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Chemical evaluation of the seeds of *Milletia obanensis*

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Abstract

A study was conduced to evaluate the nutritional potential of *Milletia obanensis* ''Odudu'' as a possible food or feedstuff and to assess the effect of various processing methods on its nutritional quality. Results of proximate analysis showed that the raw seeds contained 26.7% crude protein, 23.5% ether extract, 3.47% crude fibre, 4.37% ash and 42.0% nitrogen free extract. The protein was well supplied with essential and non-essential amino acids, though the values were low when compared with popular seed legumes. Minerals were in fair supply: P 3.10, Mg 92.30, K 45.25 and Fe 2.20 mg/100 g. Processing methods significantly ($p < 0.05$) affected the nutritional composition. While autoclaving, boiling and toasting (heat treatment) increased the protein content, it reduced the levels of anti-nutritional factors-phytate, tannins, oxalates, cyanogenic glycosides and (slightly) saponin. Thus, it was concluded that *M. obanensis* seeds, if properly processed, could serve as livestock feed or food for man.

Keywords: Chemical evaluation; Nutritional potential; *Milletia obanensis*; Processing methods; Antinutritional factors; Amino acids

1. Introduction

Milletia obanensis 'Odudu' is a legume plant of tropical origin. According to history, the plant was first discovered in Oban, Cross river State in the Southeast region of Nigeria. This is where it derives its specific name (obanensis). Presently, the plant is widely distributed all over the southeast region of the country.

Milletia obanensis is a small forest tree. It bears hairy green leaves with a rusty touch. In its terminal panicles, at the end of the branches, are usually borne very beautiful flowers. Its fruits are normally abundant during the early to mid-dry season (between November and early March). The seeds are usually dispersed, mainly by an explosive mechanism. The mature pod is golden brown in colour and measures about 10–20 cm in length and about 2.5 cm in width at the middle. Both the foliage and pod feel slightly velvety.

There is no documentation of the utilization of the seeds of *M. obanensis* 'Odudu' as food for man or livestock in Nigeria. However, its foliage has found a variety of usage as a traditional medicinal herb. In addition, mature branches of the plant are used for fencing in the villages because of their high sprouting and rejuvenating potential. Also, the nutritional importance of the seeds is yet to be investigated.

Studies by Ranjhan (1981) and Williamson and Payne (1978) show that the average proximate analysis of legume seed shows 30%, 18.3%, 4.5%, 3.9% and 5.1% of crude protein, ether extract, crude fibre, NFE and ash, respectively. This indicates that legume seeds are rich sources of nutrients. Indeed, most legume seeds have excellent nutritional value in terms of protein, calories, vitamins and minerals. In addition to their nutritional value, for both humans and livestock, legumes are also important in cropping systems because of their ability to fix nitrogen and so increase the overall fertility of the soil (Rachie, 1985). When, therefore, the nutritional potential of *M. obanensis* is successfully harnessed for livestock and human nutrition, attention will be turned to the possibility of its utilization in alley farming – a novel technique in agronomic practice.

It is, however, important to note that, despite their promising nutritional significance, legumes have been found to contain some inherent antinutritional factors,

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which limit their nutritive value by exerting certain deleterious effects. Such effects include lowering of the bioavailability of sulphur amino acids with respect to trypsin inhibitors (Kakade, Rachis, McGhee, & Puski, 1974), hemagglutinating effects of lectins (Liener, 1974), haemolytic effects of saponins (Nowacki, 1980); the ability of tannins to form insoluble complexes with proteins, thus interfering with the digestion process by inactivating the enzymes (Bate-Smith, 1973), release of hydrogen cyanide by cyanogenic glycoside on hydrolysis (Montgomery, 1980) and antivitamin effects of isoflavones (Liener, 1979).

Over the years, poultry and swine production have been among the most lucrative sectors of Agriculture in Nigeria. Indeed from inception, through the early 1980s, the industries have experienced tremendous expansion and growth. During the past decade, however, they have witnessed a serious set-back, due largely to inadequate feed supply, which stems from unavailability of protein concentrate and short supply of energy source. The problem has been aggravated by the high competition between man and livestock for some of the existing feed ingredients, such as maize, millet and soybeans. Furthermore, animal protein sources for compounding monogastric diets are in extremely short supply and unaffordable to farmers. It is in view of this trend that research was conducted to evaluate the nutritional potential of *M. Obanensis* and to assess the nutritional quality, with a view of harnessing it as a protein source for livestock or man.

2. Materials and methods

2.1. Collection and preparation of samples

Seeds of *M. obanensis* were harvested from the forest zone of Okoyong Usangabasi, Cross River State in southeastern Nigeria. The decorticated seeds were air dried for 48 h and stored in air tight plastic containers in a deep freezer prior to use.

About 100 g of dry *M. obanensis* seeds were autoclaved for 30 min at 126 °C under 15 psi. Another batch of the seeds was put through normal cooking for 60 min. Two other sample batches were similarly treated: one was soaked in water at room temperature for 12 h and the other toasted (popped) in a fry pan for 10 min. In addition, another sample was subjected to germination. This was achieved in 72 h, during which period emergence of the radicles only was observed. Apart from the toasted sample, other heat-treated samples, samples of soaked and germinated seeds were sun-dried for 24 h. All samples were milled to fine power using a laboratory mill (Wiley). Including a milled sample of the intact raw seeds, altogether six samples (autoclaved, boiled, toasted, germinated, soaked and raw) were prepared.

2.2. Proximate analysis

Proximate analysis was carried out on all the variously treated *M. obanensis* seeds. Moisture, ash, ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were determined by the methods of Association of Official Analytical Chemists (A.O.A.C, 1990). The crude protein content was then calculated by multiplying the nitrogen content by 6.25.

2.3. Amino acid determination

2.3.1. Acid hydrolysis of sample

One millilitre of 6 M HCl was added to 5 mg of raw seed sample (ground and passed through a 30 mm sieve), according to the procedure of Tkachuk and Irvine (1967), and hydrolysed at 105 °C for 16 h using Pierce-Reacti – Therm heating modules (Pierce Rockford, USA).

2.3.2. Amino acid analysis of hydrolysate

Analysis of the amino acid content of the protein hydrolysate was carried out as outlined by Spackman, Stein, and Moore (1958) using a Beckman System Gold High Performance Liquid Chromatograph (HPLC).

2.4. Mineral analysis

Total phosphorus was determined by the reaction between phosphorus and molybdovanadate to form phosphomolybdovanadate complex. The complex was measured colorimetrically at 420 nm. Other minerals were determined by, first, wet-ashing the *M. obanensis* flour (A.O.A.C, 1990). Potassium was determined by flame photometry (Corning 400). Calcium, magnesium and iron were determined by atomic absorption spectrophotometry (Perkin–Elmer 702).

2.5. Determination of some toxic compounds

2.5.1. Cyanogenic glycoside

Cyanogenic glycoside was estimated by determining the amount of HCN released on hydrolysis. *M. obanensis* seed extracts were obtained by homogenizing 30 g of seeds in 250 ml of 0.1 M orthophosphoric acid for 5 min. The homogenate was centrifuged at 2500 rpm for 20 min and clear supernatant was taken. An aliquot of the supernatant was used for estimation of hydrogen cyanide using an auto analyzer Technicon AAII, according to the method of Rao and Hahn (1984).

2.5.2. Tannic acid

The method of Hagerman and Butler (1978) was employed for the extraction of tannic acid from the *M. obanensis* seed samples. Extracts were prepared using the Folin–Denis method of Hoff and Singleton (1977) and

the absorbance was read at 760 nm in a Perkin–Elmer Lambda 3B UV–Vis spectrophotometer.

2.5.3. Phytic acid

Phytic acid was extracted using 3% trichloroacetic acid. The extract were prepared according to the method outlined by Wheeler and Ferrel (1971). The absorbance was read at 480 mm, while the phytic acid was calculated by employing the method of Sutardi and Buckle (1985), on the basis that six atoms of phosphorus are contained in one molecule of phytic acid, giving a 1:3.55 phosphorus:phytic acid molecular ratio (phytic acid = $C_6H_{18}O_{24}P_6$).

2.5.4. Saponin

Extraction and estimation of saponin were accomplished according to the procedure of Shukla and Thakur (1986).

2.5.5. Oxalate

Estimation of oxalate was done using the method described by Dye (1956).

2.6. Statistical analysis

All results were subjected to completely randomised designed (CRD) analysis of variance according to the method of Steel and Torrie (1980), while significant means were separated using Duncan's multiple range test (Duncan, 1970).

3. Results and discussion

3.1. Proximate composition

The proximate composition of the *M. obanensis* seed meal, as affected by the various seed treatment methods, is presented in Table 1. Treatment method significantly ($p < 0.05$) affected the proximate chemical composition. For instance, crude protein varied from 26.3% in soaked to 30.6% in toasted samples. Ether extract ranged from 16.85% in boiled (cooked) to 23.48% in raw samples; total ash ranged from 1.27% in soaked to 4.37% in raw *M. obanensis* samples. Crude fibre was moderate and similar in all samples, extending from 3.19% in cooked to 4.09% in germinated samples. Total ash ranged from 1.27% in soaked to 4.37% in raw intact seed meal and NFE varied from 42.0% in the raw to 50.3% in soaked samples. The mean crude protein value of 28.5% for all the seed treatments and intact raw seeds of *M. obanensis* agrees with the observation of Osagie (1998) that the protein content of legumes ranges from 20% to 40%. This study also reveals that the crude protein of *M. obanensis* compares favourably with those of groundnut, green gram, bambara groundnut and jackbean, but is lower than soybean seed (Carnovale, Marletta, Marconi, & Brosio, 1990; Akpata & Ologhobo, 1994). This indicates that *M. obanensis* seeds, if successfully processed, could be utilised as a good protein concentrate for livestock feeds. In addition, communities dependent on vegetable sources of protein could harness the protein rich seeds of this plant for their nutritional improvement.

As would be expected application of heat (autoclaving, roasting and cooking) improved the protein value of the sample. Conversely, soaking led to a reduction in crude protein value, a condition which could have arisen by solubilization and removal of some nitrogenous substances in the beans.

Ether extract values, for all the seed treatments, were quite high and compared favourably with African Locust bean (20.30%) and perhaps soybeans (19.10%), but were lower than the 50.92% reported for groundnut cake (Oyenuga, 1968). The foregoing reveals that *M. obanensis* seeds are a good source of vegetable oil.

The mean crude fibre value of 3.54% of *M. obanensis* seed agrees with the values reported for most legumes, except perhaps for African Locust bean seed with a value of 8.82% (Oyenuga, 1968) and jackbean with a value of 7.8%. The fibre values of a these two legume seeds are considered quite high for a grain feed. Udedibie (1990) attributed the high fibre content of jackbean seeds to the presence of a thick and tough seed coat. The nutritional significance of fibre in livestock feeds should not, however, be overlooked. Depending on the class and species of animal, a minimum level of fibre is needed to facilitate digestion, and sometimes it is needed for dilution of feeds. The level of fibre in *M. obanensis* seed will be suitable for monogastric rations, since these animals require low levels of fibre in their diets.

Table 1
Proximate composition of the seed meals of *Milletia obanensis* (g/100 g dry matter)

Seed samples	Dry matter	Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
Raw	92.07	26.7	23.48	3.47	4.37	42.0
Soaked	80.75	26.3	18.48	3.50	1.27	50.3
Boiled	85.70	30.2	16.85	3.19	2.73	47.0
Autoclaved	83.47	28.5	17.28	3.28	2.40	48.4
Toasted	68.30	30.6	17.18	3.70	1.43	46.9
Germinated	84.50	28.5	19.75	4.09	3.30	44.2

Values are means of triplicate determinations.

The ash content of *M. obanensis* compares closely with ash values of 3.68%, 3.22%, 3.56% and 4.11% reported for pigeon pea, lima bean, lablab bean, and mucuna bean, respectively (Aletor & Aladetimi, 1989). The ash value of a feed gives a clue to its mineral content. This implies that *M. obanensis* is a fairly good source of minerals.

The NFE value of *M. obanensis* seed is comparable to those of high carbohydrate containing legumes. According to Elegbede (1998), carbohydrate content of legumes ranges from 23% in groundnut to 66% in bambara groundnut. The 50.3% NFE value of *M. obanensis* therefore ranks with the high carbohydrate containing legume seeds. This implies that besides its potential as a protein concentrate for livestock feeds, *M. obanensis* seed can also double as an energy source.

3.2. Amino acid composition

The amino acid profile of raw *M. obanensis* seed protein is shown in Table 2. With the exception of tryptophan and cysteine, all the essential amino acids were present, though in minute quantities. The values of sulphur – containing amino acids (SAA), cysteine and methionine (0.26 and 0.15/16 g N, respectively) were quite low when compared to reference values of 2.0 g/16 g N for cysteine, and 2.2 g/16 g N for methionine (FAO/WHO, 1973). Lysine, which is generally deficient in most cereal proteins, was low in *M. obanensis* "Odudu" seed protein, being 1.26 g/16 g N. This situation might get worse with heat treatment. Autoclaving for instance, can completely destroy cysteine (D'mello, Acanoic, & Walker, 1985). However, since cysteine and methionine are fully interconvertible (Bressani & Elias, 1980), the quality of protein in food or feed whose cysteine has been destroyed by heating of the protein concentrate could be improved by supplementing with methionine. Like other oilseeds, aspartic and glutamic acids were the most abundant amino acids in *M. obanensis* seed protein.

Table 2
Amino acid composition of the protein of raw *Milletia obanensis* seeds (g/16 g nitrogen)

Amino acid	Value (g/16 g nitrogen)
Cysteic acid	0.00
Aspartic acid	2.07
Threonine	0.69
Serine	1.01
Glutamic acid	2.45
Proline	0.78
Glycine	0.74
Alanine	0.71
Valine	0.71
Methionine	0.15
Isoleucine	0.61
Leucine	1.46
Tyrosine	0.66
Phenylalanine	0.89
Histidine	0.62
Lysine	1.26
Tryptophan	0.00
Arginine	0.89
Cysteine	0.26

3.3. Mineral composition

The mineral composition of *M. obanensis* is presented in Table 3. There were significant variations among the different seed treatment techniques ($p < 0.01$). Magnesium was the most abundant of the elements considered, with values ranging from 63.2 mg/100 g in soaked to 144 mg/100 g in toasted seeds. Calcium varied from 32.5 mg in soaked to 74.3 mg/100 g in toasted seed. Potassium extended from 31.2 mg in soaked to 71.1 mg/100 g in toasted samples, phosphorus from 1.3 mg in autoclaved to 3.10 mg/100 g in raw seeds, and iron being the lowest, ranged from 1.13 mg in soaked to 4.75 mg/100 g in boiled seeds.

Except for phosphorus which was highest in raw seeds, the results generally indicated a trend of high mineral content in heat-treated seed samples, particularly in dry heat treatment (toasting). An increase in elemental composition was also noticed in germinated samples. Thus, these two processing methods may be useful for making minerals available to animals that are fed *M. obanensis* seed meal. Meiners, Derise, Lau, Ritchey, and Murphy (1976) have reported a decrease in

Table 3
Mineral element composition of the seed meal of *M. obanensis* as affected by the different seed treatment techniques (mg/100 g)

Seed samples	Elements				
	Calcium	Phosphorus	Magnesium	Potassium	Iron
Raw	47.4^{d}	3.10^{a}	92.3^{d}	45.3^{d}	2.20^{b}
Soaked	32.5^{f}	2.18^{ab}	63.2^{f}	31.2^{f}	1.13^{e}
Boiled	53.4^{c}	1.90^{b}	104^{c}	51.3^{c}	4.75^{a}
Autoclaved	41.8^{e}	1.32^{c}	81.2^{e}	40.4^{e}	1.17^{e}
Toasted	74.3^{a}	1.86^{b}	144^{a}	71.1^{a}	1.43^{d}
Germinated	65.0^{b}	2.44^{a}	126^{b}	62.2^{b}	1.90^{c}

Values are means of triplicate determinations.
Values within the same column followed by different superscripts are significantly different ($p < 0.05$).

calcium from 69.0 ± 0.9 mg/100 g in raw cowpea to 29.0 ± 1.0 mg/100 g after cooking, thus corroborating the results of this work.

Mineral compositions of legume seeds have been reported by Akpata and Ologhobo (1994) among other workers. Mineral values of *M. obanensis* obtained in this investigation are comparable to other legumes. In general, research findings have indicated that legumes contain substantial amounts of minerals in their seed meal. However, the bioavailability of these minerals, especially phosphorus, remains a problem, as it is more often than not in a complexed form.

Mineral elements play vital roles in many important processes in the body, such as enzyme systems, skeletal structures (e.g., calcium and phosphorus), important physiological compounds (such as iodine in thyroxine, sulphur in amino acids cysteine and methionine) blood (calcium is needed for neuromuscular irritability and the clotting of blood). Essentially, mineral requirements of the body are supplied by dietary mineral elements, and food legumes make a significant contribution to dietary mineral intake for some populations. An examination of 27 Indian foods (Guittikar, Damayanti, Adhkari, Ambegoakar, & Rao, 1966) revealed a high contribution of minerals, especially magnesium and copper, by the legume *Vigna catjang*.

3.4. Antinutritional factors

Antinutritional components of *M. obanensis* seeds are summarized in Table 4. There were significant differences ($p < 0.05$) in their concentrations among the different seed treatments. Heat application significantly reduced the amounts of the antinutrients. Total oxalate varied from 24.4 mg in toasted to 37.5 mg/100 g in raw samples. Soluble oxalate followed the same trend, being lowest (18.8 mg/100 g) in toasted seeds and highest (29.6 mg/100 g) in raw seeds. Phytic acid ranged from 0.82 mg in autoclaved to 3.98 mg/100 g in intact raw seeds; tannic acid varied from 0.12 mg in autoclaved to 0.44 mg/100 g in the raw sample; cyanogenic glycoside ranged from 0.82 mg in toasted to 2.60 mg/100 g in raw seeds and saponin from 6.76 mg in boiled to 9.74 mg/100 g in raw seed samples.

The presence of antinutritional factors in feeds is of significant importance, since they exert some deleterious effects on animals, for instance, oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce acute metabolic calcium deficiency (hypocalcemia) when fed as main feed to livestock (Checke & Shull, 1985). Osagie (1998) has also indicated that a high oxalate diet can increase the risk of renal calcium absorption. The concentration of oxalate in the seed meals of *M. obanensis* seems to be on the high side when compared to reported values in some crop seeds (Aletor & Ojo, 1989).

Phytic acid is present in most foodstuffs, either as a phytate salt (Oberleas, 1973) or as a complex with protein (Anderson, 1985). Phytate chelates with certain metal ions, such as calcium, magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged, thus reducing the bioavailability of these minerals (Maga, 1982). Phytates also form strong complexes with proteins and this can lead to their reduced digestibility.

Tannic acid is known to evoke growth-depressing effects in rats. In this study, however, tannin level was found to be quite low in *M. obanensis*.

Cyanogenic glycoside contents of legume seeds have been investigated. Liener (1977) reported total HCN values of 2.1, 210–312, 2.3, 2.0, 0.8 and 0.50 mg/100 g for cowpea, lima bean, field pea, kidney bean, chicken pea and pigeon pea, respectively. In this study, it has been observed that the highest HCN level was in the intact raw seeds (2.60 mg/100 g). Except for lima bean, the HCN value of *M. obanensis* is in agreement with those of the above-mentioned legumes. The concentration of the HCN decreased with application of heat, and this is because HCN is volatile and can be expelled by heat application.

Table 4
Antinutritional factors of the seed meal of *Milletia obanensis* under the different seed treatment methods (mg/100 g dry matter)

Seed sample	Total oxalate	Soluble oxalate	Phytic acid	Tannic acid	Cyanogenic glycoside	Saponin
Raw	37.5^{a}	29.6^{a}	3.98^{a}	0.44^{a}	2.60^{a}	9.74^{a}
Soaked	27.8^{c}	23.5^{b}	2.98^{a}	0.17^{b}	1.62^{b}	8.35^{ab}
Boiled	27.1^{c}	22.8^{b}	1.21^{b}	0.15^{b}	1.52^{b}	6.76^{c}
Autoclaved	25.4^{d}	21.0^{c}	0.82^{c}	0.12^{bc}	0.98^{c}	6.84^{bc}
Toasted	24.4^{d}	18.8^{c}	0.98^{bc}	0.13^{bc}	0.82^{cd}	7.43^{b}
Germinated	31.7^{b}	27.9^{ab}	2.14^{ab}	0.25^{ab}	2.04^{ab}	7.67^{b}
Mean	29.1	23.9	2.02	0.21	1.60	7.80
SE of means	±1.96	±1.69	±0.56	±0.05	±0.27	±0.46

Values are means of triplicate determinations.
Values within the same column followed by different superscripts are significantly different ($p < 0.05$).

Saponins are widely distributed in plants; and in animal nutrition are particularly important in temperate forages (Checke & Shull, 1985). They are bitter compounds affecting palatability and feed intake. They have growth-depressing properties in poultry and swine, and have been implicated in bloat in ruminants. Of all the antinutritional factors, saponin was the least affected by heat treatment, thus suggesting that, heat application might not be the best method to reduce saponin levels in foods, feedstuffs or forages.

4. Conclusion

Milletia obanensis "Odudu" is a good source of protein, fat and minerals. Heat treatment, in the form of autoclaving, toasting or boiling was the best method of processing. Whereas the antinutritional factor levels were reduced, protein level was increased by heat application. Thus, *M. obanensis*, when properly processed, could indeed be a good protein source for both man and livestock.

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