

# Effects of Processing Methods on Anti-Nutrients and Proximate Compositions of Aizien *B. Senegalensis* Seed as Alternative Protein Source for Fish Feed

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## Abstract

The study investigated the nutritional suitability *Boscia senegalensis* (Aizen) as alternative for protein in the formulation of diet of fish diet. The seeds were subjected to different processing methods to reduce levels of the most important antinutritional factors (ANFs) in the seeds. Standard methods of analysis were used for determination of anti-nutrients and proximate compositions of the processed seeds. Analyzed anti-nutritional factors of the differently processed *B. senegalensis* seeds indicated that soaking was the best in reducing the anti-nutrients with lowest levels of Saponin (2.10 mg/g), Phytic acid (0.10 mg/g), Flavonoids (72.8 mg/g), Tanin (0.6 mg/g) and Alkaloids (1.02 mg/g). Proximate analysis showed that crude protein (CP) composition of the differently processed *Boscia senegalensis* seed ranged from 28.48 – 31.50%. Soaked sample had the highest CP (31.50%) while the boiled sample was the lowest (28.48%). Decreased lipid and fibre contents were observed in the boiled, fermented and the soaked seeds respectively. The study recommends soaking as best treatment for *B. senegalensis* seeds while proximate analysis revealed that treated *B. senegalensis* seed could be good alternative source of protein for fish feed formulation.

## Introduction

Increased population growth has been associated with increased food demand especially protein which is necessary for tissue growths and repair. However, production of important sources of protein such as fish has continued to suffer setback due to high cost feed. This situation prompted nutritionists to explore alternative sources of protein for both human and fish feed. Aizien is one of such non-conventional sources with potentials for inclusion in to both human and fish feed (Diago *et al.*, 2020).

Aizien (*B. senegalensis*) is a drought resistant shrub whose seeds are used in West Africa as food. It grows wild in Algeria, Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ghana, Guinea, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, Somalia, Sudan and Togo (NRC, 2008). According to IBGR (1984) *B. senegalensis* is an evergreen shrub or small tree less than seven meters high, native to the sahilian zones of Africa. It is well known in Northern part of Nigeria as famine food. This little desert shrub seems at first to offer little direct economic benefit. But during the 1984-85 Sudan famine, its seed effectively became the staple

of market society, replacing millet and sorghum (FAO, 1989).

Aizen's nutritional content is poorly known but people existing in the extreme climates where the plant grows can rarely expect foods of high nutrition. The pulp reportedly contains good calcium, phosphorus, iron, and some B vitamins. NRC (2008) reported that its main value is in supplying vitamins A and C. Another use of *B. senegalensis* also indicates that the leaves and berries are browsed by camels, goats and sheep during the end of dry season and at the beginning of the rainy season (Edigwe, 2014). Furthermore, *B. senegalensis* is used as browse by camels, goats, and sheep during the end of the dry season and the beginning of the rainy season, as indicated by Edigwe (2014). The seeds can be eaten raw but are acidic and are usually consumed after long preparations and cooking. Berries are dried in the sun, pounded to remove the outer seed coat and then soaked again for several days. Furthermore, *B. senegalensis* is used as browse by camels, goats, and sheep during the end of the dry season and the beginning of the rainy season, as indicated by Edigwe (2014) (Loren *et al.*, 2015). The seeds are quite nutritive and contain about 25- 30% crude protein (Abdel-rahman & Ismail, 2013). Powdered seeds are mixed with millet (*Eleusine coracana* Gaertn.) flour and added to soups or cereals (NRC, 2008). However, despite their widespread use and notable value for saving lives during times of distress, these wild cereals and legumes have been largely overlooked by both food and plant scientists. This study was aimed at evaluating the nutritional composition of Aizen, *B. senegalensis* for possible use as food and in fish feed formulation.

## Materials and Methods

### Source, Preparation and Processing of the Aizen *B. senegalensis* Seeds

The *B. senegalensis* seeds were obtained from rural women in Bursari Local Government Area of Yobe State. Two kilogram (2kg) of *B. senegalensis* (Aizen) seeds was sundried for 4 days, thereafter; the seeds were then decorticated using mortar and pistil. After winnowing, the seeds were subjected to the different processing methods below:

#### Boiling

100 g of *B. senegalensis* seeds were boiled in distilled water (100°C) in a seed to water ratio of 1:10 (w/v) for 48 min (Uche *et al.*, 2014). After boiling, the water was drained off and the boiled seeds were sun dried and kept in airtight polythene bag for analysis.

#### Soaking

Approximately, 1kg of raw *B. senegalensis* seeds was soaked in 2.5 litres of water in an open container for

72 hrs with the water constantly changed after every 8 hours. The water was finally drained and the seeds were sun-dried to a constant weight and kept in polythene bag at ambient temperature until required for analysis.

#### Fermentation

1kg each of *B. senegalensis* seeds were soaked in 2.5 litres of water for 24 hrs. Thereafter, the water in the container was drained and the seeds were packed in an air-tight polythene bag allowing them to ferment for 3 days (72 hrs), until they reached a consistent weight on a concrete floor and stored in polythene bag at room temperature until required for analysis.

#### Washing with Water

Five hundred grams (500g) of the *B. senegalensis* seeds were thoroughly washed by squeezing the seeds between two palms for four minutes and the water in the container was decanted. The same process was repeated eight times and the resultant seeds were sundried to a constant weight and packaged in polythene bag for analysis.

#### Proximate Analysis

The proximate analysis of the ingredients was carried out according to the methods outlined by the Association of Official Analytical Chemists (A.O.A.C, 2000).

#### Moisture Content

Two grams of the dried ground sample were weighed into a crucible and placed in an oven at a controlled temperature of 60 °C. The sample was allowed to dry in the oven to a constant weight. The percentage moisture content was then expressed as the percentage of the original weight of the sample. The moisture content was thus calculated:

Where, W1=mass of dry crucible,  
W2= mass of crucible + sample before drying  
W3= Mass of crucible + Sample after drying

#### Crude Protein

0.6g was weighed into dried kjeldahl flask and few drops of water was added to the sample, followed by 3ml of concentrated H<sub>2</sub>SO<sub>4</sub> acid and addition of 0.5g of CuSO<sub>4</sub>. The content of the flask was then digested in a fume cupboard with occasional stirring until a clear solution was obtained. The flask was allowed to cool and a small quantity of distilled water was added. The digest was transferred into 100ml volumetric flask and the initial volume recorded. The mixture was shaken thoroughly to for distillation.

The distillation apparatus was steamed for 30 minutes. 10ml of the digest was added to the micro distillation apparatus using a funnel and 10ml of 50% NaOH solution was added. At the end of the digestion, the flask was cooled and the sample was quantitatively transferred to a 100ml volumetric flask containing 10ml of 4% boric acid and two (2) drops of mixed indicator (bromocressol green/methyl red). 10ml of the digest was pipetted into Markham steam distillation chamber and 10ml of 40% sodium hydroxide added. The sample was steam distilled, liberating ammonia into a 100ml conical flask containing 10ml of 4% boric acid and a drop of methyl red and methyl blue (2:1) until indicator changed colour from pink to green and 30ml distillate was collected. The content of the conical flask (ammonium borate) was titrated against standard 0.1M HCl until the end point was indicated by a changed from greenish to pink colour.

%Nitrogen =

Where

M = Molarity of HCl

V = Volume of acid used

Wt = Weight of sample

14 = Atomic weight of nitrogen

100 = Total volume of digest

100 = % Conversion

1000 = Conversion to litre

10 = Volume of digest taken

% Crude protein =  $6.25 \times \% N_2$

### Crude Lipid

Ten (10) grams of the dried ground sample was weighed and wrapped with a clean filter paper and placed into the thimble in a soxhlet extractor. A round bottom flask was cleaned, weighed and 120ml of food grade hexane added. The flask was connected to the sample holder of the soxhlet extractor and heated slowly on a mantle for 6 hours. Refluxed hexane was recovered and the flask containing the lipid was dried in the moisture extractor in the oven at 60°C for few minutes to remove any residual solvent. After drying, the flask containing the oil was cooled in desiccators and reweighed. The difference in mass was determined and expressed as percentage of fat.

### Crude Ash

Two grams of the dried sample was measured into a crucible and placed in the muffle furnace at 550°C until it was burnt to ash. The crucible and content were then allowed to cool in a desiccator and weighed. This was done repeatedly until a constant weight of the ash was obtained. The percentage ash content was then expressed as percentage of the original weight of the sample on dry basis. Percentage ash content was thus calculated:

Where W1= Weight of sample analyzed

W2= Weight of empty crucible

W3= Weight of crucible + Ash

### Crude Fibre

Two grams (2 g) of the defatted dried sample was transferred into a 100 ml flask, followed by addition of 200 ml of 1.25% sulphuric acid. The flask was then placed in a digest apparatus on a pre-adjusted hot plate and boiled for 30 minutes with rotation of the flask periodically to prevent solid from adhering to the bottom of the flask. At the end of 30 minutes, the mixture was allowed to stand for one minute, and filtered immediately through the Buchner funnel lined with a muslin cloth. The insoluble matter was washed into the flask for alkali digestion using 0.3 M sodium hydroxide. The digest was boiled for 30 minutes and was allowed to cool for one minute and then filtered using a muslin cloth as before.

The residue was then washed successively with 0.1M HCl and finally with boiling water until it was free of acid. It was then washed twice with alcohol and thrice with ether. The residue or insoluble matter was then transferred into a crucible and dried at 60°C in an oven for 12hrs to a constant weight, cooled and weighed. It was then ashed at 550°C, cooled and weighed. The difference in weight after ashing was then calculated as the fibre content of the sample and was expressed as a percentage of the original weight. The percentage crude fibre content was calculated as as:

$$\text{Fibre} = \frac{W3 - W2}{W1 \times 100}$$

Where, W1= Weight of sample, W2= Weight of sample and crucible after drying at 105°C

W3= Weight of sample (as ash) and crucible after ashing

### Nitrogen Free Extract (NFE)

This was estimated by subtracting the total of moisture, crude protein, crude lipid, ash and crude fibre from dry matter and the remaining value represented the NFE components of the sample.

### Analysis of Anti-nutritional Factors

Triplicate samples of the raw and processed seeds of *B. senegalensis* were analyzed for some an-nutritional factors according to the method described by AOAC (1990) as follows:

### Tannin

Few grams of the ground samples were defatted for 2 hrs using soxhlet extraction apparatus. The residue

was placed in an oven for 24 hrs, retrieved and boiled at 100 °c with 300 ml of distilled water, diluted to 500 ml in a standard volumetric and filtered through non-absorbent cotton wool. A volume of 25 ml of the infusion were measured in to 2litre porcelain dish and titrated with 0.1N oxalic acid until blue solution changes to green, then few drops of 0.1 potassium permanganates was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in the sample, since 0.1N oxalic acid = 0.006235 g tannin.

### Saponin

To determine the saponin content in the samples, a gravimetric method employing the use of Soxhlet extractor and two different organic solvents were used. The first solvent extracted lipids and interfering pigments while the second solvent extracted the Saponin proper.

A known weight of the dried ground sample was weighed and fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol was poured into the flask. The methanol was enough to cause a reflux. The saponin was then exhaustively extracted for 3hours. The flask was re-weighed and difference in weight represents the weight of saponin extracted.

### Phytic Acid (phytate)

To determine the Phytate content of the sample, a known weight of each the ground sample was soaked into 100 ml of 2% HCl in a conical flask followed by addition of 50 cm<sup>3</sup> of 0.3% potassium thiocyanate solution. The mixture was titrated in a standard solution of FeCl<sub>3</sub> until a brownish-yellow colour persists for 5 minutes. The concentration of the FeCl<sub>3</sub> is 1.04% w/v.

Mole ratio of Fe to Phytate = 1:1, Concentration of phytate = Titre value/1000x weight of sample

### Alkaloids

Analysis of the alkaloids content of the seeds samples followed gravimetric method (Harbone, 1973)

as described by Adeniyi *et al.* (2009). Five gram (5 g) was weighed using sensitive weighing balance and dispersed in to 50 ml of 10% acetic acids solution in ethanol. The mixture was well shaken and allowed to stand for 4 hrs before it is filtered. The filtrate was then evaporated to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. Pre-weighed filter paper was used to filter off the precipitate. One percent (1%) ammonium hydroxide was used to wash the filter paper and the filter paper containing the precipitate was dried in an oven at 60 °C for 30 min before been transferred in to desiccators to cool down and reweighed several times until constant weight was achieved and recorded. The weight of the alkaloids was determined by the weight difference of the filter paper expressed as percentage of the sample weight analyzed.

### Results and Discussion

The processing methods used in the present study, *viz a viz* fermentation, boiling, soaking and washing with water, generally reduced the antinutrient compounds in *B. senegalensis* seed meals. The tannin, flavonoids, saponin and alkaloid contents of the samples of differently processed *B. senegalensis* seeds were significantly lower than those recorded for the raw seeds. Among the processing methods, soaking was more effective towards eliminating the anti-nutrients and was able to reduce the tannin and the saponin content of the seed by 62.50 and 65.10% respectively compared to washing with water which was observed to reduce these compounds by 21.88 and 6.82% respectively. The variation may be due the leaching effects of water on these anti-nutritional compounds. Similar trend has been previously reported by Oke *et al.* (2013) on grain legumes (African Yam Bean and Lima bean flours) under different processing methods. Likewise, Osunbitan *et al.* (2015) reported a significant reduction in the identified anti-nutrients across various Cowpea varieties through soaking at different time intervals.

In this study, no significant reductions were observed in the phytic acids composition of the processed seed compared to the raw seeds but values

**Table 1.** Anti Nutritional Contents of Raw and Processed *B. senegalensis* Seed

Anti-nutrients	RBS	BBS	FBS	SBS	WBS
Saponin(mg/g)	6.45±0.01 <sup>a</sup>	4.40±0.03 <sup>c</sup>	4.00±0.10 <sup>d</sup>	2.10±0.03 <sup>e</sup>	6.01±0.02 <sup>b</sup>
Reduction (%)	--	22.50	33.44	65.10	6.82
Phytate(mg/g)	0.63±0.27 <sup>a</sup>	0.23±0.16 <sup>a</sup>	0.12±0.00 <sup>a</sup>	0.10±0.00 <sup>a</sup>	0.40±0.27 <sup>a</sup>
Reduction (%)	--	63.50	80.92	84.13	36.51
Flavonoids(mg/g)	90.55±0.02 <sup>a</sup>	86.08±0.10 <sup>b</sup>	86.00±0.10 <sup>b</sup>	72.80±0.10 <sup>c</sup>	71.45±0.24 <sup>d</sup>
Reduction (%)	--	4.94	5.02	19.60	21.10
Tannin(mg/g)	1.60±0.01 <sup>a</sup>	1.50±00 <sup>a</sup>	0.70±0.01 <sup>c</sup>	0.60±0.01 <sup>c</sup>	1.25±0.01 <sup>b</sup>
Reduction (%)	--	6.25	50	62.50	21.88
Alkaloids(mg/g)	1.70±0.03 <sup>a</sup>	1.14±0.01 <sup>d</sup>	1.31±0.01 <sup>b</sup>	1.02±0.01 <sup>e</sup>	1.22±0.01 <sup>c</sup>
Reduction (%)	--	32.94	22.94	40.00	28.23

Values with different superscripts across each row differed significantly (P≤0.05).

Key: RBS = Raw Boscia Seed, BBS = Boiled Boscia Seed, FBS = Fermented Boscia Seed, SBS= Soaked Boscia Seed. WBS = Washed Boscia Seed

obtained were slightly lower than those determined from the raw seeds. Phytic acid is the major storage form of phosphorus in cereals and legumes which chelates minerals and prevents their intestinal absorption in fish.

Several pre-processing treatments such as soaking, fermentation, germination, treatment of grains with phytase enzyme has been reported to reduce the phytic acid content in grains (Gupta, *et al.*, 2015; Rasane, *et al.*, 2015a; Rasane *et al.*, 2015b). Furthermore, soaking methods reduced the phytate content by 84.92% which could be due to the leaching of this compound to the soaking water. Vidal-Valverde *et al.* (1997) reported significant decreased of 40% in phytate content of soaked Faba beans. Similarly, Yasmin *et al.* (2008) and Wang *et al.* (2009) reported the effects of soaking on phytate content of seeds.

Among the processing methods employed in this study, boiling and washing with water were less effective in eliminating or reducing most of the anti-nutrients determined. However, boiling has been reported to reduce the saponin and tannin content in some other leguminous seeds (Olanipekun *et al.*, 2015). The tannin contents obtained in this study were similar to those reported for some several common legume seeds (Martin-Cabrejas *et al.*, 2009; Embaby, 2010; Pele *et al.*, 2016). The phytic acid content (0.10 - 0.63mg/100g) was within the range reported for other leguminous seeds (Embaby & Mokhtar, 2011) and lower than the previously reported values determined for fermented Soya bean meals. Soaking process has also been reported to enhance the action of naturally occurring phytase in legumes (Kumar *et al.*, 2010). When legumes are soaked in water overnight, the phytate which is water-soluble is considerably removed into the water (Mahmod *et al.*, 2014). This may be a good reason for the use of this seed for food and fish feed formulation.

The crude protein contents of soaked and fermented seeds were significantly higher than the boiled samples (Table 2). This could be due to the effect of heat on protein and other heat labile nutrients thereby decreasing the crude protein. Abdullahi & Abdullahi (2005) reported decreased nutrient contents of *Delonix regia* seeds after boiling in water. The nutritional values recorded could be used in food as well as fish feed. The substitution of dietary fish Meal with

*Solea senegalensis* in a similar study did not influence growth and feed intake of the reared fish (Abderrahim *et al.*, 2019).

However, the crude protein values obtained in this experiment were generally higher than values (20.02% and 27.3%) reported for debittered *B. senegalensis* seed meals (Parkouda *et al.* 2007; Abdel-rahman *et al.*, 2013), respectively. The variations could be due to differences in geographical locations, processing methods and maturity of the seed at the time of harvesting. Variation due to geographical location was previously reported by Taheer, *et al.* (1997) in which the nutrient composition of *B. senegalensis* seed differed significantly with those grown in Sudan. Kajihaua *et al.* (2014) reported that moisture, crude protein, and crude fibre contents of soaked sesame seeds were found to increase with an increase in soaking time.

A significant decrease has been observed in the crude lipid content of the seeds after processing. The boiled seeds showed the lowest lipid composition compared to the raw and the fermented seeds respectively. These variations may be attributed to the effects of heat on oil, microbial and biochemical processes during processing, as well as the increased activity of lipases during soaking. These variations may be attributed to the effects of heat on oil, microbial and biochemical processes during processing, as well as the increased activity of lipases during soaking

In a related experiment Ragab *et al.* (2002) and Banja *et al.* (2016) attributed decreased lipid content of soya bean meal to biochemical reaction and dissociation of lipid complexes. Dissimilar assertion has been made by Agume *et al.* (2017), who reported decreased lipid content of soya bean meal after processing including soaking. The lipid contents in this study is similar to the values (3.7%) reported for *B. senegalensis* seed meals (Kim *et al.*, 1997) but higher than values reported by Edwige *et al.* (2014). This variation could be due to differences in geographical location, maturity of the seeds at time of harvesting, processing methods adopted and to some extent, the genetic composition of plant.

## Conclusion and Recommendation

Fermentation and Soaking were found to be the best methods to reduce most of the anti-nutrients in the

**Table 2.** Proximate Composition of Raw and Processed *B. senegalensis* Seed

	RBS	BBS	FBS	SBS	WBS
CP	31.90±0.01 <sup>a</sup>	28.48±0.29 <sup>d</sup>	30.48±0.43 <sup>c</sup>	31.51±0.41 <sup>b</sup>	31.42±0.31 <sup>b</sup>
CL	10.17±0.17 <sup>a</sup>	4.50±0.20 <sup>c</sup>	5.90±0.57 <sup>bc</sup>	6.77±0.24 <sup>b</sup>	10.00±0.17 <sup>ab</sup>
CF	8.99±0.01 <sup>a</sup>	7.40±0.06 <sup>b</sup>	6.30±0.06 <sup>c</sup>	7.50±0.05 <sup>ab</sup>	8.99±0.01 <sup>a</sup>
Ash	5.81±0.01 <sup>d</sup>	6.46±0.03 <sup>c</sup>	6.10±0.06 <sup>c</sup>	9.48±0.29 <sup>a</sup>	6.81±0.01 <sup>d</sup>
NFE	40.55±0.61 <sup>c</sup>	49.20±0.05 <sup>a</sup>	47.23±0.05 <sup>ab</sup>	40.27±0.18 <sup>c</sup>	45.78±0.61 <sup>b</sup>
DM	97.42±1.15 <sup>a</sup>	96.20±0.58 <sup>a</sup>	96.30±1.20 <sup>a</sup>	95.53±0.29 <sup>b</sup>	97.00±1.15 <sup>a</sup>

Values with different superscripts across each row differed significantly (P<0.05).

Key: CP = Crude Protein, CL= Crude Lipid, CF = Crude Fibre, DM = Dry Matter, RBS = Raw Boscia Seed BBS = Boiled Boscia Seed, FBS = Fermented Boscia Seed, SBS= Soaked Boscia Seed, WBS = Washed Boscia Seed

*B. senegalensis* seed, while boiling and water washing were relatively less effective. Soaking and fermentation affected the proximate composition of *B. senegalensis* seed.

Anti-nutrients and proximate compositions of soaked *B. senegalensis* seed revealed relatively higher CP (31.5%) and lower levels of anti-nutrients of 0.60mg/g tannin and 0.10mg/g phytate, respectively. Soaking is therefore recommended for better debiterization and improved nutrient composition of *B. senegalensis* seed for inclusion in fish feed formulation.

Further research is warranted to explore the optimal soaking conditions for *Boscia senegalensis* seeds in order to maximize the reduction of antinutritional factors (ANFs) and enhance their nutritional suitability for fish diets. Investigating the effects of incorporating treated *B. senegalensis* seeds into actual fish feed formulations and conducting feeding trials with different fish species would provide valuable insights into the practical application and efficacy of these seeds as an alternative protein source. It would be beneficial to investigate the long-term storage stability and shelf life of processed *B. senegalensis* seeds, considering factors such as moisture content, microbial growth, and preservation techniques, to ensure the quality and nutritional value of the seeds for fish feed production in the future.

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