JOURNAL OF SCIENCE, TECHNOLOGY AND EDUCATION (JSTE) VOL. 8. NO. 8

JSTE PUBLICATIONS, 2024 www.nsukjste.com/

ISSN: 2651-5539

EFFECT OF BOILING AND SOAKING ON LEVELS OF ANTI-NUTRIENT IN *Brachystegia eurycoma, Detarium microcarpum* AND *Mucuna sloanei*

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Citation: Amah, G. H., Ogunbiyi, B. T., Esiaba, I., Dada, T. O., Akinpelu, A., Ogbonnaya, F. C., Adetayo, M. O. and Olajide, L. O. (2024 Effect of boiling and soaking on levels of anti-nutrient IN *Brachystegia eurycoma, Detarium microcarpum* and *Mucuna sloanei*. *Journal of Science, Technology, and Education (JSTE)*; www.nsukjste.com/ *8*(8), 83- 96

Abstract

Seeds of Brachystegia eurycoma, Detarium microcapum, and Mucuna sloanei are nutrient rich and medicinal. They are also thickening and flavoring agents commonly used for native broth soup preparation in tropical Africa. However, the characteristic high levels of antinutrients in these leguminous seeds necessitate investigation into possible minimal processing approach that could be easily adopted at household level to improve their nutritional value. Therefore, the seeds of B. eurycoma, D. microcapom and M. sloanei were soaked and boiled at varied durations respectively, as possible approaches to reducing their antinutrient contents. Boiling for three hours reduced the level of phytate (mg/kg) in B. eurycoma, D. microcarpum and M. sloanei by 43.69%, 46.19%, and 50.98% respectively. After 18 hours of soaking, the oxalate content (mg/100g) was reduced to 33%, 45%, and 48% whereas level of cyanogenic glycoside (mg/kg) was reduced by 67.69%, 66.01, and 32.20%; in B. eurycoma, D. microcarpum, and M. sloanei, respectively. Whereas, soaking was more effective in reducing the levels of tannin and cyanogenic glycoside, boiling was more efficient in reducing the levels of trypsin inhibitors, phytate and oxalate. Therefore, soaking and boiling are effective in reducing antinutrients in the seeds studied

Keywords: *Brachystegia eurycoma*, *Detarium microcapum*, *Mucuna sloanei*, Soaking, Boiling.

Introduction

In West Africa, dietary pattern varies and this is influenced by the vegetation belt components. It was observed that in Northern Nigeria, cereals dominate, while in the south legumes, nuts, seeds an/d starchy roots or tubers are the main food consumed (Petrkova et al., 2023). However, there is a general practice of processing the seeds and starch roots into a paste and eaten with soups of varied types (Amah et al., 2017). Among the legumes that form components of the soups are *Brachystegia eurycoma* locally known by the Igbos as 'Achi', 'Akolodo' by the Yorubas and 'Okweri' by the Benins; *Detarium microcapum* known locally as 'Ofor' by the Igbos, 'Ogbogbo' by the Yorubas and 'Taura' by the Hausas and *Mucuna sloanei* (commonly called Horse eye bean, velvet bean and devil bean) called 'Ukpo' by the Igbos, 'Karasuu' by Hausa and 'Yerepe' by the Yorubas (Adewale & Mozie, 2010).

B. eurycoma is a plant found in Western Africa, native to tropical Africa, and used as food additive or medicine. *B. eurycoma* seeds when used in soup, serve as thickener and as flavoring

agents. The seed is a good source of dietary fibre, crude fat, protein and carbohydrate (Enwere, 1998; Ikegwu, Okechukwu, & Ekumankana, 2010; Ndukwe, 2021). The fatty acid composition of the seed oil is approximately: palmitic acid 26%, stearic acid 7%, lignoceric acid 13%, linoleic acid 6%, oleic acid 32% (total saturated fatty acids 59%, total unsaturated fatty acids 41%) (Oyen, 2012).

daily requirement of protein and fats (Abdalbasit, Elwathig, Bustaman, & Abdelwahab, 2009; Florence, Godwin, Abubakar, & Kudirat, 2014). *D. microcarpum* seed oil has low biogenic and oxidative rancidity which is a desired property in oils meant for consumption, industrial purposes and pharmaceutical applications (Okorie, Okonkwo, Nwachukwu, & Okeke, 2010).

M. sloanei, commonly called the 'Horse-eye' or 'Hamburger' bean, is an annual leguminous plant widely used among the various ethnicities in Nigeria (Obute, 2010). The *M. sloanei* seeds are rich in proteins, carbohydrates, crude fat, fiber, essential amino acid content such as lysine, and minerals (Akpata & Muachi, 2001; Ojiako et al., 2012; Nwosu, 2011; Emiri & Enaregha, 2020). They have also been reported to contain important phytochemicals such as alkaloids, phytic acid, tannins, flavonoids, haemoglutinin and oligosaccharides (Ijeh et al., 2004; Obute & Adubor, 2007). Similarly, lectin from *M. sloanei* seeds has been reported to serve as an effective and suitable cell receptor signal inducer due to its ability to agglutinate blood cells of humans, goat, cow and chicken (Obochi, Malu, Effiom, & Bassey, 2007). They also contain many other bioactive substances such as L-3, 4-dihydroxyphenylalanine (L-DOPA), which has been reported to be a potent precursor of the brain neurotransmitter, dopamine (Adebowale, Adeyemi, & Oshodi, 2005; Hornykiewicz, 2002; Kostrzewa et al., 2005; Nagatsua & Sawadab, 2009).

The high water binding capacity of these soup thickeners (*B. eurycoma*, *D. microcapum* and *M. sloanei)* due to the formation of hydrogen bonds between water and polar residues of the protein molecules gives more palatability and good mouth feel in culinary application. The soup thickeners differ in species and have their individual characteristic flavors which they impart to soups (Obochi et al., 2007; Uhegbu, Onwuchekwa, Iweala, & Kanu, 2009).

D. microcarpum is a local African legume that grows naturally in the drier regions of West and Central Africa and widely distributed in semi-arid sub-Saharan Africa. *D. microcarpum* is a good source of carbohydrate, fiber, minerals, vitamin C, vitamin $B_{2,}$ and folic acid and could also contribute to the

Antinutritional factors are primarily compounds or substances of natural or synthetic origin, which interfere with the absorption of nutrients, and act to reduce nutrient intake, digestion, and utilization and may produce other adverse effects. Conversely, such chemical compounds can be evidently advantageous to humankind when consumed wisely. It has been evidently observed that plants primarily use antinutrients for self-defense (Popova & Mihaylova, 2019). Unhealthy antinutrient levels militate against mineral bioavailability in the gastrointestinal tract (Suleiman, Mohammed, Elhadj, Babiker, & Eltinay, 2007) by forming complexes with such minerals as iron, zinc, and magnesium and rendering them unavailable for absorption (Masum Akond, Crawford, Berthold, Talukder, Hossain, 2011). Phytates also inhibit digestive enzymes like pepsin, trypsin and amylase, to some degrees (Kumar et al., 2010). The three legumes have been shown to satisfactorily complement the quality of soup produced when added to different soups; yet, they reportedly contain varied amounts of diverse antinutrients (Amah et al., 2017; Obute & Adubor, 2007; Peace & Adekunle, 2018). Thus, this study was aimed at determining the effect of two processing methods (boiling and soaking) on the antinutrients present in the seeds of *B. eurycoma*, *D. microcapum* and *M. sloanei*.

Materials and Methods

Collection and preliminary treatment of *B. eurycoma, D. microcarpum* and *M. sloanei*

Mature dry seeds of *B. eurycoma, D. microcarpum*, *M. sloanei* (500 g each) were obtained from Oyigbo Market in Oyigbo, Lagos, Nigeria (6° 28' 35" N · 3° 22' 56" E · 6.47647, 3.38231). The samples were separately packed in black zip lock polyethylene bags and transported to the laboratory for analyses. Each sample of the soup thickeners was sorted to remove dead seeds, debris and other extraneous matter and rinsed in

plentiful tap water for cleansing. The samples were separately dried in hot air oven at 30 °C for 24 hours to remove adsorbed water. The samples were then separately packed in black zip lock polyethylene bag, lidded in airtight container and stored at -15±3 °C until required for analyses.

Sample Grouping and Processing

300 g each of the samples of *B. eurycoma, D. microcarpum, M. sloanei* was divided into three portions of 100 g each for processing into raw, boiled and soaked samples, respectively.

Raw

100 g each of *B. eurycoma, D. microcarpum, M. sloanei* was weighed out for analyses, without being subjected to any form of processing and was considered as the raw samples. The seeds were differently broken into small pieces, pulverized using a laboratory hammer mill and mechanical blender, sieved to ensure consistent particulate size of approximately 30 mm in diameter and packed in black zip-lock polyethylene bag for analyses.

Boiling

100 g each of the three different seed types (*B. eurycoma, D. microcarpum* and *M. sloanei*) were divided into three groups and boiled separately in distilled water at 100 °C for 1 hour, 2 hours and 3 hours, respectively. A total of nine treatment groups were obtained (three groups of boiled sample per seed type). All these treatments were carried out before dehulling, which is the established culinary preparation protocol of folklore users of these legumes. The water was then drained off and each boiled sample dehulled, dried at 40 °C in dry air oven for 24 hours, broken into smaller pieces and pulverized to fine powder using a mechanical blender. The fine powder of each sample was then packed in a labelled airtight container and stored at 0±2 °C until required for analyses.

Soaking

Each of the three different seeds (*B. eurycoma, D. microcarpum* and *M. sloanei*) were divided into three groups and soaked in distilled water for 6 hours, 12 hours and 18 hours respectively, at room temperature. The water was then drained off and the soaked samples respectively dehulled, dried at 40 °C for 24 hours, broken into smaller pieces and pulverized to fine powder using mechanical blender. Each powdered sample was then packed, labelled and stored at 0±2 °C until required for analyses.

Determination of Antinutrient Composition

Determination of Phytate: Phytate was determined by the procedure of Lucas and Markakas (1975). Two grammes portion of each sample powder was soaked in 100 mL 2% concentrated HCl for three hours and then filtered with Whatman No. 1 filter paper. 50 mL of the filtrate was pipetted into a 250 mL beaker and 107 mL of distilled water was added in each case to improve acidity. 10 mL 0.3% ammonium thiocyanate solution was measured into the solution to serve as an indicator and titrated against 0.00195 g/mL standard iron (III) chloride solution. The end point was observed to be a brownish yellow colour which persisted for five minutes. The percentage Phytate was calculated thus:

% Phytic acid (g/kg) = 0.00195 × (volume of $FeCl_3$ consumed / Sample weight)

Estimation of Tannins: Determination of Tannin was carried out following the method of AOAC (1980)**.** One gramme of each sample was weighed into a flask and 10 mL of distilled water was added followed by agitation. It was left to stand for 30 minutes at room temperature after which it was centrifuged at 2500 rpmto obtain a clear supernatant. Two mL of the supernatant was dispensed into a 10 mL volumetric flask and 1 mL of Folin Ciocalteu reagent was added followed by addition of 2 mL saturated $NaCO_3$ solution. The mixture was diluted to 10 mL using distilled water and incubated for 30 minutes at room temperature. Absorbance was read at 725 nm using Spectrumlab 23A (LengGuang Tech, Shanghai, China) UV visible spectrophotometer. Tannin content of the sample was obtained by extrapolation on the standard tannic acid curve obtainable from same procedure.

Determination of Oxalate: The method of **Ohlweiler and Schneider (1972)** as modified by **Karamad et al. (2019)** was adopted in quantifying oxalate content in each of the samples. Briefly, 150 mL of 3 N H_2SO_4 was added to 1 g of each sample and left for 30 minutes. The mixture was then filtered through

Whatmann number one filter paper. 50 mL of filtrate was pipetted into another conical flask and 2 drops of methyl red indicator added. The filtrate containing the indicator was then brought to boiling and titrated immediately against 0.05 M $KMnO_4$ solution until a faint pink colour appears and persisted for at least 30 seconds. The oxalate content was calculated by taking 1 mL of 0.05 M $KMnO_4$ as equivalent to 2.2 mg/kg Oxalate.

Determination of Trypsin Inhibitors: One gramme of sample was weighed into a conical flask containing 50 mL of NaCl. It was shaken properly and left to stand for about 30 minutes, the mixture was then centrifuged at 1500 rpm for 5 minutes. 10 mL of the supernatant was transferred into another flask and 2mL of known concentration of standard trypsin inhibitor was added to spike the trypsin in the sample. The absorbance of the solution was read at 410nm using a spectrophotometer. The concentration was then obtained by comparing on a trypsin standard graph **(AOCS, 1997)**.

Determination of Cyanogenic Glycosides: Modified method of Association of Official Analytical Chemist (AOAC) (1990) was applied to quantify cyanogenic glycoside in the respective samples as reported by Moriasi et al. (2017). Five grammes of pulverized sample was weighed into a conical flask containing 50 mL of distilled water. The mixture was shaken and allowed to stand for 24 hours (as against one hour) to allow all the cyanide present in the sample to solubilise in the water before it was filtered with Whatman filter paper number 1. Two (2) mL of the filtrate was pipetted into a test tube and 4 mL (as against 2 mL) of alkaline picrate solution was added. The tube was incubated for 15 minutes at 37 ºC in a water bath. The reaction was terminated by adding 15 µL of concentrated sulphuric acid in order to stabilise the reading. The colour changed from yellow to reddish brown after incubation. The absorbance of the reddish brown coloured solution was read at 510 nm using a UV visible spectrophotometer against 2 mL of distilled water mixed with 4 mL alkaline picrate solution as the blank. The cyanide concentration was obtained from a standard KCN calibration curve. Amount of cyanide in each test sample was calculated using the mathematical relation below:

$$mg/kg\ cyanide = \frac{\mu g/mL\ of\ cyanide \times final\ volume\ (L)}{weight\ of\ sample\ (kg)}$$ (AOAC, 1990)

Where: µL/mg CN is the HCN equivalent obtained from the KCN standard calibration curve; final volume represents the volume of sample obtained from sample filtrate; and sample weight represents the weight of sample extracted in kg.

Statistical Analysis

Mean values of triplicate readings were reported as mean ± SEM. One-way Analyses of Variance was used to analyse for variations in mean values. The means was separated using least significant difference (LSD) at 95% confidence limit. Tables were used in result presentation. All statistical computations were carried out on SPSS version 20.

Result.

Effect of boiling and soaking time on anti-nutrient levels in B. *eurycoma*

Table 1 showed the level of phytate, tannins, oxalate, cyanogenic glycoside, and trypsin inhibitors at different boiling and soaking conditions. It was observed that boiling and soaking, two major traditional processing methods commonly adopted in processing B. eurycoma for native culinary in Nigeria and other West African subsumes, reduced the anti-nutrients in the seeds considerably and variedly depending of the antinutrient and processing method in view.

The level of tannins reduced (from 185.18±1.55 mg/100 g) significantly ($p<0.05$) with increasing boiling time to mean values of 125.24 ± 0.55 mg/100g, 75.585 ± 0.47 mg/100 g and 56.58 ± 0.51 mg/100 g for 1 hour, 2 hours and 3 hours respectively. Boiling at 100 ℃ for 3 hours showed 69% reduction in tannins and therefore, it is the most effective boiling approach to reducing tannins. Also, the level of tannins reduced significantly ($p<0.05$) with increasing soaking time to mean value of 82.56 ± 0.29 mg/100g, 68.57 ± 0.61 mg/100g and 42.15 ± 0.81 mg/100g after durations of 6 hours, 12 hours and 18 hours respectively, compared to 185.18 ± 1.55 mg/100g observed in the raw sample. The foregoing clearly demonstrated that soaking *B.*

eurycoma seeds at room temperature for 6, 12 and 18 hours led to a reduction of 55%, 63% and 77% respectively of tannins when compared with the raw seeds.

The level of phytate reduced significantly ($p<0.05$) with increasing boiling time and soaking time. Boiling at 100 °C for 3 hours led to 43% reduction (to 34.44 ± 0.27 mg/Kg) in phytate levels of the processed seeds compared with the raw samples (61.18 ± 0.55 mg/Kg). Soaking at room temperature for 18 hours showed 38% reduction of the phytate level (37.68 ± 0.46 mg/Kg). Furthermore, boiling at 100 °C for 3 hours (4.91 ± 0.11 mg/Kg) significantly ($p<0.05$) reduced the cyanogenic glycosides content of *B. eurycoma* (about 21% reduction) when compared with its content in the raw seed (6.23 ± 0.11 mg/Kg) while soaking for 18 hours (2.37 ± 0.06 mg/Kg) at room temperature significantly ($p<0.05$) reduced the cyanogenic glycosides level of *B. eurycoma* by about 62% when compared with its content in the raw seed sample (6.23 ± 0.11 mg/Kg).

The level of oxalate reduced significantly ($p<0.05$) with increasing boiling time. Boiling at 100 °C for 3 hours (36.30 ± 0.19 mg/100g) showed 33% reduction of the oxalate content as compared to the raw sample (54.06 ± 0.93 mg/100g). Also, the level of oxalate reduced significantly ($p<0.05$) with increasing soaking time. Soaking at room temperature for 6, 12 and 18 hours showed 26%, 32% and 33% reduction of the oxalate content respectively, as compared to the raw seed. Meanwhile, boiling at 100 °C for 3 hours (38.35 ± 0.26 mg/100g) significantly ($p<0.05$) reduced the trypsin inhibitors level in *B. eurycoma* when compared with its level in the raw seed (50.70 ± 0.58 mg/100g). Soaking at room temperature for 18 hours (110.49 ± 0.37 mg/100g) significantly ($p<0.05$) reduced the trypsin inhibitors content in *B. eurycoma* by 50.24% when compared with the raw sample (219.89 ± 2.03 mg/100g).

Effect of boiling and soaking time on anti-nutrient composition of *Detarium microcarpum*

Table 2 showed the level of phytate, tannins, cyanogenic oxalate, glycoside, and trypsin inhibitors at different boiling and soaking conditions. The level of tannins reduced significantly ($p<0.05$) with increasing boiling time of 1 hour, 2 hours and 3 hours to mean values of 121.56 ± 0.85 mg/100g, 114.78 ± 1.32 mg/100 g and 107.48 ± 0.74 mg/100 g respectively when compared with the raw seed sample (257.23 ± 0.41 mg/100g). Boiling at 100 °C for 3 hours showed 58% reduction in tannins. Also, the level of tannins reduced significantly ($p<0.05$) with increasing soaking time to mean values of 128.13 ± 0.06 mg/100g, 89.34 ± 0.32 and 57.28 ± 0.62. Soaking at room temperature for 6, 12 and 18 hours showed around 50%, 65% and 78% reduction in the tannin content respectively compared with the raw seed sample.

Furthermore, the level of phytate reduced significantly ($p<0.05$) with increasing boiling time. Boiling at 100 °C for 3 hours (21.30 ± 0.08 mg/Kg) showed 53.8% reduction in phytate content compared to the raw seed (39.59 ± 0.75 mg/Kg). Similarly, the level of phytate reduced significantly ($p<0.05$) with increasing soaking time. Soaking at room temperature for 18 hours (29.44 ± 0.54 mg/Kg) showed 74.3% reduction in phytate content compared to that of the raw seed (39.59 ± 0.54 mg/Kg). Boiling at 100 °C for 3 hours (19.30 ± 0.23 mg/Kg) significantly ($p<0.05$) reduced the cyanogenic glycosides content in *D. microcarpum* by 57.78% when compared with its content in the raw seed (33.40 ± 0.48 mg/Kg) just like soaking for 18 hours (10.79 ± 0.32 mg/Kg) at room temperature significantly ($p<0.05$) reduced the cyanogenic glycosides content in *D. microcarpum* by 32.30% when compared with its content in the raw seed.

Oxalate level reduced significantly ($p<0.05$) with increasing boiling time. Boiling at 100 °C for 3 hours (44.87 ± 0.06 mg/100g) showed 28% reduction of the oxalate content as compared to the raw seed (62.73 ± 0.28 mg/100g) while boiling at 100 °C for 3 hours (64.22 ± 0.23 mg/100g) significantly ($p<0.05$) reduced the trypsin inhibitors activity of *D. microcarpum* showing around 71% reduction when compared with its level in the raw seed (219.89 ± 2.03 mg/100g). Additionally, the level of oxalate and trypsin inhibitors reduced significantly ($p<0.05$) with increasing soaking time. Soaking at room temperature for 6, 12 and 18 hours showed 21%, 34% and 45% reduction of the oxalate content respectively when compared with oxalate content in the raw seed. Soaking at room temperature for 18 hours significantly ($p<0.05$) reduced the trypsin inhibitors activity of *D. microcarpum* by 50% to 110.49±0.37 mg/100 g when compared

with 219.89±2.03 mg/100 g observed in the raw sample.

Effect of boiling and soaking time on anti-nutrient composition of *Mucuna sloanei*

Table 3 shows the level of the anti-nutrients studied in *M. sloanei* at different boiling and soaking conditions. It demonstrated that the level of tannins reduced significantly ($p<0.05$) with increasing boiling time of 1 hour, 2 hours and 3 hours to mean values of 582.59 ± 0.09 mg/100g, 556.59 ± 0.34 mg/100g and 510.67 ± 0.45 mg/100g respectively, when compared with the value observed in the raw seeds (652.91 ± 1.35 mg/100g). Boiling at 100 ℃ for 3 hours showed about 22% reduction of the tannin content as compared to the raw seed just like the level of tannins reduced significantly ($p<0.05$) with increasing soaking time to mean values of 545.19 ± 0.51 mg/100 g, 473.92 ± 0.46 mg/100 g and 411.26 ± 0.36 mg/100g for 6 hours, 12 hours and 18 hours of soaking as compared to the raw seed (652.91 ± 0.68 mg/100 g). Soaking at room temperature for 6, 12 and 18 hours showed about 16%, 27% and 37% reduction of the tannin content respectively, when compared to the value observed in the raw seed sample.

The phytate content in *M. sloanei* reduced significantly ($p<0.05$) following boiling for 3 hours. Significant reduction in phytate content was not observed after boiling for 2 hours. Boiling at 100 ℃ for 3 hours (21.24 ± 0.29 mg/Kg) showed about 51% reduction of the phytate level as compared to the raw seed (43.33 ± 0.17 mg/Kg). Soaking at room temperature for 18 hours (26.66 ± 0.12 mg/Kg) showed 38% reduction of the phytate value as compared to the raw seed (43.33 ± 0.17 mg/Kg). Boiling at 100 °C for 3 hours (36.27 ± 0.06 mg/Kg) significantly ($p<0.05$) reduced the cyanogenic glycosides content of *M. sloanei* by 20% when compared with its content in the raw seed. Similarly, soaking for 18 hours (30.56 ± 0.45 mg/Kg) at room temperature significantly ($p<0.05$) reduced the cyanogenic glycosides level in *M. sloanei* by 32% when compared with its level in the raw seed (45.08 ± 0.62 mg/Kg).

The oxalate and trypsin inhibitors levels of *Mucuna sloanei* were also shown to decrease with different boiling and soaking conditions. The level of oxalate reduced significantly ($p<0.05$) with increasing boiling time. Boiling at 100 ℃ for 3 hours (65.73 ± 0.52 mg/100g) reduced the oxalate content by 49% as compared to the raw seed (127.75 ± 0.16 mg/100g). Similarly, boiling at 100 ℃ for 3 hours (81.01 ± 1.42 mg/100 g) significantly ($p<0.05$) reduced the trypsin inhibitors level in *M. sloanei* by 61% when compared with its level in the raw seed (209.71 ± 0.21 mg/100g). The level of oxalate reduced significantly ($p<0.05$) with increasing soaking time. Soaking at room temperature for 6, 12 and 18 hours showed 41%, 44% and 48% reduction in the oxalate content respectively when compared with the value in the raw seed. Soaking at room temperature for 18 hours (104.92 ± 6.74 mg/100 g) significantly ($p<0.05$) reduced the trypsin inhibitors content in *M. sloanei* by 50% of its content in the raw seed sample (209.71 ± 0.21 mg/100g).

Table 1: Antinutrient composition of *B. eurycoma* as influenced by varied duration of boiling and soaking.

Method of processing	Antinutrient	Antinutrient Level			
Boiling Duration (Hour)		0	1	2	3
BOILING	Phytates (mg/kg)	61.18±0.55[a]	57.24±0.78[b]	54.39±0.19[c]	34.45±0.28[d]
	Tannins (mg/100g)	185.18±1.55[a]	125.23±0.55[b]	75.59±0.47[c]	56.58±0.51[d]
	Oxalates (mg/100g)	54.06±0.93[a]	51.95±0.97[b]	42.19±0.11[c]	36.30±0.19[d]
	Cyanogenic Glycosides (mg/kg)	6.23±0.11[a]	5.93±0.09[b]	5.56±0.14[c]	4.91±0.11[d]
	Trypsin Inhibitors (mg/100g)	50.70±0.58[a]	50.17±0.16[a]	43.00±0.06[b]	38.35±0.26[c]
Soaking Duration (Hour)		0	6	12	18
SOAKING	Phytates (mg/kg)	61.18±0.55[a]	50.11±0.03[b]	46.42±0.55[c]	37.68±0.47[d]
	Tannins (mg/100g)	185.18±1.55[a]	82.56±0.29[b]	68.57±0.62[c]	42.15±0.81[d]
	Oxalates (mg/100g)	54.06±0.93[a]	39.65±0.02[b]	36.81±0.51[c]	36.20±0.05[c]
	Cyanogenic Glycosides (mg/kg)	33.40±0.48[a]	23.20±0.51[b]	16.07±0.38[c]	10.79±0.32[d]
	Trypsin Inhibitors (mg/100g)	219.89±2.03[a]	155.05±0.83[b]	126.90±0.19[c]	110.49±0.37[d]

Note: Values are expressed as mean ± standard error of the mean. Different superscripts on same row indicate significantly different mean values.

Table 2: Antinutrient composition of *D. microcarpum* as influenced by varied duration of boiling and soaking

Method of Processing	Antinutrient	Level of Antinutrients			
Boiling Duration (Hour)		0	1	2	3
BOILING	Phytates (mg/kg)	39.59±0.75 [a]	28.11±0.25 [b]	25.95±0.08 [c]	21.30±0.08 [d]
	Tannins (mg/100g)	257.23±4.41[a]	121.56±0.85 [b]	114.78±1.32 [c]	107.48±1.74[d]
	Oxalates (mg/100g)	62.73±0.28 [a]	46.96±0.13 [b]	45.70±0.06 [b]	44.87±0.06 [c]
	Cyanogenic Glycosides (mg/kg)	33.40±0.48 [a]	28.58±0.04 [b]	26.75±0.57 [c]	19.30±0.23 [d]
	Trypsin Inhibitors (mg/100g)	219.89±2.03	158.69±1.22	74.05±0.95	64.22±0.83
Soaking Duration (Hour)		0	6	12	18
SOAKING	Phytates (mg/kg)	39.59±0.75 [a]	37.19±1.01 [a]	34.25±0.65 [b]	29.44±0.54 [c]
	Tannins (mg/100g)	257.72±4.55 [a]	128.13±1.96 [b]	89.34±0.52 [c]	57.28±0.61 [d]
	Oxalates (mg/100g)	62.73±0.28 [a]	49.77±0.30 [b]	41.56±0.29 [c]	34.32±0.99 [d]
	Cyanogenic Glycosides (mg/kg)	33.40±0.48 [a]	23.20±0.51 [b]	16.07±0.38 [c]	11.35±0.32 [d]
	Trypsin Inhibitors (mg/100g)	219.89±2.03 [a]	155.05±0.83 [b]	126.90±1.19 [c]	110.49±0.37[d]

Note: Values are expressed as mean ± standard error of the mean. Different superscripts on same row indicate significantly different mean values.

Table 3: Antinutrient composition of *M. sloanie* as influenced by varied duration of boiling and soaking

Method of Processing	Antinutrient	Level of Antinutrients

Boiling Duration (Hour)		0	1	2	3
BOILING	Phytates (mg/kg)	43.33±0.17[a]	35.39±0.13[b]	21.88±0.43[c]	21.24±0.29[c]
	Tannins (mg/100g)	652.91±1.35[a]	582.59±3.09[b]	556.59±4.34[c]	510.67±3.75[d]
	Oxalates (mg/100g)	127.75±0.86[a]	92.28±0.65[b]	70.32±0.80[c]	65.73±1.52[d]
	Cyanogenic Glycosides (mg/kg)	45.08±0.62[a]	44.11±0.22[a]	38.29±0.36[b]	36.27±0.42[b]
	Trypsin inhibitors (mg/100g)	209.71±3.21[a]	151.30±3.00[b]	111.00±0.87[c]	81.01±1.42[d]
Soaking Duration (Hour)		0	6	12	18
SOAKING	Phytates (mg/kg)	43.33±0.17[a]	38.54±0.39[b]	30.99±0.41[c]	26.66±0.12[c]
	Tannins (mg/100g)	652.91±3.68[a]	545.19±4.51[b]	473.92±4.46[c]	411.26±3.36[d]
	Oxalates (mg/100g)	127.75±1.16[a]	76.01±0.47[b]	71.08±0.69[c]	66.13±0.47[d]
	Cyanogenic Glycosides (mg/kg)	45.08±0.82[a]	42.11±0.97[b]	40.18±0.60[c]	30.56±0.45[d]
	Trypsin Inhibitors (mg/100g)	209.71±2.21[a]	137.35±2.13[b]	120.97±2.19[c]	104.92±1.74[d]

Note: Values are expressed as mean ± standard error of the mean. Different superscripts on same row indicate significantly different mean values.

Discussion

Results gotten from the study demonstrated that boiling and soaking, as processing methods, reduced the levels of anti-nutrients in *B. eurycoma*, *D. microcarpum* and *M. sloanei.* Tannins are oligomers of flavan-3-ols and flavan-3, 4-diols that are concentrated in the bran fraction of legumes (Obiakor-Okeke & Oly-Alawuba, 2014). Tannins exhibit antinutritional properties by impairing the digestion of various nutrients and preventing the body from absorbing beneficial bioavailable substances (Hendek-Ertop & Bektaş, 2018). Soaking of these various seeds; *B. eurycoma*, *D. microcarpum* and *M. sloanei*, significantly ($p<0.05$) reduced the concentration of tannin present in them. The highest time frame of significant reduction of tannin by soaking was seen in the eighteenth hour with 77% reduction in *B. eurycoma*, 78% reduction in *D. microcarpum* and 37% reduction in *M. sloanei.* These are indications that soaking these seeds for a longer period might reduce the tannin content to a non-detectable amount, except that such prolong soaking may result into production of fermentation products and possible nutrient leaching. The significant reduction in tannin level in soaked sample may be due to removal of seed coat and leaching of tannins into the soaking medium. It has been observed that soaking reduced the tannin content of some cowpea varieties; African yam beans, bambara groundnut, hyacinth and rajina beans to non-detectable levels (Mazahib, Nuha, Salawi, & Babiker, 2013).

Furthermore, boiling of these seeds significantly reduced the tannin contents. Boiling at 100 °C for 3 hours brought about 69%, 58% and 22% decrease in tannin contents in *B. eurycoma*, *D. microcarpum* and *M. sloanei* respectively. The tannins in cooked seeds were also observed to be reduced in earlier investigations on plant foodstuff and may be due to solubilization or degradation of tannins at high temperatures (Habiba, 2002; Nithya, Ramachandramurty, & Krishnamoorthy, 2007). Tannins are water soluble phenolic compounds with a high molecular weight and with the ability to form complexes which are not readily digestible. Tannins can also bind proteins and affect protein conformations. Tannin-protein complexes may cause digestive enzymes inactivation and reduced protein digestion caused by protein-substrate interaction (Salunkhe, Chavan, & Kadam, 1990). Tannin is generally present in the seed coat and dehauling should eliminate this toxic constituent and improve the nutritive value of the food products. Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional properties depend upon the structural presentation and

dosage (Ndulaka, Onuh, Okoro, Oriaku, & Ekaiko, 2017).

The highest phytate content in the raw samples was observed in *B. eurycoma* (61.18mg/kg) among the three soup thickeners studied, tailed by *D. microcarpum* (39.59 mg/kg). However, the phytate content was reduced by 38%, 26% and 38% in *B. eurycoma* (37.68mg/kg), *D. microcarpum* (29.44mg/kg) and *M. sloanei* (26.66mg/kg) respectively after 18 hours of soaking. The reduction in phytate level after soaking for graded duration agreed with similar studies that observed reduction in phytic acid contents of faba beans and kidney beans following soaking in water as reported by Alonso et al. (2000) and Bauhinia Vijayakumari et al. (2007). Additionally, Duhan et al. (2002) reported that the reduction of phytic acid content was more effective when soaking is combined with dehulling compared with soaking alone. Ene-Obong and Obizoba also reported that a decrease in phytate content might be due to leaching of phytate ions into soaking water (Ene-Obong & Obizoba, 1996). Boiling at 100 °C for 3 hours brought about 43%, 46% and 51% decrease in phytate content in *B. eurycoma, D. microcarpum* and *M. sloanei* respectively. According to El Maki et al. (2007), loss of phytic acid from food during boiling could probably be explained on the basis that phytate may degrade from inositol hexaphosphate to the pentaphosphate or lower molecular weight forms at temperature of 40-55 °C. It was also reported that phytic acid content decreased during boiling due to formation of insoluble complexes between phytate and other compounds, resulting into reduction in the amount of free phytate (Mazahib, Nuha, Salawi, & Babiker, 2013).

After 18 hours of soaking, the oxalate content was reduced to 33% in *B. eurycoma*, 45% in *D. microcarpum* and 48% in *M. sloanei*. After 3 hours of boiling, the levels of oxalate were decreased to 36.3 mg/100g, 44.86 mg/100g and 65.73 mg/100g in *B. eurycoma, D. microcarpum* and *M. sloanei* respectively. The decreases in oxalate content of the samples after boiling agreed with the results reported by Olaleye et al. (2013); that boiling reduced the oxalate content of bambara groundnut seeds. Oxalate combines with calcium to form calcium oxalate which passes through the intestine without being absorbed. Calcium oxalate is responsible for most of the kidney stone formation. Formation of these stones frequently reflects dysfunctional alkalinity of bladder and renal pelvic urine caused by bacteria that hydrolyses urea, releasing ammonia (Olaleye, Adeyeye, & Adesina, 2013). Most people can tolerate limited amounts of oxalate in foods, while individuals with certain conditions, such as enteric and primary hyperoxaluria, need to lower their oxalate intake (Natesh, Abbey, & Asiedu, 2017).

The levels of trypsin inhibitors after soaking reduced significantly ($p<0.05$) with increase in soaking time, With soaking for 18 hours being most effective in reducing trypsin inhibitors in the seeds studied. The results agreed with that of Mubarak (2005) who reported that trypsin inhibitor activity was significantly decreased by soaking and dehulling mung bean seeds. However, the present results are contrary to those of other investigators who reported a significant increase in trypsin inhibitors activity following soaking of certain legume seeds (Wang, Hatcher, & Gawalko, 2008; Martín-Cabrejas et al., 2009). Also, soaking had no significant effect on trypsin inhibitor content of sweet lupin seeds, but significantly increased it after 72 to 96 hour in bitter lupin seeds (Embaby, 2010). Three hours of boiling, is also, very effective in reducing trypsin inhibitor content of the seeds studied and could be applied to subsistent processing of these seeds for household consumption and industrially. This result agrees with the observations of other investigators. For instance, Embaby (2010) reported that ordinary cooking of bitter lupin and autoclaving of sweet lupin were the most effective treatments in destroying the trypsin inhibitor (by 60.8 and 86.8%, respectively). Furthermore, similar observations of partial and complete inactivation of trypsin inhibitor in cooked, microwave cooked, and autoclaved samples of Dolichos lablab beans, vegetable peas, soybean (Glycine max Merr.), cowpea (*Vigna unguiculata* L. Walp), and ground bean (*Macrotyloma geocarpa Harms*) were reported (Osman, 2007; Habiba, 2002; Egounlety & Aworh, 2003). Adewusi *et al.* (2008) reported that boiling reduced the level of trypsin inhibitors after 60 minutes and 90 minutes by 49.1% and 50.1% in *M. flagellipes*. Trypsin inhibitors induce lowered activities of trypsin and chymotrypsin in the gut, thus limiting protein digestion. Excess trypsin synthesis and associated burden on sulfur-containing amino acids may concomitantly result due to the release of cholecystokinin triggered by

ingestion of diet high in trypsin inhibitors (Shahidi, 1997).

Cyanogenic glycosides are chemical compounds contained in foods that release hydrogen cyanide when chewed or digested. The act of chewing or digestion leads to hydrolysis of the substances, causing cyanide to be released (Kwok, 2008). Hydrolysis of cyanogenic glycosides usually occurs when cyanogenic plants are chewed by herbivores or when the plants are disintegrated during processes, such as grinding, pounding or in the presence of water, for example during soaking or fermentation (Bolarinwa, Oke, Olaniyan, & Ajala, 2016). The cyanogenic glycosides contents in *M. sloanei* were not as much as other antinutrients. The levels of cyanogenic glycoside after soaking reduced significantly ($p<0.05$) with increase in soaking time. Also, the levels of cyanogenic glycoside reduced ($p<0.05$) with increase in boiling time, implying that boiling for 3 hours effectively reduced the level of cyanogenic glycoside in these seeds. It was reported that soaking of cassava root decrease its total cyanide content by 13-52% after 24 hours, 73–75% after 48 hours and 90% after 72 hours (Kemdirimi, Chukwu, & Anchinewhu, 1995) while steaming of a cassava product (akyeke) was reported to result in a 74–80% reduction in total cyanide levels. Cyanogenic glycosides are generally water soluble. During cooking, significant amount of cyanogens are leached into cooking water (Bolarinwa et al, 2016).

Conclusion

From the study, the different processing methods used reduced the levels of the anti-nutrients in *B. eurycoma*, *D. microcarpum* and *M. sloanei*, with increasing treatment time yielding better results. Soaking was more effective in reducing the levels of tannin and cyanogenic glycoside whereas boiling was more efficient in reducing the levels of trypsin inhibitors, phytate and oxalate. Notwithstanding, treatment duration is an important variable to consider in making the processing methods optimally effective as increased treatment duration gave better results. Therefore, these processing methods are empirically validated as being effective in managing the antinutrient content of *B. eurycoma*, *D. microcarpum* and *M. sloanei* for culinary purposes.

Acknowledgement: The Food and Nutritional Biochemistry Laboratory of Department of Biochemistry, Babcock University, Ilisan-Remo, Ogun State, Nigeria is acknowledged for providing bench space used for this study

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