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EFFECTS OF DIFFERENT PROCESSING METHODS ON THE PROXIMATE COMPOSITION AND ANTI-NUTRIENT OF *PHILIOSTIGMA RETICULATUM* SEED MEAL

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Abstract
This study investigated the proximate composition and anti-nutrient content of Philostigma reticulatum seed meal processed through different methods: raw, toasted, fermented, and soaked. Seeds were obtained from Njimtilo, Konduga LGA, Borno State, and authenticated at the University of Maiduguri. The seeds were cleaned and processed to reduce contamination. The proximate composition was analyzed using the Association of Official Analytical Chemists (AOAC, 2010) method by measuring moisture content, crude protein, ether extract, ash, crude fiber, and nitrogen-free extract. Data collected was analyzed using one-way analysis of variance. The results revealed that the highest moisture content (7.90%) was observed in soaked seeds, whereas the lowest (2.72%) was observed in toasted seeds. The harvested seeds had the highest crude protein (33.85) and the lowest raw protein (25.36%). The highest extract (8.40%) was obtained from raw seeds, whereas the lowest (4.38%) was obtained from fermented seeds. Toasted seeds had the highest ash content (5.09%), whereas raw seeds had the lowest ash content (4.30%). The highest crude fiber content (19.33%) was obtained from raw seeds, whereas the lowest fermented seed content (13.41%) was obtained. The highest nitrogen-free extract (42.22%) was obtained from fermented seeds and the lowest was obtained from toasted seeds (35.47%). The anti-nutrient contents (phytic acid, tannins, flavonoids, alkaloids, saponin, and trypsin inhibitors) were also analyzed. The results revealed significant reductions in anti-nutrient levels through various processing methods. Fermentation was the most effective method for reducing phytic acid content, achieving a 55.58%

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reduction, followed by soaking and toasting. Tannin content was significantly reduced by fermentation (54.60%) and soaking (55.87%), whereas toasting was less effective. Flavonoid content was reduced by 36.96% by fermentation, with soaking and toasting also contributing to the reduction. The alkaloid content showed no significant difference across processing methods, with a reduction of 37.11%. The saponin content was most effectively reduced by toasting (50.00%), followed by soaking and fermentation. Trypsin inhibitor levels were reduced by 43.63% by toasting, with fermentation and soaking also contributing to the reduction. These findings suggest that processing methods, particularly fermentation and toasting, significantly improve the nutritional quality and digestibility of Philostigma reticulatum seed meal, making it more suitable for use in animal feeds.

1. Introduction

Philostigma reticulatum is a leguminous medium–sized tree that grows wild in the tropics and is one of the most common species of Piliostigma (Hochst) in the northern part of Nigeria Keay (1987), where it is locally known as Kargo or Kalgo in Hausa, it is also known as Okpo-atu in Igbo (Eastern Nigeria), Abafe in Yoruba (Western Nigeria (Burkill, 1995). Other names that are frequently used are "camel food" in English, "pied de chameau," "semallier," in France, and "Musacanca" in Portuguese (Vodouhe et al., 2010). It yields a great deal of seed pods, which are now not used for poultry—all except ruminants, which consume the full pod. According to Musa and Bichi (2015), the crude protein content of pilostigma seeds ranges from 20% to 34%. There is limited information on the use of P. reticulatum seeds as ingredients in animal feed. Philostigma reticulatum (kargo), which is a non-conventional feed ingredient that is less exploited as a source of protein in fish production. Studies have shown that Philostigma reticulatum is a potential alternative for the replacement of some conventional feed ingredients because of its ability to supply high protein (CP: 30.3% undefatted) Bah et al. (2023). Philostigma reticulatum is one of the non-conventional feed ingredients referred to as a browse plant. Browsing plants are an important component of plants beside grass.

Bah et al. (2023) reported that the proximate composition of Philostigma reticulatum showed that raw seed had less moisture (11.13%) compared to the boiled sample (15.52%). Saulawa et al. (2014) reported moisture content (boiled sample: 15.52%) while (6.25%) was reported by Musa and Bichi (2015). It was also revealed that the boiled sample had more moisture than the raw Philostigma reticulatum and this may be attributed to the processing method that involved wet heating for 60 minutes. Bah et al. (2023) reported a percentage crude protein (CP) of 26.45% for boiled and 28.34% for raw Philostigma reticulatum,. The CP of raw Philostigma is also higher than the CP of raw sunflower seeds (27.02) as reported by Adesina, (2018) and is in the range of the 28.53% CP in pumpkin seed meal as reported by Wafar et al., (2017).

Anti-nutritional factors are compounds that reduce the nutrient use and/or feed intake of plants or plant products used as human foods. They play a vital role in determining the use of plants by humans and animals. Some vitamins in food may be destroyed by anti-nutritional substances. These anti-nutritional factors must be inactivated or removed if the values of food substances are to be fully maintained.

(Thakur et al., 2019) Plants that produce seeds rich in energy supplies (carbohydrates, lipids, proteins) usually

accumulate potent chemical defense compounds. This also applies to grain legumes with comparably large and protein-rich seed that often contain substantial amounts of "anti-nutritive" factors (ANF), such as lectins, protease inhibitors, non-protein amino acids (NPAAs), alkaloids, cyanogenic glycosides, pyrimidine glycosides, saponins, tannins, isoflavone, oligo- saccharides, erucic acid, or phytates (Thakur et al., 2019).

Abdulrahman et al. (2018) revealed some anti-nutritional factors found in Philostigma reticulatum pod as cyanide, oxalate, phytate, saponins, and tannins, where oxalate content contains a reasonable amount of calcium, magnesium, and phosphorus. Ca and carbon are also released from the hydrolysis of Ca Oxalate, some of which is either absorbed or excreted by the ruminant animals (Njidda, 2010). In ruminants, dietary tannins of 2%–3% have been shown to have beneficial effects because they reduce protein degradation in the rumen by the formation of a protein-tannin complex (Njidda, 2010). Haruna et al. (2015) reported that the antinutritional factors (ANFs) in Philostigma reticulatum seeds indicate the presence of saponins, tannins, cyanides, oxalates, phytates, and phenols.

The presence of saponins, phenolics, and cyanides in Philostigma reticulatum seeds was also reported by Jimoh and Oladiji (2005). Awe and Omojasola (2009) reported the presence of tannins, alkaloids, phenolics, triterpenes, and phiobatininsin in the Mackenzie barck extract of P. reticulatum Plant. The aim of this study was to determine the effects of different processing methods on the anti-nutrient content of P. reticulatum seed meal.

Materials and Methods

Study Area

The study was conducted at the Teaching and Research Fish Farm of the Department of Fisheries, University of Maiduguri, Borno State, Nigeria. The study area falls within the semi-arid zone of Northeastern Nigeria, located between latitude 11° 48° and 16° North and longitude 13° 12°and 12° East. The mean monthly temperature is 40.2°C prior to the onset of the rain in June and the lowest 31.3°C during the peak of the rainy period in August. The area has an average annual rainfall of about 519.34 mm (Abatcha et al., 2023)

Processing of Philostigma reticulatum seeds

The seeds were processed using three (3) methods: Soaking, Toasting and Fermentation. The method that gave the best result in terms of Crude protein was selected for formulation of the experimental diet. The processes used for seed processing are as follows:

i. Raw: A total of One Hundred and Fifty (150g) grams of raw seeds were labeled and kept in a polytene container and labeled as raw.

ii. Soaking: A total of One Hundred and Fifty (150g) grams of raw seeds were soaked in water to a ratio of 1:3 for 48hrs, spread on a tray to be sun-dried, milled, and stored in an airtight polythene container and labeled as soaked.

iii. Toasting: A total of One Hundred and Fifty (150g) raw seeds were toasted for 10minutes at 850C using an electric hot plate according to Oladele et al. (2009), milled, stored in an airtight polythene container, and labeled as toasted.

iv. Fermentation: A total of One Hundred and Fifty (150g) grams was moistened with water, kept in a container, and covered to ferment for 72hrs. The fermented seeds were spread on a tray to be sun dried, milled, stored in an airtight polythene container, and labeled as Fermented.

Proximate Composition of Philostigma reticulatum Seed Meal

The raw and processed seeds were used for proximate composition analysis using the method described by (AOAC, 2005). The analysis was replicated three times.

Moisture content

Two (2) grams of the ground sample were thoroughly mixed to determine the water content. The sample was

weighed into a glass Petri dish that had been previously dried. The dish, including the sample, was placed in a hot-air oven at 105°C for 5 hours. The sample was then dried to a constant weight and cooled for ten minutes in a desiccator before each weighing, and the moisture content was calculated using the below formula.

% moisture = 3% (Weight loss on drying)/(Weight test portion,g) x 100

Crude protein

Two (2) grams of the ground sample were placed in a Kjeldahl flask, and about 200 milligrams of a catalyst mixture (potassium sulfate, copper sulfate, and selenium powder) were added. Then, 10.0ml of concentrated sulfuric acid was added to the flask. Heat was gently applied for a few minutes until frothing ceased, then digested the sample for 1 hour. The mixture was allowed to cool before being diluted to a known volume with distilled water (100 ml).

A 10.0 ml aliquot of the diluted digest solution was pipetted into the distillation chamber of a micro Kjeldahl distillation apparatus. Next, 10.0 ml of 40% sodium hydroxide solution was added, and the mixture was steam distilled into 10.0 ml of 4% boric acid containing a mixed indicator (noting the color change from red to green). The distillate was titrated with standard 0.01 N or 0.02 N hydrochloric acid to a gray endpoint.

The percentage of nitrogen was estimated using the formula below, and the crude protein (%) was calculated by multiplying the percentage of nitrogen by a conversion factor as follows:

%N = ((a-b) x 0.01 x 14.0057 x c x)/(d x e) x 100

Where:

a = titer value for the sample, b= titer value for the blank; c = Volume of digest made up of distilled water; d = Aliquot taken for distillation; e = Weight of dried sample (mg).

% Cude protein = % N x 6.25 (factor for feeds)

Ash

Ash content was determined by weighing a 2-g test solution into a porcelain crucible placed in a muffle furnace preheated to 600°C. The sample was held at this temperature for 2 hours. The crucible was then directly transferred to a cooling desiccator. After cooling, the crucible was immediately weighed, and the percentage of ash was reported to two decimal places.

The percentage of ash was estimated as follows:

%Ash= (Weigth of Ash)/(Weight of Sample (on dry matter basis)) x 100

Ether extract

The ether extract was determined using a Soxhlet extraction apparatus and a 250 ml Quickfit flask previously dried in the oven. Five (5) grams of the sample were weighed and transferred to a fat-free extraction thimble, which was then lightly plugged with absorbent cotton.

The thimble was placed in the extractor, and about 150 cm^3 of petroleum ether (B.P. 40-60°C) was added to the flask until it was siphoned over once. The heat source (electrothermal heating mantle) was adjusted so that the ether boiled gently, and the extraction was allowed to continue for at least 6 hours.

After extraction, the flask containing the oil was detached. The extract (oil) was filtered through Whatman filter paper into a weighed beaker, and the filter paper was washed with a small portion of hot fresh ether. The solvent was evaporated at 100°C, and the beaker containing the residue was dried in an air oven for 1 hour at 100-105°C. The oil content is reported as a percentage in two decimal places.

The percentage of fat was calculated as follows:

% Crude fat = (W2 – W1) x 100/S,

Where, W1 = Weight of empty flask (g), W2 = Weight of flask and extracted fat (g) and S = Weight of sample

Crude fiber

This procedure was performed with slight modifications. The defatted ground sample from the fat determination was transferred into a 250 ml Quickfit flask, and 150 ml of 1.25% sulfuric acid was added. The flask was fitted to a reflux condenser and refluxed for 30 minutes. After cooling, the mixture was filtered through a Buchner funnel fitted with Whatman filter paper. The residue was rinsed three times with hot distilled water, dried, and carefully transferred back into the Quickfit flask. Next, 150 ml of 1.25% sodium hydroxide was added, and the mixture was refluxed for another 30 minutes. The solution was then filtered using a Buchner funnel and rinsed three times with hot distilled water, once with 1.25% sulfuric acid, and finally with 95% ethanol. The filter paper containing the residue was transferred into a porcelain crucible and dried in an oven for 2 hours at 130°C. After cooling in a desiccator, the sample was ashed at 550°C ± 10°C in a muffle furnace, cooled in a desiccator, and weighed.

The percentage of Crude fiber was estimated as follows:

% Crude fiber = Weight of oven-dried sample weight of Ash/Weight of Sample X 100

Determination of Anti-Nutritional Factors

The Anti-nutritional factors were determined using the standard procedure of (AOAC, 2005) and were replicated twice.

Alkaloid determination

The total alkaloids content was determined according to the method described by Biradar and Racheti (2013), with some modifications.

First, 5 (5) grams of each sample was added to 50 ml of a solution containing 10% acetic acid in ethanol and mildly stirred for 48 hours. After filtration, the extracts were concentrated to one-quarter of the original volume. Then, 2 ml of 3% sulfuric acid and approximately 8 ml of water were added to reach a pH of 2.5. This solution was transferred to a separator funnel in which 10 ml of a petroleum ether: diethyl ether (1:1) solution was added. The bottom phase was collected and added to concentrated ammonium hydroxide solution until precipitation was complete (pH 8.0). The entire solution was allowed to settle, and the precipitated phase was collected and washed again with ammonium hydroxide and chloroform. This phase was first dried with sodium sulfate, then completely dried using a rotavapor, and weighed to estimate the percentage of alkaloids.

% Alkaloids = Ove-dried paper and precipitate Weight of filter paper X 100 Weight of dry matter samples

Determination of Phytic acid

Phytic acid was determined using a method reported by Yahaya et al. (2013). Four (4) grams of the ground sample were soaked in 100 ml of 2% hydrochloric acid (HCl) for 3 hours and then filtered through two layers of filter paper.

Twenty-five (25) ml of the filtrate was placed in a 250 ml conical flask, and 5 ml of 0.3% ammonium thiocyanate (NH4SCN) solution was added as an indicator. Then, 53.5 ml of distilled water was added to reach the proper acidity. This mixture was titrated against ferric chloride (FeCl3) solution, which contains approximately 0.00195 g of iron per ml of FeCl3 solution, until a brownish-yellow color that persisted for 5 minutes was obtained (Ileke, 2014). The result was multiplied by a factor of 1.95 to obtain phytate P. The phytate P result was then multiplied by a factor of 3.55 to convert it to phytate.

Determination of tannin

An extract of 2 grams of the sample was prepared by extraction with anhydrous ether for 20 hours. The residue was then boiled for 2 hours with 300 ml of water, cooled, diluted to 500 ml, and filtered. Measure 25 ml of this infusion into a 2-L porcelain dish, add 20-ml Indigo solution, and 750 ml of water. Add standardized potassium

permanganate solution (1 ml at a time) until the blue solution changes to green; then add a few drops at a time until the solution becomes golden yellow. Similarly, titrate a mixture of 20 ml of the Indigo solution with 750 ml of water.

Multiply the difference between the two titrations by the desired factor to obtain Quercitannic acid, where the Reagents: are (a) an oxalic acid solution – 0.1N. 1 ml = 0.006235 g Quercitannic acid or 0.0008 g absorbed. (b) Potassium Permanganate Standard Solution:

dissolve 1.333 g $KMnO_4$ in 1 L H_2O and standardize against (c) Indigo solution: dissolve 6 g Sodium Indigotin disulfonate in 500 ml water by heating; cool, add 50 ml H_2SO_4, dilute to 1 L, and filter. 1 ml of 0.1 N $KMnO_4$ = 0.006235.

Determination of flavonoids

One (1) gram of the sample was weighed and repeatedly extracted with 100 cm^3 of 80% methanol at room temperature. The mixture was then filtered through filter paper into a 250 cm^3 beaker. The filtrate was transferred to a water bath and allowed to evaporate to dryness. The residue was then weighed. The percentage of flavonoids was calculated according to the method described by Krishnaiah et al. (2009).

Determination of saponin

Saponin content (percent yield) was determined using the gravimetric method as described by Kaur et al. (2015). The methanolic extracts from each plant (1 g in 10 ml) were macerated for 24 hours and then partitioned in a water and n-butanol (1:1 ratio) solution. This solution was poured into a separator funnel and left for 2 hours. The upper no-butanol layer was separated, and the solvent was evaporated to obtain the crude saponin extract.

Data Analysis

The data were subjected to One-way Analysis of Variance (ANOVA). Differences between the means were determined using Least Significant Difference (LSD) at the 95% confidence level (P= 0.05) with the aid of Statistix 8.0.

1. Results and Discussions

Results

Table 1. Mean Proximate Compositions of Raw and Processed *Philostigama reticulatum* seed Meals

	Processing Methods				
Components (%)	**Raw**	**Toasted**	**Fermented**	**Soaked**	**SEM(P<0.05)**
Moisture Content	5.47^{b}	2.72^{c}	5.63^{b}	7.90^{a}	0.09
Crude Protein	25.36^{d}	33.85^{a}	29.41^{b}	28.8^{d}	0.18
Ash	4.30^{c}	5.09^{a}	4.71^{b}	4.36^{c}	0.05
Crude Fiber	19.33^{a}	17.43^{b}	13.41^{a}	15.50^{c}	0.18
Ether extract	8.40^{a}	5.46^{b}	4.38^{c}	5.70^{b}	0.07
Nitrogen-free extract	37.16^{b}	35.47^{b}	42.22^{a}	38.31^{b}	0.38

Key: Means with the same superscripts in the same row are not significantly different (P>0.05)

SEM: Standard error of the mean

Mean Proximate Compositions of Raw and Processed Philostigama reticulatum seed Meals

The proximate composition of Philostigma reticulatum seed meal with different processing methods (Raw, Toasted, Fermented, and Soaked) is presented in Table 1. The components measured include moisture content, crude protein, Esther extract, ash, crude fiber, and nitrogen-free extract. The highest (7.90%) moisture content was recorded in soaked Philostigma reticulatum seed meal, whereas the lowest (2.72%) was recorded in

toasted seed meal. There was no significant difference (P>0.05) in moisture content between raw and fermented samples but differed significantly (P<0.05) from other processing methods. Toasted P. reticulatum seed meal had the highest (33.85%) crude protein and the lowest (25.36%) in raw. There was no significant difference (P>0.05) in crude protein between raw and soaked samples, but it differed significantly (P<0.05) from other processing methods.

The highest ash content (5.09%) was obtained in toasted seeds, whereas the lowest (4.30%) was recorded in raw seeds. There was no significant difference (P>0.05) in ash content between raw and soaked samples, but it significantly differed (P<0.05) from other processing methods. The crude fiber content was highest (19.33%) in raw seed meals and lowest (13.41%) in fermented seeds. There was no significant difference (P>0.05) in crude fiber content between raw and fermented but differs significantly (P<0.05) between toasted and soaked processing methods. The highest nitrogen-free extract (42.22%) was recorded in fermented seeds and the lowest (35.47%) was recorded in toasted seeds. There was no significant difference (P>0.05) in nitrogen-free extract content between raw and soaked extracts and significant differences (P<0.05) from other nitrogen-free extracts in other treatments.

Table 2: Anti-nutrients of raw and processed *Philostigma reticulatum* seed Meals

Parameters	Raw	Toasted	Fermented	Soaked	SEM(P<0.05)
Phytic Acid (g/100g)	667.27^{a}	405.57^{c}	296.43^{d}	510.21^{b}	0.77
Percentage Reduction		39.21	55.58	23.54	
Tannin (mg/100g)	20.44^{a}	15.15^{b}	9.28^{c}	9.02^{c}	0.76
Percentage Reduction		25.88	54.60	55.87	
Flavonoids (mg/100g)	3.22^{a}	2.78^{c}	2.03^{d}	2.98^{b}	0.03
Percentage Reduction		13.66	36.96	7.45	
Alkaloid (mg/g)	8.11^{a}	7.78^{a}	6.24^{a}	5.31^{a}	1.18^{ns}
Percentage Reduction		4.07	23.06	37.11	
Saponin (mg/g)	0.44^{a}	0.22^{c}	0.33^{b}	0.16^{c}	0.12
Percentage Reduction		50.00	25.00	28.00	
Trypsin (TIU)	18.06^{a}	10.18^{d}	13.34^{c}	15.68^{b}	0.09
Percentage Reduction		43.63	26.14	13.18	

Key: Means with the same superscripts in the same row are not significantly different (P>0.05)

SEM: Standard error of the mean

Anti-nutrients of Raw and Processed Philostigma reticulatum seed Meals

The anti-nutrient contents (Phytic Acid, Tannin, Flavonoids, Alkaloid, Saponin, and Trypsin) of raw and processed Philostigma reticulatum seed meal is shown in table 2. The result revealed the highest (667.27mg) phytic acid was recorded in raw seeds, followed by 510.21 mg in soaked seeds and 296.43 mg in fermented seeds. There were significant differences (P<0.05) in phytic acid among all processing methods with Phytic acid reduction of up to 55.58%. The highest (20.44mg) Tannin contents were recorded in raw seeds and the lowest (9.02mg) in soaked seeds. There was no significant difference (P>0.05) in tannin value between fermented and soaked, while a significant difference (P<0.05) is recorded between the other processing methods, and tannin

reduction was observed from 25.88% to 55.87%. The highest flavonoid content (3.22 mg) was recorded in raw seeds, followed by 2.98 mg in soaked seeds, and the lowest (2.03mg) was obtained in fermented seeds. There were significant differences ($P<0.05$) in flavonoids among all processing methods, with a percentage reduction of 36.96%.

Alkaloid is similar across all methods, showing no significant difference ($P>0.05$) between processing methods with alkaloids reduction of 37.11%. The highest Saponin content of 0.44 mg was recorded in raw seeds, followed by 0.33 mg in fermented seeds, and the lowest (0.16mg) was recorded in soaked seeds. There was no significant difference ($P>0.05$) in the saponin content between toasted and soaked but significantly differed ($P<0.05$) from other processing methods. The highest (18.06mg) trypsin inhibitor was recorded in raw seeds, followed by 15.68 mg in soaked seeds, and the lowest (10.18mg) in toasted seeds. There were no significant differences ($P>0.05$) in the values of trypsin inhibitor among processing methods. Up to 50% and 43.63% reductions in the levels of saponin and trypsin inhibitors were observed. The various processing methods significantly reduced the levels of anti-nutrients in Philiostigma reticulatum seed meal. Fermentation is the most effective method for reducing phytic acid content, achieving a 55.58% reduction, whereas toasting and soaking also reduce phytic acid content to a lesser extent. Both fermentation and soaking are highly effective in reducing tannin content, with reductions of 54.60% and 55.87%, respectively, whereas toasting is less effective.

Fermentation significantly reduces flavonoid content by 36.96%, with toasting and soaking also contributing to reductions but to a lesser extent. Soaking is the most effective method for reducing alkaloid content, achieving 37.11% reduction, followed by fermentation, whereas toasting has a minimal effect. Toasting is the most effective method for reducing saponin content, achieving a 50.00% reduction, while soaking and fermentation also reduce saponin content to a lesser extent. Finally, toasting is the most effective method for reducing trypsin inhibitor units, achieving 43.63% reduction, with fermentation and soaking also contributing to reductions but being less effective.

Discussion

Proximate Composition of Philiostigma reticulatum

The moisture content of Philiostigma reticulatum seeds in this study ranged from 2.72% to 7.90%, with the highest (7.90%) recorded in processed (soaked) seeds and 5.47% in raw seeds in a similar study. Bah et al. (2023) revealed the moisture content of Philiostigma reticulatum in a raw seed sample of moisture (11.13%), which is higher than the moisture content in this research. Haruna and Bichi (2015) also reported a moisture content of 7.27% in raw Philiostigma reticulatum seeds, which is within the range of the present study.

Similarly, Adeyeye (2014) found the moisture content of raw ground nut (Arachis hypogaea) seeds to be around 5.5%. Chibudike and Adeyoju (2021) also reported a moisture content of approximately 6.0% for raw cowpea (Vigna unguiculata) seeds, which is within the range observed in this study. The moisture content of 2.72% of toasted seeds in the research is slightly higher than the 1.91% reported by Haruna and Bichi (2015) for seeds toasted for 40 minutes but revealed for longer toasting durations (5.16% for 80 minutes and 10.24% for 120 minutes). This suggests that the duration and temperature of toasting significantly affected the moisture content. The 5.63% moisture content in fermented seeds is consistent with the findings of Ntso et al. (2016). The crude protein content of seeds in this study ranged from 25.36% to 33.85%, which Haruna and Bichi (2015) revealed 34.20% in similar study of Philostigma reticulatum. This difference could be due to variations in seed sources, environmental conditions, and processing methods (Bake et al., 2021) revealed 28.61% to 35.82 % crude protein in germinated Canavalia gladiate seed, and the increasein protein concentration may be associated with a decline in the dry weight due to the breakdown of fats and carbohydrates through respiration, whereas some amino acids

would have been formed during germination (Jan et al., 2017). Yusuf et al. (2019) reported 22.87 grams of crude protein in boiled S. obtusifolia seeds, which is slightly lower than that in this study. Balogun et al. (2016) reported similar results for S. obtusifolia, S. siamea, and soaked Bauhinia monandra seed meals. Aliyu et al. (2020) reported 29.22% crude protein in processed sorrel seed. In another research corroborated the range of values (31.75 – 41.57%) previously reported by other authors on differently processed Parkia biglobosa seed (Michael and Mathias, 2020). Augustine et al. (2018) reported crude protein of 26.95 to 28.79% which falls within the range of this study. The crude proteins were reported to be 30.3% and 40.4% for the seeds otherwise known as unspoiled or inedible and defatted seeds, respectively, by Akin-Osanaiye et al. (2009), indicating that the seeds are high in both crude protein (40.4%) and lipids (27.9%).

This is supported by the study of Haruna and Bichi (2015), who reported an increase in ash content in toasted P. reticulatum seeds. Higher ash content indicates a higher mineral content, which is beneficial for the nutritional value of the seed meal. Akin-Osanaiye et al. (2009) reported a raw ash value of 4.0%, which was rather lower than the 4.30% found in this study in respect of the raw seeds of the same plant. This corroborates the results obtained by Musa and Bichi (2015), who reported similar readings for the toasted seed meal of Philostigma reticulatum.

The crude fiber content was highest in raw seeds (19.33%) and lowest in fermented seeds (13.41%). This reduction is beneficial for improving the digestibility of seed meal. Alaba et al. (2016) reported a decline in crude fiber content in fermented P. reticulatum. Similarly, (Aliyu et al., 2020) reported 12.80% to 14.50% reported in Hibiscus sabdariffa seed meal.

The extract content of Philiostigma reticulatum seeds in this study was highest in raw seeds (8.40%) and lowest in fermented seeds (4.38%). The significant differences among the processing methods indicate that fermentation effectively reduces fat content. This reduction is advantageous for producing low-fat seed meals, which are desirable for certain dietary requirements.

Similar results were observed by Alaba et al. (2016), who found that fermentation significantly reduces the extract content in Philiostigma reticulatum seeds. Haruna and Bichi (2015) reported ether extract contents of 5.51% to 11.21% for Philiostigma reticulatum seeds at different temperatures, which falls within the range of the study, while Balogun et al. (2016) reported lower ether extract in Senna obtusifolia.

The nitrogen-free extract was highest (42.22%) in fermented seeds and lowest in toasted seeds (35.47%). This is beneficial for providing a readily available energy sources. Alaba et al. (2016) also reported an increase in nitrogen-free extract in fermented P. reticulatum pods. (Adesina et al., 2023) revealed lower NFE values of 24.60% – 31.45% almost corresponded to 13.16 – 28.73% obtained by Michael and Mathias (2020), as well as by Michael et al. (2021). 40.29 - 45.72% NFE found in soybean pulp-supplemented diets reported by (Zulhisyam et al., 2021) is within this range. The experimental values obtained were similar and accorded with the range of 31.56-32.86% reported by Iheanacho et al. (2018) thereby lending credence to the nutritional potential of melon seed peel meal as an alternative energy feed source.

Anti-nutrients of Raw and Processed Philostigma reticulatum seed Meals

Analysis of anti-nutrient content in Philostigma reticulatum seed meals processed using different methods revealed significant variations. The phytic acid content in this study was highest (667.27 mg) in raw seeds and lowest (296.43 mg) in fermented seeds. This significant reduction in phytic acid through fermentation might be due to enzymatic activity, which is in agreement with the work of Agbai et al. (2021), who showed that fermentation plays a significant role in the reduction of phytic acid in rubber seed meals.

The reduction in phytic acid is beneficial as it enhances mineral bioavailability, particularly for iron and zinc.

Hassan et al. (2023) reported significant reductions in tannin, flavonoids, saponin, and alkaloid content in processed B. senegalensis seed meals.

Similarly, According to Osunbitan et al. (2015), anti-nutritional factors significantly decreased in different cowpea varieties soaked for varying durations.

Several pretreatment methods, such as soaking, fermentation, germination, and the use of the phytase enzyme on grains have been reported toreduce grains' phytic acid levels (Gupta et al., 2015). The study revealed that fermentation was the most effective method for reducing phytic acid content, achieving a (55.58%) reduction. Toasting and soaking also reduce phytic acid content, but to a lesser extent. This is consistent with the findings of Haruna et al. (2015), who reported significant reductions in phytic acid through various heat-processing methods. Hassan et al. (2023) reported that the soaking methods led to a reduction of 84.92% of phytate, which is higher than that observed in the current study. A reason for this difference could be the leaching of the compound into the soaking water. The lack of significant differences between fermented and soaked seeds in this study suggests that both methods effectively reduce tannin levels.

This was supported by Onainor et al. (2018), who found that soaking and fermentation significantly reduced tannin content in sesame seeds. Lower tannin levels are advantageous because tannins can interfere with protein digestibility and mineral absorption.

Both fermentation and soaking are highly effective for reducing tannin content, with reductions of 54.60% and 55.87%, respectively. Toasting also reduces tannin content, which is similar to results observed by Johnson (2016), who found that fermentation significantly reduced tannin levels in legume seeds. According to Olanipekun et al. (2015), boiling reduces the levels of saponins and tannins in different leguminous seeds. The tannin levels recorded for these seeds in this study compared well with those noted for other well-known legume seeds (Pele et al., 2016).

Thakur and Kumar (2019) noted that processing methods like soaking and fermentation can alter flavonoid content in cereals and pseudocereals. Flavonoids are important for their antioxidant properties, and their reduction through processing may impact the overall antioxidant capacity of seeds. Fermentation significantly reduces flavonoid content by 36.96%, whereas toasting and soaking also contribute to reductions but to a lesser extent. This is consistent with the findings of Bassi (2005), who noted that fermentation effectively reduces flavonoid content in seeds. The alkaloid content in the study remained relatively stable across all processing methods, with the highest levels observed in raw seeds and the lowest in soaked seeds. This indicates that alkaloids are less affected by the processing techniques used. Agbai et al. (2021) reported similar results, noting minimal changes in alkaloid content in processed rubber seed meals. Soaking is the most effective method for reducing alkaloid content, achieving a 37.11% reduction. Fermentation also significantly reduces alkaloids, and toasting has a minimal effect. This reduction is crucial because alkaloids can be toxic at high levels, as highlighted by Devic et al. (2018).

The highest saponin content (0.44 mg) was recorded in raw seeds, followed by 0.33 mg in fermented seeds, and the lowest (0.16mg) was recorded in soaked seeds. Toasting is the most effective method for reducing saponin content, achieving a (50.00%) reduction while soaking and fermentation also reduce saponin content to a lesser extent.

This is supported by studies on the effects of heat processing on saponin levels in seeds. Olanipekun et al. (2015) reported that boiling is likely to considerably reduce the levels of saponins and tannins in various leguminous seeds.

Trypsin inhibitors are relatively stable across different processing methods. However, the reduction observed in toasted seeds aligns with the findings of Onainor et al. (2018), who noted that heat treatment effectively decreases

trypsin inhibitor activity in sesame seeds. Toasting is the most effective method for reducing trypsin inhibitor units, resulting in a 43.63% reduction. Fermentation and soaking also reduce TIU but are less effective. This reduction is important for improving protein digestibility, as noted by Sankian et al. (2017).

Conclusion

The results showed that all processing methods reduced anti-nutritional factors. However, the toasted seed meal was particularly notable for providing the highest crude protein content and comparatively fewer anti-nutrients although some of the anti-nutrients remained

Recommendation

Use of other processing methods or combination or used of culture medium for growth performance of Clarias gariepinus. A laboratory investigation on anti-nutrients and anti-nutrients should be conducted on not less than two (2) different laboratories for result reproducibility.

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