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# Proximate Composition and Functional Properties of Some Defatted Calabash Seeds

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***ABSTRACT***

The ever-increasing cost of animal protein coupled with poor standard living in terms of per capital income in developing nation has necessitated the search for alternative sources of dietary protein. Two local varieties of calabash seeds were extracted using soxhlet method and n-hexane as refluxing solvent. The defatted cakes were subjected to Isoelectric precipitation to obtain protein concentrate which was characterized by analysing their moisture, ash, crude protein, crude fibre, available carbohydrate, water absorption capacity, oil absorption capacity, Foam capacity and stability and Bulk density. The results of the analysis revealed high amount of protein (69-70%) and also high water and Oil absorption capacities (84.6-134.1%) and (87.2-129.8%) respectively.

**Keywords**: Cake, Calabash, Proximate, Functional properties

## 1.0 INTRODUCTION

Looking at the projected world population in few decades to come, coupled with the shortage and high cost of animal protein then, there is need for a complementary and alternative source to animal protein that is relatively cheaper and richer. To satisfy this quest, harnessing the not-well explored legumes such as *Lagenaria*siceraria which is readily available for plant protein becomes desirable. Though, a lot of information is known on themedicinal aspects of bottle gourd (Katare et al., 2014). However, its potential as a possible food security crophas been lowly documented (Chimonyo and Modi, 2013). 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 *(Lagenaiasiceraria)* are under-utilized and are almost going extinct (Ibeabuchi, 2014).

Bottle gourd (*Lagenariasiceraria*) 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 (Chimonyo and Modi, 2013). The plant is believed to be a native of Africa and is commonly known as *Luddai* (Hausa). Its stem is prostrate, angular, ribbed, thick, brittle, hairy, up to 9m long and produces no sap when cut. Leaves are simple, soft and hairy, up to 40cm long and 40cm broad. (Hassan et al., 2008, Ileola*et al.,* 2019). In Nigeria, the plant is commonly grown in Sudan savanna, particularly in Zamfara State, where it is planted as cash crop (Hassan et al., 2008).The fruits, leaves, oil, and seeds are edible and used by local people as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases (Ileola*et al.,* 2019).Given such benefits, it is surprising that bottle gourd is the cucurbit with the least amount of scientific research directed at enhancing utilization, letalone productivity (Chimonyo and Modi, 2013).

Ibeabuchi (2014) investigated the proximate and functional properties of raw and fermented bottle gourd seed (*Lagenariasiceraria*). The result of the proximate composition showed that moisture, protein and fat content increased from 9.07 to 10.50 g, 6.43 to 7.11 g and 50.56 to 60.64 g for raw and

fermented bottlegourd seeds respectively. Significant difference (P < 0.05) existed in their proximate composition, except for ash which had no significant difference. The result of the functional properties for the fermented sample showed an increase in the foam capacity, emulsion capacity and foam stability, but opposite effect was observed in the case of wettability, swelling index, gelation and pH. There was a significant difference (P < 0.05) in the functional properties of the samples, but shows no significant difference pH of the fermented sample. The result from this study is an indication that a good manipulation can improve the fermented seeds so that they can be more desirable for use as an alternative food condiment in the future.

Popoola *et al.* (2016) studied the optimization of oil extraction from giant bushel gourd seeds using response surface methodology. The results obtained in this study showed that gourd seedis a potential source of vegetable oil. Roasting duration and temperature combinations influenced both oil yield and quality significantly at 95% confidence level. Models developed showed that gourd oil yield and oil quality (FFA, color, specific gravity, saponification value, moisture, and refractive index) were influenced by roasting temperature and duration. Six possible optimum solutions were found with desirability ranging from 0.65 to 0.67. The best of the six conditions was roasting at 100°C for 20 min, which gave optimum oil yield (27.62%) and good quality attributes (FFA: 0.61%, color: 3.47 abs, specific gravity: 0.90, saponification value: 289.66 mL, and refractive index: 1.47).

Ogunbusola (2018), examined the nutritional and antinutritional composition of calabash and bottle gourd seed flours (var*L*agenariasiceraria). The result indicated that Calabash and bottle gourd seed flours are good sources of dietary protein, nutritionally important minerals and they exhibit very fair levels of antinutritional factors. The seed flours may be an economic and alternative source of protein, thereby alleviating the problem of protein malnutrition and enhance the utilization of otherwise underexploited agricultural produce.

In another study, Patel *et al.* (2018) investigated the effect of fortification of de-oiled bottle gourd (*Lagenariasiceraria*) seed on the functional and chemical characteristics of the biscuit: anutritional assessment. The result showed that de-oiled bottle gourd (*Lagenariasiceraria*) seed is a highly nutritious by-product of edible oil industries. The seed contains a substantial amount of quality protein, dietary fiber, minerals, and essential amino acids. Nutritional assessment of optimized biscuit heightened a significant (p < 0.05) rise in the amount of crude protein, ash content, and soluble fiber as in comparison with the control group sample. BGSCP fortification revealed significant improvement (43.22) in terms of essential amino acid availability ascompared to control biscuit. However, the fatty acid compositions of optimized biscuit were insignificantly (p > 0.05) different.

Since plant proteins play a significant role in human nutrition, particularly in developing countries where average protein intake is less than that require (Ayodele and Aladesanmi, 2015). Then to solve the problem of protein malnutrition, more researches need to be done. Hence, this research was designed to isolate and characterize protein concentrate from different varieties of *Lagenaria*seceraria seeds.

## 2.0 MATERIALS AND METHOD

### 2.1 Sample Collection and Treatment

Dried samples of two varieties of Calabash, *Lagenaria*seceraria (African wine kettle gourd) and *Lagenaria*seceraria II (Basket ball gourd) seedswere purchased fromZauma village inBukkuyum Local Government, Zamfara State-Nigeria. The seeds were then sortedto remove grits, damaged and unripe seeds after which the two varieties of calabash weregrinded using pestle and mortar and kept for subsequent analysis.

### 2.2 Preparation of Calabash Seeds Protein Isolate

Defatted flour samples of the two varieties of gourd seeds were prepared by subjecting the seed to soxhletextraction using n-hexane as refluxing solvent at temperature of (40-60) ºC for nine hours. The defatted flour sample was dried at ambient temperature (28±4 ºC), packed in polythene bag, and kept in an air-tight plastic container and labeled appropriately.

### 2.3 Analytical Procedure

#### 2.3.1 Production of Isolate/Concentrate from the Seeds.

Boye et al 2010 and Ogundele et al 2013 were used in the preparation of the concentrated and isolate with slight modification.

#### 2.3.2 Proximate Analysis

The Association of Official Analytical Chemists (AOAC, 1990) was used for the determination of moisture, ash, crude lipid, crude fibre and crude protein content.

#### 2.3.3 Estimation of Percentage Carbohydrate

Estimated carbohydrate was calculated by difference by subtracting total sum of crude protein, crude lipid, crude fibre and ash from 100% dry weight sample.

#### 2.3.4 Determination of Moisture Content

Exactly 0.5g of the sample was weighed and placed in an oven at 100°C for 24 hours after which the sample was removed and cooled in desiccator and then weighed. The weight lost was obtained by subtracting the weight of the dried sample from the original weight of the sample. This is calculated using equation 1.

$$\text{Percentage Moisture Content} = \frac{\text{loss in weight due to drying}}{\text{weight of fresh sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

#### 2.3.5 Determination of Ash Content

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600°C in muffle furnace for 4h(AOAC 1990).The powdered sample(0.5g) was weighed and transferred into an empty crucible. The crucible containing the sample was then heated in a muffle furnace at a temperature of 600°C for 2hours. It was then removed and allowed to cool in a desiccator, weighed and recorded. The percentage ash was determinedusing equation 2.

$$\text{Percentage ash} = \frac{\text{Weight of ash}}{\text{weight of sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

#### 2.3.6 Determination of Crude Protein

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crudeprotein is determined by Kjeldahl method. The method involves: Digestion, Distillation and Titration (AOAC 1990).

#### 2.3.7 Digestion

Exactly (0.5 g) of the sample was weighed into Kjeldahl flask and 20 mL of concentrated sulphuric acid, 0.5 g of $CuSO_4$, 5 g of $Na_2SO4$ and a speck of selenium tablet were added. Heat was applied in a fume cupboard slowly at first to prevent undue frothing; it continued to digest for 45 min until the digest became clear pale green. It was left to completely cool and 100 mL of distilled water was added.

#### 2.3.8 Distillation

Markham distillation apparatus is used for distillation and 10ml of aliquot of digestion was put into Kjeldahl flask, distilled water was added to make the volume up to 50ml. 40% of NaOH solution was also added to extract the ammonia which was evaporated into 20ml of boric indicator. The ammonia liberated into the boric acid until the volume is made up to 40ml in the conical flask, then the color changes from pink to green. Then the ammonia produced was trapped in the acid.

#### 2.3.9 Titration

The collected sample with ammonia was then titrated against 0.01M of HCl to end point, which give the ammonia content in the sample. The color changes from green to pink as the end point and then the titer value was recorded. The percentage of nitrogen was calculated using equation 3.

$$\%N_2 = \frac{\text{T.V} \times \text{Conc.}(0.01\text{N}) \times 0.014 \times 50\text{ml}}{\text{Aliquot (10ml)} \times \text{weight of sample (0.5g)}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

% Crude protein = %N2 x 6.25

#### 2.3.10 Determination of Percentage Crude Lipid Procedure

Ten grams (10 g) of the dried sample was weighed and transferred into a thimble which has been dried and weighed. The thimble containing the powdered sample was weighed and the mouth of the porous thimble was covered with fat free absorbent cotton wool in order to distribute the draping petroleum ether. The thimble was then placed in a soxhletextractor fitted to a round bottom flask containing 120ml of petroleum ether. The apparatus was switched on for 5 to 6 hours at 50°C.

After this, the thimble was removed and from the soxhlet and weighed. The flask was thenremoved with care and the organic solvent was evaporated. Finally, the extracted flask containing the oil was weighed to know the content of the crude lipid (AOAC,1990).

The percentage crude lipid is calculated using equation 4.

Percentage crude lipid content = $\frac{\text{Weight loss by flask}}{\text{weight of sample}} \times 100$....…………………….(4)

### 2.3.11 Determination of Percentage Crude Fiber

The organic residue left after extraction of sample with ether was used to determine the crude fibre.0.5g of the extracted sample from the extraction of lipid was weighed and put into a conical flask and added to it, 200ml of 1.25% $H_2SO_4$ solution and distilled water then boiled gently for 30mins to maintain constant volume. The sample was then filtered and rinsed well with hot distilled water. Using spatula, the material was scraped back into the flask. 200ml of 1.25% NaOH was added with distilled water, the residue was heated again for 30mins and filtered then washed thoroughly with hot distilled water. After allowed to drain, the residue was scrape into a crucible and dried overnight in an oven at 105$^{O}$C. Then cooled in a desiccators and weight ($W_1$) was taken. After that, it was then put into a muffle furnace to burn to ash for 90mins at 550$^{o}$C. And allowed to cool in a desiccator and weighed again ($W_2$). The percentage crude fiber is calculated using equation 5.

$\%\text{crude fiber} = \frac{W1-W2}{W0} \times 100$ ……………… (5)

### 2.3.12 Determination of Carbohydrate Content

This was determined by difference in percentage. (% Carbohydrate = 100 - total weight of other nutritional factors).

## 2.4 Analysis of the functional properties

### 2.4.1 Determination of water absorption capacity

The modified procedure as described by Rodriguez *et al.* (2005) was adopted to determine the water absorption capacity. One gram (1 g) of each sample was weighed out into 13 cm$^3$ pre-weighed centrifuge tube. Ten (10 cm$^3$) of distilled water was added into the tube under continuous stirring with a glass rod at room temperature for 25 min. to make a suspension. The tube was then centrifuged for 20 min at the speed of 3500 rpm for 15 mm. After which, supernatant was cast-off then the tube and its content re-weighed and taken. The gain in weight is the water absorption capacity of the test sample.

### 2.4.2 Determination of oil absorption capacity

Fat absorption capacity was determined using the method described by Lin and Zayas et al (1987) with little modifications. A small gram (0.5 g) of each sample was weighed into 13 cm$^3$ pre-weighed centrifuge tube and thoroughly mixed with 5cm$^3$ vegetable oil. The emulsion was incubated at room temperature for 25 min and then centrifuged for 15min and the supernatant was carefully removed, and the tube was reweighed. The gain in mass is the oil absorption capacity of the sample and was calculated using equation 6

$$Oil\ Absorption\ Capacity = \frac{F_2 - F_1}{F_0} \ldots\ldots\ldots.(6)$$

Where $F_0$ is the weight of the sample (in gram), $F_1$ is the weight of the tube plus the sample (in gram), and $F_2$ is the weight of the tube plus the sediment (in gram).

### 2.4.3 Determination of foam capacity and foam stability

Foaming capacity (FC) and stability (FS) were determined by the method described by Sze-Tao and Sathe (2000), with certain modification. One gram (1 g) of each sample was isolated in 50 cm$^3$ of distilled water. The solution was stirred at a speed of 160 rpm for 10 min. The blend was immediately transferred into a 100cm$^3$ graduated cylinder. The volumes were recorded before and after stirring using AbbeyandIbeh (1988) formulasin equations 7 and 8.

$$Foam\ capacity\ (\%) = \frac{volume\ after\ whipping - volume\ before\ whipping}{volume\ before\ whipping} \times 100\%.(7)$$

$$Foam\ stability\ (\%) = \frac{Volume\ after\ standing - Volume\ before\ whipping\ ml}{Volume\ before\ whipping} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..\ldots(8)$$

## 3.0 RESULTS AND DISCUSSION

The results for the proximate composition of the two varieties of the Gourd seeds

(*Lagenariasiceraria I & II*) are shown in Table 3.1& Table 3.2 respectively and represented in Figure 3.1. The functional properties of the two concentrates are shown in table 3.2 and represented in Figure 3.2.

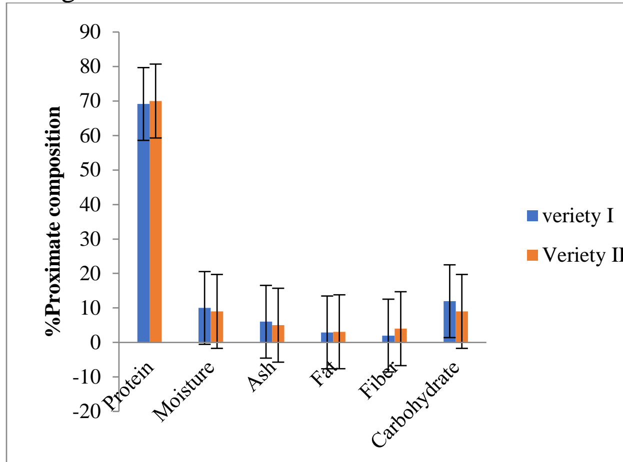


**Figure 3.1: Proximate compositions of the two concentrates from variety I and II**

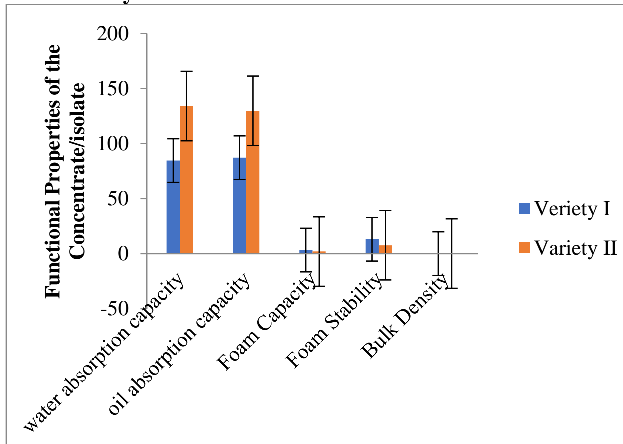


**Figure 3.2: Functional properties of the concentrates.**

**Table 3.3: Functional properties of protein isolate from calabash seeds**

| Functional properties | (*Lageneriasiceraria I* ) | ( *Lageneriasiceraria II*) |
|---|---|---|
| Water absorption capacity (%) | 84.6 | 134.1 |
| Oil absorption capacity (%) | 87.2 | 129.8 |
| Foaming capacity (%) | 3.30 | 1.92 |
| Foaming stability (%) | 13.11 | 7.69 |
| Bulk density (g/cm$^3$) | 0.07 | 0.11 |

## 4.0 DISCUSSIONS

### 4.1 Proximate composition

Out of the six parameters analysed for proximate analysis, protein account for 70% composition in all the samples (69 .14- 70.0 %). This shows good level of beneficiation compared to the 35% in the raw seed as reported by Hassan et al 2008. The result obtained is in agreement with that reported by Boyeetel 2010 for Desi chick pea and kabuli chick pea concentrates.

Moisture content of the concentrates, ranges from 9.0 to 10.0 %, with variety I having higher value. This index depicts the storage capacity of the concentrate. Samples with high moisture content are susceptible to bacterial attack and hence got spoiled easily.

Ash is index of mineral composition in samples. The higher the ash content the higher the percentage mineral composition of the concentrate. The values are relatively high when compared with other isolates and seed samples reported by Boye et al 2010 on green pea, lentil and *also lagenariasiceraria* reported by Hassan et al 2008.

As expected, the concentrates registered low fat (Crude lipid) content. This is because the concentrates were derived as a result of soxhlet extraction of the seed flower followed by iso electric precipitation of the two samples.

Crude fiber content of variety II is twice that of variety I which are all below the recommended daily allowance (RDA) for fiber is 18-35g, which means consuming 100g of the two concentrate cannot provide up to 50% of daily fiber for the body(Gulthrine, 1989). Foods with high crude fibre content are good in the treatment of conticipation. This indicates the samples are not promising sources of fibre.

Available carbohydrate content ranges between 9.0 -11.96 % with concentrate from variety II having higher value. The result obtained in this study is comparable to …

### 4.3 Functional properties

Optimal extraction condition used were adopted from Boye et al 2010(i.e pH: 9.5 with 1/15 solid/liquid ratio at 35$^0$C). The functional properties analysed are water absorption capacity, oil absorption capacity, foaming capacity, foaming

stability and bulk density. The parameters (Table 3.2) were analysed to ascertain the applicability of the concentrates in food processing and formulation. The samples were found to have high water absorption capacity (84.6 – 134.1 %) and low oil absorption capacity (87.2-129.8 %) compared to 0.6 -2.7g/g and 226-177% respectively reported for red lentis and yellow pea protein (Boye 2010). The concentrates were found to have poor percentage foaming capacity, stability and bulk densities(g/cm$^3$).

## 5.0 CONCLUSION

From foregoing, it evident that, the concentrates proximate compositions, especially crude protein have increased drastically compared to dehulled seed. This makes it potential source of protein source for dietary formulation considering its improved functional properties. However, more research on the amino acid profile, amino acid scores need to be carried out to ascertain level of essential amino acid in the concentrates.

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