

PHYSICO-CHEMICAL PROPERTIES OF MODIFIED *Blighia sapida* SEED STARCH

Abiodun O. A.^{1*}, Dauda, A. O.¹, Ojo, A.², Adepeju A. B.³, Odenibi, D. E.¹ and Mudi F. O.¹

¹Department of Home Economics and Food Science, University of Ilorin, Kwara State, Nigeria.

²Department of Food Science and Technology, Osun State Polytechnic, Iree, Nigeria.

³Department of Food Science and Technology, Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.

*Corresponding email address: funmiabiodun2003@yahoo.com, abiodun.aa@unilorin.edu.ng

Abstract

Physicochemical properties of modified *Blighia sapida* seed starch was determined. Starch was extracted and subjected to modification using four methods. Physicochemical properties of the starches were analyzed. Acetylated starch was lighter in colour than other starches. *Blighia sapida* seed starch had irregular shapes ranging from oval, flat, round, elliptical and dome-shaped. *Blighia sapida* seed granules were smaller in sizes with values ranging from 3.38-6.53 µm while amylose contents ranged from 18.29 to 24.83 %. There was a rapid increase in swelling power at 80 °C and 90 °C in acetylated and alkali treated starches. Hypoglycin A ranged from 38.8 to 57.5 mg/kg while hypoglycin B ranged from 71.80 to 84.90 mg/kg. Pregelatinized starch had higher paste clarity at 4 °C and at ambient temperatures which reduced with storage time. Alkali and acid treated starches had low freeze-thaw stability than the native and other modified starches. Peak viscosity ranged from 543.00 to 3974 cP. Pregelatinized starch had low viscosity while alkali treated starch had higher stability ratio. Modification of *Blighia sapida* seed starch provides starches with different properties for different applications in food and non-food products.

Keywords: *Blighia sapida* seed, Hypoglycin, Modification, Physicochemical properties, Starch

Introduction

Ackee (*Blighia sapida*) is a tropical fruit belonging to the Sapindaceae family, the fruit originated from West Africa, and can be found widely distributed throughout the island of Jamaica (Onuekwusi et al., 2014). It is a woody perennial multipurpose tree with broad and pinnate leaves (Nwoso et al., 2014) with shining black, bigger seeds than that of legume seeds. The fruit is cultivated for the aril which is used as meat substitute in soup while the seed is discarded as a waste product. Abiodun et al (2015a,b) worked on the use of the discarded seeds for flour and starch production but observed higher starch contents in the crop. This starch was reported as a cheap source of raw material which could be used in food and non-food applications and thereby reducing wastage of the crop during its season. Native starch had limitations in their use due to low shear resistance, thermal resistance, thermal decomposition and high tendency towards retrogradation (Otegbayo et al., 2013) therefore modification of starch had been reported to improve the functional properties of starches for different applications (Yadav et al., 2013; Palacios-Fonseca, 2013).

However, according to Alcazar-Alay and Meireles (2015), starch is modified to enhance its positive attributes and eliminate deficiencies in its native characteristics. Jyothi et al., (2013) also observed that modification of starch widens the exploitation of

starch in industrial products due to its better viscosity, clarity, higher gel strength, improved film forming capacity and lower retrogradation tendency. Lots of researchers have reported toxicity in consumption of ackee apple seed (Blake et al., 2006; Brown et al., 1992). According to Blake et al. (2006), the seed contains a natural toxin, which exists as the cyclic amino acid, hypoglycin A and its gamma-glutamyl derivative, hypoglycin B which causes vomiting and death. Presence of hypoglycin in the seed of *Blighia sapida* limits the use of the seed in food production. The seed had been used in feeding rat and the acute dose had been established (Blakes et al., 2006). A by-product from the seed is the starch which had been observed to be high in the seed (Abiodun et al., 2015). Extraction and modification of the starch could also have impact on the toxicity level of the starch product. Hence, this work presents hypoglycin and physicochemical properties of modified *Blighia sapida* seed starch.

Materials and Methods

Blighia sapida seeds were obtained from Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria. The seeds were manually dehulled and milled. Starch extraction was done using method of Akinwande et al. (2007). Centrifugation method was used to obtain the starch content. Acid, Alkali, acetylated, oxidized and heat treated starches were done according to the methods of Okunola &

Akingbala (2013), Gutierrez *et al.* (2014), Iheagwara (2012) and Oladebeye *et al.* (2011) respectively. The colour attributes (L, a and b values) of the yam starch was measured using a Minolta portable chroma-meter. The colour coordinates system L* a* and b* values were recorded. Morphological structure of starch was done using light microscope (LM) and stained with drops of potassium iodide (KI) solution. The images were viewed at a magnification of X400 (AmScope Binocular Digital Microscope version x86, 3.74183). The area, perimeter and diameter of the starch granules were measured and used to calculate the granule sizes and form factor. Golden *et al.* (2002) method was used to determine the hypoglycin contents of the starches. Amylase determination was done with the method of Juliano (1971). The starch yield was also calculated. The stability and clarity of starch pastes were determined at 25°C and 4°C following the method of Gutierrez *et al.* (2014). The percentage (%) of transmittance at 650 nm was determined in spectrophotometer (BK-UV1600PC) using distilled water as a blank. Method of Lutfi and Hasnain (2013) was used for freeze thaw stability of 5% starch solution. Moisture and swelling power determination were carried out using the procedure of AOAC method 934.01 (AOAC, 2006) and Peroni *et al.* (2006) respectively. The pasting profile of the starch sample

was studied using a Rapid Visco-Analyzer (RVA) (Newport Scientific Pty Ltd, 1998) with the aid of a thermocline for windows version 1.1 software.

Statistical Analysis

All procedures were carried out in triplicates. The mean and standard deviation of the data obtained were calculated. The data were evaluated for significant differences in their means using Analysis of Variance at p

Results and Discussion

Effect of Modification of Colour of *Blighia Sapida* Seed Starch

The lightness (L*) value of *Blighia sapida* seed starches were very similar across all treated starches, except for pre-gelatinized starch (PB) which was significantly different ($p < 0.05$) from other samples (Table 1). This indicated that PB was tending towards reddish brown and yellowish as it had the highest values for both redness (a*) and yellowness (b*) of 1.17 and 9.02 respectively. Other starches have negative values in a* coordinates which denote that the starches tend toward greenish in colour. The relatively high L* values of starches is an index of purity (Oyeyinka *et al.*, 2015). Acceptability of starch also depends on the purity of whiteness as this is required for suitability in industrial applications.

Table 1: Effect of Modification on the Color of *Blighia sapida* Seed Starch

Treatment	L*	a*	b*
RB	94.81 ^{ab} ±0.45	-0.35 ^b ±0.10	5.33 ^b ±0.33b
AEB	95.66 ^a ±0.27	-0.27 ^b ±0.11	4.12 ^d ±0.06d
ADB	94.26 ^{ab} ±0.83	-0.22 ^b ±0.16	4.77 ^c ±0.32c
AKB	94.58 ^{ab} ±0.23	-0.28 ^b ±0.10	4.80 ^c ±0.09c
OB	92.44 ^b ±1.86	-0.26 ^b ±0.13	4.74 ^c ±0.10c
PB	79.55 ^c ±0.65	1.17 ^a ±0.18	9.02 ^a ±0.06a

Values in the same column with different superscripts are significantly different ($P < 0.05$)

RB- Raw starch, AEB- Acetylated starch, ADB- Acidified starch, AKB- Alkalized starch, OXB- Oxidized starch, PB- Pre-gelatinized starch

Effect of Modification on the Morphological Structure, Granule Size and Amylose Contents of *Blighia sapida* Seed Starch

Fig.1 and Table 2 showed the morphological structure, granule size, form factor and amylose contents of *Blighia sapida* seed starches. *Blighia sapida* seed starch demonstrated oval, flat shaped granules with a few granules round, elliptical, dome-shaped and irregular shape. Oxidized (OXB) starch had irregular shapes with whitish coating surrounding the starch granules. Acetylated starch granules were deformed and the shape different from other modified starches. The starch structures were flat, dome and irregular shapes. This report corroborates the findings of Olayinka *et al.* (2013) and Sodhi and Singh (2005) that acetylation led to alteration and deformation of starch granules. The granules of the starches were smooth except in pregelatinized starch which showed fragments of the starch granules. This report corroborates the findings of Alcázar-Alay and Meireles (2015) that pre-

gelatinization led to destruction of the granular structure of the starch thereby resulting in complete granular fragmentation of the starch. The form factor of the starch ranged from 0.31- 0.64. The values observed by Abiodun *et al.* (2015a) for *Blighia sapida* seed starch ranged from 0.75-0.93 which was within the range observed in this study. Form factors of the starches were less than one which signified that they were not perfect circle. Form factor is a measure of grains roundness (Sarka *et al.*, 2011). *Blighia sapida* seed starches were smaller in sizes with values ranging from 3.38-6.53 µm and were within the mean range reported by Abiodun *et al.* (2015a). Smaller granule sizes are more digestible than larger particle size due to the large surface area and provides better mouth feel as a lipid substitute (Yuan *et al.*, 2007; Whistler *et al.*, 1990). Granule size is important in determining the mesh size for application and purification sieves and also affects the possibilities of utilization and processing (Leonel *et al.*, 2003).

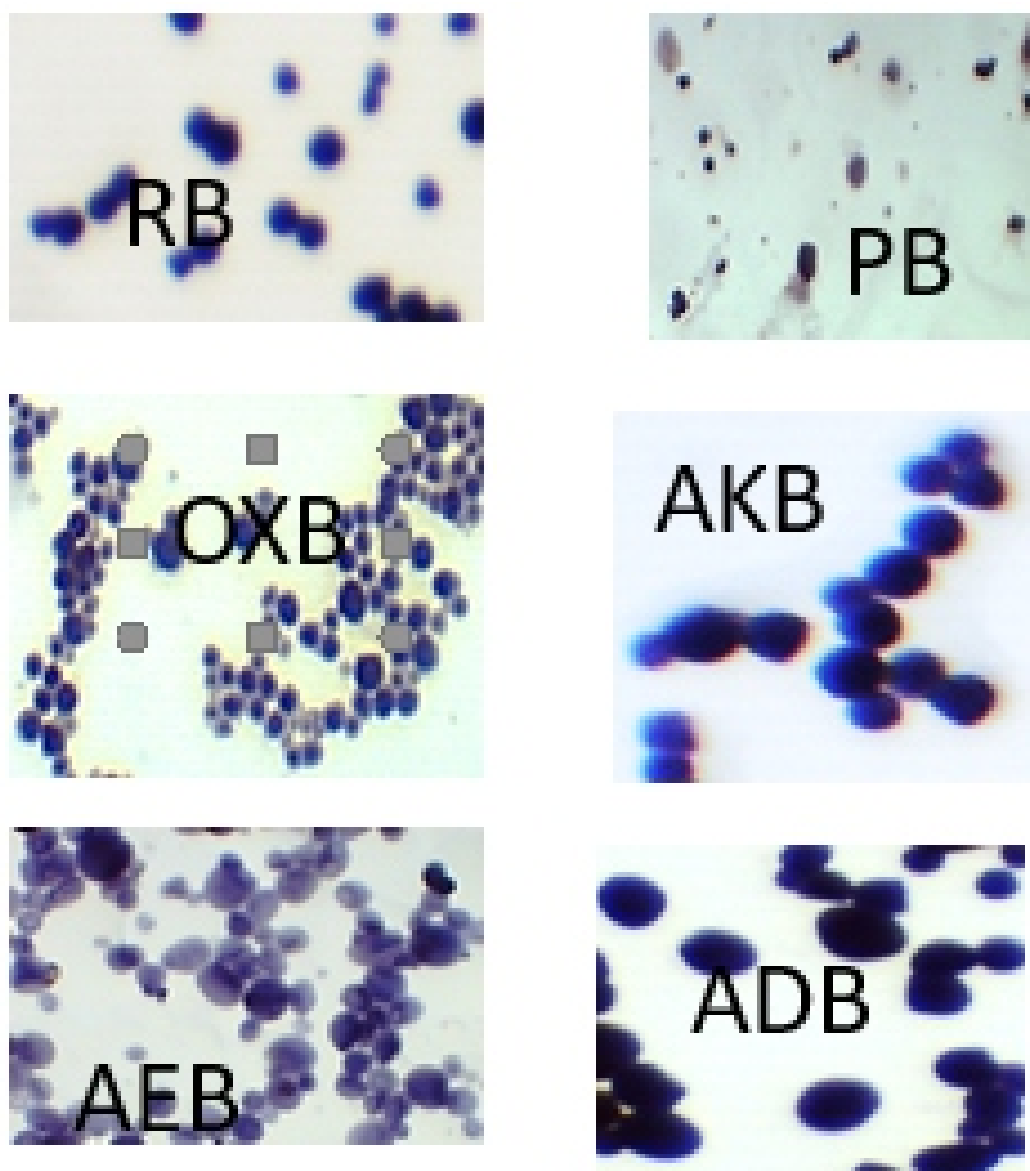


Fig. 1: Light micrographs of native and modified *Blighia sapida* seed starch (x400). ADB- Acidified starch, AKB- Alkaline starch, AEB- Acetylated starch, RB- Raw starch PB- Pre-gelatinized starch, OXB- Oxidized starch.

Table 2: Effect of modification on the granule size, form factor and amylose contents of *Blighia sapida* seed starch

Treatment	Granule size (μm)	Form factor	Amylose (%)	Yield (%)
RB	3.38 ^d \pm 0.21	0.32 ^c \pm 0.03	22.05 ^b \pm 0.46	49.62 \pm 4.12
AEB	5.07 ^b \pm 0.03	0.44 ^b \pm 0.04	18.29 ^c \pm 0.80	-
ADB	4.98 ^b \pm 0.11	0.56 ^{ab} \pm 0.10	24.83 ^a \pm 0.22	-
AKB	6.53 ^a \pm 0.89	0.64 ^a \pm 0.07	18.82 ^c \pm 0.14	-
OB	4.53 ^c \pm 0.65	0.50 ^b \pm 0.04	21.18 ^b \pm 0.72	-
PB	4.21 ^c \pm 0.73	0.31 ^c \pm 0.04	18.29 ^c \pm 0.11	-

Values in the same column with different superscripts are significantly different ($P < 0.05$)

RB- Raw starch, AEB- Acetylated starch, ADB- Acidified starch, AKB- Alkaline starch, OXB- Oxidized starch, PB- Pre-gelatinized starch

Acid-modified starch (ADB) had the highest amylose content (24.83%) among all modified starches. There were no significant differences ($p>0.05$) in the amylose contents of native and alkali treated starches. There were no significant differences ($p>0.05$) in the amylose contents of AEB, AKB and PB in amylose contents. Lower amylose contents lead to higher solubility and swelling power. Amylose content reported for *Blighia sapida* seed starch was higher than the values observed in this study by Abiodun et al. (2015). This may be due to the cultivar of the seed, location, method of analysis etc. Higher amylose content was reported in alkaline steeping than acid steeping corn starch (Palacios-Fonseca et al., 2013). According to Tester et al. (2004); Copeland et al. (2009) the amylose content of a starch depends on the sample of starch, the source of the starch, the method used for determining the amylose content and the chemical composition of the starch. The yield of starch obtained from the seed was 49.62 % of the dry matter higher than the value (43.44%) reported by Abiodun et al. (2015). The starch yield is considerably higher when compared to other crops. *Blighia sapida* starch yield depends major on the cultivar of the crop.

Effect of Modification on swelling Power of *Blighia sapida* seed Starch

The swelling power of the starches was determined at temperature of 60 °C to 90 °C and was represented in Fig. 2. *Blighia sapida* seed starches showed a

progressive increase in swelling power as temperature increased. At lower temperatures, there were gradual swellings of the all the starches. However, a sharp increase in swelling power was observed between the temperature range of 80 °C and 90 °C in acetylated (AEB) and alkali (AKB) treated starches. AEB had the highest swelling value of 2.92 at 90 °C due to the rupturing of the starch granules which breaks down the sizes of the starch and increases the surface area. Increase in acetylated starch was also observed by Olayinka et al. (2013) for sorghum starches and decreased during acid thinning and oxidation. Native starch had lower swelling power than the modified starches at all temperatures. Among the modified starches, oxidized starch (OXB) had the least swelling value. Hua-Xi et al. (2012) attributed the reduction in the swelling power in OXB to be due to structural disintegration within the granules of the starch during the modification process. Adebowale et al. (2002) explained low swelling power observed in OXB as a result of hypochlorite oxidation which weakens the internal structures of the starch granules making the starch more soluble and leading to reduced swelling. Berski et al. (2011) observed increase in swelling capacity and solubility of capacity of acetylated starch when compared to native starch. Tester et al (1990) reported swelling of starch as a function of amylopectin, the magnitude of interaction within the amorphous and crystalline regions.

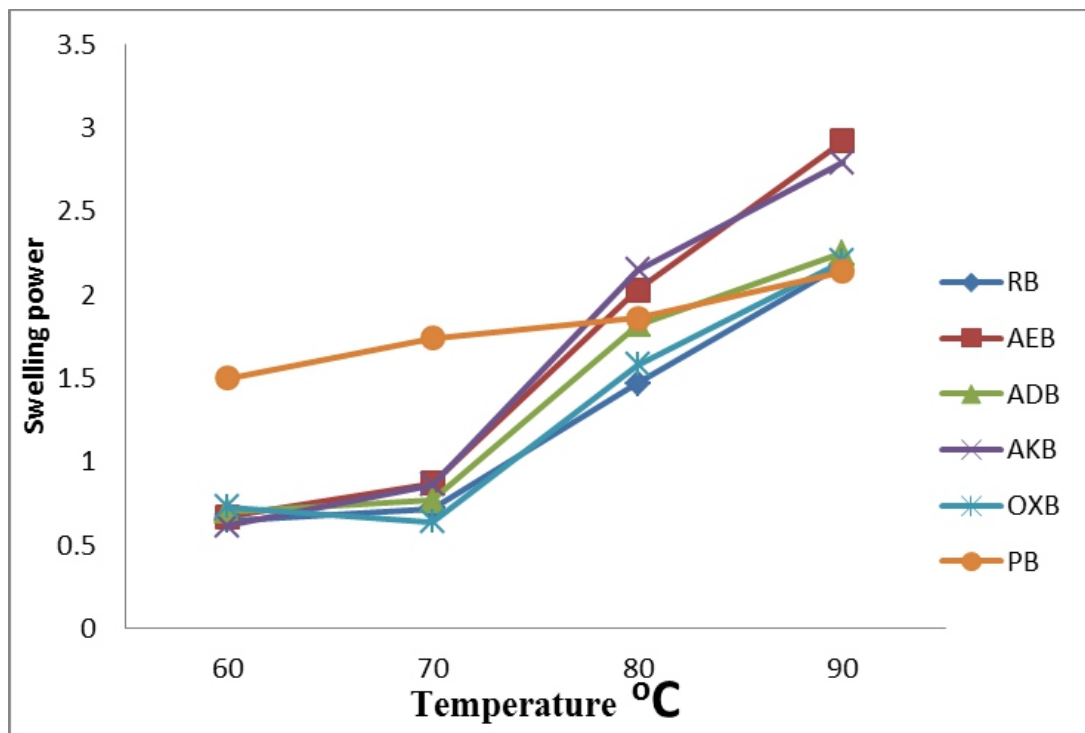


Fig. 2 : Effect of modification on the swelling power of *Blighia sapida* seed starch
 RB – Native starch, ADB – Acidified starch, AKB – Alkalized starch, OXB – Oxidized starch, AEB – Acetylated starch, PB – Pregelatinized starch.

Effects of Modification on Hypoglycin Content of *Blighia sapida* Seed Starch.

Table 3 showed the effect of modification on hypoglycin contents of ackee seed starch. Hypoglycin A ranged from 38.8 to 57.5 mg/kg while hypoglycin B ranged from 71.8 to 84.9 mg/kg. Alkali-modified starch had higher hypoglycin A content (57.5 mg/kg) but was not significantly different ($p < 0.05$) from acetylated, alkalized and oxidized starches. The least value of hypoglycin A was observed in pregelatinized starch which may be as a result of heat treatment which the starch was subjected to thereby destroying the component. The values obtained for hypoglycin B were higher than that of hypoglycin A for all the starches. Alkali-treated starch also had the highest hypoglycin B. Hypoglycin contents were high in acid, alkali, oxidized and acetylated starches as this may be as a result of the reactions with the chemical constituents used for modification which caused hydrolysis of the starch thereby releasing the bound hypoglycin compound making them free thus increase the hypoglycin content. Golden et al. (2002) observed

124.4 mg/100g and 6.40 mg/100g hypoglycin A in unripe and ripe fruits of Ackee apple respectively. The value reported for hypoglycin B were 142.8mg/100g and 106.0 mg/100g for unripe and ripe ackee apple fruits respectively.

These values were higher than the values observed for the native and modified starches. According to Blake *et al.* (2006), hypoglycin A had been the major toxic compound in *Blighia sapida* seed with the acute dose of 231.19 mg/kg BW per day for male rat and 215.99 mg/kg BW per day for female. Hypoglycin had been reported to caused vomiting, sickness or even death according to Tanaka *et al* 1976. The values obtained for the all the starches were low when compared to the acute dose. Heat treatment reduced the hypoglycin content than other treatments. Reduction of hypoglycin in the starches may be due to extraction process which involved several washing of the starch and decanting of the medium. *Blighia sapida* seed starch could be used in food and non-food applications.

Table 3: Effects of modification on hypoglycin test of *Blighia sapida* seed starch.

Samples	Hypoglycin A (mg/kg)	Hypoglycin B (mg/kg)	Total Hypoglycin (mg/kg)
RB	44.6±0.14 ^c	80.2±0.14 ^d	124.8±0.13 ^d
AEB	57.3±0.14 ^a	84.1±0.04 ^a	141.2±0.14 ^b
ADB	56.5±0.14 ^{ab}	81.3±0.07 ^c	137.8±0.21 ^c
AKB	57.5±0.14 ^a	84.8±0.14 ^a	142.1±0.04 ^a
OXB	57.1±0.14 ^a	83.9±0.21 ^b	141.0±0.35 ^b
PB	38.8±0.07 ^d	71.8±0.11 ^e	110.6±0.04 ^e

Values with the same letter down the column are not significantly different ($p < 0.05$) from each other.

RB – Native starch, ADB – Acidified starch, AKB – Alkalized starch, OXB – Oxidized starch, AEB – Acetylated starch, PB – Pregelatinized starch.

Effect of Modification on the Paste Clarity of *Blighia Sapida* Seed Starches.

Paste clarity of starches chemically modified with different methods is presented in Fig. 3. Paste clarity value of the starches at 0 hr ranged from 0.7 to 0.9%, acetylated and oxidized starches had the highest values, while acidified starch and alkalized starch had lower values. At 4 °C, the values obtained were constant at 24 hrs to 72 hrs for the starches except for

pregelatinized starch. Increase in paste clarity was noticed at 72 hrs in native, acid and alkali treated starches at ambient temperature but constant values were obtained for AEB and OXB. Paste clarity increased sharply in pregelatinized starch at 24 hrs and at both 4 °C and 27 °C, and declined at 48 and 72 hrs. Acid-treated starch had higher paste clarity (1.70 %) at 72 hrs than other starches at ambient temperature.

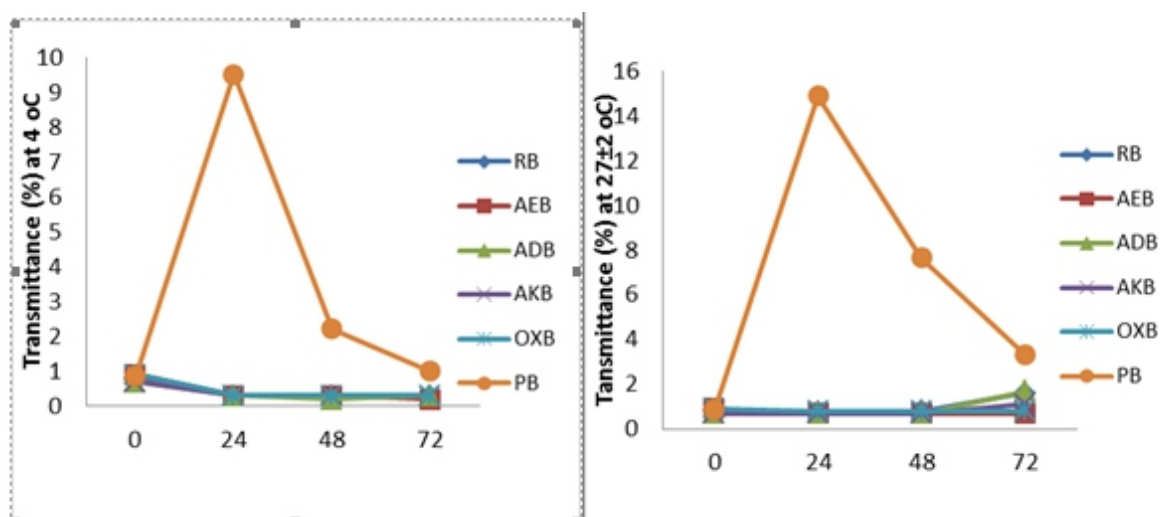


Fig. 3: Paste clarity of modified *Blighia sapida* seed starches
 RB – Native starch, ADB – Acidified starch, AKB – Alkalized starch, OXB – Oxidized starch, AEB – Acetylated starch, PB – Pregelatinized starch.

According to Khan et al. (2014), acid-treated potato starch also had an increase in paste clarity. Khan et al. (2014) further explained that paste clarity of native and modified starches decreased during storage at refrigeration temperature due to amylose leaching, amylopectin chains, granular remnants, granular swelling, amylose and amylopectin chain length (Jacobson et al., 1997).

Effect of Modification on Freeze-thaw Stability of *Blighia sapida* seed Starches at 5 % (w/v) Concentration.

The result of freeze-thaw stability of *Blighia sapida* seed starch using different chemical modification

method is shown in Table 4. Freeze-thaw stability of the native starch increased with increase in number of days and ranged from 30 to 63%. Likewise, freeze-thaw of acetylated starch, acidified starch, oxidized starch and pregelatinized starch also increased with increase in number of days and ranged from 18 to 73%, 31 to 58%, 20 to 65%, 20 to 65%, 50 to 76% respectively. Alkali-treated starch had a different trend; the amount of water exudate increased from day 0 – 5, then reduced back to 58% and became constant from day 7 – 10.

Table 4: Effect of modification on freeze-thaw stability of *Blighia sapida* seed starches at 5 % (w/v) concentration.

Samples	No of freeze-Thaw cycles (days) % of water thawed after storage					
	0	3	5	7	9	10
RB	30	60	62	63	63	63
AEB	18	60	64	73	73	73
ADB	31	57	58	58	58	58
AKB	32	62	70	58	58	58
OXB	20	60	65	65	65	65
PB	50	74	75	76	76	76

Values with the same letter down the column are not significantly different ($p < 0.05$) from each other.
 RB – Native starch, ADB – Acidified starch, AKB – Alkalized starch, OXB – Oxidized starch, AEB – Acetylated starch, PB – Pregelatinized starch

Other starches also became constant in values from day 7 – 10. For day 0 the result showed that the values ranged from 18 to 50% with acetylated starch having the lowest and pregelatinized starch having the highest value. Pregelatinized starch had the highest value for all cycles, since freeze-thaw is a factor of

storability it means the sample undergo syneresis faster when compared to other starches. This may be as a result of it being subjected to heat treatment which caused fragmentation of the starch granules thereby reducing the ability of the starch to form spongy network. Acidified and alkalized starches have low freeze-thaw

Table 5: Effect of modification on pasting properties of *Blighia sapida* seed starch.

Sample	Peak Viscosity (cP)	Trough (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Pasting Time (min)	Pasting Temperature (°C)
RB	3685.0±1.97 ^{ab}	2751.5±6.57 ^b	933.50±8.55 ^b	3890.50±5.72 ^a	1139.0±1.23	4.88±0.01 ^c	80.74±0.16 ^b
AEB	3217.0±2.23 ^{bc}	2050.5±5.44 ^c	1161.5±1.54 ^a	2934.00±	878.50±1.92	5.08±0.07 ^d	80.25±0.71 ^b
AKB	3973.0±7.63 ^a	3506.5±4.73 ^a	466.50±2.89 ^c	3741.00±	234.50±3.88	5.90±0.04 ^c	84.40±0.56 ^{ab}
ADB	3259.0±8.48 ^{bc}	2852.0±2.40 ^b	407.00±3.25 ^c	3056.00±	204.00±3.95	6.04±0.04 ^b	84.83±0.03 ^{ab}
PB	543.00±2.12 ^d	451.00±1.97 ^d	92.00±1.41 ^d	848.00±3	397.00±1.83	7.00±0.01 ^a	89.45±7.70 ^a
OXB	2970.5±1.18 ^c	2363.0±1.00 ^b	404.00±9.89 ^c	2890.00±	323.50±1.02	5.86±0.09 ^c	84.48±0.03 ^{ab}

Values with the same letter down the column are not significantly different ($p < 0.05$) from each other.

RB – Native starch, ADB – Acidified starch, AKB – Alkalized starch, OXB – Oxidized starch, AEB – Acetylated starch, PB – Pregelatinized starch.

Peak viscosities obtained were lower than the peak viscosity observed for *Blighia sapida* seed starch (Abiodun et al., 2015a). This may be majorly due to the species of *Blighia sapida* seed used, location, soil type and method of analysis. It had been observed that starch paste viscosities are reduced due to depolymerization of the starch granules (Pérez and Bertoft, 2010; Ulbrich et al., 2015). AKB had higher holding strength (trough) (3506.5 cP) which was significantly different ($p < 0.05$) from the native and modified starches. The stability ratio of the starch pastes revealed higher stability in AKB and ADB than other starches. AEB had the least stability ratio which indicated low ability to withstand shear stress during cooking. Stability ratio denotes the resistance of a starch paste to viscosity breakdown as shear stress is applied.

Native starch had higher final viscosity value (3890.5 cP) but was not significantly different ($p < 0.05$) from AKB. Likewise, there were no significant differences ($p < 0.05$) in the final viscosity values obtained for AEB, ADB and OXB while PB had the lowest value. Native starch of *Blighia sapida* had higher tendency to retrograde due to the higher setback value (1139.0 cP) which was significantly different ($p < 0.05$) from all the modified starches. Among the modified starches, acid-treated starch had low setback value (204.00 cP) which was not significantly different ($p < 0.05$) from alkali treated starches. Pasting time ranged from 4.88 to 7.00 min. The native starch formed paste at short time (4.88 min) than the modified starches. Pregelatinized starch formed paste at longer time (7.00 min) and at high temperature (89.45 °C).

Conclusions

Modification of *Blighia sapida* seed starch was determined in this study. Pregelatinized starch was significantly different ($p < 0.05$) from other starches in colour. A rapid increase in swelling power was observed in acetylated starches at high temperatures. Pregelatinized starch had low hypoglycin contents than other starches. The hypoglycin contents observed in this study for the starches were low to the acute doses reported in the literatures indicating that the

starches could be used in food applications. Alkali and acid treated starches had higher freeze-thaw stability due to their low water exudate. Alkali-treated starch had higher peak viscosity and stability ratio while pre-gelatinized starches had low viscosities. Alkali-treated starch could be employed in applications where high viscosity is required while pre-gelatinized starch could be useful in low viscosity food products. Therefore, *Blighia sapida* starch could also be used in textile and adhesive industries.

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