

Chemical and Functional Properties of Raw and Cooked Under Utilized *Blighia Sapida* Seed Flour

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Abstract

Chemical and functional properties of raw and cooked under-utilized *Blighia sapida* (Ackee) seeds flour were determined. The seeds were divided into two portions: portion A was peeled and dried while portion B was cooked, peeled, dried and milled. The flours were subjected to chemical, physico-chemical and anti-nutritional analyses. Higher protein content (14.97 %) was observed in the raw flour. Calcium and potassium contents were the major mineral in the seed flour. Amino acids in the flour include glutamic acid, aspartic acid, leucine, arginine and lysine in decreasing order. Raw flour had higher peak viscosity (78.25 RVU), holding strength (53.66 RVU), breakdown (24.59 RVU), final viscosity (66.79 RVU), setback (13.13 RVU) and pasting temperature (82.43 °C). The swelling power of raw flour increased drastically at 80-90 °C than the cooked flour. Swelling power of the flours increased with increasing temperature. Cooked *Blighia sapida* flour had low tannin, oxalate and alkaloid contents but high in phytate content.

Keywords: Amino acids, Anti-nutritional factor, *Blighia sapida* seed, Cooked flour, Pasting properties

Introduction

Blighia sapida, commonly known as *Ackee* (English), *Gwanja Kusa* (Hausa; Northern Nigeria), *Isin* (Yoruba; Western Nigeria), *Okpu* (Igbo; Eastern Nigeria) and *Yila* (Nupe; North Central Nigeria) is a soap berry plant of the family *Sapindaceae*. It is a perennial herbaceous plant that is prominently found in Western

Tropical Africa (Onuekwusi *et al.*, 2014). *Blighia sapida* is a woody perennial multipurpose fruit tree species native to the Guinean forests of West Africa. The fleshy arils of the ripened fruits are edible (Ekueet *et al.*, 2010). At maturity, arils are consumed directly fresh, added to sauce to replace sesame (*Sesamum indicum* L.). Arils are also fried in peanut (*A. hypogaea*) or oil palm (*Elaeis guineensis* Jacq.) oil (Ekue *et al.*, 2010). Ackee arils could serve as an appreciable source of natural antioxidants which could play a key role in health maintenance and prevention of the chronic and degenerative diseases (Dossou *et al.*, 2014). The fruits splits open while still on the tree to reveal three glassy black seeds surrounded by a thick, yellow and oily aril (Nwoso *et al.*, 2014). The physical properties, fatty acid composition and phytochemical contents of *Blighia sapida* aril and seed oil have been determined (Omobuwajoet *et al.*, 2000; Omosuli, 2013; Onuekwusi *et al.*, 2014). The arils are eaten or used to make sauce while the seeds are discarded and under-exploited. 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 (Blake *et al.*, (2006). Due to this, there is a dearth of information on the properties and usefulness of the seed. Therefore, this paper studied the chemical and functional properties of raw and cooked flours obtained from the under-utilized *Blighia sapida* seed.

MATERIALS AND METHODS

Sample Collection and Preparation

Blighia sapida seeds were obtained at a farm in Iree, Osun State, Nigeria. The seeds were peeled manually with knives and the cotyledons were separated. The peeled seed was dried in the oven at 60 °C to obtain the raw flour while whole seed was cooked at 100±2 °C for 20 min, peeled, dried in hot air oven at 60 °C and milled into flour to obtain cooked flour. The dried seeds were milled and packaged.

Proximate, mineral and anti-nutritional composition

Proximate composition (Crude protein, fat, ash and crude fibre contents) analyses were done following AOAC methods (AOAC, 1990). The phytate contents of *Blighia sapida* seeds were determined using the method of Dairo (2008). Alkaloid, oxalate and tannin were quantified using the method of Alexis and Georges (2012).

Determination of amino acid profile

The Amino Acid profile in the known sample was determined using methods described by Benitez (1989). The sample was defatted using chloroform/methanol mixture of ratio 2:1. Sample (4g) was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen determination

Sample (200 mg) was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10mL) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate (CuSO_4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100mL in standard volumetric flask. Aliquot (10mL) of the diluted solution with 10mL of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10mL 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70mL of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured. The amount of nitrogen present was determined following the equation below

$$\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times v \times 100}{W \times C}$$

Where: a=Titre value of the digested sample, b=Titre value of blank sample, v.=Volume after dilution (100 mL), W=Weight of dried sample (mg), C.=Aliquot of the sample used (10mL), 14=Nitrogen constant in mg.

Hydrolysis of the sample

Sample (30 mg) was weighed into glass ampoule. 7 ml of 6NHCl was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^\circ\text{C} \pm 5^\circ\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered. The filtrate was then evaporated to dryness at 40°C under vacuum using a rotary evaporator. The residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into Amino acid analyzer (TSM)

Ten microlitre of the hydrolysate was loaded and dispensed into the cartridge of the analyzer. The TSM analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis was 76 minutes.

$$\% N = \frac{(a - b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Determination of Amino Acid Values from the Chromatogram Peaks.

An integrator attached to the Analyzer auto-calculates the peak area proportional to the concentration of each of the amino acids. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norceucine peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{\text{std}} = NE_{\text{std}} \times \text{Molecular weight} \times \mu\text{MAA}_{\text{std}}$

Finally, the amount of each amino acid present in the sample was calculated

Concentration (g/100g protein) = $NH \times W \times NH/2 \times S_{\text{std}} \times C$

Where

$$C = \frac{\text{Dilution} \times 16}{\text{Sample weight (g)} \times N(\%) \times 10 \times \text{vol. loaded}} / NH \times W (nleu)$$

Where: NH = Net height

W = Width @ half height

nleu = Norleucine

Determination of swelling power

This was determined as described by Appiah *et al.* (2011). One gram of the flour sample was mixed with 10 mL distilled water in a centrifuge tube and heated in a hot water bath at 60, 70, 80 and 90°C for 30 min while continuously shaking the tube. After heating, the suspension was centrifuged at 1000 g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as:

Swelling power = Weight of the paste/weight of dry flour

Determination of Pasting Properties

Pasting profile of the flour sample was studied using a Rapid Visco-Analyzer (RVA) (Newport Scientific Pty. Ltd) with the aid of a thermocline for windows version 1.1 software. The RVA was connected to a PC where the pasting properties and curve were displayed directly. Flour suspension was prepared by addition of the equivalent weight of 3.0 g flour to distilled water to make a total of 28.0 g suspension in the

RVA sample canister. The flour suspension temperature was held at 50 °C for 1 min and later heated to 95 °C for 3 min. It was held at 95 °C for 3 min before the sample was subsequently cooled to 50 °C over a 4 min period. This was followed by a period of 1 min where the temperature was kept at constant temperature of 50 °C.

Statistical analyses

The data obtained were in triplicate and the mean and standard deviation were calculated.

RESULTS AND DISCUSSION

Chemical composition of *Blighia sapida* flour

Chemical compositions of *Blighia sapida* flour are shown in Table 1. Moisture contents of the raw and cooked *Blighia sapida* seed flour were 8.29 and 8.57 % respectively. Higher protein content (14.97 %) was observed in the raw flour but there was slight reduction in the protein value (14.68 %) of the cooked flour. The protein contents of *Blighia sapida* seed flours were comparable to protein contents of *Monodora myristica* (12.83 %) and *Afzelia Africana* seed (9.56 %) (Ojiako et al., 2010). Boiling and blanching methods were reported to reduce the protein contents of *Sphenostylis stenocarpa* (African yam bean), *Sesamum indicum* (Sesame seed), *Monodora myristica* (Calabash nutmeg), *Afzelia Africana* (African oak) and black bean flour (Ojiako et al., 2010; Vongsumran et al., 2014). These may be due to denaturation and leaching of the protein in the seed during cooking. Raw *Blighia sapida* seed flour also had higher ash, fat and crude fibre contents of 2.70, 13.14 and 1.71% respectively. Carbohydrate content of cooked *Blighia sapida* seed flour was 60.01 % which was higher than raw flour. Energy compositions of the raw and cooked flour were 414.91 and 412.93 kcal/100g. Energy compositions observed in the raw and cooked flour were higher than that of yam (210.5 kcal/100g), cornstarch (376.87 kcal/100g) and sweet potato flour (344.56 kcal/100g) (Akaninwor and Okechukwu, 2006; Adenuga, 2010). The high energy values of *Blighia sapida* seed flours signified its potential to meet the energy requirements of both infants and adults. Calcium and potassium contents were the major mineral in the seed flour. Raw flour contained higher calcium (834.32 mg/kg), sodium (64.84 mg/kg) and iron (0.05 mg/kg) contents while the cooked flour had higher magnesium (23.78 mg/kg) and potassium (641.38 mg/kg) contents. Increase in magnesium content of the cooked flour may be due to migration of the nutrient from the peel into the seed endosperm during cooking. Likewise, high potassium content observed in cooked flour may be due to reduction in the anti-nutrients which bind with the mineral. Flour high in calcium are useful in the diet for formation of strong bone and teeth, while the presence of high potassium makes it valuable due to its ability to aid digestion.

Composition	Raw seed flour	Cooked seed flour
Moisture (%)	8.29±0.04	8.57±0.05
Protein (%)	14.97±0.21	14.68±0.15
Ash (%)	2.70±0.02	2.43±0.04
Fat (%)	13.14±0.06	12.69±0.03
Fibre (%)	1.71±0.02	1.64±0.01
Carbohydrate (%)	59.21±0.09	60.01±0.02
Energy (kcal/100g)	414.91±0.15	412.93±0.14
Ca (mg/kg)	834.32±0.56	811.31±0.52
Mg (mg/kg)	23.64±0.17	23.78±0.20
K (mg/kg)	615.59±0.10	641.38±0.04
Na (mg/kg)	64.84±0.07	42.14±0.02
Fe (mg/kg)	0.05±0.08	0.04±0.10

Mean± SD

Amino acid compositions of *Blighia sapida* seed flour are presented in Table 2. Major amino acids in the flour include glutamic acid, aspartic acid, leucine, arginine and lysine. Boiling of the seeds led to increase in the amino acid values of the cooked flour. This was contrary to the result obtained for protein content of cooked seed flour. Slight reduction in protein content was observed in the cooked seed flour. Limiting amino acids in the raw flour were cysteine and methionine. All the essential amino acids in the cooked flour were greater than approved standards by FAO/WHO (1991). This showed that cooked *Blighia sapida* seed flour meet the amino acid requirement for proper growth and development. Amino acids content obtained in *Blighia sapida* seed flour were higher than those of *Bosqueia angolensis* but compares with *Luffacyl indrica* seed flour (Nwosu et al., 2008, Oyetayo and Ojo, 2012). Total essential amino acid in the cooked flour was higher than the raw flour and the FAO/WHO (1991) reference pattern. This was also higher than the total amino acid in the *Luffacyl indrica* seed flour (38.76g/100g) (Oyetayo and Ojo, 2012) but comparable to *Pisumsativum* (Achat pea) (40.96 g/16g N) (Amarakoon, 2012).

Table 2: Amino acid compositions of raw and cooked flour of *Blighiasapida* seed

Amino Acid	Raw (g/100g protein)	Cooked (g/100g protein)	FAO/WHO (1991)
Lysine	4.59	6.00	5.8
Histidine	2.02	2.59	1.9
Arginine	5.18	6.82	1.1
Aspartic acid	9.37	10.17	
Threonine	3.37	4.03	3.4
Serine	3.47	3.58	
Glutamic acid	11.66	12.57	
Proline	2.64	3.46	
Glycine	3.70	3.70	
Alanine	3.57	4.03	
Cysteine	0.93	1.32	
Valine	3.53	4.23	3.5
Methionine	0.99	1.20	2.5+cysteine
Isoleucine	2.66	3.17	2.8
Leucine	6.88	7.54	6.6
Tyrosine	3.17	3.25	
Phenylalanine	4.13	4.47	6.3+tyrosine
Tryptophan	ND	ND	1.1
Total EAA	33.35	40.05	35
Total NEAA	38.51	42.08	
Total amino acid	71.86	82.13	

ND=Not determined

EAA= Essential amino acid, NEAA= Non-essential amino acid

Pasting properties of *Blighia sapida* flour

Pasting properties of *Blighia sapida* seed flour are shown in Table 3. Raw flour had higher peak viscosity (78.25 RVU), holding strength (53.66 RVU), breakdown (24.59 RVU), final viscosity (66.79 RVU), setback (13.13 RVU) and pasting temperature (82.43 °C) than the cooked flour. Peak and final viscosities observed in the seed flours were lower than the values reported for legume starches (Sandhu *et al.*, 2008) and maize flour (Bolade, 2009). Low peak viscosities are desirable for high-calorie food formulations.

Longer pasting time (6.86 min) was required for cooked flour but at low pasting temperature (49.83 °C). Breakdown value (0.52 RVU) was low in the cooked flour. Yadav *et al.* (2011) explained the causes of breakdown in flour as disintegration of gelatinized starch granules structure during continued stirring and heating leading to shear thinning property of starch. A low breakdown value suggests higher stability of the flour under hot conditions. There was increase in the final viscosity of the cooked flour which may be due to the aggregation of the amylose molecules according to Bhupender *et al.* (2013). Cooked *Bligha sapida* seed flour showed higher stability ratio (0.91) than raw flour. Stability ratio indicates the ability of the cooked flour to retain its viscous properties after cooking and cooling. Pasting properties of the cooked flour suggests the feasible production and utilization as weaning food which require low viscosities.

Table 3: Pasting properties of *Blighia sapida* and flour

Parameter	Raw flour	Cooked flour
Peak viscosity (RVU)	78.25±0.35	5.51±0.02
Holding strength (RVU)	53.66±0.37	5.00±0.00
Breakdown (RVU)	24.59±0.01	0.52±0.02
Final viscosity (RVU)	66.79±0.54	8.41±0.01
Setback (RVU)	13.13±0.18	3.41±0.01
Pasting time (min)	4.72±0.02	6.86±0.01
Pasting Temperature (°C)	82.43±0.04	49.83±0.04
Stability ratio	0.69	0.91

Mean± SD

Swelling properties of *Blighia sapida* flour

Swelling properties of *Bligha sapida* seed flour are shown in Fig. 1. At low temperature (60-70 °C), cooked flour swelled than the raw flour. This may be as a result of the treatment given to the flour which led to rupturing and gelatinization of the starch thereby facilitating its ability to imbibe water at low temperatures. At 80-90 °C, the raw flour increased significantly than the cooked flour. Raw flour had higher swelling power (8.3) at 90 °C. Swelling power of the flours increased with temperature. Swelling power observed in *Bligha sapida* seed flour was lower than that of cassava and potato at 90 °C (Nuwamanya *et al.*, 2011). According to Iwuoha

(2004) and Nuwamanya *et al.* (2011) swelling power depends on amylose/amylopectin ratio, presence of lipid and starch granule size. This corroborates the finding of Kauret *et al.* (2007) that starches with lower amylose content swell more than those with higher amylose content. Swelling takes place when the amylose content had been leached into the cooking medium during processing. Low swelling power observed in cooked seed flour at high temperatures resulted into low breakdown viscosity as confirmed by Yuan *et al.* (2007).

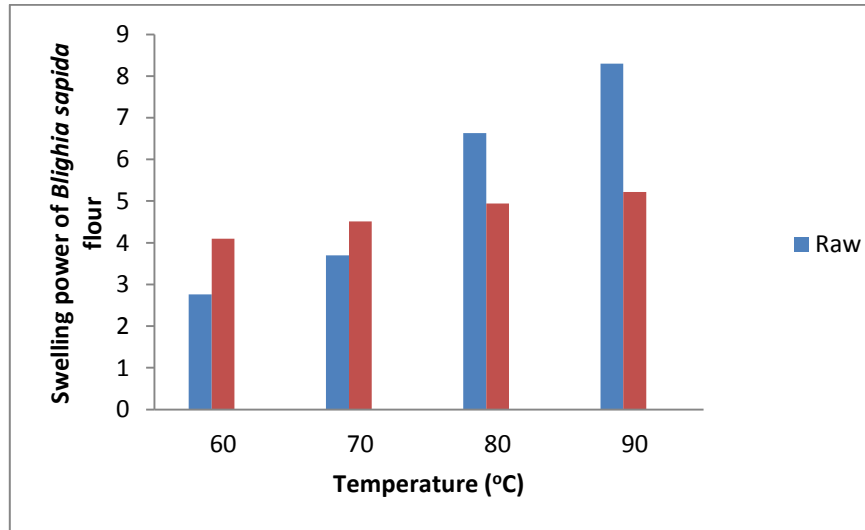


Fig. 1: Swelling power of raw and cooked *Blighia sapida* seed flour

Anti-nutritional factors in *Blighia sapida* flour

Anti-nutritional contents of *Blighia sapida* flour are shown in Table 4. *Blighia sapida* flour had low tannin (0.22-0.51 mg/kg), oxalate (0.43-0.57 mg/kg) and alkaloid (0.15-0.27 mg/kg) contents but high in phytate content (5.23-6.18 mg/kg). Cooked flour had lower values in all the anti-nutrients determined. Cooking caused reductions in the anti-nutritional contents of the *Blighia sapida* flour. Oxalate and alkaloid had been reported to be water soluble (Eka, 1998) and according to Albihn and Savage (2001) boiling may cause considerable skin (epidermal) rupture and facilitate the leakage of soluble oxalate into cooking water. Kumar *et al.* (2010) also reported partial phytate dephosphorylation of the food during processing. Reduction in the anti-nutritional factors may be as a result of leaching into the cooking medium.

Anti-nutritional factor	Raw flour	Cooked flour
Tannin (mg/g)	0.51±0.01	0.22±0.01
Phytate (mg/g)	6.18±0.04	5.23±0.02
Oxalate (mg/g)	0.57±0.03	0.43±0.10
Alkaloid (mg/g)	0.27±0.02	0.15±0.11

Table 4: Anti-nutritional factors in *Blighia sapida* flour

Mean± SD

Conclusions

Cooking for twenty minutes caused reduction in the chemical, anti-nutrients and pasting properties of the flour while the amino acids contents were improved. Cooking also increased the swelling power of the flour at lower temperature. The flour exhibited properties that are useful in the food industry. *Blighia sapida* seed flour could be extensively researched using *in vitro* and *in vivo* methods in order to ascertain its potential toxicity in animal model.

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