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Physicochemical and Functional Properties of Starch from Ackee (*Blighia sapida*) Seeds

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Abstract: *Seeds of the ackee fruit are high in starch content and are a major waste product of the ackee aril canning industry. The objective of this study was to investigate the physicochemical and functional properties of isolated ackee seed starch. De-hulled seeds were dried and milled into 'flour' which was defatted by Soxhlet extraction using petroleum ether. Starch extraction was carried out using 0.2% w/v NaOH solution (24°C, 6 h) and the starch residue soaked in aqueous NaOH (0.05% w/v) for 12 h to remove soluble impurities and then subjected to a bleaching treatment (HCl, 0.01 N). Solubility, swelling power, water absorption, oil absorption and extent of syneresis of the starch were measured and hypoglycin content was determined by reversed phase HPLC. Pasting, thermal properties, crystalline pattern, granule morphology and gel texture were determined, and the gelatinised starch used to prepare retrograded resistant starch. Ackee seed starch comprised small granules which exhibit a C-type diffraction pattern. The starch showed restricted swelling, moderate peak viscosity, and low breakdown compared with commercial corn and potato starches, while the water absorption and oil absorption values were similar to the commercial starches. Ackee starch had a high setback, high syneresis, produced opaque pastes and formed a hard gel texture. Apparent amylose content and the content of retrograded starch were high. Based on the properties, the starch may be suitable in manufacturing of noodles and to produce retrograded resistant starch and may have applications in fat replacers, dusting/face powders and bioplastics.*

Keywords: *Ackee seed, starch, properties, physicochemical, functional*

1. Introduction

The ackee plant is a tropical to sub-tropical tree, originated from West Africa, and it can be found in most islands of the West Indies, Central America and Southern Florida. When the fruit is mature, the red or yellow pod splits open to reveal cream-coloured or light-yellow arils attached to a glossy, spherical black seed (see Figure 1).

Only the mature arils are edible as the immature fruits contain the toxic cyclopropyl non-protein amino acid hypoglycin A (HGA). The seeds of the fruit contain HGA as well as hypoglycin B (HGB), a glutamyl conjugate of HGA (Hassall and Reyle, 1955).

Canned mature ackee arils are produced in Jamaica, Haiti and Belize, and exported to the United States of America (USA), Canada and the United Kingdom (UK). The value of ackee exports from Jamaica averaged 15.6K USD in 2018 (Statistical Institute of Jamaica (STATIN), 2019). Unlike ackee arils, the seeds have no commercial value and are often discarded as a waste residue of the ackee canning industry (Hyatt, 2006). The seeds are, however, rich in starch (44.2%), protein (22.4%) and fat (21.6%) (Djenontin et al., 2009). Ackee seeds comprise a shiny black protective outer shell that strongly adheres to the cream-coloured cotyledons (Morton, 1987) (see Figure 2).

A few pioneering works have investigated the properties of the ackee seed 'flour' (from whole and de-hulled seeds) and the extraction of starch from the ackee seeds (Abiodun et al., 2015a, 2015b; 2018). Abiodun et al. (2015a) reported on several properties of ackee seed starch isolated from de-hulled seed flour, including amylose content, granules size, swelling properties and paste clarity and viscosity. They reported that ackee seed starch has a relatively high amylose content (41.5 ± 1.0%), small granules (6.5 µm), restricted swelling (9.68 g gel/ g starch at 90°C) and produced opaque pastes (light transmission 0.70 ± 0.11%). Additionally, the starch was found to have a low viscosity breakdown (1978 cP), but setback was high (4664 cP).

Figure 1. Mature Ackee Fruit (left) and Immature Ackee Fruit (right)

a b

Figure 2. Ackee Seed (a) whole (b) colyledon and shell

Abiodun et al. (2015b) reported the whole seed flour to have a carbohydrate content of 59.2% and a swelling power of 8.3 g gel/g flour at 90°C. Additionally, the flour was found to have a lower setback (157.6 cP) compared with the corresponding starch.

In a further study, Abiodun et al. (2018) studied the effect of chemical modification (acid, alkali, acetylation and oxidation) and physical modification (pre-gelatinisation) on the properties of ackee seed starch isolated from de-hulled seed flour. Paste clarity was improved by pre-gelatinisation; however, none of the chemical modifications resulted in increased light transmittance of the starch paste nor improved the freeze-thaw stability of the starch. The starch treated with alkali (2.5% NaOH, pH 10.5) was found to have a higher peak viscosity than the native starch, and the starch was more stable towards heat and shear when treated with acid or alkali, oxidised or pre-gelatinised. All the modifications investigated resulted in an improvement (reduction) in the setback values of the starch.

Previous works on starch isolation were based on an extraction method used for yams and starchy tubers, which do not contain significant quantities of lipids and proteins. Ackee seeds, however, have high lipid and protein contents (up to 20% dry weight each) (Djenontin et al., 2009; Esuoso and Odetokun, 1995). Previous works did not report on the protein content of the isolated ackee seed starch and did not include a defatting step to remove lipids. Lipids are reported to restrict swelling and solubility of granules, and reduce light transmission of starch pastes, hence resulting in opaque pastes (Alcazar-Alay and Meireles, 2015; Chinma et al., 2012).

The objective of this study was to deepen the characterisation studies of native ackee seed starch through the isolation of starch from defatted seed flour and assessment of physicochemical and functional properties of the starch. Based on the results of the characterisation of the ackee seed starch, possible applications are presented. All analyses (physicochemical and functional properties) were repeated using commercial corn and potato starches for comparison purposes.

2. Materials and Methods

Ackee seeds were collected directly from canned ackee processors. Seeds were manually de-hulled and dried (60°C, 24h) in a forced draft convection oven (Environette, Lab Line Instruments Inc., Illinois). Dried seeds were milled (Model 4-E Quaker City Mill, The Straub Company, Philadelphia) to pass through a sieve (pore size 0.5 mm) and the flour was defatted by Soxhlet extraction using petroleum ether (b.p. 60-80°C).

Starch was isolated from ackee seeds according to a method developed by Falloon (2019, unpublished) to determine an optimum steeping condition in terms of starch recovery and colour. Starch extraction consisted of steeping the defatted flour in an extracting solution of 0.2% sodium hydroxide (NaOH, 1:10 w/v, 24°C, 6 h), soaking in aqueous NaOH (0.05% w/v, 12 h) to remove contaminating proteins, hypoglycin toxins and other soluble impurities, and washing the starch residue in hydrochloric acid (0.01 N) to improve starch whiteness. The starch slurry was dried in a convection oven (37°C ± 2°C, 48 h), ground to a powder (0.5 mm) and stored in re-sealable LDPE bags at 4°C. The process steps are shown in Figure 3.

Chemical composition (g/100 g starch wet weight basis) of ackee seed starch and commercial corn and potato starches was determined using standard analytical methods: moisture (AOAC (2012) Official Method 930.15), total starch (AOAC (2012) Official Method 996.11), crude protein (AOAC (2012) Official Method 2001.11), crude fat (AOAC (2012) Official Method 945.16), damaged starch (AOAC (2012) Official Method 942.05), and apparent amylose (AACC (1999) Method 61-03). Hypoglycin A (HGA) and Hypoglycin B (HGB) contents of starch from ackee seeds were determined by reversed phase HPLC as described by Sarwar and Botting (1994). The analyses were done using four replicates.

Granule size and morphology of ackee seed, corn and potato starch granules were determined using a light microscope and a scanning electron microscope (SEM) according to the method of Pérez et al. (2011). For light microscope, a 2% aqueous starch suspension was stained using iodine solution (3.5 x 10-3 N). The granules were viewed on a light microscope at x 40 and x 100

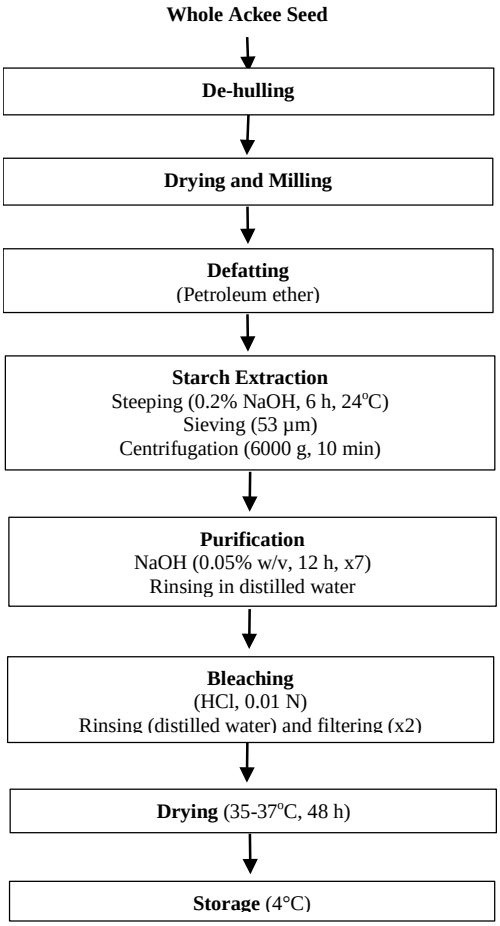


Figure 3. Starch Isolation from Ackee Seed Defatted Flour

magnifications. In the case of SEM (Phillips SEM 515,Denton), starch samples were placed on an electrically ground adhesive tape and coated (EM Sputter Coater) with a thin layer (15 - 40 nm) of gold in an argon atmosphere. The starch granules were viewed at a magnification of x 2500 at 30 kV. Granular diameters were measured using a Gatan Microscopy Suite (GMS 3) software (Gatan, Inc., Pleasanton, CA 94588).

X-Ray diffraction pattern of ackee seeds, corn and potato starches was determined based on the method described by Nwokocha and Williams (2011). Starch samples were heated in a convection oven (Thelco Laboratory Oven, Thermo Electron Corporation, Winchester, Virginia) at 50°C for 24 h. Step-scanned X-ray diffraction patterns for starches were collected on a diffractometer (D2 Phaser, Bruker Corporation, Billerica, Massachusetts) using the DIFFRAC.SUITE V. 3.0 software (Bruker Corporation). The X-Ray source operated at 40 keV and 20 mA with a Cu target and graphite – monochromator radiation Kα radiation (λ = 1.5406). Data were collected by a step-scanned method between 2° to 40° in 2θ angle (1.2°/min).

Thermal properties of ackee seed, corn and potato starches were determined according to Hussain (2015) using a Differential Scanning Calorimeter (DSC) (Setaram Micro DSC III). A 1:3 starch/water slurry was prepared, equilibrated to ambient temperature and heated in the DSC from 5°C to 110°C at 2°C/min. Distilled water was used as reference and data analysed using Setsoft 2000 software V. 3.0.6 (Setaram Inc, Cranbury, NJ 08512). Melting enthalpy and temperature axis were calibrated with standard metals. Onset of gelatinisation, peak temperature (°C), conclusion gelatinisation temperature (°C), and gelatinisation enthalpy (J/g) were determined from the resulting thermograms.

Water absorption capacity (WAC) of starches was determined according to the method described by Yadav et al. (2016). Aqueous starch suspensions (1:10 w/v) were stirred for 30 minutes at 25°C on a magnetic stirring plate and the mixtures centrifuged at 2000g for 10 min. WAC (g H_2O/g starch) was calculated according to Equation (1). Oil Absorption Capacity (OAC) was calculated in the same manner except that oil was used instead of water.

$$WAC = \frac{(Weight\ Starch\ g_{final} - Weight\ Starch\ g_{initial})}{Weight\ Starch\ g_{initial}} \quad (1)$$

Swelling power (g gel/g starch) and solubility (%) of the starches were based on the method described by Torruco-Uco and Betancur-Ancona (2007). Aqueous starch suspensions (1:10 w/v) were heated in a water bath at 30°C ± 1°C for 30 minutes with constant agitation, centrifuged (12,000 g, 10 minutes) and the supernatants dried in a convection oven at 120°C for 4 h. The weight (g) of the water-saturated starch sediment and the dried soluble starch were recorded. The experiment was repeated at temperatures of 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 95°C. Solubility and swelling power were calculated using equations (2) and (3) respectively, using four replicates.

$$\%\ Solubility\ (S) = \left[\frac{(Weight\ Dried\ Solubilised\ Starch\ g)}{Weight\ Starch\ Initial\ g}\right] \cdot x\ 100 \quad (2)$$

$$Swelling\ Power\ (g\ gel/g\ Starch) = \frac{(Weight\ swollen\ starch\ granules\ g}{Weight\ Starch\ Initial\ g - [(\%\ Solubility/100)\ x\ Weight\ Starch\ Initial\ g]} \quad (3)$$

Pasting properties (peak viscosity (cP), breakdown (cP), set back (cP) and pasting temperature (°C) of the starches were determined, in triplicates, using a Rapid Visco Analyser, RVA 4 Stand-alone (Newport Scientific, Warriewood, Australia) according to Method 76-21 STD1 of the American Association of Cereal Chemists (AACC) (1999). The effects of pH on pasting properties were also investigated. The pH of the mixture was adjusted to 3.0, 5.0, 7.0 or 9.0 by dropwise addition of HCl (0.1N) or NaOH (0.1N).

Paste clarity (% transmission) was determined according to the method described by Hassan et al. (2013). Aqueous starch suspensions (1% w/v) from ackee seed, corn and potato were prepared in triplicate and heated (95°C, 1 h) and cooled to 25°C. Initial paste

clarity was determined by measuring the percentage light transmission of the pastes at 640 nm using a UV-VIS spectrophotometer (Evolution 60S, Thermo Scientific, Madison, WI). Starch pastes were stored at 4°C, and light transmittance measured every 24 h for 6 days.

Starch syneresis (%) was determined based on the method described by Torruco-Uco and Betancur-Ancona (2007). Aqueous starch suspensions (6% w/v) were first heated to 95°C for 15 min, held at 50°C for 15 min, then cooled to 25°C. The starch pastes were centrifuged (8000g, 10 minutes). The starch gels were stored at 4°C, and the extent of syneresis determined after 48 h, 72 h, 96 h and 120 h. Percentage syneresis was calculated according to equation (4). Freeze-thaw stability (% syneresis at -18°C) was assessed using the procedure described for syneresis except that gels were stored at -18°C.

$$\%\,Syneresis = \frac{(Weight\ gel\ initial\ g - Weight\ gel\ after\ storage\ g)}{Weight\ gel\ Initial\ g} \times 100 \quad (4)$$

Gel Texture was assessed using the method described by Sun et al. (2014). Aqueous starch suspensions (10% w/v) were heated in the Rapid Visco Analyser (RVA) according to Method 76-21 STD1 of the AACC (1999) to produce starch pastes. The pastes were cooled to ambient temperature and sealed with paraffin film and stored at 4°C for 8 h. Texture parameters were analysed using the Brookfield QTS-25 Texture Analyser using a cylindrical probe (dia 12 mm) at a penetration depth of 10 mm (0.5 mm/s). Hardness (N), adhesiveness (gs), chewiness (gs), springiness (mm), cohesiveness and gumminess (g) were calculated using the TexturePro Version 2.0 software (CNS Farnell, Borehamwood WD61RX, UK). The experiment was repeated by storing the starch pastes at 25°C for 8 h. Analyses were done using three replicates.

Retrograded starch was prepared according to the method described by Sajilata et al. (2006). A 10% starch suspension was heated in a water bath at 95°C for 30 minutes with constant agitation. The resulting starch pastes were further heated in an autoclave at 121°C for 20 minutes, cooled to 25°C and immediately stored at -18°C for 24 h. A portion of the starch paste (15g) was thawed at 25°C for 2 h, dried at 50°C (24 h) in a Precision convection oven (J'Quan Inc., Winchester, Virginia), milled and stored at 4°C. The remaining starch paste was used for two additional autoclave/freeze-thaw cycles. The resistant starch content of the retrograded starches (% dry weight) was determined according to the AOAC (2012) Official Method 2002.02. The experiment was done in triplicate.

Statistical analyses were performed using IBM SPSS Statistics Version 21 (2015) (IBM Corporation, Armonk, New York) and Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, Washington).

3. Results and Discussion

3.1 Chemical Composition

The starch and moisture contents (% wb) of whole and de-hulled seeds and defatted flour are presented in Table 1. The moisture content of de-hulled seeds averaged 50.46 % (wb) or 1.23 g H_2O/g dm. The starch content of whole seeds, de-hulled seeds and defatted flour ranged from 15.42 to 56.31% (wb) or 0.34 to 0.60 g/g DM, respectively. The dry matter content of de-hulled ackee seed flour reported by Abiodun et al. (2015a) was 32.94% (wb).

Table 1. Starch and Moisture Content of Ackee Seed

Component	Ackee Sample		
	Whole Seeds	De-hulled Seeds	Defatted flour
Moisture %wb	55.24 ± 0.89[c]	50.46 ± 1.11[b]	5.99 ± 0.55[a]
Starch %wb	15.42 ± 0.27[a]	21.74 ± 0.58[b]	56.31 ± 1.51[c]

Values represent mean ± standard deviation, N = 3;
[a-c] Values sharing at least one letter in a row are not significantly different (95% CI)

In this study, the starch yield (%) from the defatted flour averaged 45.13 ± 1.75% which is higher than the 14.31% reported by Abiodun et al. (2015a) for de-hulled seed flour, possibly because the seed flour used in that study was not defatted. In this study, the starch yield (%) from the whole seeds with shell averaged 13.47 ± 0.51%.

The chemical composition of the isolated ackee seed starch and commercial starches is given in Table 2. The moisture content of the isolated starch averaged 12.03% (wb) or 0.14 g H_2O/g dm and was within the normal range expected for starches (Thomas and Atwell, 1999). For all starches, crude fat and protein was less than 0.1% (wb). The process used to isolate starch from ackee seeds was therefore very effective in removing both fat and protein.

Table 2. Chemical Composition of Starches

Component (% wb)	Ackee Sample		
	Ackee Seed	Corn	Potato
Moisture	12.03 ± 0.55[b]	9.51 ± 0.19[a]	13.19 ± 0.27[c]
Crude Protein	0.08 ± 0.01	0.09 ± 0.015	0.10 ± 0.01
Crude Fat	0.07 ± 0.01	0.09 ± 0.021	0.08 ± 0.01
Ash	0.13 ± 0.01[a]	0.12 ± 0.010[a]	0.28 ± 0.03[b]
Total Starch (Purity)	82.59 ± 2.09	82.54 ± 2.31	82.90 ± 1.49
Apparent Amylose	34.26 ± 0.20[b]	27.62 ± 0.60[a]	34.25 ± 0.40[b]
Damaged Starch	1.59 ± 0.09[c]	1.23 ± 0.22[b]	0.40 ± 0.07[a]

Values represent mean ± standard deviation, N = 3
[a-c] Values sharing at least one letter in a row are not significantly different (95% CI)

Minute quantities of proteins and lipids are chemically bonded to starch granules and are difficult to remove (Thomas and Atwell, 1999). Starch from ackee seed and commercial corn starch had a similar ash content of 0.13% (wb), but the quantity was more than twice as high for potato starch. This may be due to higher quantities of phosphorous in potato starches (Singh et al., 2003).

The purity of starch from the ackee seed averaged 82.59% (wb) (or 0.94 g/g dm) which was similar to values obtained for commercial corn and potato starches. The quantity of damaged starch granules in ackee seed starch was found to be 1.59%, which is within the range reported by Pérez et al. (2011) for waxy yam varieties (0.41-2.95%) and Simsek et al. (2009) for different pea varieties (1.54-1.80%). Lower values were obtained for commercial corn and potato starches. Starch granules may become damaged as a result of milling during starch isolation, or degradation by endogenous amylases in the starting material (Tran et al., 2011; Williams, 1967).

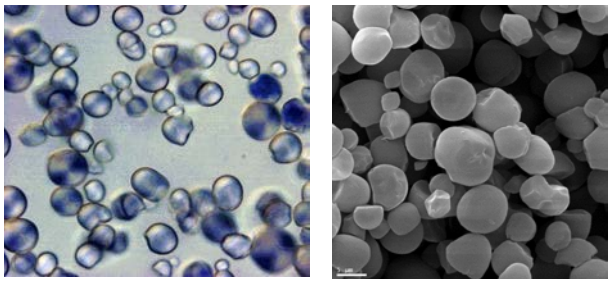
The apparent amylose contents of ackee seed starch and commercial potato starch were similar, but apparent amylose content of commercial corn starch was lower ($p > 0.05$). Abiodun et al. (2015a) reported an amylose content of 41.47% for ackee seed starch extracted from de-hulled seed flour, similar to the findings of this study. However, in a subsequent study, they reported a much lower amylose content of 22.1% for the native starch isolated from de-hulled seed flour, and even lower quantities (18.3 - 21.2%) for the acetylated, alkaline treated and pre-gelatinised starch (Abiodun et al., 2018).

Jane et al. (1999) reported that the apparent amylose content of potato starch was much higher than its absolute amylose content (36% vs 19%); for corn starch, there was a 7% difference between apparent amylose and absolute amylose (29% vs 22%). The disparity between apparent and absolute amylose for potato starch could be as a result of amylopectin chains with relatively fewer branches and intermediate materials; these are known to bind iodine resulting in an overestimation of amylose (Jane et al., 1999). High amylose starches are known to have restricted swelling properties and tend to retrograde rapidly resulting in opaque pastes, hard gels, high syneresis and high setback (Alcazar-Alay and Meireles, 2015).

In this study, no hypoglycin toxins were detected in the ackee seed starch samples. Both compounds are water-soluble (Sarwar and Botting, 1994; Hassall and Reyle, 1955) and would have been leached into solution by repeated washing of the starch residue during isolation. Abiodun et al. (2018) reported HGA and HGB content of native and modified starch obtained from de-hulled ackee seed flour ranged from 38.8 - 57.5 ppm and 71.8 - 84.8 ppm, respectively. In all cases, the values were below the regulatory limits of 150 ppm and 100 ppm set by the Bureau of Standards, Jamaica (BSJ) and the United States Food and Drug Administration (USFDA) (Gordon et al., 2015).

3.2 Size and Shape of Starch Granules

Ackee seed starch granules had a round shape, and some were truncated (see Figure 4a). Regions of darker stains indicated higher amylose content and therefore represented amorphous sections of the granules (Thomas and Atwell, 1999). When viewed under a scanning electron microscope (SEM), some granules appeared round, truncated or dome-shaped (see Figure 4b).



a b

Figure 4. Ackee Seed Starch Granules; a: x 100; b: x 2500

Granule diameter was smaller (6.89 ± 1.89 µm, $N = 407$) compared with corn (10.74 ± 2.24 µm, $N = 90$) and potato starches (28.56 ± 14.52 µm, $N = 90$). Size distribution pattern revealed that approximately 95% of ackee starch granules have a diameter less than 10 µm. Abiodun et al. (2015a) reported similar morphologies and size distribution for granules of ackee seed starch. Abiodun et al. (2018) further reported that chemical modification by acetylation resulted in granules with deformed shapes, while in the case of the pre-gelatinised starch, the granules appeared as fragments. These modifications thus resulted in damaged starch granules which tend to have a higher water absorption capacity and are more susceptible to attack by amylases (Hossen et al., 2011).

Small granule starches are suitable for use in foods requiring creamy smooth texture and may serve as fat replacers and are desirable for use in biodegradable plastic films (Lindeboom et al., 2004). High amylose starch-based films have higher tensile and impact strengths as well as higher modulus and are less likely to absorb moisture compared with films from waxy starches (Wittaya, 2012). Jane et al. (1992) stated that small-granule starches are suitable for use in dusting, face and baking powders, and as a laundry stiffening agent.

3.3 X-Ray Diffraction Pattern

Starches produce characteristic peaks when subjected to X-ray diffraction because of their semi-crystalline properties (Singh et al., 2003). Starch granules in which chains are closely packed and accommodate relatively few water molecules produce an A-type diffraction pattern while B-type starches have a more open structure accommodating more water molecules (Hizukuri et al., 2006). Starches of the C-type are considered intermediate, comprising granules of both A and A types (Hizukuri et al., 2006). A, B, and C-Type starches are typical of cereal, tuber and legume starches respectively.

The X-Ray diffraction pattern for ackee seed starch,

not previously reported, is shown in Figure 5. A strong peak was observed at 17° (2 θ), moderate peaks were observed at 15° and 22.5° in 2θ, and weak peaks were observed at 6°, 10.5°, 20° and 26° in 2θ. These peaks are characteristic of the C-Type crystalline structure (Nwokocha and Williams, 2011). In the case of the commercial corn starch, strong peaks (in 2θ) were observed at 15°, 17°, 18° and 22°, while weak peaks were observed at 11°, 20°, and 26°. This is characteristic of an A-type diffraction pattern (Nwokocha and Williams, 2011). For the commercial potato starch, a strong peak was observed at 17°, a moderate peak at 22° and weak peaks at 15° and 20° suggested that this is a B-Type starch (Nwokocha and Williams, 2011).

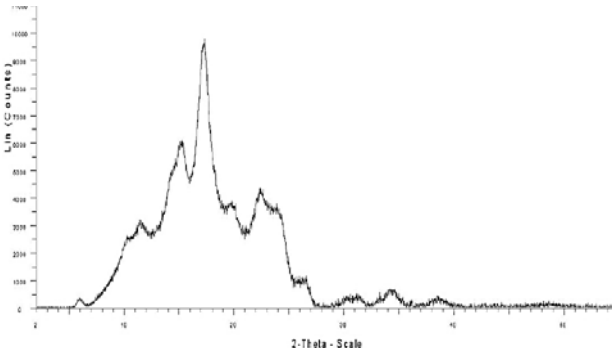


Figure 5. X-Ray Diffraction Pattern for Ackee Seed Starch

3.4 Thermal Properties

The DSC thermogram of ackee seed starch is presented in Figure 6. Ackee seed starch had highest onset of gelatinisation (T_o) (66.67 ± 0.09 °C)), peak gelatinisation temperature (T_p) (71.45 ± 0.03°C) and conclusion gelatinisation temperature (T_c) (77.62 ± 0.10°C) ($p < 0.05$), slightly lower values were recorded for commercial corn starch (T_o = 65.75 ± 0.08°C; T_p = 70.09 ± 0.09°C and T_c = 77.18 ± 0.18°C, respectively) and even lower values for commercial potato starch (T_o = 59.19 ± 0.05°C, T_p = 63.63 ± 0.05°C and T_c = 72.63 ± 0.13°C, respectively). Thermal properties of ackee seed starch using DSC have not been previously reported.

A similar gelatinisation temperature of 71.50°C for ackee seed starch based on microscopic analyses of the granules was reported by Abiodun et al. (2015a). Yuan et al., 2007) stated that starches with higher melting temperatures have a higher level of crystallinity. Additionally, the gelatinisation temperature of a starch is further influenced by the "molecular architecture" of crystalline regions rather than simply the proportion of crystalline and amorphous regions (Huang et al., 2007).

Enthalpy of gelatinisation (Δ_{gel}) was highest for potato starch (15.98 ± 0.43 J/g) followed by ackee seed starch (13.65 ± 0.12 J/g) and corn starch (12.34 ± 0.60 J/g). Variation in Δ_{gel} represents differences in bonding forces between double helices that form amylopectin crystallites and relates to loss of double helical structures rather than crystalline order (Bhupender et al., 2013). Aggarwal et al. (2004) stated that high Δ_{gel} of starches implies the presence of many large size and irregular granules, while lower Δ_{gel} is indicative of small-sized oval granules. In this study, potato starch granules were found to be larger than ackee seed and corn starches and had highest Δ_{gel}. Simsek et al. (2009) stated that starches having mainly B-polymorph have higher gelatinisation enthalpy than those comprising A-polymorph; this was consistent with findings of this study where potato starch had higher Δ_{gel} compared with corn starch.

Peak Height Index (PHI), the ratio of enthalpy of gelatinisation to gelatinisation temperature range (R), is a measure of uniformity in gelatinisation (Aggarwal et al., 2004). PHI appears to increase as the size of starch granules increases (Aggarwal et al., 2004; Bhupender et al., 2013). There was no significant difference in PHI for ackee seed (2.86 ± 0.08 $J/g^{-1}\,{}^{o}C^{-1}$) and corn starches (2.85 ± 0.17$J/g^{-1}\,{}^{o}C^{-1}$) but the value was significantly higher ($p < 0.05$) for potato starch (3.60 ± 0.10 $J/g^{-1}\,{}^{o}C^{-1}$), possibly due to larger granular diameter.

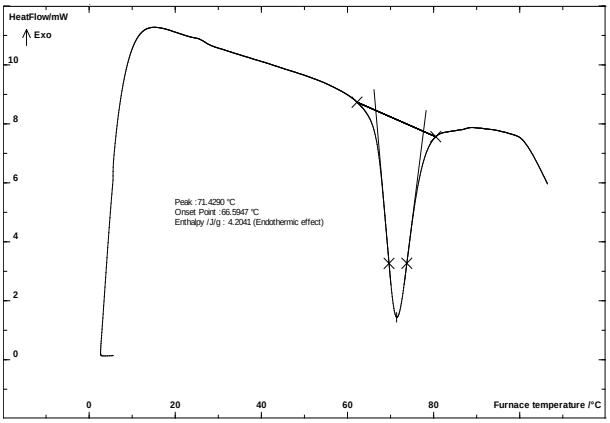


Figure 6. Differential Scanning Calorimeter (DSC) thermogram of ackee seed starch

3.5 Physical Properties

There was no significant difference in water absorption capacity (WAC) at 25°C between ackee seed starch (1.09 g H_2O/g starch) and potato starch (1.10 g H_2O/g starch), but corn starch had a significantly lower ($p < 0.05$) WAC (0.84 g H_2O/g starch). Reasons for this are not clear but could be as a result of differences in granule structure, steric factors, hydrophilic-hydrophobic balance and extent of association between amylose and amylopectin chains (Henríquez et al., 2008). Oil absorption capacity (OAC) for ackee seed starch (0.75 g oil/g starch) was similar ($p > 0.05$) to those recorded for corn (0.75 g oil/g starch) and potato starches (0.72 g oil/g starch). WAC and OAC of ackee seed starch have not been previously reported in the literature.

The swelling power of ackee seed starch at 95°C

was 19.49 ± 0.59 g gel/g starch; this implied a restrictive swelling property. The swelling powers of starches at 95°C can be classified as high (> 30), moderate (20 - 30), restricted (16 - 20) and highly restricted (< 16) (Shimelis et al., 2006). The commercial corn and potato starches were found to have moderate (25.91 ± 0.37 g gel/g starch) and high (40.57 ± 0.92 g gel/g starch) swelling properties respectively at 95°C. For all three starches, swelling power increased exponentially beyond their respective gelatinisation temperatures (see Figure 7). Starches with relatively low amylose content, large granule size and low gelatinisation temperature tend to have high swelling power (Alcázar-Alay and Meireles, 2015; Singh et al., 2003). Swelling occurs primarily as a result of hydration of amylopectin chains; amylose chains reduce swelling because they form insoluble complexes with lipids and proteins (Singh et al., 2003; Shimelis et al., 2006).

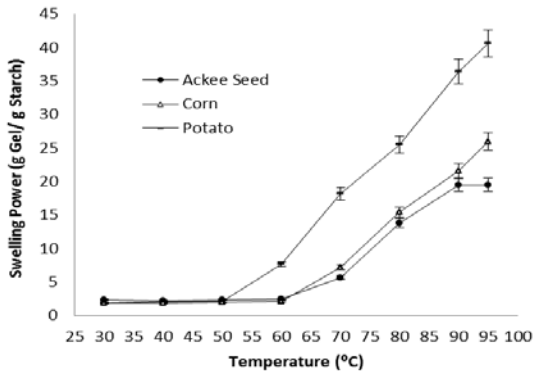


Figure 7. Swelling Power of Ackee Seed Starch and Commercial Corn and Potato Starches

Abiodun et al. (2015a) reported a swelling power of 9.68 at 90°C for ackee seed starch which is lower than the 19.41 ± 0.12 g gel/g starch reported in this study at that temperature. In a further study, Abiodun et al. (2018) reported an increase in swelling power of the ackee starch by chemical modification (acetylation, oxidation, alkali-treated, acid treated) as well as pre-gelatinisation. However, the increase was only marginal, and in all cases, the swelling of the starch was still highly restricted. The starch extraction method reported by Abiodun et al. (2015a) did not involve a defatting process. Thus, the isolated starch might have had a higher lipid content thus reducing granule swelling (Alcázar-Alay and Meireles, 2015). Starches with restricted swelling properties are suitable for use in foods such as noodles where much swelling is not desired (Shimelis et al., 2006).

At 95°C, solubility of ackee seed starch was significantly lower ($p < 0.05$) (12.24 ± 0.60%), when compared with potato starch (15.62 ± 0.30%) and corn starch (29.34 ± 0.09%). The solubility of the starches increased rapidly beyond their gelatinisation temperature. Like swelling power, solubility is reduced by the presence of bound lipids in starch (Shimelis et al., 2006; Singh et al., 2003). No prior works have been published on the solubility of ackee seed starch.

3.6 Pasting Properties

The pasting curve of ackee seed starch is presented in Figure 8. Ackee seed starch was found to have lower breakdown values and higher setback compared with commercial corn and potato starches (see Table 3). Pasting temperature was also higher for ackee seed starch perhaps due to higher gelatinisation temperatures (see Section 3.3).

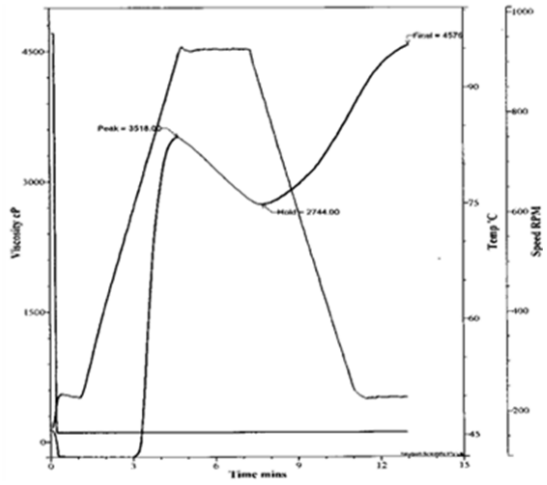


Figure 8. Pasting Curve of Ackee Seed Starch

Table 3. Pasting Properties of Ackee Seed Starch and Commercial Corn and Potato Starches

Pasting Property	Starch Sample		
	Ackee Seed	Corn	Potato
Pasting Temperature (°C)	75.10 ± 0.05 ^{c}	74.22 ± 0.08 ^{b}	66.20 ± 0.05 ^{a}
Peak Viscosity (cP)	3760 ± 142 ^{b}	1937 ± 33 ^{a}	8348 ± 113 ^{c}
Hot Paste Viscosity (cP)	3038 ± 136 ^{c}	625 ± 7 ^{a}	2727 ± 123 ^{b}
Breakdown (cP)	721 ± 53 ^{a}	1312 ± 31 ^{b}	5621 ± 53 ^{c}
Relative Breakdown (% of Peak Viscosity)	19.19 ± 1.39 ^{a}	67.73 ± 0.50 ^{b}	67.34 ± 1.09 ^{b}
Cool Paste Viscosity (cP)	5230 ± 113 ^{c}	1265.33 ± 20.82 ^{a}	3297 ± 88 ^{b}
Setback (cP)	2191 ± 46 ^{b}	640 ± 18 ^{a}	570 ± 51 ^{a}
Relative Setback (% of Peak Viscosity)	58.37 ± 3.31 ^{c}	33.05 ± 0.41 ^{b}	6.84 ± 0.65 ^{a}
Pasting Time (min)	4.55 ± 0.04 ^{c}	4.05 ± 0.04 ^{b}	3.33 ± 0.00 ^{a}

Values represent mean ± standard deviation, N = 3; $^{a-c}$Values sharing at least one letter in a row are not significantly different (95% CI)

The lower breakdown of ackee seed starch suggests that it may be suitable as a thickening agent in foods processed at high temperature such as gravies and soups. However, a high setback means that ackee starch has a high tendency to retrograde. The consequences of starch retrogradation include exudation of water from gels and staling of bread (Alcázar-Alay and Meireles, 2015). A high setback and low breakdown of ackee starch were also reported by Abiodun et al. (2015a), although peak viscosity was higher (i.e., 501.25 RVU or approximately 6015 cP). Highest peak viscosity was recorded for potato starch, but breakdown was also the highest suggesting that this starch paste is not stable towards heat and shear.

With regards to chemical modification of ackee seed starch, Abiodun et al. (2018) found that peak viscosity was significantly higher for the alkali treated starch (3973 cP) compared with the native starch (3685 cP). However, peak viscosity was lower (2970 - 3259 cP) for the acid-treated, acetylated and oxidised starches. As expected, peak viscosity was the lowest for the pre-gelatinised starch (543 cP). The authors found that acetylation increased the tendency of the starch to breakdown; however, the thermal stability of the starch improved significantly when modified with alkali or acid, oxidised, or pre-gelatinised. Abiodun et al. (2018) reported a setback of 1139 cP for native ackee starch; with acid and alkali treatments resulting in lower setback (204 cP and 235 cP, respectively), thus reducing the tendency of the starch to retrograde.

When the effects of pH (3.0, 5.0, 7.0 and 9.0) on the pasting properties were investigated, ackee seed starch paste appeared more stable towards acidic conditions compared with corn and potato starches. Peak viscosity of ackee seed starch was largely unaffected by pH changes (see Figure 9), though relative breakdown (33.68 ± 0.67% of peak viscosity, or 1133.67 ± 26.08 cP) was slightly higher at pH 3.0 (see Figure 10).

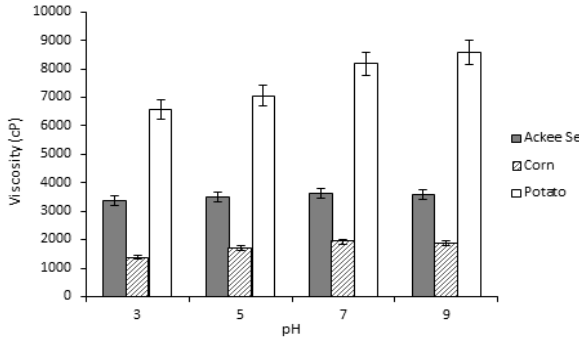


Figure 9. Effect of pH on Peak Viscosity of Starch Paste from Ackee Seed, Corn and Potato

Ackee seed starch relative setback was significantly lower at pH 3.0 (40.16 ± 1.39 % of peak viscosity, or 1351.33 ± 37.10 cP) compared with that at higher pH values (56.58 - 66.58 % of peak viscosity). These results imply that ackee seed starch might be more suitable for use in acidic foods compared with corn and potato starches. No previous studies have been reported concerning the effects of pH on pasting properties of ackee seed starch.

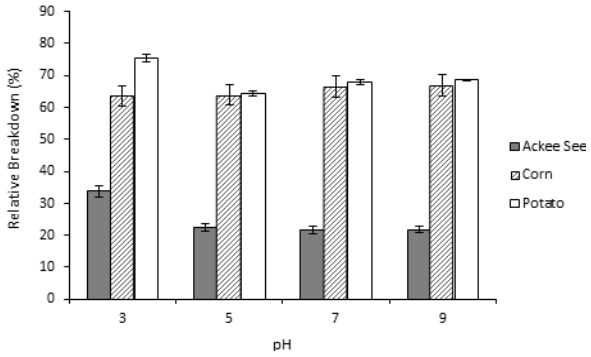


Figure 10. Effect of pH on Relative Paste Viscosity Breakdown

3.7 Paste Clarity

The changes in paste clarity of the starches with storage time are shown in Figure 11. Ackee seed starch formed an opaque paste; recording an initial light transmission (T) of 11.10 ± 1.00%. For corn starch, a translucent paste was produced (T = 29.88 ± 0.88%) while potato starch formed a clear paste (T = 74.80 ± 6.46%). The clarity of the pastes from ackee seed and potato starches decreased rapidly during the first three to four days of refrigerated storage and tapered off thereafter. In the case of corn starch, paste clarity decreased slowly during the first three days of storage at 4°C (from 29.88 to 23.76%) but showed very little change after that.

Lower light transmittance of ackee seed starch paste was reported by Abiodun et al. (2015a), averaging 0.70 (after 24 h), 0.62 (48 h) and 0.53 (72 h), possibly due to a higher lipid content of the starch. In a subsequent study in 2018, the authors found that chemical modifications of the starch did not result in any increase in paste clarity. However, the percentage transmittance of the pre-gelatinised starch increased to 9-14% within 24 h of storage but decreased on further storage.

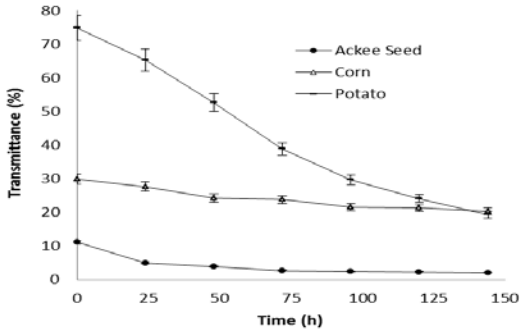


Figure 11. Effect of Storage Time (at 4°C) on % Transmittance of starches from Ackee Seed, Corn and Potato

Starches that produce opaque pastes have relatively high amounts of phospholipids that form insoluble complexes with amylose and long chain amylopectin, which reduce light transmittance (Alcázar-Alay and Meireles, 2015; Singh et al., 2003). Potato starches are known to contain phosphate-amylopectin monoesters which are responsible for the high paste clarity (Alcázar-Alay and Meireles, 2015; Singh et al., 2003). An implication of these findings is that ackee seed starch pastes would be more useful in dark-coloured products such as sauces, salad dressings and puddings (Alcázar-Alay and Meireles, 2015; Torruco-Uco and Betancur-Ancona, 2007).

3.8 Starch Gel Syneresis

Water loss (syneresis) of starch gels from ackee seed, corn and potato at 4°C and -18°C is illustrated in Figure 12 and 13, respectively. A high level of syneresis for ackee starch was observed at 4°C (see Figure 12), with values ranging from 31.35 (g H_2O/100 g gel) within 24 h to 49.18 after storage for 120 h. The starch gels would, therefore, be unsuitable for use in foods typically stored at refrigerated temperature. A lower syneresis was observed with gels from corn and potato starches. The realignment of amylose chains on cooling of starch gels results in exudation of water molecules; this process is accelerated when gels are stored at refrigerated or frozen temperatures (Lee et al., 2002). Syneresis of ackee seed starch gels has not been previously reported.

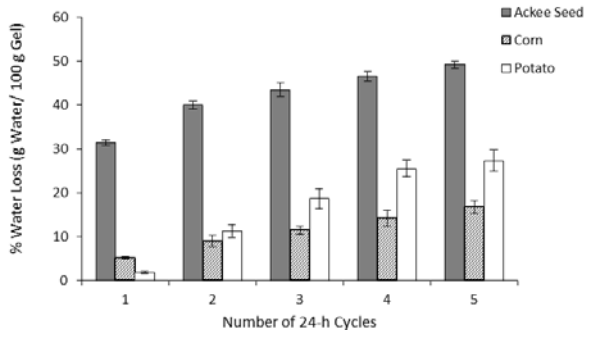


Figure 12. Syneresis of Starch Gels from Ackee Seed, Corn and Potato at 4°C

When the starch gels were stored at frozen temperature (-18°C) and subjected to five freeze-thaw cycles, high syneresis was observed for all starches (see Figure 13). Several authors have reported poor freeze-thaw stability of gels from native starches including corn, amaranth, plantain, banana, rice, potato, and cassava (Torruco-Uco and Betancur-Ancona, 2007; Bello-Pérez et al., 1999). Abiodun et al. (2018) attempted to improve the freeze-thaw stability of ackee starch by chemical modification (acid-treated, alkali-treated, acetylation and oxidation) and pre-gelatinisation. However, all the modifications failed to improve the freeze-thaw stability of the starch paste.

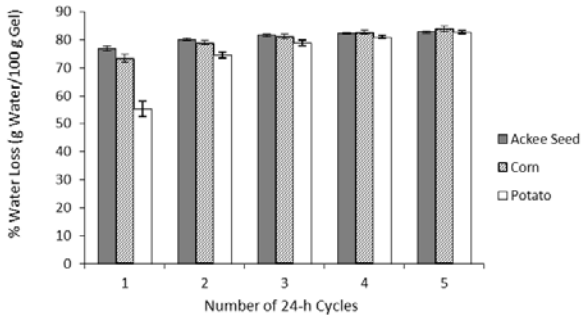


Figure 13. Syneresis of Starch Gels from Ackee Seed, Corn and Potato at -18°C

3.9 Starch Gel Texture

The textural properties of the starch gels are given in Table 4. Starch gels from ackee seed had a significantly harder texture compared to gels from corn and potato starches. Hardness values (N) were higher when the gels were stored at 4°C compared with 25°C. Starches that produce hard gel texture have a high tendency to retrograde, and this effect is more pronounced at refrigerated and frozen temperatures (Herceg et al., 2010).

Cohesiveness describes the strength of internal bonds within the gel (Trinh and Glasgow, 2012). It was not significantly different among the starch gels when incubation was done at 25°C. However, when ackee seed starch gels were stored at 4°C, cohesiveness was significantly lower (0.48, $p<0.05$) compared with storage at 25°C (0.64). Springiness, which describes the elasticity of a food sample (Trinh and Glasgow, 2012), ranged from 7.00 to 8.44 mm and was generally similar regardless of starch source or incubation temperature.

The values suggest that gels from ackee seed and potato starches that were set at the ambient temperature will require more energy to chew compared with gels set at 4oC. A reverse in this trend was observed for corn starch gels. Overall, chewiness (gs) was lowest for corn starch gels due mainly to lower hardness. Gels from ackee seed starch had significantly higher gumminess than gels from corn and potato starches incubated at 25oC. Potato starch gel incubated at 4°C showed similar gumminess to gels from ackee seed starch. Higher gumminess suggests that ackee starch could be used in the manufacturing of gummy candies.

Adhesiveness, the work required to overcome sticky forces between the samples and probe (Trinh and Glasgow, 2012), was significantly higher for ackee seed starch gels, incubated at 25°C, compared with other starch gels. Ackee seed starch may, therefore, be suitable for use in the manufacturing of adhesives. No prior works have been reported regarding the textural properties of ackee seed starch gel.

Table 4. Effect of Storage Temperature on Texture Properties of Gels of Ackee Seed, Corn and Potato Starches

Property	Starch Sample					
	Ackee Seed		Corn		Potato	
	4°C	25°C	4°C	25°C	4°C	25°C
Hardness (N)	109.67 ± 5.13[d]	91.00 ± 2.65[c]	42.67 ± 5.03[a]	36.67 ± 1.15[a]	94.00 ± 4.36[c]	61.00 ± 1.00[b]
Cohesiveness	0.48 ± 0.02 [a]	0.64 ± 0.04[b]	0.64 ± 0.05[b]	0.65 ± 0.03[b]	0.60 ± 0.01[b]	0.65 ± 0.02[b]
Springiness (mm)	7.68 ± 0.68[a,b]	8.44 ± 0.19[b]	8.20 ± 0.19 [b]	7.98 ± 0.25[a, b]	7.64 ± 0.24[a,b]	7.00 ± 0.56[a]
Chewiness (gs)	398.76 ± 49.40[c]	486.95 ± 18.99[d]	255.40 ± 42.29[a,b]	191.34 ± 18.28[a]	436.45 ± 29.55[c,d]	278.45 ± 24.94[b]
Gumminess (g)	51.82 ± 1.82[c]	57.67 ± 1.85[c]	27.47 ± 4.96[a]	23.95 ± 1.73[a]	57.12 ± 2.50[c]	37.41 ± 4.50[b]
Adhesiveness (gs)	-92.15 ± 2.54[b]	-166.94 ± 15.34[c]	-104.05 ± 25.93[b]	-92.91 ± 20.99[b]	-38.28 ± 6.13[a]	-0.17 ± 0.30 [a]

Values represent mean ± standard deviation, N = 3; Values sharing at least one letter in a row are not significantly different (95% CI)

3.10 Retrograded (Resistant) Starch

Resistant starches are not broken down by human digestive enzymes and can be used as a more palatable source of dietary fibre compared with traditional sources (Öztürk and Köksel, 2014; Sharma et al., 2008). These starches are thus used to fortify products such as cereals, and baked and fried goods, while maintaining or even improving sensory attributes such as taste, crispiness, texture and mouthfeel (Fuentes-Zaragoza et al., 2010; Raigond et al., 2015). The production of retrograded resistant starch from ackee seed starch is being reported for the first time.

The native starches of ackee, corn and potato were found to have a resistant starch content of 44.42, 8.81 and 77.31 % (dry weight basis), respectively. The types of resistant starch found in native starches are RS1 (physically inaccessible starches locked within cell walls) and RS2 (starches having rigid crystalline structures), both of which become completely digestible when freshly cooked (Raigond et al., 2015; Sharma et al., 2008). Such starches are therefore not suitable as a functional ingredient in baked or cooked products. Retrograded resistant starch (RS3) is stable to gelatinisation up to 150°C and hence ideal for use in thermally processed foods (Raigond et al., 2015; Leszczyñski, 2004). The resistant starch contents of retrograded starches (RS3) (up to three autoclave/freeze-thaw cycles) from ackee, corn and potato are shown in Figure 14. The process used to produce the retrograded starches would have completely destroyed the native RS1 and RS2 starches.

The highest amount of RS3 (11.61% db) was produced from ackee retrograded starch at cycle 3. Additionally, in the case of ackee and corn retrograded starches, the amount of RS3 formed increased as the number of autoclave/free-thaw cycles increased, but more so for corn. However, for potato, the quantity of RS3 decreased as the number of cycles increased. The quantity of RS in ackee seed retrograded starch was higher than values reported for waxy maize (2.5% db), potato starch (4.4% db), maize starch (7.0% db) and wheat starch (7.8% db) (Sievert and Pomeranz, 1989). Similar resistant starch content was reported for pea retrograded starch (10.5% db) (Sievert and Pomeranz, 1989) and a slightly higher value was reported for rice (13.9% wb) (Ha et al., 2012). Higher yields of RS3 might have been possible through modifications of process variables such as autoclave temperature and time, starch/water ratio, freezing temperature/time, amylose content and the number of heating/cooling cycles (Ha et al., 2012; Calixto and Abia, 1991).

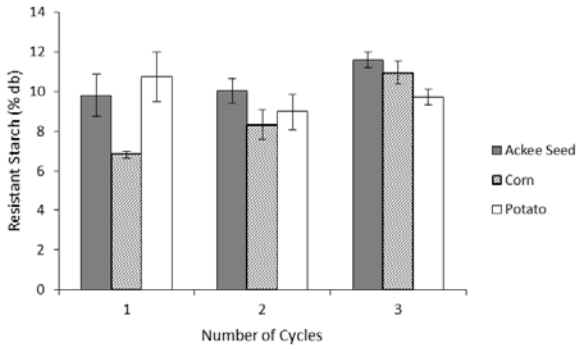


Figure 14. Resistant Starch Content of Retrograded Starches from Ackee Seed, Corn and Potato

4. Conclusions

High purity starch was successfully isolated from defatted ackee seed flour. Isolated starch was characterised as having small-sized granules, high apparent amylose content, C-type crystalline structure, high gelatinisation and pasting temperatures, restricted swelling, moderate peak viscosity, low breakdown and a high tendency to retrograde. Because of its tendency to readily retrograde, ackee seed starch could be used to produce retrograded resistant starch (RS3).

The low breakdown of ackee seed starch suggests that it could be used as a thickening agent in foods processed at high temperatures such as gravies and soups. The restricted swelling of the starch makes it suitable for use in the manufacturing of noodles. Because of its small granular size, ackee seed starch could be used as fat replacers in foods and other products such as dusting powders and baking powders. The starch may be suitable in the production of biodegradable plastic films because of its high apparent amylose content and small granule size. Further research is recommended to investigate the actual behaviour of the starch in these specific applications.

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