ADAN J. Agric. 2023, 4: 53-63 DOI:10.36108/adanja/3202.40.0160

ISSN 2736-0385

Determination of the chemical composition of the ripe and unripe ballnut kernel (*Calophyllum inophyllum*) meals

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Abstract
This research was carried out to evaluate the chemical composition of ripe and unripe ballnut kernel meals. The ripe and unripe samples were harvested, decorticated and the kernels oven dried at 50^{o}C for 24 hours before milling and storage at room temperature in labelled containers. The proximate, mineral, amino acids and phytochemical composition of the ballnut kernel meals were determined. Data generated were subjected to analysis of variance procedures using SPSS 17.0 Windows computer software package with t-test as a statistical tool. The results showed that unripe ballnut kernel was superior in metabolizable energy (3707.60±2.64 kcal/kg), sodium (0.25%), potassium (0.88%), magnesium (0.29%), calcium (0.19%) and iron (107.45%) to ripe ballnut kernel meal. The phytochemicals in the two samples were phytic acids, oxalic acid, alkaloids, saponins, tannins and hydrogen cyanides. The concentration of the phytochemicals in the two samples was not significantly (P>0.05) different. The concentration of amino acids between the two samples was statistically (P>0.05) similar. From this study, it was concluded that the ripe and unripe ballnut kernel meal was similar in their composition and could therefore serve as potential animal feed resources owing to high energy level, amino acids profile as well as low fibre and other anti-nutritional factors.

Keywords: *Ripe ballnut kernel, unripe ballnut kernel, crude protein, phytochemicals, minerals*

Introduction

Feeding of animals by livestock farmers in Nigeria has become very difficult recently, due to the high cost of raw materials meant for feed compounding. The prices of conventional energy concentrates (maize, millet and guinea corn) and plant protein concentrates (soybean meal, cotton seed meal and groundnut cake) which constitute about 50-65 and 30-35%, respectively in compounded feeds have tremendously risen recently due to drought, poor yield and massive export out of the country by grain farmers. This condition has created a big gap between the demand and supply of these conventional feed resources for feed mill industries in the country. Utilizing non-conventional feed resources has been one of the approaches used in solving this problem but the traditional non-conventional feed resources (crop residues, agro-industrial by-products, slaughterhouse by-products, leaf and seed meals) are no longer sustainable (Amata, 2014, Andhale, 2024).

Therefore, animal nutritionists must search for more alternative feed resources to support the livestock industry. It is for this reason that the ballnut (*Calophyllum inophyllum*) kernel has been considered. The ballnut (*Calophyllum inophyllum* Linn) tree is valued for its hardiness and beauty as an ornamental tree that provides shade and shelter from the wind on streets, parks, andcoastal areas. The wood is a prized timber for carving, cabinet making and boat building (Friday and Okano, 2006). The tree grows to a height of 8-20 m, sometimes reaching up to 35 cm, the canopy width which is often greater than the tree's height when grown in an open location. It has a broad spreading crown mostly with large, gnarled, horizontal branches. According to (Chavan *et al*., 2013), ballnut tree is made of elliptical, shiny and tough leaves and flowers arranged in axillaries cymes with a sweet, lime-like fragrance. The tree bears flowers twice a year with numerous fruits which are spherical drupes and arranged in clusters. Once ripe, the smooth, yellow epidermis discloses a thin layer of pulp, with a taste similar to apple. The grey, ligneous and soft nut contains a pale-yellow kernel, which is odourless when fresh but when chewed, it coats the mouth and emulsifies saliva and its insipid taste turns bitter.

Different parts of the plant, such as the leaves, bark and seeds have medicinal potential and have been used for many purposes. Ballnut kernel contains different compounds which include coumarins, xanthones, flavonoids, steroids, triterpenoids and oil, having about 40-75% of it on dry weight basis. A mature tree may produce about 1-10 kg of oil/year which according to Atabania *et al.* (2011) depends on the productivity of the tree and the oil extraction efficiency. The ballnut kernel oil has a greenish-yellow colour, has benzoic and oxy-benzoic acids, a small amount of vitamin E, phosphoamino lipids, free fatty acids, glycerides and steroids (canophyllal, canophyllol, canophyllic acids) as it constituents. The seed oil has also been found to exhibit good physio-chemical properties andcould be utilized as a biodiesel feedstock (Atabania *et al.*, 2011; Chavan *et al*., 2013; Arumugam and Ponnusami, 2019).

According to Chavan *et al.* (2013), the oil demonstrates anti-inflammatory activity with 4-Phenylcoumarin calaphylloidea and a group of xanthones, including dehydrocycloguandin, chlorophyllin-B, Jacareubin, mesuaxanthone-A, mesuaxanthone-B and euxanthone. The presence of xanthones explains the reduction of rashes, sores, swelling and abrasions with topical application of the oil. In addition, the presence of xanthones and coumarins in ball nut seed oil demonstrated anti-oxidant properties, specifically inhibiting lipid peroxidation and protecting skin cells from damage by reactive oxygen species. Praveena *et al*. (2013) observed that the oil of ballnut is rich in bioactive activities, such as anti-HIV, anti-cancer, anticoagulant, anti-inflammatory, anti-platelet aggregation. It is also used traditionally for medicine, cosmetics and lamp oil (Prabakaran and Britto, 2012).

There were no reports in the literature highlighting the nutrient composition of ballnut kernel meal as well as comparing the nutrient composition of the ripe and unripe ballnuts, therefore, this study was designed to determine the chemical composition of ripe and unripe ballnut kernel (*Calophyllum inophyllum* Linn) meals.

Materials and methods

Sample collection

The ripe ballnut kernels were gathered from the floor, and unripe kernels were plucked in their greenish stage from the ballnut tree in the Department of Forestry and Wildlife Management plantation, University of Calabar, Calabar (Fig. 1, 2).

Fig. 1: Unripe ballnut seed

Fig. 2: Ripe ballnut seed

Sample preparation and analysis

The hard layers covering the kernel were manually decorticated using stone to expose the kernels and the kernels were oven-dried at 50^{o}C for 12 hours before milling using an industrial blender (Kenwood) to a fine flour. The milled samples were stored at room temperature using air-tight plastic containers in preparation for the laboratory analysis. The proximate and phytochemicals analysis was conducted at the Department of Animal Science Laboratory, University of Calabar, Nigeria and the mineral and amino acids profile was carried out at Animal Care Laboratory, Lagos, Nigeria

The proximate composition of the ballnut kernel meal was carried out using the methods of (AOAC 2005). The components analysed were crude protein, ether extract, ash, and crude fibre. Nitrogen-free extract was obtained by subtracting the total crude protein, crude fibre, ether extract ash and moisture from 100. The percentage crude protein content (N×6.25) was determined by the micro-Kjeldahl method and the metabolizable energy was estimated using the equation: **ME (kcal/ kg) = 37 (%CP) +81.8 (%E.E)+35.5 (NFE) (**as described by Abeke *et al*. 2012)

Atomic Absorption Spectrometer (model 703 Perkin Elmer, Norwalk, CT, USA) was used in the determination of calcium, manganese, magnesium, sodium, potassium and iron. Sodium and potassium were determined using a flame photometer (Sherwood flame photometer 410, Sherwood Scientific Ltd., Cambridge, UK), while Phosphorus was determined using Vanadomolybdate method AOAC (2005).

Amino acids were determined by Ion Exchange Chromatography (AOAC, 2005) using the Technicon Sequential Multi-sampling (TSM) amino acid analyzer. The period of analysis was 76 min for each sample. The gas flow rate was 0.50 mL minG1 at 60EC with reproducibility consistent within ±3 min. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of three determinations. Nor-leucine was the internal standard.

The phytochemical analysis was carried out to determine the composition of cyanogenic glycosides, tannins, oxalate and alkaloids. Phytate was determined by the anion-exchange method as described by AOAC (2005) using phosphate as the standard, while saponin composition was determined using the spectrometric method as described by Brunner (1984). The total flavonoids and alkaloids were determined according to the methods outlined by Harborne (1973).

Data from the chemical analysis obtained in triplicates values were tested for significance using the SPSS 17.0 Windows computer software package with a T-test as a tool for comparison between the two samples.

Results and Discussion

Proximate composition of ballnut kernel meal

The result of the proximate composition of the test samples showed that the percentage dry matter composition of ripe ballnut kernel (97.02%) and unripe ballnut kernel (98.50%) were not different (P>0.05) from each other. The high dry matter content observed in the two samples implied that they contain less moisture with a higher proportion of other nutrient components and could have longer shelf life, if well packaged and stored properly. The knowledge of the dry matter content of the feedstuff is essential as it helps to determine the feeding standards of the animal. An increase or decrease in feed dry matter content results in over or under-feeding of nutrients (World Ostrich Association, 2013).

The crude protein content of ripe ballnut kernel meal (8.16%) was similar (P>0.05) to 8.52% observed in the unripe sample. The crude protein of the test samples had a close resemblance with those of maize (8.75%) and sorghum (9.16%) as stated by Ape *et al*. (2016). Percentage crude protein content of ballnut kernel meal was low compared with some nuts like walnuts, containing 28.85% (Chijioke *et al.*, 2015), and Jatropha seed with 29.4% (Ugbogu *et al*., 2014). The results compared favourably with the earlier report of 9.99-11.89% for the de-oiled ballnut cake but higher than 8.40 and 3.77% reported by Ibironke *et al*. (2015) and Chijioke *et al.* (2015) for areca nut and walnut seeds, respectively.

The total fat contents in the ripe ballnut kernel (10.10 %) and unripe ballnut kernel meal (9.30%) did not differ (P>0.05) significantly from each other. Fats are organic compounds and an important constituent of plants and animal tissues, supplying energy to the body. A diet is said to provide 1-2% of fat as its calories of energy as excess fat consumption is implicated in certain cardiovascular disorders (Osabor *et al*., 2009). Fat is important in diets because it promotes fat-soluble vitamin absorption. It is a high-energy nutrient and does not add to the bulk of the diet (Osabor *et al*., 2009).

The crude fibre content was 6.00% for ripe ballnut kernel meal and 4.50% for the unripe sample. Variation in the crude fibre content between the two samples was significant (P<0.05). The percentage crude fibre values obtained in this experiment were higher, compared with2.0% reported for maize and 2.57% for Jatropha (Osabor *et al*., 2009) but similar to 6.01% for walnut seed (Chijioke *et al.*, 2015). Ibironke *et al*.(2015) reported 34.25% as crude fibre content of areca nut, a value quite higher than those obtained in this study. Crude fibre is a significant component of diets, playing a useful role in providing roughage that aids digestion and reduces the accumulation of carcinogens in the body (Jun *et al.*, 2023). It has been reported that the presence of fairly high crude fibre in food material decreases the dry matter digestibility in animals (Zhang, 2013). The crude fibre content of the ballnut kernel meal reported in this study is within the safety limit of monogastric animals.

The ash content of the ballnut was 2.20% for the ripe kernel meal and 2.05% for unripe meal. The results showed no significant difference between the two samples. The values for ash recorded in this study were lower than the range (5.44 - 6.07%) earlier reported for de-oiled seed cake of ballnut but comparable to 1.76% reported for areca nut by Ibironke *et al*. (2015). The ash content of food is reflective of its mineral content and is an index for assessing the quality of feeds and feedstuffs. The ash content of 1.5-2.5% has been recommended by Omosuli *et al*. 2009) as the suitable range in nuts, seeds and tubers for animal feeds. The ash contents of the test samples were within this range and, therefore, could be recommended for use in animal feed production.

Nitrogen-free extract (NFE) was 71.36% for the ripe sample and 74.13% for unripe sample. Variation in the NFE composition between the two samples was not significant. Nitrogen-free extract content is an index of the potential of such feed material in supplying energy for animals. Ibironke *et al*. (2015) reported 79.18% as NFE value of areca nut.

The metabolizable energy values differed (P<0.05) significantly, with unripe ballnut kernel meal having the highest value corresponding to 3707.60ME (kcal/kg) relative to 3661.38 (ME) (kcal/ kg) observed for the ripe ballnut kernel meal. The metabolizable energy recorded in the two samples was higher than 3430.00, 3300.00 and 3000.00 (kcal/kg) of metabolizable energy reported for maize, guinea corn and cassava meal, respectively. The above results indicated that ball nut seed meal has the potential to replace conventional feed resources as an energy source in poultry and livestock feeds.

Table 1: Proximate composition of ballnut kernel meal

Composition	Ripe	Unripe
Dry matter (%)	97.02±0.11	98.50±0.11
Crude protein (%)	8.16±0.30	8.52±0.21
Crude fat (%)	10.10±1.99	9.30±0.18
Crude fibre (%)	6.00±0.65*	4.40±0.32
Ash (%)	2.20±0.23	2.05±0.23
Nitrogen free extract (%)	71.36±0.02	74.13±0.02
Metabolizable energy (kcal/kg)	3661.38±0.02	3707.60±0.70*

[*]Values are Means ± SD (n=3)

* Significant means

Phytochemical composition of ballnut kernel meal

The results of the phytochemical composition (Table 2) indicated that the ripe and unripe ballnut kernel meal contained phytic acid, oxalic acid, alkaloids, saponins, tannins and hydrogen cyanide. The stage of harvesting did not (P>0.05) affect the composition of these compounds in the two samples. The phytochemicals are synthesized for plant defense and other biological functions, with multiple health benefits at lower concentrations (Thurber and Fahey, 2009). Phytic acids have been reported to possess anti-oxidant, anti-cancer, hypo-cholesterolemic, and hypolipidemic effects, thereby preventing oxidative browning in fruits and vegetables by inhibiting polyphenol oxidase (Fang *et al*. 2022, Chao *et al*. 2023). On the other hand, saponins reduce the absorption of certain nutrients like glucose and cholesterol in the gut, thereby preventing diabetes and cardiovascular diseases (Thurber and Fahey 2009, El Barky *et al*. 2017). Alkaloids were also known to show medicinal as well as physiological activities (Akinpelu and Onakoya, 2006). Tannins and flavonoids have been shown to have biological activities that were beneficial in the prevention and treatment of many ailments (Obasi *et al*. 2011 and Oduse *et al*. 2012). Despite the beneficial effects of phytochemicals, Bernhoft (2010) observed that they are detrimental to human and animal health at high dosage. Tannins is known to depress growth by decreasing protein quality and digestibility, causing liver damage, and inhibiting absorption of minerals such as iron which leads to anaemia (Singh, 2023). Studies have shown that areca nut, walnut, and jatropha contain higher concentrations of phytic acid, oxalic acid, alkaloids, saponins, and tannins when compared with those of ripe and unripe ballnut kernel meals (Sari *et al*., 2020). The hydrogen cyanide concentration in the ballnut meals was lower compared to that of jatropha meal which was reported as 60.70 mg/kg (Ugbogu *et al*., 2014). Lower concentrations of the phytochemicals/anti-nutrients in the ripe and unripe ballnut kernel meals imply that they can be used in compounding feeds for animals without detrimental effects on performance.

Table 2: Phytochemical composition of ballnut kernel meal (mg/100 g)

Composition	Ripe	Unripe
Phytic acids	1.86	1.83
Oxalate	6.30	6.12
Alkaloids	3.06	3.64
Saponins	1.70	1.70
Flavonoids	-	-
Tannins	0.02	0.02
Cyanide	10.26	10.58

*Values are Means ± SD (n=3)

Mineral composition of ballnut kernel meal

The results of the mineral constituents presented in Table 3 indicated the presence of sodium, potassium, magnesium, phosphorus, calcium and iron in both the ripe and unripe ballnut kernel meals. Unripe ballnut kernel meal was observed to be richer (P<0.05) in sodium, potassium, magnesium and calcium composition relative to ripe ball nut kernel meal. In contrast, the composition of the remaining minerals was similar (P>0.05) between the two samples. The result indicated that unripe ballnut kernel meal was richer in some minerals and it becomes so important when the usefulness of such minerals like sodium, potassium, magnesium phosphorus, and calcium in the body of animals is considered. For instance, calcium and phosphorus are essential for bone metabolism and assist in teeth development (Serna and Bergwitz 2020). Calcium is a cofactor of the three important enzymes, pyruvate dehydrogenase complex, isocitrate and ketoglutarate dehydrogenase complex in the citric acid cycle. Iron plays important roles in many proteins and enzymes, notably in haemoglobin to prevent anaemia (Osabor *et al.* 2009), while magnesium is also a cofactor for many enzymes. Sodium and potassium are very important for nerve transmission and osmolality of body fluid. The presence of minerals in the diet is generally required for normal growth, activities of muscle and skeletal development, cellular activity and oxygen transport, chemical reaction in the body and intestinal absorption, fluid balance and nerve transmission as well as the regulation of acid-base balance (Morris and Mohiuddin, 2023). Low mineral consumption could lead to in proper proportion, reduction in production, infertility problems, weakness of the bone and increased incidences of non-infectious diseases. The minerals composition of ball nut seed meals observed in this experiment were lower than 2.89% for iron, 265.95% for phosphorus, 59.77% for magnesium, 44.99% for calcium, 24.08% for potassium and 9.59% for sodium reported by (Chijioke *et al*. 2015) for walnut seed.

Table 3: Mineral composition of ballnut seed meal (%)

Minerals	Sodium	Potassium	Magnesium	Phosphorus	Calcium	Iron
Ripe	0.22±0.02	0.69±0.01	0.24±0.01	0.35±0.090	0.17±0.01	93.05±0.20
Unripe	0.25±0.01*	0.88±0.02*	0.29±0.01*	0.40±0.019	0.19±0.01*	107.45±0.09*

*Values are Means ± SD (n=3)

* Significant means (P>0.05)

Amino acids profile of ballnut seed meal

The amino acids composition of the ballnut kernel meal presented in Table 3 showed that ripe and unripe ballnut kernel meals were rich sources of amino acids. The essential amino acids in the two samples were histidine, threonine, methionine, phenylalanine, isoleucine, leucine, tryptophan, arginine and lysine. Tryptophan, glutamic acid, cysteine, phenylalanine, lysine, leucine and alanine were abundant in the ballnut used for this study. The concentration of amino acids between the two samples was (P>0.05) similar. According to FAO and WHO (2013) recommendation for some nuts and seeds, the ballnut kernel contains lower levels of aspartic acid, arginine, asparagine, glycine valine, threonine, methionine, serine, histidine and hydroxyproline but adequate in tyrosine, norvaline, isoleucine, sarcosine and proline. Compared to other oil seed cakes and cereal grains, it is essential to supplement the protein of the ballnut kernel meal with synthetic amino acids to take care of the deficiency for effective absorption of the other amino acids present in the meal. The inability to meet the requirement of any of these essential amino acids has serious health implications and can result in the degradation of the body's proteins. The concentration of amino acids reported by Monforte-Braga *et al*. (2006) showed that maize contained 3.7g/kg of cysteine, 4.2g/kg of arginine, 4.3g/kg of phenylalanine, 3.1g/kg of histidine, 2.5g/kg of isoleucine, 8.8 g/kg of leucine, 4.8g/kg of lysine, 2.1g/kg of methionine, 1.8g/kg of threonine and 3.5g/kg of valine as essential amino acids. Similarly, the authors also reported 1.9g/kg of cysteine, 5.6g/kg of arginine, 5.9g/kg of phenylalanine, 4.2g/kg of histidine, 4.2g/kg of isoleucine, 13.6g/kg of leucine, 4.7g/kg of lysine, 1.4g/kg of methionine, 2.1g/kg of threonine and 5.3g/kg of valine as essential amino acid composition in sorghum.

Table 4: Amino acids profile of ripe and unripe ballnut kernel meal (%)

Amino acid	**Ripe**	**Unripe**
Aspartic acid	1.07	1.18
Glutamic acid	6.11	6.38
Asparagine	0.65	0.77
Serine	0.78	0.89
Glutamine	5.23	5.46
Histidine*	1.08	1.17
Glycine	1.58	1.76
Threonine*	0.97	1.13
Arginine*	1.18	1.34
Alanine	3.45	3.53
Tyrosine	2.28	2.37
Cysteine	5.06	5.15
Valine*	1.59	1.78
Methionine*	1.08	1.35
Norvaline	2.15	2.28
Tryptophan*	18.23	18.47
Phenylalanine*	3.79	3.88
Isoleucine*	2.35	2.52
Leucine*	3.67	3.78
Lysine*	3.77	3.93
Hydroxyproline	1.07	1.16
Sarcosine	2.17	2.33
Proline	2.36	2.57

Values are Means ± SD (n=3)
*Essential amino

Conclusion and Recommendation

From the results of this study, it was concluded that ripe and unripe ballnut kernel meals could be utilized as an alternative energy source in animal diets, owing to their high energy level, amino acids profile, and low anti-nutritional factors. A preliminary feeding trial using animals to determine the feeding value of this promising feed resource is hereby recommended.

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