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Proximate and mineral composition of raw and toasted calabash seed (*Lagenaria vulgaris*) cake for aquaculture feeds

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Abstract

This research study was carried out to harness the potential benefits of calabash seed (*Lagenaria vulgaris*) cake by assessing the nutritional value on the raw and toasted calabash seed cake. The study was carried out in the department of fisheries and aquaculture research farms Usmanu Danfodiyo University Sokoto. The proximate and mineral analysis was carried out in the laboratory, department of soil, faculty of Agriculture, Usmanu Danfodiyo University, Sokoto. The results of proximate composition obtained for raw and toasted calabash seed cake are; the crude protein in the raw calabash seed cake ($20.87 \pm 3.31\%$) was higher than the value recorded in toasted calabash seed cake ($20.43 \pm 2.70\%$), the moisture content in the raw calabash seed cake ($4.00 \pm 0.29\%$) was higher than the value recorded in toasted calabash seed cake ($2.50 \pm 0.87\%$), the lipid content in the toasted seed cake ($12.25 \pm 5.50\%$) was higher than the value obtained in raw seed cake ($2.5 \pm 0.23\%$), the fibre content in the toasted seed cake ($3.75 \pm 0.32\%$) was significantly higher when compared with the value obtained in the raw seed cake ($3.1 \pm 0.23\%$), the ash content in the toasted seed cake ($23.50 \pm 6.35\%$) was significantly higher when compared with the value obtained in raw seed cake ($19.25 \pm 6.21\%$), the nitrogen free extra in the raw seed cake ($50.28 \pm 9.75\%$) was higher than the value recorded in toasted seed cake ($37.57 \pm 5.19\%$). From the proximate composition obtained in this study there were significantly ($p < 0.05$) difference between the two samples. Results of the elemental analysis revealed that, the raw and toasted seed cake contained the following minerals (in mg/kg) Na, K, Mg, Ca, Cu, Zn, Fe and Mn. The results showed significantly ($p < 0.05$) difference between the two samples. The results from this study indicate that the seed cake contains nutrients that may be useful in the aquaculture feeds.

Keywords: Calabash seed, proximate composition, minerals, processing, aquaculture feed

Introduction

For good nutrition in an animal production system is essential to economically produce a healthy high-quality product (Abubakar *et al.*, 2013). In fish farming, nutrition is critical because feed represents 60 -80% of the production cost ^[1]. Fish nutrition has advanced dramatically in recent years with the development of new, balanced diets that promote optimal fish growth and health ^[1]. The development of new species-specific diet formulation supports the fish farming industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products ^[1]. Nigerian feedstuffs have been on the decline in recent years because of the diminishing output of certain traditional crops ^[2]. Recent statistics demonstrated that Nigeria relies on imports to meet the need for an expanding livestock and aquaculture industry ^[2]. This has increased the price of food and feed resources in the country which aggravated the high cost of fish feed which has been a major problem of fish farmers in Nigeria ^[2].

Global food security is becoming unstable with increasing dependence on a few major staple crops, which has resulted in an alarming reduction not only in crop diversity but also in the variability within crops. Many indigenous leguminous crops including the bottle gourd *Lagenaria vulgaris* are under-utilized and are almost going to extinction ^[3]. Bottle gourd (*Lagenaria vulgaris*) is one of the most important crops in the Cucurbitaceae family, although it is considered as a poor man's crop due to the socioeconomic restrictions governing its production and use.

It has a pan-tropical distribution with regional economic importance and is used as a vegetable, container, musical instrument or float while its seeds are used for oil and protein [4].

In general, non-conventional feed resources (NCFRS) are feeds that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production [5, 6, 7]. NCFRS are credited for being non-competitive in terms of human consumption, very cheap to purchase, by product or waste from agriculture, farm made feeds and processing industries and are able to serve as a form of waste management in enhancing good sanitation [5, 6, 7]. The nutrient quality of feed ingredients is one of the major prerequisites apart from availability (which some time is a function of cost and season) for production of good quality feeds [8]. The basic nutrient that cannot be compromised in the choice of ingredient for feed formulation is protein [8]. Hence it became imperative to research into the nutrient composition of some of the easily culturable animal protein sources [9]. Research on the nutritive value of some fish feed ingredients of both plant and animals' sources such as plantain [10], poultry offal [11], maggot meal [12], calabash seed [13], calabash seed meal [14], processed pumpkin seed [15] and water hyacinth [16] were reported. There was dearth of information on the nutritional value of some oil seed such as calabash seed subjected to processing method. The main objective of this research is to assess the nutritional value of the raw and the toasted calabash seed as feed ingredients for aquaculture feeds

Materials and Methods

Study area

The experiment was conducted at Agricultural Chemical Laboratory, Department of Soil Science and Agricultural Engineering Faculty of Agriculture, on latitude 13° 07' 78" N and longitude of 05° 12' 25" E at 275 m above sea level [17], at the Permanent site of Usmanu Danfodiyo University, Sokoto. The site is located in Sudan Savanah agro-ecological vegetation zone of Nigeria. The climate is characterized by a long dry season, cool dry air during the harmattan from November to February and hot season from March to May. Annual rainfall in the area ranged from 500 to 724 mm [18]. The mean relative humidity was 14.9% and 40% between March and June and 41 °C as maximum temperature [18].

Calabash Seed collection

Calabash (*Lagenaria vulgaris*) seeds were purchased from Sokoto central market, Sokoto State.

Identification of Calabash Seeds

Identification was carried out at the Herbarium of Botany Department, Usmanu Danfodiyo University, Sokoto.

Processing of calabash seeds

Raw Seed

The oil of the raw and toasted calabash seed was extracted using a mini oil screw press extraction machine (Prasa DO, model DL- 2YJ02) with a capacity of 10kg per hour (Plate 1). After oil extraction the cake was used for the analysis.

Toasting

1kg of calabash seeds was placed in a hot oven pan which was pre-heated on fire for 5 minutes to ensure the sufficient and uniform heat is obtained for toasting [19].



Fig 1: Mini oil screw press extraction machine

Chemical Analysis

The chemical analysis was conducted at the Agric. Lab., department of soil science, faculty of Agriculture, Usmanu Danfodiyo University, Sokoto. The analysis was carried out using [20].

Moisture content

One gram of sample in pre-weighted crucible was placed in an oven (105 °C) for 24 hours, cooled, and weighted. The percentage moisture was calculated as follows:

$$\text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 is the weight of the crucible, W_2 is the weight of the crucible and sample after drying at 105°, and W_3 is the weight of the crucible and sample after cooling in airtight container [20].

Crude protein

The procedure to be followed in the determination of crude protein was involving Kjeldahl method. Samples of each of the feed ingredients, diets and experimental fish will be weighed (2 g) and wrapped in a filter paper together with 1/4 of mercury tablet and placed into the flask. Then 10 ml concentrated H_2SO_4 acid was poured into the De211p Dalton digester and left to boil until the solution became clear. The mixture of each of the samples was allowed to cool overnight. This followed by adding of 130 ml of water and 13 ml of caustic soda to each of the labelled flasks. The solution formed was introduced into an electric distiller and fumes of ammonia from each of the bottles was allowed to bubble into the beaker containing some concentrated H_2SO_4 acid, and 2 drops of methyl orange was used as indicator. The solution obtained from the distillation was cooled and was titrated with NaOH. The end point of titration was recorded. To obtain the crude protein, the nitrogen value was multiplied by 6.25

The value of the protein was expressed by

Volume of acid x molarity x 100 x 0.014 x 6.25
Where Molarity = 0.0025 x 100

Crude fibre

A weighted crucible containing 1kg of defatted sample was attached to the extraction unit (in Kjeldahl, D-40599; Behr Labor-Technik GmbH, Dusseldorf, Germany) and in this 150 ml of hot 1.25% H_2SO_4 was added and digested for 30 minutes, the acid was drained and sample washed with hot distilled water for 1 hour 30 minutes. Percentage extracted fibre was calculated as:

Crude fibre = $\frac{\text{weight of digested sample} - \text{weight of ashes}}{\text{sample weight}} \times 100$

Crude Lipid

The crude lipid determination of the feed ingredients, experimental diet and fish was carried out by using the solvent extraction, Soxhlet, testator otherwise known as Soxhlet method. This was achieved by weighing and wrapping of the samples in a filter paper and introduced into weighed flask, these was done in line with number of samples, each containing 80 ml petroleum ether. These were then introduced into Soxhlet extractor to extract the fat content for 18-20 °C. After the ether had become clear, the wrapped samples were then removed from the flask and the petroleum ether recovered. The flask containing the extracted oil was weighed. The fat content was then expressed as follows;

Weight of flask and fat-weight of flask/Weight of flask and sample-weight of flask

Ash

Ash was determined as follows:

$$\text{Ash (\%)} = \text{Ash (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

W_1 is the weight of cleaned, dried, ignited and cooled crucible, W_2 is the weight of the crucible and sample after incinerating at 600 °C, and W_3 is the weight of the crucible and sample after cooling in an airtight homogenized vessel.

Carbohydrate

Carbohydrate content was determined by difference, that is, addition of all the percentage of moisture, fat, crude protein, ash and crude fibre subtracted from 100%. This gives the amount of nitrogen-free extract (NFE)

% Carbohydrate = 100 - (% moisture + % Fat + % Crude fibre + % Crude protein)

Minerals Analysis

The absorbance was read on atomic absorption spectrophotometer (Buck Science Model 200A) at different wavelength for each mineral element (Zn-213.9 nm, Ca-422.7 nm, Fe-248.3 nm, Mg-285.2 nm, Mn-279.2 nm, Na-589.0 nm and K-766.5 nm) [21].

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and, the treatment means were separated for significant differences following the procedure of Duncan's Multiple Range Test [22]. All the analyses were carried out using the computer software Statistical Package for the Social Sciences Version 20 for Windows [23].

Results and Discussion

Proximate composition of calabash seed cake

The results of proximate composition for raw and toasted calabash seed cake are presented in Table 1. The crude protein content of the raw sample (20.87±3.31%) was higher than the value recorded in the toasted calabash seed cake (20.43±2.70%). These variations in crude protein content of the calabash seed samples could be attributed to the processing methods employed and the displacement of oil from the seed thereby reducing the nutrient content of the seed. The high crude protein values recorded in the raw samples are in agreement with the work of [15] who obtained

higher values 24.50, 37.25, and 26.33% of raw pumpkin, calabash and cotton seed meal, respectively [24]. Reported that feed ingredients with crude protein greater than 20% are considered as protein sources.

This qualifies calabash seed as an alternative protein source for aquaculture feed [25] reported that protein formed the principal component of the embryo, thus this could be the reason for the high amount of it in the seed. The plant seed can therefore be a potential food protein supplement.

The moisture content of the raw calabash seed cake (4.00±0.29%) was higher than the value recorded in the toasted calabash seed cake (2.50±0.87%). This finding is in agreement with the work of [15] who obtained closed values of toasted and raw calabash seed meal at 3.70 and 2.37%, respectively, and also the toasting of calabash seed meal suggests that processing was able to reduce the moisture content. This might prolong the shelf life of calabash seed. This is similar to the observation of [26] who reported a decrease in the moisture content of cooked *Canavalia cathartica* seeds. These values were lower than the values obtained by [3] who analysed fermented Calabash seed (27.35±0.20%). The relatively low moisture content of the samples is of advantage since high moisture content is associated with an increase in bacterial action during storage [27].

The lipid content of toasted seed cake (12.25±5.50%) was higher than the value obtained in raw seed cake (2.5±0.23%). The lipid content for both raw and processed seed was lower when compared with the values obtained in the raw soybean (23.55%) reported by [28], toasted calabash seed meal (42.27%), raw calabash seed meal (41.28%) [15] and also the lipid content obtained in this study for both raw and processed seed was lower than 42.88±0.99% in the fermented calabash seed meal reported by [3]. The lower lipid content obtained in the present study could be attributed to the displacement of oil from the seed thereby reducing the nutrient content of the seed.

The fibre content of toasted seed cake (3.75±0.32%) was significantly higher when compared with the value obtained in raw seed cake (3.1±0.23%). This observation is in line with the finding by [29] who analysed raw calabash seed (3.65%). The finding in this study is also lower than the values obtained by [15] who obtained higher values of fibre content in the raw and the toasted calabash seed meal 5.59 and 4.17%, respectively.

The ash content for toasted seed cake (23.50±6.35%) was significantly higher when compared with the value obtained in raw seed cake (19.25 ± 6.21%). There was an increase in the ash content of the processed seed. Since ash content represents the index of mineral elements present in the seed, this indicates that, the processing has significance effects on the nutritional values of calabash seeds, this finding is also generally higher than 3.90% and 3.78% reported as the ash content of raw and fermented seed, respectively [3].

The nitrogen-free extra for raw seed cake (50.28±9.75%) was higher than the value recorded in toasted seed cake (37.57±5.19%). The reduction in the NFE of the processed (toasted) seed could be attributed to the higher heat during the processing. The value of NFE obtained in this study was higher than 13.45± 0.95% for fermented seed as obtained by [3] Ibeabuchi (2014). From the proximate composition obtained in this study, there were significant ($p < 0.05$) differences between the two samples.

Table 1: Proximate composition of calabash seed cake; Mean \pm SE

Proximate composition	Calabash seed cake (%)	
	Raw	Toasted
Moisture	4.00 \pm 0.29 ^a	2.50 \pm 0.87 ^b
Ash	19.2 \pm 6.21 ^b	23.50 \pm 6.35 ^a
Lipid	2.5 \pm 0.23 ^b	12.25 \pm 5.50 ^a
Fibre	3.1 \pm 0.23 ^b	3.75 \pm 0.32 ^a
Crude protein	20.87 \pm 3.31 ^a	20.43 \pm 2.70 ^b
Carbohydrate	50.28 \pm 9.75 ^a	37.57 \pm 5.19 ^b

Values with same letters are not significantly ($p < 0.05$) different.

Mineral composition of calabash seed cake

The results of mineral composition for raw and toasted calabash seed cake are presented in Table 2. The value of sodium ranged from 1200 \pm 2.34 raw seed cake to 1154 \pm 0.93 mg/100 g toasted seed cake, the value of sodium obtained in this study was higher when compared with the value obtained in whole seed meal (69.60 mg/kg) reported by [29]. The variations in the value of sodium might be due to processing method, seasonal variation and other environmental factors. Sodium is an element associated with potassium and is responsible for the maintenance of body fluids [30]. The potassium element ranged from 2900 \pm 1.85 raw seed cake to 2452 \pm 4.83 mg/100 g toasted seed cake, these values were higher than 113.12 mg/100 g reported by Ibeabuchi (2014) who analysed fermented calabash seed and it was also higher than 73.12 mg/kg in the whole calabash seed reported by [29].

The magnesium element ranged from 567 \pm 1.52 raw seed cake to 370 \pm 3.00 mg/100 g toasted calabash seed cake, the values are higher when compared with the values obtained by [3] Ibeabuchi (2014) who reported magnesium levels of 56.80 to 56.74 mg/100 g fermented and raw calabash seed respectively, 49.72 mg/kg whole calabash seed [29] and 205 mg/kg whole calabash seed [13]. Magnesium is involved in enhancement of activities of metabolic enzymes [29].

The value of calcium ranged from 3.11 \pm 0.36 raw seed cake to 3.78 \pm 0.44 mg/100 g toasted seed cake, these values were lower than the ranged from 8.42 fermented calabash seed to 3.10 \pm 0.36 mg/100 g raw calabash seed reported by [3], and 49.72 mg/kg whole calabash seed as obtained by Chinyere *et al.* (2009). The value of calcium obtained in this study compared well with the recommended ratio of 2.2 [31]. Supplementation with calcium may be necessary if the seed meal is to be used for diet formulation particularly as fry feeds [3].

The value of copper ranged from 14.3 \pm 0.23 mg/100 g raw seed cake to 40.75 \pm 1.07 mg/100 g toasted seed cake, the values were higher than 0.53 mg/100 g raw pumpkin seed flour, 0.56 mg/100 g fermented pumpkin seed flour, 0.45 mg/100 g germinated pumpkin seed flour and 0.12 mg/100 g roasted pumpkin seed flour reported by [32]. The differences in this finding with the earlier findings could be attributed to the different in the species, maturity, processing methods, displacement of oil from the seed, and some other factors. The value of zinc ranged from 0.15 \pm 0.02 raw seed cake to 0.20 \pm 0.03 mg/100 g toasted seed cake, the values were lower than 0.27 mg/100 g raw pumpkin seed flour, 0.30 mg/100 g fermented pumpkin seed flour, 0.25 mg/100 g germinated pumpkin seed flour and 0.43 mg/100 g roasted pumpkin seed flour reported by [32].

The iron ranged from 57.4 \pm 3.24 raw calabash seed cake to 57.6 \pm 1.578 mg/100 g toasted seed cake, the values were higher than 14.88 raw calabash seed to 11.76 mg/100 g fermented calabash seed reported by [3, 29] reported low value

(14.40 mg/100 g) of iron content in the raw calabash seed. The results of iron obtained in these studies were higher when compared with other findings. Differences observed between the results of these studies can be attributed to different processing methods employed, differences in geographical location, soil composition, cultivation, climate, maturity stage and harvesting time of the seeds. Iron is involved in the formation of hemoglobin, myoglobin, growth, and boost immune system, as cofactor for enzymes and so many other functions. Iron is the most abundant micro element in the plant seed. In this study, calabash seed has shown to be rich in iron, which makes it a good source suitable for pregnant women. This is an indication that the seeds could serve as a source of iron for both man and domestic animals including fishes. Iron and copper showed consistent levels across both samples, suggesting the seed's viability as a source for these vital minerals.

The value of Manganese ranged from 26.4 \pm 0.40 raw seed cake to 26.0 \pm 0.88 mg/100 g toasted seed cake, the finding of this study was higher when compared with the finding of [32] who obtained 0.00 mg/100 g in raw and different processed pumpkin seed flours analysed. Zinc and manganese are dietary trace elements needed for a healthy immune system among other functions.

Table 2: Mineral composition of calabash seed cake; Mean \pm SE

Mineral composition	Calabash seed cake (mg/100 g)	
	Raw	Toasted
Sodium	1200 \pm 2.34 ^a	1154 \pm 0.93 ^b
Potassium	2900 \pm 1.85 ^a	2452 \pm 4.83 ^b
Magnesium	567 \pm 1.52 ^a	370 \pm 3.00 ^b
Calcium	3.11 \pm 0.36 ^b	3.78 \pm 0.44 ^a
Copper	14.3 \pm 0.23 ^b	40.75 \pm 1.07 ^a
Zinc	0.15 \pm 0.02 ^b	0.20 \pm 0.03 ^a
Iron	57.4 \pm 3.24 ^b	57.6 \pm 1.578 ^a
Manganese	26.4 \pm 0.40 ^a	26.0 \pm 0.88 ^b

Values with same letters are not significantly ($p < 0.05$) different.

Conclusion

In conclusion, the toasted sample of *Lagenaria vulgaris* exhibited noticeably higher levels of nutrients when compared with the raw seed cake. Generally, the processing methods used produced cake with better nutritional values than the raw sample.

Recommendation

Based on the finding of this study the following recommendations were made;

1. The results of this finding could serve as baseline studies for aquaculture industry
2. The use of calabash seed as source of dietary protein in fish feed must be considered
3. Therefore, it's utilization in fish feed formulation should be encouraged, though feeding trials still need to be conducted to determine the level of inclusion, productive and economic efficiency.

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