recorded the least value. Hence, the highest  $\Delta H$  value of Saba starch that melts during thermal diffusing was resistant and linked to its native structure. The  $\Delta H$  refers to the energy required to fuse the segments of double helices in the crystalline region of native starch [25]. Besides, it has been reported that the highest crystallinity of starch reflects the higher enthalpy value (ΔH) during of disintegration both the double helices and the bonding forces between double helices. In this study, starches with high crystallinity level reflect their high  $\Delta H$  values. In the case of Popoulu starch, it was found that higher crystallinity level with low  $\Delta H$  value, indicates lose packing of amylose and amylopectin in the crystalline region. The thermal behavior of starch plays a significant role and indicates their potential applications in foods that requires thermal processing. This is important in products where delayed pasting is required, like retorted canned foods. Popoulu starch with higher temperature tolerance with higher RS content makes it suitable in the preparation of noodles, baked products, and fried snacks [2].

#### 6.5. Pasting properties by RVA

Fig. S2 and Table 5 illustrate the pasting behavior of various starches. The pasting temperature (PT) significantly varied among the varieties (76.7–85.7 °C) (Fig. S2). However, varieties Saba, Nendran, and Popoulu had higher pasting temperatures with negligible differences, which can be validated by amylose content and amylopectin ratio, as this slows starch swelling and thus raises the PT. Higher PV values were exhibited for Grand Naine (7951 cP) starch whereas minimum peak viscosity (PV) was recorded with Popoulu (3111 cP). These values were associated with the water binding ability and the rate of starch granule disintegration [26,31]. In addition, the amylopectin arrangement and the mass influence the PV value [35]. Later with the disintegration of granules, the trough viscosity is reduced with shear forces. Nevertheless, of banana varieties, corn starch recorded the lowest pasting properties like trough viscosity, break down viscosity, and final viscosity. However,

**Table 4** Thermal profiling of banana starches and corn starch.

Varieties	T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	T <sub>c</sub> (°C)	GEL <sub>R</sub> (°C)	ΔH <sub>gel</sub> (J/g)
Grand Naine	$66.23 \pm 0.95^{\rm c}$	$70.61\pm1.0^{\mathrm{f}}$	$\textbf{78.55} \pm \textbf{1.85}^{\text{f}}$	$12.32 \pm 1.09^{\rm d}$	$12.36 \pm 1.25^{\mathrm{e}}$
Monthan	$69.60 \pm 0.83^{\rm b}$	$85.36 \pm 0.93^{\rm b}$	$96.81 \pm 2.25^{\mathrm{b}}$	$27.21 \pm 1.00^{\rm a}$	$22.46 \pm 2.15^{\rm b}$
Saba	$72.95 \pm 0.60^{a}$	$87.67 \pm 1.11^{a}$	$99.35 \pm 2.26^{a}$	$26.40 \pm 0.86^{\rm b}$	$24.40 \pm 1.65^{a}$
Nendran	$66.13\pm0.85^{\mathrm{c}}$	$81.78 \pm 01.22^{\rm d}$	$92.65 \pm 1.25^{c}$	$26.52 \pm 0.91^{\rm b}$	$20.52 \pm 2.21^{c}$
Popoulu	$70.30 \pm 0.75^{\rm b}$	$83.02 \pm 1.05^{\rm c}$	$90.38\pm2.45^{\rm d}$	$20.08\pm0.76^{\mathrm{c}}$	$19.26 \pm 1.10^{ m d}$
Corn	$65.71 \pm 0.90^{d}$	$73.43 \pm 0.98^{e}$	$79.98\pm1.25^{\mathrm{e}}$	$12.31\pm0.56^{\mathrm{d}}$	$11.49\pm1.20^{\mathrm{f}}$

 $T_o = \text{onset}$  temperature,  $T_p = \text{peak}$  temperature,  $T_c = \text{conclusion}$  temperature,  $GEL_R = \text{gelatinization}$  range  $(T_c \cdot T_o)$ , and  $\Delta H_{gel} = \text{gelatinization}$  enthalpy. Data presented are mean value  $\pm$  standard deviation (n = 3). Different superscripts in the same column are significantly different (p < 0.05) by Duncan's test.

**Table 5**Pasting profiles of banana starches and corn starch.

Varieties	PT (°C)	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PTm (min)
Grand Naine Monthan	$76.75 \pm 2.0^{e}$ $81.65 \pm 2.39^{b}$	$7951 \pm 18.19^{a} \\ 7504 \pm 57.44^{b}$	$3613.6 \pm 9.5^{d}$ $4250.3 \pm 8.0^{c}$	$4348.0 \pm 62.0^{a} \\ 3223.0 \pm 29.51^{b}$	$4585.0 \pm 15.7^{\rm d} \\ 5779.0 \pm 14.8^{\rm b}$	$972.0 \pm 2.0^{\rm f} \\ 1530.0 \pm 4.6^{\rm e}$	$4.40 \pm 0.2^{\rm cd} \\ 4.47 \pm 0.09^{\rm cd}$
Saba	$85.71 \pm 1.93^{a}$	$6726 \pm 39.5^{\circ}$	$4286.6 \pm 5.5^{\mathrm{b}}$	$2438.3 \pm 18.9^{d}$	$5871.0 \pm 12.0^{a}$	$1583.0 \pm 3.6^{d}$	$4.67 \pm 0.14^{bc}$
Nendran Popoulu	$85.60 \pm 1.99^{a}$ $85.73 \pm 1.52^{a}$	$6014 \pm 85.3^{d}$ $3111 \pm 43.71^{e}$	$4584 \pm 6.2^{a}$ $2541.3 \pm 6.8^{e}$	$1466.0 \pm 10.53^{\rm e} \\ 570.1 \pm 10.2^{\rm f}$	$4639.0 \pm 17.3^{c}$ $4534.0 \pm 9.5^{e}$	$1908.6 \pm 2.8^{b}$ $1993.3 \pm 5.1^{a}$	$4.92 \pm 0.17^{b}$ $5.27 \pm 0.27^{a}$
Corn	$78.40 \pm 1.99^{bc}$	$4377 \pm 40.95^{\mathrm{f}}$	$1734.0 \pm 6.2^{\mathrm{f}}$	$2642.6 \pm 15.5^{c}$	$3544.0 \pm 31.0^{\rm f}$	$1809.3 \pm 4.7^{c}$	$4.25 \pm 0.17^{c}$

PT: pasting temperature, PV: peak viscosity, TV: trough viscosity, BD: breakdown viscosity, FV: final viscosity, SB: setback viscosity, and PTm: Pasting time. Data presented are mean value  $\pm$  standard deviation (n = 3). Different superscripts in the same column are significantly different (p < 0.05) by Duncan's test.

the lowest setback viscosity was recorded with Grand Naine, a dessert banana. The rigidity of swollen granules positively influenced the breakdown viscosity [4]. Cooking bananas like Monthan and Saba recorded lower BD viscosity than dessert banana. With higher amylopectin, cooking banana resulted in lesser dispersion and produce as a network that resists the temperature and shear and thus lower BD viscosity.

This clearly showed that cooking varieties like Monthan and Saba behaved similarly and recorded higher pasting properties whereas the varieties like Nendran and Popoulu behaved similarly and were moderate on their pasting properties. This can be due to the amylopectin branch chain-length distribution and amylose molecular size [32,29]. Distinctively, the setback viscosity of Grand Naine (972 cP) was lower than other varieties and in the order of Monthan < Saba < Corn < Nendran < Popoulu (1993 cP). With reference to the pasting time, Popoulu required a higher time than other varieties including corn starch.

## 7. Morphological and structural properties

# 7.1. Scanning electron microscopy (SEM), light microscopy (LM), and particle size distribution

The microscopic studies of starch granules were extensive array of shapes like elongated, oval, and irregular shapes and different sizes (Fig. 5). However, the smaller starch granules mostly appeared circular. Through SEM analysis, it was inferred that starch granules also possessed smooth surface, dense flat, and without any grooves indicates the purity of the starch (Fig. 6). Starch granules from Monthan appeared more elongated (55.09  $\pm$  1.25  $\mu M)$  than other starch granules whereas Nendran starch granules were elongated and had flattened surfaces. Previous reports also ascribed the varying shapes, lengths, and diameters of banana starch granules [32,29]. These variations in granular morphology might be accredited to innate characteristics of the varieties and arrangement of amyloplast [16]. The sizes of the starch granules were measured by volume mean diameter and a presentative curve of particle size distribution is presented in Fig. 7. The starches from Grand Naine and Nendran showed a unimodal particles distribution with an average diameter of 13.59  $\pm$  6.90  $\mu m$  and 8.56  $\pm$  2.31  $\mu m$ , respectively. The starch granules from Monthan appeared multimodal distribution with the larger particle size of 40.19  $\pm$  5.57  $\mu m$  whereas bimodal dispersion with a mean particle size of 23.01  $\pm$  5.65  $\mu m$  and 29.60  $\pm$ 6.19 µm was exhibited with the Saba and Popoulu starch granules, respectively. The starch granules from Monthan, Popoulu, and Saba with higher particle size could have positively influenced the high SP, WHC, gelatinization, and textural properties.

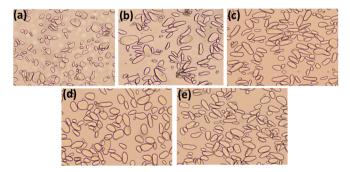


Fig. 5. Light microscope (LM) (40× magnifications) images (10  $\mu m)$  of banana starches.

Grand Naine (a), Monthan (b), Saba (c), Nendran (d), and Popoulu (e).

7.2. ATR-FTIR short-range molecular order, X-ray diffraction pattern, and relative crystallinity

ATR-FTIR spectra of starches are illustrated in Fig. 8(a). It is observed that broad bands at 3000–3500 cm<sup>-1</sup> and 2800–3000 cm<sup>-1</sup> were initiated by the vibrational type of O—H stretching due to hydrogen-bonded OH groups of amylose and amylopectin. The peaks observed around 1643–1646 cm<sup>-1</sup> represented the bending vibration of O—H (water absorbed) in the amorphous region. The peaks at 575, 861, and 930 cm<sup>-1</sup> reflected the skeletal vibration of the pyranoid ring. The absorption range from 800 to 1200 cm<sup>-1</sup> corresponded to variation in double helical polymer structure and chain conformation in starch [36]. The bands around 800–1000 cm<sup>-1</sup> reflected the C—C skeletal mode of

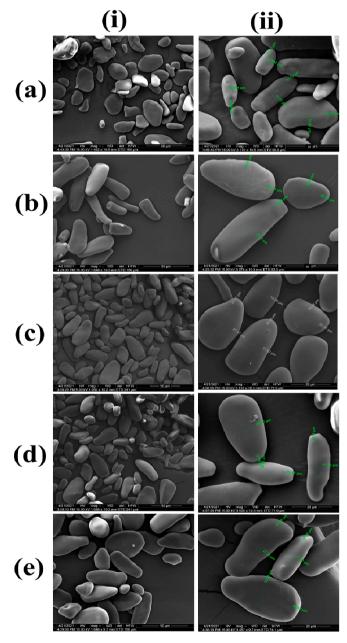


Fig. 6. Scanning electron microscope (SEM) images of banana starches at 50  $\mu m$  (i) and 20  $\mu m$  (ii).

Grand Naine (a), Monthan (b), Saba (c), Nendran (d), and Popoulu (e).

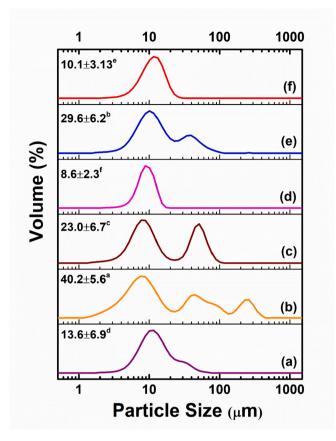
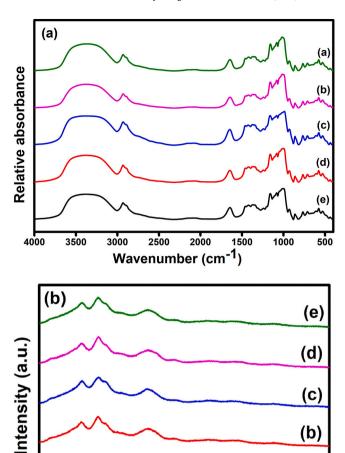


Fig. 7. Particle size distribution curve of banana starches and corn starch. Grand Naine (a), Monthan (b), Saba (c), Nendran (d), Popoulu (e), and Corn (f).

 $\alpha$ -(1–4) glycosidic linkage, whereas peaks at 1081 and 1157 cm<sup>-1</sup> were assigned to C-O bond stretching. The entire peaks around1000-1200 cm<sup>-1</sup>, arose from C—O, C—C, C-O-C, C-O-H stretching mode, and C-O-H bending mode [37]. Deconvoluted FTIR spectrum was used to understand the short-range molecular order. Particularly the absorbance intensity at 1045, 1022, and 995 cm<sup>-1</sup> are sensitive to confirmation of changes in the starch molecule [38]. The absorbance peaks at 1045 and 995 cm<sup>-1</sup> were related to the molecular order of starch, while the peak at 1022 cm<sup>-1</sup> was associated with the amorphous region [39]. The absorption peak ratios 1045/1022 cm<sup>-1</sup> and 995/1022 cm<sup>-1</sup> were used to quantify the differences in degree double helix and degree of order, respectively. The degree of double helix was evident by IR ratios of  $1045/1022 \text{ cm}^{-1}$  and  $995/1022 \text{ cm}^{-1}$  which varied from 0.91 to 0.95 and 0.97 to 1.04, respectively (Table 6). The IR ratios of 1045/1022 and 995/1022 cm<sup>-1</sup> were found to be higher in Saba (cooking banana) with 0.95 and 1.04, respectively. The high values of absorbance ratio 1045/ 1022 cm<sup>-1</sup> exhibit a higher level of ordered structure exterior [38].

The XRD behavior of starches is presented in Fig. 8(b). The diffractogram showed several peaks (2θ) ranging from 14° to 34°. In our studies, the highest peak was located between 17 and 18° for all banana starches, while other minor peaks (2θ) were obtained approximately at 14–15° (Shorter), 22–23° (broader), which were typical of a B-type and C-type (a combination of A and B) [22]. The additional peak (2θ) at 31° (Grand Naine, Popoulu) and 34° (Nendran) indicated the differences in the crystalline structure of these starches. It is inferred that Monthan, Saba, and Popoulu exhibited B-type polymorph whereas Grand Naine and Nendran displayed C-type polymorph showing a combination of A and B type [40]. The B-type polymorph is comprised of double p-glucose molecules. This led to an open structure linking the macromolecules and water molecules existing among the double helices of amylopectin. It has been reported by Marta et al. [35] that cooking bananas have B-type



**Fig. 8.** ATR-FTIR absorption spectrum (a) and XRD- Patterns (b) of banana and corn starches.

Degree (2θ)

30

35

(a)

45

40

(a) Grand Naine, (b) Monthan, (c) Saba, (d) Nendran, and (e) Popoulu.

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**Table 6**IR ratios and Relative crystallinity (%) of banana starches.

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Varieties	IR ratio	Relative Crystallinity (%)	
	1045/1022 cm <sup>-1</sup>	995/1022 cm <sup>-1</sup>	
Grand Naine Monthan Saba Nendran Popoulu	$0.92 \pm 0.05^{c}$ $0.91 \pm 0.04^{d}$ $0.95 \pm 0.01^{a}$ $0.93 \pm 0.03^{b}$ $0.93 + 0.00^{b}$	$0.97 \pm 0.02^{b}$ $1.00 \pm 0.03^{a}$ $1.04 \pm 0.02^{a}$ $1.02 \pm 0.00^{a}$ $1.02 + 0.01^{a}$	$21.19 \pm 0.12^{e}$ $35.11 \pm 0.10^{c}$ $52.02 \pm 0.17^{a}$ $25.03 \pm 0.18^{d}$ $48.10 \pm 0.15^{b}$

Data presented are mean value  $\pm$  standard deviation (n = 3). Different superscripts in the same column are significantly different (p < 0.05) by Duncan's test.

pattern and dessert varieties have C-type pattern. But, Nendran (plantain banana) exhibited C-type pattern in this study. In addition, the crystallinity (%) level for all tested banana starches significantly varied from 21.19% to 52.02% and is presented in Table 6. Cooking bananas such as Saba (52.02%), Popoulu (48.10%), and Monthan (35.11%) exhibited higher relative crystallinity level than Grand Naine (21.19%). The degree and difference of crystallinity is a crucial factor that determined starch digestibility [26]. These results implied that all banana starches of this study have a combination of both B-type and C-type

**Table 7**Texture profile of banana and corn starches.

Varieties	Hardness (g)	Cohesiveness	Gumminess (g)	Springiness (s)	Resilience	Chewiness (gs)	Adhesiveness (gs)
Grand Naine Monthan	$\begin{array}{c} 120.23 \pm 2.88^{b} \\ 91.97 \pm 2.80^{c} \end{array}$	$\begin{array}{l} 0.66 \pm 0.01^{b} \\ 0.45 \pm 0.01^{e} \end{array}$	$\begin{array}{l} 72.27 \pm 2.92^a \\ 40.22 \pm 2.63^c \end{array}$	$\begin{array}{c} 77.30 \pm 1.73^{a} \\ 66.80 \pm 1.55^{b} \end{array}$	$\begin{array}{c} 37.15 \pm 2.17^b \\ 13.37 \pm 2.95^d \end{array}$	$\begin{array}{c} 55.98 \pm 1.65^a \\ 27.28 \pm 1.71^e \end{array}$	$\begin{array}{l} -126.10 \pm 2.14^d \\ -49.77 \pm 2.04^b \end{array}$
Saba	$91.55\pm1.14^{\rm c}$	$0.54\pm0.01^{\rm d}$	$57.14 \pm 1.87^{b}$	$73.57 \pm 2.45^a$	$15.31 \pm 2.94^{d}$	$34.76 \pm 1.78^{d}$	$-38.11 \pm 1.18^{a}$
Nendran	$127.92 \pm 1.69^a$	$0.60\pm0.01^{\rm c}$	$72.70 \pm 2.06^{a}$	$77.70 \pm 6.76^{a}$	$26.40 \pm 2.76^{c}$	$51.62 \pm 2.08^{\mathrm{b}}$	$-67.82 \pm 1.53^{\circ}$
Popoulu Corn	$\begin{array}{c} 89.30 \pm 2.85^c \\ 66.15 \pm 1.92^d \end{array}$	$\begin{array}{c} 0.61 \pm 0.01^c \\ 0.87 \pm 0.01^a \end{array}$	$54.02 \pm 2.01^{\mathrm{b}} \\ 43.25 \pm 2.36^{\mathrm{c}}$	$76.47 \pm 2.18^{a} \\ 74.60 \pm 3.41^{a}$	$22.35 \pm 1.45^{c} \\ 63.96 \pm 3.15^{a}$	$44.59 \pm 2.08^{c} \\ 35.60 \pm 1.43^{d}$	$-48.87 \pm 1.43^{\mathrm{b}} \\ -36.45 \pm 2.52^{\mathrm{a}}$

Data presented are mean value  $\pm$  standard deviation (n = 5). Different superscripts in the same column are significantly different (p < 0.05) by Duncan's test.

patterns with significant crystallinity differences.

#### 7.3. Texture properties of starches

The textural profile is presented in Table 7. Nendran and Grand Naine starches recorded harder gel (127.92 and 120.23 g) and gumminess (72.70 and 72.27) than other starches. This was due to the lower particle scattering which caused rapid retrogradation, reaggregation, and related syneresis behavior [2]. The Springiness values of all starch gels were found similar except for Monthan starch. Grand Naine recorded the higher value for adhesiveness and cohesiveness, whereas it was lower with Saba. This showed the resilience of the Grand Naine starch to the applied force, its capability to withstand the deforming force up to certain strength, and maintaining soft texture [35,40]. Soft desserts could be therefore made with Grand Naine and Nendran starch. In our study, the resilience property of starch gel varied significantly. The resilience was found high in corn starch and Grand Naine while starches from Saba, Nendran, and Popoulu did not differ significantly. Our results are in line with Sszczesniak [41], who reported that the resilience property frequently varied with the formation of a network among the macromolecules of different food products.

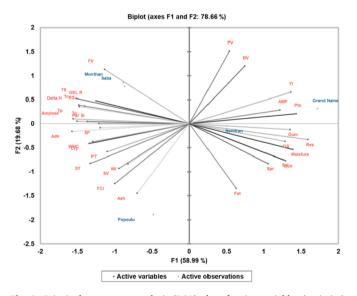


Fig. 9. Principal component analysis (PCA) plot of active variables (varieties) and observations (different properties). AMP (Amylopectin), TS (Total starch), DS (Digestible starch), RS (Resistant starch), Ptn (Protein), WI (Whiteness index), YI (Yellowness index), FCI (Flour colour index), To (Onset temperature), Tp (Peak temperature), Tc (conclusion temperature), GEL R (gelatinization range), Delta H ( $\Delta$ Hgel), PT (Pasting temperature), PV (Peak viscosity), BD (Breakdown viscosity), FV (Final viscosity), Cry (Crystallinity), Adh (Adhesiveness), Res (Resilience), Coh (Cohesiveness), Spr (Springiness), Gum (Gumminess), Par Si (Particle size), Sol (Solubility), SP (Swelling power), WHC (Water holding capacity), and SY (Syneresis).

#### 7.4. Multivariate analysis

Principal component analysis (PCA) of starch samples depending upon their physicochemical characteristics, textural and thermal properties is plotted (Fig. 9). Results of the study demonstrated that overall, first and second principal component (PC1 and PC2) accounts for 58.9% and 19. 68% of variations with cumulative 78.66% variation among the active variables and influenced the starch properties whereas PC3 and PC4 contributed 15.10 and 6.2% on the overall cumulative impact. It is evident that the varieties Saba and Monthan were grouped together based on their functionality whereas the starch of Popoulu was very distinct from others. On the other hand, the intermediate amylose content in Nendran and Grand Naine makes them at a differential distance away from the PC1. Properties such as amylose, total starch,  $\Delta H$ and final viscosity and textural parameters like adhesiveness, springiness and starch yield contributed more to the PC1; whereas gumminess, amylopectin, moisture, cohesiveness contributed significantly to PC2; parameters like fat, whiteness index, setback viscosity contributed to PC3. It is not unusual to note that starch parameters like amylose, swelling powder, resistant starch content weighed and contributed heavily on the grouping of various starches. Colour values and the proximate parameters contributed lesser to the overall weight among the parameters. This relationship was consistent as studied in the early reports in different banana starches [16-18,24,32]. They reported that starch functionality is highly correlated with the amylose, resistant starch content and XRD behavior. The results of the PCA provided way to identify the factors that could be targeted to modify the starches to enhance the functional properties.

# 8. Conclusion

In a nutshell, the isolated starch recorded a significantly lesser amount of fat, protein, and ash. Furthermore, Saba and Monthan starches have higher resistant starch content with lower glycemic index, making them potential varieties for low glycemic food products such as extruded snacks and bakery products. In addition, Monthan, Popoulu, and Saba starches could be used for industrial applications requiring stronger swelling power, water holding capacity, and higher gelatinization qualities, such as ready-to-cook food products, canned foods, and as a thickening agent in soup and pie fillings. The retrogradation qualities of Grand Naine, Popoulu, and Nendran suggested their suitability for usage in ice cream, dessert, and freeze-thawed items. Based on their intrinsic features, we believe that banana starches could be employed as a supplement to existing commercial starches in food and non-food applications due to their improved structural and functional qualities.

# Declaration of competing interest

The authors declare that there was no conflict of interest.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.09.172.

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