



Impact of processing (toasting and fermentation) on the proximate, anti-nutrient and mineral composition of sickle pod (*Senna obtusifolia*) seeds

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Abstract

Background: The plant *Senna obtusifolia* is of the kingdom plantae, family fabaceae, found Northern Nigeria. It uses are in management of dysentery and diarrhea. **Objective:** To propose an effective processing method for safe utilization of sickle pod seeds. **Methods:** For five days, *S. obtusifolia* seeds were fermented using *Aspergillus niger* at room temperature. *S. obtusifolia* seeds were also toasted for 10 minutes at 85°C. **Result:** 7.45 mg/100g moisture, 33.33 mg/100g crude protein, 17.25 mg/100g lipid, 9.49 mg/100g dietary fiber, 4.50 mg/100g ash, 37.99 mg/100g nitrogen free extract were all found in raw *S. obtusifolia* seeds. Raw *S. obtusifolia* seeds had 3.95 mg/100g tannins, 3.74 mg/100g phytates, 2.13 mg/100g oxalates and 2.62 mg/100g saponins, 11.23 TUI/mg trypsin inhibitor and 5.77 IC₅₀ (mg/mL) alpha amylase inhibitor. Fermentation increased the crude protein of *S. obtusifolia* seeds but reduced NFE, moisture, lipid and dietary fibre similar to toasting plus fermentation. Toasting reduced moisture, dietary fibre, crude protein but increased lipid, ash and NFE. Fermentation reduced the tannins by 38 %, phytates by 84 %, oxalates by 36 %, saponins by 37 %, trypsin inhibitor by 12 % and α -amylase inhibitor to 6.40 IC₅₀ (mg/mL) while toasting reduced tannins by 63%, phytates by 89%, oxalates by 61 %, saponins by 66 %, trypsin inhibitor by 40 % and α -amylase inhibitor to 13.05 IC₅₀ (mg/mL). Toasting/fermentation reduced tannins by 70 %, phytates by 93%, oxalates by 54 %, saponins by 91 %, trypsin inhibitor by 43 % and α -amylase inhibitor to 19.54 IC₅₀ (mg/mL). Fermentation reduced all the total mineral contents, toasting increased Fe, Mg, K and Na but reduced Ca and Zn. Toasting/fermentation reduced Ca, Mg, Zn, K and Na but increased Fe. **Conclusion:** The proximate composition, anti-nutrients, and mineral content of *S. obtusifolia* seeds were all influenced by fermentation.

Keywords: Anti-nutritional factors, fermentation, minerals contents, proximate analysis, *Senna obtusifolia* seeds and toasting

1. Introduction

Nigeria have long relied on soybeans as a source of protein for both humans and animals (Vadivel *et al.*, 2008), but production is insufficient to meet the ever-increasing population and livestock businesses (Vijayakumari *et al.*, 2007). Pugalenthi *et al.*, (2004) reports that under-utilized legumes of wild plants have great potential for commercial exploitation yet remain ignored. Wild plants are drought tolerant and are collected both in periods of abundance and in times of scarcity. Soaking, sprouting, frying, and fermentation are some of the ways to increase the nutritional value of plant grains (Mohamed Nour *et al.*, 2010; Yagoub & Abdalla, 2007). Fermentation and heat treatment have been shown to make foods containing natural toxicant edible as reported by Ganiyu, (2005). As reported by Frolich (1995) some phytochemicals decrease the bio-accessibility and bioavailability by chelating dietary minerals in the gastrointestinal tract.

For essential part of the global diet, fermented foods account for 20-40% of all human food. Fermented foods has also been discovered to be more palatable around the world (Etong *et al.*, 2014 & Idowu *et al.*, 2013). It has been documented that toasting raw soybeans can effectively reduce the anti-nutritional factors linked to chicken growth retardation. (Akpodietee *et al.*, 2001).



To facilitate the efficient exploitation of legume grains by animal's anti-nutritional factors must be eradicated thus improving their nutrient value. Establishing processing technique(s) is critical to ensuring that it is used to its full potential. Reports from Muhammad *et al.*, (2002) & Ingwey *et al.*, (2010) showed that the proximate composition of *S. obtusifolia* seeds have promising nutritional values as cheaper substitute protein for feedstuff. There appears to be a scarcity of data on the chemical composition of *S. obtusifolia*'s response to various processing treatments.

Hence this research seeks to look at the influence of different treatments on the nutrient value in *S. obtusifolia* Seeds. Data from this paper will aid nutritionists understand the impact of various treating approach on the nutrient content of *S. obtusifolia* seeds also helping them choose the best processing methods to use.

2. Experimental

Collection of matured *S. obtusifolia* seeds within Hayin Mallam Zangon Shanu Sabon Gari Local Government Area Kaduna State was done in the first week of October. The seeds remained dried under the sun which is the conventional and cheap method of drying cereals and legumes in Northern Nigeria and threshed. The identity of the plant was confirmed at the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna state, Nigeria, and a voucher number was deposited (V/N:-573). Grinding of the dry seeds was done with a research laboratory mortar and sieving was done with a 1mm mesh size.

2.1 Processing of Sickle Pod Seeds

Seeds from Sickle Pods are processed according to Oladele *et al.*, (2009) method of toasting was used. The seeds were toasted in an open pan with a hot plate for 10 minutes at 85°C. The seeds were fermented as defined by Gabriel *et al.* (2015) by combining *S. obtusifolia* seeds with water in a 2:1 ratio (1 part *S. obtusifolia* seed to 2 parts water); 0.25 mL cultured *Aspergillus niger* was prepared. The *Aspergillus niger* was obtained from the Ahmadu Bello University Zaria of Microbiology Departmental laboratory, pipetted with a foster pipette, and mixed with water. Before being covered with cotton wool, the mixture was packaged using a plastic bag, then preserved in a laboratory with a constant temperature of 25°C.

The fermentation process took five days to complete. After that, the fermented samples were washed and dried in a room, where they were dried for six (6) days to about 90% dry matter. A hammer mill was used to ground the seeds into powder. To detoxify the seeds again, they were toasted and then fermented for 5 days before being ground into powder in a hammer press.

2.2 Proximate Analysis

2.2.1 Moisture determination

AOAC's (2000) method was used in calculating the presence of moisture in the seeds based on measuring weight loss due to desiccating at a high temperature of about 105°C. Six crucibles were cleaned and dried up in a 105°C furnace before being cooled and weighed empty. Every sample was weighed into their respective watch glasses at a weight of two grams. The watch glasses and their contents were dried to a stable weight in an air circulated oven at 105°C. The crucibles and what they contain were weighed after cooling via desiccating. Using the phrase, the percentage moisture content of each sample was measured.

$$\% \text{ moisture} = \frac{\text{Changes in weight of dried seeds (g)} \times 100}{\text{Incipient seeds weight}} \quad (1)$$

2.2.2 Ash material estimate

AOAC (2000) method for determining ash was followed. Six tiny pots were heated at 550°C in a muffle incinerator. Every crucible was weighed after cooling in a desiccator. Weighing of 1g of the ground seeds into the crucibles was done. Then crucibles and what they contain were then put in a muffle incinerator and heated to 550 °C up to 5 hours. The contents of the crucible were weighed and registered. The following formula was used to measure the percentage of ash.

$$\text{Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of dry sample}} \quad (2)$$



2.2.3 Crude lipid content estimate

AOAC's (2000) method for evaluating the lipid content of each sample was used.

Anti-bumping granules were used in a clean and dry round bottom flask. In a flask equipped with a soxhlet extraction unit, precisely 210cm³ of petroleum ether (60–80 °C) was added. Weighed sample was put in a thimble that had been pre-attached to the Soxhlet extraction device. The circulation of cold water was begun. After that, the heat apparatus was turned on also the rate of heat was changed until the solvent was refluxing at a constant frequency. It took 8 hours to remove the material. The sample was taken out and dried in an oven to a constant weight, then cooled in a desiccator before being weighed again.

As a result, the percentage crude lipid content was calculated;

$$\% \text{ lipid} = \frac{\text{Changes in lipid extraction Weight} \times 100}{\text{Weight of dry seeds}} \quad (3)$$

Changes after extraction, oven dried samples and cooling using a desiccator was used to measure the weight of lipid extracted.

2.2.4 Crude fiber quantification

The AOAC (2000) method was used to calculate crude fiber. A round bottom flask was filled with two grams of the ground sample and a total of 100mL of 0.25M hydrogen sulphuric acid, and then boiling for 30 minutes at a low temperature was done. The acid-free insoluble matter was washed severally in hot water (C1). (C1) was put into a flask with 100 mL of 0.25 M NaOH reagent. After 30 minutes of boiling under reflux, filtration of the mixture was done by suctioning. The residue was made base free with warm water washing, then was dried using oven at 105°C for 24 hours and tagged (C2). (C2) was ashed in a 550°C furnace for two hours. (C2) was then removed from the furnace and measured after cooling using a desiccator and tagged (C3). After that, the content of crude fiber was measured as a weight loss in ashing. The initial sample's weight was used as W.

$$\% \text{ crude fiber} = \frac{C2 - C3 \times 100}{W} \quad (4)$$

2.2.5 Dietary fiber determination

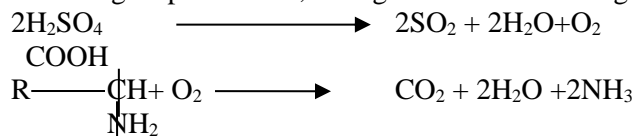
The AOAC (2000) approach was used to assess dietary fiber. In the presence of heat stable alpha amylase, samples were gelatinized after defatting and protease in addition with amyloglucosidase were used enzymatically to digest the sample to eliminate any starch and protein that is digestible. Precipitation of soluble dietary fibre, was done using four volumes of ethanol. Acetone and ethanol was used in filtering and washing off the overall residue. After drying, the residue was measured. Protein and ash content were determined in the remaining material. Complete dietary fiber content was determined by subtracting the quantities estimated for ash, protein and a blank control from the dry weight of the filtered residue.

2.2.6 Nitrogen content and crude protein determination

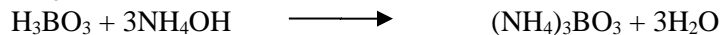
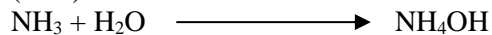
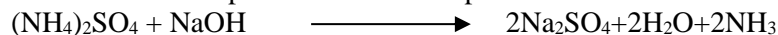
Proteins are large molecules that contain nitrogen, mainly as amino acids, which are the bedrock of proteins. When compared to true protein, nitrogen is used as an index called crude protein. For crude protein determination, the AOAC (2000) Kjeldahl method was used.

Procedures for evaluating

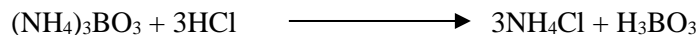
In boiling sulphuric acid, an organic material undergoes mineralization.



B. Ammonium sulphate distillation steps after alkalisation of the Boric acid solution



C. Titration of ammonium with a uniform concentration of hydrochloric acid





Methodology:

Using 100 mL Kjeldahl flask, along with some anti-bumping granules we measured two grams of the sample into it. 15 mL of concentrated sulphuric acid and a gram of catalyst mixture (CuSO₄ and K₂SO₄ in an 8:1 ratio) were added into the flask. Kjeldahl digestion rack was used in heating the flask till the solution turn visible.

After digesting the content of flask it was allowed to cool down and the sample was move quantitatively in a 100 mL volumetric flask, using distilled water to fill it to mark. A Markham semi micro nitrogen steel tube was used to hold 10 ml of the digest, then followed by 10 milliliters of 40 percent NaOH solution. Ammonia was removed from the sample by steam distillation, the sample was then moved to a 100 mL conical flask containing 10 mL of 4% boric acid and a methyl blue indicator was dropped once, a color change was seen from pink to green indicating neutralization reaction. After that, we obtained 30 mL of sample volume. The sample in the conical flask was then titrated with 0.1 Molar HCl. A color change was seen from green to pink indicating an end point and the titre value of each acid distillate was recorded. The following expression was used to measure the nitrogen in each sample:

$$\%Nitrogen = \frac{M \times v \times 14 \times 100 \times 100}{Weight\ of\ sample \times 1000 \times 10} \quad (6)$$

Where M is the HCl molarity and 14 is the nitrogen atomic weight. The cumulative volume of the digest is equivalent to 100 ml. Conversion rate of 100 percent, 10 is the sum of digested food 1000 = litre conversion. The crude protein was measured using the formula: percent Protein = 6.25 x nitrogen.

2.2.7 Nitrogen free extract determination

Subtraction of total percentages of moisture, crude protein, crude lipid, ash, and crude fibre from 100%, we calculated nitrogen free extract (carbohydrate).

Percentage NFE = 100 – (moisture* + protein + lipid + ash + fiber**).

* Indicating moisture was omitted by using a dry matter basis.

** Indicates including fibre here is the best reflect carbohydrate content that is theoretically usable.

2.3 ANTI-NUTRIENT ANALYSIS

2.3.1 Assay for the inhibition of alpha-amylase

A McCue and Shetty protocol (2004) was used. In a tube, 250 liters of extract were combined with 250 liters of 0.02 m sodium phosphate buffer pH 6.9 that contains amylase solution. This solution was pre-incubation of the solution was done for 10 minutes at 25°C before adding 250L of 1% starch solution in 0.02 M sodium phosphate buffer pH 6.9 at timed intervals and incubating at 25°C for another 10 minutes.

After incubation, it was stopped by mixing 500 mL of dinitrosalicylic acid (DNS) reagent.

After that, the tubes were boiled for 5 minutes and then cooled down at ambient temperature. The absorbance was taken at 540 nm with a spectrophotometer after the reacting mixtures were diluted using 5 mL distilled water. A regulation was rendered by adopting the same method but substituting distilled water for the extract. Individual blanks were made individually needed for correcting the background absorbance.

The mixture was instantly inserted into a water bath containing a color reagent and a starch solution. Activity inhibition for alpha-amylase was measured using inhibition proportion. The absorbance was taken at 540 nm using a spectrophotometer after the reaction mix was watered down with 5 mL distilled water.

$$\%Inhibition = \left\{ \frac{A\ control - A\ samples}{A} \right\} \times 100 \quad (7)$$

Where A *control* denotes each control's absorbance and A *samples* denotes each sample's net absorbance. The following equation was used to measure the net absorbance of each sample:

A sample is the same as a test – a void.

Each test's absorbance is A test, and each blank's absorbance is A blank.

The extract concentrations that inhibit enzyme activity by 50% (IC₅₀) was calculated.

2.3.2 Inhibition of trypsin determination

Onwuka's (2005) procedure for evaluating trypsin inhibition was used.



Sample extraction: Ground sample of 1 g was taken and mixed with 50 cm³ of 0.5 M NaCl solution. The mix was then centrifuged after 30 mins of stirring at ambient temperature. Whatman filter paper was used to filter the supernatant. The assay was performed on the filtrate (extract volume).

Normal trypsin and substrate preparation:

1 mg/mL trypsin in 0.1 M HCl was prepared, as well as 1 percent casein substrate in 0.1 M phosphate buffer pH 7.7. 1 cm³ of trypsin inhibitor was applied to 2 cm³ of trypsin regular solution and incubated for 10 minutes at 37°C. In a test tube, a blank of 5 cm³ substrate was prepared (without trypsin inhibitor extract added). A 3 cm³ 5% TCA was used in stopping the reaction in the test tube after 10 minutes. The absorbance was then measured spectrophotometrically at 410 nm after it was filtered.

The equation below was used for calculating trypsin inhibition (TUI) per unit weight of the sample.

Method of Calculation:

$$(b-a)/0.1 \times F = \text{TUI/mg}$$

Where b represents the absorbance of the test sample solution.

A = the blank's absorbance

$$(1 \times V_f/V_a \times D)/W \quad F = (1 \times V_f/V_a \times D)/W$$

Where W denotes the sample weight.

V_f denotes the total amount of extract used in the experiment.

V_a = regular volume

D = dilution factor

2.3.3 Phytic acid determination

Lucas and Markaka's method for evaluating phytic acid was used (1975). Two grams of the powdered seeds was taken into a 250 mL conical flask, drenched in 100 mL of 2 percent HCl acid for three hours, then filtering using a thick layer of harden filter paper, 50 mL of each filtrate in a 250 mL beaker, and 100 mL of distilled water added in each case to achieve proper acidity.

Each solution was titrated with a regular iron (III) chloride solution containing 1.95 mg of iron/mL and ten milliliters of 0.3 percent ammonium thiocyanide solution as an indicator. The end result was a brownish color that lasted for 5 minutes. The formula below was used in calculating phytic acid:

$$\text{Phytic acid} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{Y \times 1.19 \times 100}{\text{Sample weight}} \quad (8)$$

Where Y is the titre value multiplied by 1.95mg.

2.3.4 Oxalates determination.

Oke's procedure was used to assess oxalate (1965). Into a 250 mL capacity conical flask, 2g of the sample was measured into it for calculating total oxalic acid content. After which, 10 mL 6M HCl acid was added alongside 190 mL distilled water. The mix was warmed inside a boiled water for about a one hour, and it was allowed to cool down, poured inside a 250 mL volumetric flask, followed by dilution to volume, and then filtered. Four drops of methyl red indicator were added; ammonia was then added making the solution turn to faint yellow. Heating of solution was done at 100°C, allow to settle down and ferrous iron-containing precipitate was extracted via filtration. The filtrate was then boiling of the filtrate was done with the addition of 10mL of 5% CaCl₂ alongside 10mL of water with constant shaking. The solution was kept overnight. Filtering of the reaction mixture was done with Whatman No. 4 filter paper.

Washing of the precipitate was done using distilled water severally, before transferring it to a beaker and 5mL of 25% sulphuric acid was dissolved inside the beaker. The solution gotten was kept at 80°C and used for titration against 0.5 percent potassium permanganate until a pink color that lasted for about one minute was observed. A blank was run for the test study. The sample oxalate content of was determined with the equation below, this was based on the amount of used potassium permanganate: where 2.24 mg oxalate is equal to 1 mL potassium permanganate.



2.3.5 Tannins determination

The amount of tannins in the sample was measured using Glick and Joslyn's process (1970). Two grams of the powdered seeds (2 g) were poured into a 50 mL distilled water inside a beaker and was heated to 60°C. It was then purified and the residue was thrown away. A ten (10mL) solution made of 4 percent copper acetate was added onto the hot filtrate and boiling was done for another extra ten minutes.

Filtering was done to obtain precipitate after which the filtrate was thrown away. The residue was dried using filter paper, and the dried sample was scraped off the filter paper into a crucible that is pre-weighed (W). A muffle furnace was used in incinerating the sample crucible at 550°C, allowed to cool down inside a desiccator and reweighed as (W1). The tannin was calculated as the difference between the weight before ashing and the ash residue after ignition.

2.3.6 Saponins' Determination

AOAC (1990) approach was used to determine the saponin content of *S. obtusifolia* seeds. Acetone was used in extracting crude lipid from the sample, while methanol was used in extracting saponins. Filter paper was used to fold 2g of the sample and then placed in a thimble before being collected in a Soxhlet extractor by refluxing. A 250 cm³ round bottomed flask containing a 200 cm³ acetone was used for extraction for three hours, after which another extraction using 100 cm³ methanol in a 150 cm³ flask was done for another 3 hours. The difference between weight of the flask before and after the second extracting procedure was recorded. The flasks were oven-dried after the second extraction; it was cool to a room temperature and weighed to recover the methanol. The equation below was used in calculating the saponins content of the sample:

Saponins: (mg/100g) = $A - B \times 100/S_m$

Where A is the mass of the flask, B is the mass of the empty flask, and S_m is the mass of the sample.

2.4 Minerals Analysis

Atomic absorption spectrometry was used to evaluate iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg), while flame photometry was used to determine sodium (Na) and potassium (K) according to the AOAC process (2003).

DIGESTION OF SAMPLE: Roughly (1.0 g) of powdered sample was put in a digesting glass tube for wet digestion. The samples were combined with 12 milliliters (12 mL) of HNO₃ and left at ambient temperature overnight. The sample mix was then combined with 4.0 mL perchloric acid (HClO₄) and left in the fumes chamber to undergo digestion. The temperature was steadily raised from 50°C to 250–300°C. The digested mixture took about 70-85 minutes, as seen by the presence of white fumes. The reaction mixture in the tubes were moved to 100 mL volumetric flasks after cooling, and made to 100 mL with distilled water. The wet digested solution was transferred to bottles that were clearly branded and processed for mineral analysis. The elements' absorption measurements for the samples were read out as part of the procedure. Each mineral was analyzed with different electrode lamps.

Before and during the determination, the AAS apparatus was checked for regular solutions of each mineral to ensure that it was operating properly. For all minerals except Mg, the dilution factor was 100. To calculate Mg, the solution was further diluted using 0.5 mL original solution and adequate distilled water was added to make up the volume to 100 mL. In order to differentiate Ca from Mg for calcium (Ca) determination, 1.0 mL lithium oxide solution was applied to the initial solution.

The following formula was used to measure the mineral concentration:

$$M_w = \text{absorbance (ppm)} \times \text{dry wt.} \times D / \text{wt. of sample} \times 100$$

Where M_w is the Concentration of minerals

The dilution factor is denoted by the letter D.

For the determination of Na and K, the same wet digested sample solutions were used as in AAS.

For both Na and K, standard solutions of 20, 40, 60, 80, and 100 milli equivalent/L were used.

The method for calculating total minerals is the same as that defined in AAS.

2.5. Statistical Analysis

The data obtained were presented as mean \pm standard deviation (SD). The data recorded were analyzed using (ANOVA). Duncan multiple range test was used for multiple mean comparison tests to determine differences between the means. Significance at 0.05 level of probability ($p < 0.05$) was accepted.

3. Results

As shown in table 1, contained in the raw seeds, the proximate composition of *S. obtusifolia* seeds shows that percentage crude protein increased in fermented seeds but decreased significantly in toasted and toasted followed by fermentation processed seeds. There appeared to be a large alteration in crude protein between processing technique ($< P0.05$) in all of the processing methods. When toasted seeds were compared to raw and fermented seeds, the percentage moisture decreased in the toasted seeds but increased in the toasted/fermented seeds.

In contrast to raw *S. obtusifolia* seeds, the percentage crude lipid within fermented, toasted, and toasting plus fermented seeds decreased significantly. When toasted, toasting plus fermented seeds were compared to fermented and raw seeds, the percentage ash increased dramatically. In contrast to raw *S. obtusifolia* seeds, the percentage crude fibre within toasted and toasting plus fermented seeds increases significantly while the fermented seeds decrease significantly. When compared to the raw seeds, the percentage dietary fibre in fermented, toasted, and toasting plus fermented seeds is significantly lower. When compared to raw seeds the percentage nitrogen free extract shows a significant increase in toasted seeds, toasting plus fermented seeds, while fermented seeds showed a significant decrease.

Table 1: Impact of Processing on Proximate Composition of *S. obtusifolia* Seeds.

	Moisture (g/100g)	crude lipid (g/100g)	ash (g/100g)	Crude protein (g/100g)	Crude fibre (g/100g)	Dietary fibre (g/100g)	Nitrogen free extract (g/100g)	Calorific value (kcal/100 g)
Raw	7.45 \pm 0.1 3 ^b	17.25 \pm 0.0 5 ^d	4.50 \pm 0.3 5 ^{ab}	33.33 \pm 0.0 2 ^c	15.38 \pm 0.3 5 ^b	9.49 \pm 0.0 2 ^d	37.99 \pm 0.1 6 ^b	440.53
Fermented	7.44 \pm 0.1 4 ^b	16.37 \pm 0.3 0 ^c	4.10 \pm 0.0 7 ^a	36.86 \pm 0.0 6 ^d	13.39 \pm 0.0 1 ^a	6.72 \pm 0.0 6 ^a	35.06 \pm 0.2 1 ^a	435.01
Toasted	3.23 \pm 0.3 5 ^a	10.86 \pm 0.0 6 ^a	4.82 \pm 0.0 3 ^{bc}	31.17 \pm 0.3 7 ^b	23.00 \pm 0.1 4 ^d	8.27 \pm 0.0 8 ^c	50.03 \pm 0.3 8 ^d	422.54
Toasted/ Fermented	7.79 \pm 0.0 8 ^c	15.21 \pm 0.1 5 ^b	5.33 \pm 0.1 3 ^c	25.39 \pm 0.6 2 ^a	19.95 \pm 0.5 0 ^c	7.68 \pm 0.2 2 ^b	46.04 \pm 0.0 8 ^c	422.61

Values are expressed as mean \pm standard deviation and values with distinct superscript on the same column are significantly different ($p < 0.05$).

As seen in table 2, anti-nutrients were found to be reduced to extremely low levels after toasting.

Raw *S. obtusifolia* seeds had 3.95 mg/100g tannins, 3.74 mg/100g phytates, 2.13 mg/100g oxalates and 2.62 mg/100g saponins, 11.23 TUI/mg trypsin inhibitor and 5.77 IC₅₀ (mg/mL) alpha amylase inhibitor. Fermentation reduced the tannins by 38 %, phytates by 84 %, oxalates by 36 %, saponins by 37 %, trypsin inhibitor by 12 % and α -amylase inhibitor to 6.40 IC₅₀ (mg/mL) while toasting reduced tannins by 63%, phytates by 89%, oxalates by 61 %, saponins by 66 %, trypsin inhibitor by 40 % and α -amylase inhibitor to 13.05 IC₅₀ (mg/mL). Toasting/fermentation reduced tannins by 70 %, phytates by 93%, oxalates by 54 %, saponins by 91 %, trypsin inhibitor by 43 % and α -amylase inhibitor to 19.54 IC₅₀ (mg/mL).

Amylolytic action was appraise on the base of IC₅₀ it was found to be 5.77, 6.40, 13.05 and 19.54 for the raw, fermented, toasted and toasted/fermented seeds respectively. Processing remarkably influenced the amylyolytic activity of *S. obtusifolia* seeds.

Table2: Effect of processing on the Anti-nutritionalContents of *S. obtusifolia* Seeds

	Phytate (g/100g)	Tannins (g/100g)	Oxalate (g/100g)	Saponins (g/100g)	Trypsin Inhibitor (TUI/mg)	α -amylase inhibitor IC ₅₀ (mg/mL)
Raw	3.74±0.03 ^a	3.95±0.21 ^a	2.13±0.01 ^a	2.62±0.68 ^a	11.23±0.03 ^a	5.77±0.50 ^a
Fermented	0.58±0.09 ^a	2.45±0.49 ^{bc}	1.36±0.28 ^b	1.65±0.35 ^{ab}	9.92±0.01 ^b	6.40±1.57 ^b
Toasted	0.42±0.06 ^{ab}	1.48±0.71 ^{bc}	0.83±0.07 ^c	0.90±0.07 ^b	6.72±0.01 ^c	13.05±0.88 ^c
Toasted/ Fermented	0.26±0.07 ^{ac}	1.18±0.11 ^c	0.99±0.15 ^c	0.23±0.04 ^{bc}	6.39±0.01 ^d	19.54±1.46 ^d

Values are expressed as mean ± standard deviation and values with distinct superscript in the column are statistically significant (p<0.05).

From table 3, when we compare the raw seeds, the iron content of toasted and toasted/fermented seeds is significantly higher, while the iron content of fermented processed seeds is remarkably lower. In contrast to raw seeds, the processing techniques three resulted in a substantial decrease in calcium content. When toasted seeds were compared to raw seeds, the Mg value of the toasted and toasted/fermented seeds increased significantly, while the Mg content of the raw seeds decreased remarkably. When compared to toasted and toasted/fermented seeds, the Zn content of fermented seeds is slightly lower, whereas raw seeds display no substantial difference. When toasted seeds were compared to raw seeds, the K value was remarkably higher in the toasted seeds, but significantly lower in the fermented and toasted/fermented seeds. There is no substantial difference in Na between the processed and raw seeds.

Table 3:Mineral Contents and the Impact of Different Processing Methods of *S. obtusifolia*Seeds (ppm)

	Fe	Ca	Mg	Zn	K	Na
Raw	0.88±0.046 ^a	81.60±0.01 ^a	29.98±0.21 ^a	0.49±0.03 ^b	0.94±0.01 ^a	0.32±0.39 ^a
Fermented	0.62±0.02 ^b	64.05±0.51 ^b	23.06±0.40 ^b	0.30±0.01 ^a	0.72±0.02 ^b	0.04±0.01 ^a
Toasted	1.48±0.04 ^c	39.65±0.66 ^c	31.06±0.37 ^c	0.43±0.13 ^b	1.16±0.07 ^c	0.34±0.39 ^a
Toasted/ Fermented	2.87±0.05 ^d	79.98±0.20 ^d	24.67±0.35 ^d	0.46±0.01 ^b	0.53±0.01 ^d	0.07±0.00 ^a

Values are expressed as mean ± standard deviation and values with different superscript in the column are statistically significant (p<0.05).

Table 6 displays the measured phytate/zinc molar ratios for raw and processed *S. obtusifolia* which were found to be above the suggested critical level (15 being the best for zinc absorption) (Morris and Ellis, 1989). Low zinc bioavailability is linked to ratios below 15 (Morris and Ellis, 1989). Phytic acid to zinc mole ratios of greater or equal to 15, 5–15, and 5 is equal to zinc bioavailability as low (10–15%), moderate (30–35%), and high (50–55%), respectively, are the WHO cut-offs (Goicoechea and W.H.O, 1996).Molar Proportion of Calcium× phytate/ zinc molar proportions of raw and processed *S. obtusifolia* were higher than dangerous threshold (0.5 mol/Kg) as indicated in Table 6 insinuating that calcium interfering does occurs thereby impairing zinc bioavailability.

Table 4: Raw and Processed Anti-nutrient/Mineral Molar Ratios *S. obtusifolia* Seeds

	[Phy]/[Zn] ¹	[Ca]/[Phy] ²	[Phy]/[Fe]	[PhyXCa]/[Zn]	[OX]/[Ca]
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RAW	756.06	0.34	359.62	622.82	11.87
FERMENTED	191.51	1.82	79.16	123.83	9.67
TOASTED	96.75	1.55	25.37	38.73	9.53
TOASTED/FERMENTED	55.99	5.07	7.67	45.21	5.64
CRITICAL VALUES	15	6.0	1.0	0.5	2.5

¹(mg phytate/MWofphytate :mg Zn/MWof Zn)

²(mgCa/MWofCa :mgphytate/MWof phytate)

(mg phytate/MWofphytate :mg Fe/MWof Fe)

(mol/Kg of phytate × mol/Kg of Ca : mol/Kg of Zn)

(mg oxalate/MW of oxalate :mg Ca/MW of Ca).

4. Discussions

The fermented seeds had the highest mean value of crude protein among the processed *S. obtusifolia* seeds. This pattern may be due to fermentation which activated hydrolysis, thereby increasing the quality of crude protein (CP), which associated with microbial fermentation process. According to Yashimet *et al.* (2009) and Tamburawa (2010), fermentation duration usually increases the crude protein levels. The smaller crude protein value in toasted seeds perhaps was due to protein adulteration. When the seeds of mucuna and parkia were roasted, Vadivel and Pungalenthi (2007) and Tamburawa (2010) found lower crude protein values. This may be as a result of an unfavorable effect caused by denaturing of the protein in the seeds during heating process, which changes the chemical composition of proteins and reduces their nutritional value (Liener, 1980; Van der Poel, 1989; Norton, 1991).

Related study of Obadinaet *al.*, (2013) that fermenting soymilk seeds decreases their lipid content. The amount of lipid in the sample decreased when it moved from raw to toasted, but it decreased even further when it was fermented and processed twice. Tamburawa (2010) discovered that toasting lowers crude lipid levels. The loss of volatile lipid compounds in the toasted seeds may be responsible for the lowest fat content recorded. Since the seeds have low moisture content, they will store well and nutrients will be well retained. According to Onyeikeet *al.* (1995), increased moisture content causes food spoilage due to microbial behavior. Low moisture content inhibits microbial growth and thus serves as a food preservation indicator (Chew *et al.*, (2011).

The moisture content of toasted *S. obtusifolia* seeds was lower than that of fermented seeds, which we were expecting owing to heat application. These findings suggest that toasting can help preserve the quality of *S. obtusifolia* seeds. The moisture content of the toasted/fermented *S. obtusifolia* seeds sample was higher than that of raw seeds, which is expected given that seeds are exposed to higher temperatures during toasting and fermentation. This may be because the seed coat became softer after fermentation, which allows them to absorb more water. The increase in moisture content after fermentation is consistent with findings of Adegbehingbeet *al* (2017) and this could be due to secretion of free water molecules attributed to the activities of the *Aspergillus niger* in the medium. The result of the crude ash content revealed that the fermented seeds of *S. obtusifolia* recorded the lowest ash contents this may be ascribed to straining. This corresponds to the work of Igbabulet *al.*, (2014) that documented a decrease in the ash content of cocoyam flour and ascribed it to straining of dispersible mineral contents within fermenting environment. The small percentage of crude ash within the raw seeds is perhaps caused by anti-nutrients' impact on the mineral content of the seeds, where anti-nutrients such as phytate can chelate mineral elements and render them metabolically inaccessible. Anti-nutrients may interfere with mineral bioavailability, according to previous research (Alonso *et al.*, 2001; Anigoet *al.*, 2009). However, since anti-nutritional factors, like lectin and protease inhibitors, are heat labile, processing methods like toasting have decreased anti-nutrient levels, improving



mineral bioavailability as evidenced by the increase in crude ash values of toasted and toasted/fermented seeds. Toasting was found to increase crude fibre content, which may be attributable to insoluble dietary fibre being converted to short length chains or units (Yuan *et al.*, 2005). The decrease in crude fibre in fermented seeds may be attributable to enzymatic breakdown of the fibre by the fermenting *Aspergillus niger*, which is consistent with the findings of Ojokoh *et al.*, (2015), who found a reduction in crude fibre in a sorghum and pumpkin blend after fermentation.

The nitrogen free extract of *S. obtusifolia* seeds was significantly reduced after fermentation. activation of amylolytic enzymes, usually leads to hydrolysis of starch and other multiplex carbohydrates to glucose, may explain the apparent decrease in nitrogen free extract. This is consistent with the findings of (Ojokoh&Fagbemi 2016), who found that the carbohydrate content of cowpea-plaintain flour blend and popcorn-groundnut flour was reduced after fermentation. The nitrogen free extract of toasted and toasted/fermented *S. obtusifolia seeds* increased in this research, which is linked to the softening and breaking down of the cellulose, making the starch more available (Agiang *et al.*, 2010).

Heat treatment was the most effective approach for reducing the tannin content of the crop. The existence of microbes having tendency of liberating tannase enzyme, which can deteriorate tannin constituents of the seeds to a certain value, may be the reason for the decrease in tannin. This is similar to the report of (Enujiugh&Akanbi 2015) who found the tannin content of African oil bean seed reduced. The secretion of the phytase enzyme by *Aspergillus niger* per chance is behind the decrease in phytate constituent of fermented seeds. Phytase can hydrolyze phytate (Oboh *et al.*, 2003), and phytase activity has been observed in a broad variety of microflora (Ojokoh 2005), lowering the phytate content of seeds. Since more moisture was lost during toasting, the phytate content was also reduced significantly. This is consistent with Khan *et al.* (1990) and (Onimawo and Akpojowo's., 2006) findings for findings for roasted maize and toasted pigeon pea, which found that the higher the moisture content, the greater the phytate loss. The lowest phytate content was found in the toasted/fermented seeds, which could be due to the dual action of heat during toasting and phytase during fermentation.

The results show that the fermentation method reduced saponin content, which is consistent with Akande and Fabiyi's (2010) paper, which stated that germination has been shown to be an effective treatment for removing anti-nutritional factors in legumes by mobilizing secondary metabolic compounds that are considered reserve nutrients. However, heat was to greater extent efficacious in lessening the saponin constituent of *S. obtusifolia* seeds, as seen in the toasted and toasted/fermented seeds, indicating that saponins are heat labile.

Fermentation greatly reduced oxalate, while toasting was the most effective process for reducing oxalate. Obasi and Wogu (2008) found a major reduction in the oxalate content of yellow maize after soaking it in water for 12 hours. Toasting, on the other hand, greatly decreased oxalate content; the higher oxalate loss in toasted, toasted/fermented *S. obtusifolia* seeds could be due to oxalate annihilation at high temperature.

The seeds trypsin inhibitor activity was decreased after fermentation. Fagbemi *et al.* (2005) found a similar pattern in the parameter for bread nut, cashew nut, and fluted pumpkin. Previous research has found substantial reductions in trypsin inhibitor activity content in cereal and legumes (Mbithi-Mwikya *et al.* 2001; Ojokoh *et al.* 2012). Trypsin inhibitors have been reported to be reduced or removed by *R. oligosporus* (Fadahunsi and Sani, 2010). Toasted and toasted/fermented seeds had a significant effect in the reduction of trypsin inhibitor which could be attributed to the unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes (Sathe *et al.*, 1984).

The decreased amylolytic activity of the fermented seeds with a higher IC₅₀ value than the raw seeds coincides with previous reports that the amount of anti-nutrients in legume seeds decreases during soaking (Rao and Deosthale, 1982; Mulimani and Supriya, 1993), which may account for increased amylolytic activity, i.e. increased starch



digestibility of the soaked seeds. Swelling and rupturing of starch granules, which facilitate more randomized configuration for alpha-amylase to affect hydrolysis, may also be responsible for the increased amylolytic activity of the toasted and toasted/fermented seeds with higher IC_{50} values. With similar findings from Kataria and Chauhan (1988) and Kataria *et al.* (1992) that observed a 35 to 48 percent increase in amylolytic activity in amphidiploids (black gram x mungbean) after cooking. Sorghum amylase inhibitory activity was found to be destroyed by overnight soaking followed by heat treatment (Mulimani and Supriya, 1993).

The substantial low value of potassium and iron constituent of the seeds after fermentation has been recorded in several studies and is ascribed to their utilization by some fermenting microbes for growth development. The reduction of minerals is consistent with the results of (Alemu, 2009; Afify *et al.* 2012; Difo *et al.* 2015), who attributed it to mineral leaching in fermenting water. It may also be as a result of fermenting microorganisms using these minerals (Zamora and Fields, 1979). Heat application causes a decrease in minerals, which is consistent with previous research (Saharan *et al.* 2001; Alemu 2009 and Afify *et al.* 2012). This may be due to an increase in the solubility of these minerals when heat is applied (Saharan *et al.*, 2001).

The critical phytate:zinc molar ratio may also be influenced by dietary calcium levels because of the kinetic synergism between calcium and zinc ions, which results in a Ca : Zn : Phy complex that is less soluble than phytate complexes formed by either of the ions alone (Lopez *et al.*, 2002), signifying that Ca : phy/Zn molar ratio is a superior forecaster of zinc bioavailability than Phy : Zn molar ratio alone. Molar Proportion of Phytate to Iron as seen in Table 6, phytate/iron molar proportion were >1 (a sign of low iron bioavailability) for all raw and processed *S. obtusifolia* seeds. This ascribing to excessive phytate content and inadequate phytate breakdown following heat application, as related by Akin-Idowu *et al.* (2009). Molar Proportion of Calcium to Phytate: The calcium/phytate molar proportions as seen in both raw and processed *S. obtusifolia* seeds were <6 , this is thought to be detrimental to calcium absorption. Akin-Idowu *et al.* (2008), predicting a low calcium bioavailability

In addition to phytic acid, insoluble oxalic acid, which forms insoluble salts, interferes with the absorption of divalent metals, especially calcium (Hassan *et al.*, 2011). All of the raw and processed seeds had oxalate/calcium molar ratios above 2.5, which is established to remarkably reduce calcium bioavailability, implying that they are poor calcium bio-resources for the local population.

5. Conclusion

S. obtusifolia seeds had low fat and moisture content, suggesting that they could be preserved for a long period, according to the results of this report. Processed *S. obtusifolia* seeds are high in microelements and macro elements, especially protein, and can be utilized in places where other legumes are not available or affordable. The anti-nutritional components were found to be reduced by the processing treatment used in this research. However, heating can inactivate enzyme inhibitors, and heating can also result in the development of insoluble protein-tannin complexes, which is likely to be the basis of the low *in vitro* protein content.

Fermentation in the presence of *A. niger* greatly increased the sum of all amino acids as well as crude protein content in the seeds. Using *S. obtusifolia* seeds as a mixture in recipe creation could aid in tackling malnutrition in Nigeria's agrarian areas, particularly during times of food scarcity. Other heating methods (boiling, steaming, microwave, and autoclaving) should also be examined to see what impact they have on the nutrients composition of *S. obtusifolia*.

CONFLICT OF INTERESTS

The authors declared no conflict of interests



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