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NUTRITIONAL EVALUATION OF DIFFERENTLY PROCESSED *Moringa oleifera* SEED BEFORE AND AFTER OIL EXTRACTION FOR INCLUSION IN *Clarias gariepinus* BASED DIETS

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ABSTRACT

The present study aims to evaluate the effect of different processing methods on the nutrient and anti-nutrient profiles of *Moringa oleifera* seed before and after oil extraction. Four processing methods involving toasting at 10, 20 and 30mins, boiling at 30, 60 and 90mins, soaking at 8, 16 and 24 hours and combination of boiling (30, 60 and 90 minutes) and soaking for 72 hours were employed. The results showed that **t**he three toasting treatments significantly increased the crude protein content while revealing a significant ($p<0.05$) increase in the crude protein (55.05±0.17) and ash (6.01±0.10) contents of the processed seed meal and significantly ($p<0.05$) lower fat (9.39±0.11), moisture (1.50±0.07) and crude fibre (3.93±0.05) contents after oil extraction. The anti-nutritional factors detected in the raw moringa seeds were (Oxalate, saponins, alkaloids, phytic acid, tannin, cyanide and phytate) with the following contents; 0.86±0.13, 1.50±0.06, 2.32±0.06, 269.84±1.62, 1.21±0.14, 0.56±0.04 and 69.82±0.86, respectively. Combination of boiling and soaking treatments (B30mins/S72hrs, B60mins/S72 hrs, B90mins/S72hrs) significantly ($p<0.05$) reduced the anti-nutritional factors. Processing, B60mins/S72hrs and B90mins/S72hrs were able to reduce all the anti-nutritional factors to acceptable levels with the exception of phytic acid which remained significantly ($p<0.05$) higher. All the toasting treatments significantly increased the crude protein content while boiling and soaking did not. However, displacement of oil after extraction greatly enhanced the crude protein level of the treated (B90mins/S72hrs) seed. It could be concluded that this processing method coupled with oil extraction could effectively improve on the crude protein content of the seed.

Key words: *Moringa oleifera* seed; proximate; anti-nutritional factors; processing; oil extraction

INTRODUCTION

Aquaculture is one of the most rapidly expanding food production industries (FAO, 2014). Though the main constraint remains the reliance of aqua feed on fishmeal. The high cost, inconsistent availability, and varying quality of fishmeal has prompted the search for alternate protein sources for fish feed production. Therefore, to ensure an economically viable, ecologically friendly and profitable fish feed production; it is necessary to direct research efforts towards the evaluation and utilization of non-conventional sources of plant protein sources such as *M. oleifera* seed, which could be less expensive and more easily available, compared to fishmeal.

Previous findings on different fish species including African catfish that were fed with a high percentage of plant protein were reported (Falaye and Oloruntuyi, 1998; Fasakin *et al.*, 2006; Goda *et al.*, 2007). However, plant protein sources were reported to have some limiting factors such as crude fiber and anti-nutritional factors (Alegbeleye, 2005).

Anti-nutritional factors are known for their diverse roles, ranging from antioxidant and immune-modulating properties to potential antimicrobial effects. These compounds are naturally present in plant-based feed ingredients and can contribute positively to the overall health of animals. However, when the intake of phytochemicals surpasses a certain level, they interfere with nutrient use, animal health, and production by themselves or by their metabolic products, or by reducing nutrient intake, digestion, absorption, and utilization and perhaps other harmful effects (Akande and Fabiyi, 2010). Different processing methods result in the decrease of anti-nutritional factors ensuring improved growth performance in fish (Fagbenro, 1999; Siddhuraju and Becker, 2003; Francis *et al.*, 2006).

Therefore, the usage of plant protein source in fish feed requires proper investigation on different processing methods to ensure effective utilization (Francis *et al.*, 2001). The choice of *Moringa oleifera* as the focal point for investigating various processing methods is grounded in its nutritional richness, sustainability, anti-nutrient properties, economic viability, and identified research gaps (Francis *et al.*, 2001). The objective of this research was to determine the effect of different processing methods on the nutrient and anti-nutrient profiles of *M. oleifera* seed before and after oil extraction.

MATERIALS AND METHODS

Experimental Site

The research was carried at the laboratory of Fisheries and Aquaculture Department, Usmanu Danfodiyo University, Sokoto on latitude 13° 07' 47.6'' N and longitude 05° 12' 11.3'' E at 275m above sea level (Google Map, 2005).

***Moringa oleifera* Seed Collection and Preparation**

Dried mature fruits of *M. oleifera* were obtained from Maiyafe farm at *Kududdufawa* in Ungoggo Local Government, Kano state on latitude 12º 05' 26º N and longitude 8º 29'48º E. The seed was identified in the herbarium by comparing it with the one on the shelf and was confirmed by an expert from the Department of Forestry and Environment, Usman Danfodio University, Sokoto. The seeds were properly cleansed to remove dirt, stones, and those that had damaged. The cleaned seeds were manually shelled to remove the kernels, then dried in the shade at 38°C until needed.

Processing and Chemical Analyses of *Moringa oleifera* Seed

Four processing methods involving toasting at 10, 20 and 30mins, boiling at 30, 60 and 90mins, soaking at 8, 16 and 24 hours and combination of boiling (30, 60 and 90 minutes) and soaking for 72 hours were employed. Boiling and soaking were done in accordance to the procedure of Vavidel and Pugalenthi (2007), while toasting was conducted as described by Eyo (2001).

The dried *Moringa oleifera* seeds were ground into a coarse powder using grinding machine. The ground seed powder was subjected to mechanical pressing to extract the oil. This was done using an oil expeller machine (mini), which applies pressure to the seeds to release the oil. The extracted oil was collected, leaving behind the seed cake. The extracted oil was packaged into containers.

The proximate and anti-nutritional composition of the raw and processed seeds were carried out in triplicate according to the standard methods (AOAC, 2006). Although toasting for 30 minutes (T30min) gave the highest crude protein, it also resulted in higher levels of anti-nutritional factors. The combination of boiling for 90 minutes and soaking for 72 hours (B90/S72hrs) yielded the least amount of anti-nutritional factors. Hence, the choice of B90/S72hrs.

Statistical Analysis

Data obtained from proximate and anti-nutritional compositions were subjected to two-way analysis of variance (ANOVA). Means were separated using Duncan's multiple range test (Duncan, 1955) using SPSS computer software version 23. Significant difference between mean values was accepted at the 0.05 level of probability.

RESULTS

Proximate Composition of Raw and Processed *Moringa oleifera* Seed before Oil Extraction

Table 1 shows the proximate composition of the raw and processed seed prior to oil extraction. The three toasting treatments significantly increased the crude protein content. Boiling for 90 minutes (B90) and soaking for 16 and 24 hours. (S16 and S24) did not lead to a significant increase in the crude protein compared to the raw seed. However, boiling for 30 and 60 minutes and soaking for 8 hours, significantly reduced the crude protein content. Toasting for 20 and 30 minutes (T20 and T30), soaking for 24 (S24) hours, boiling and soaking for 60 minutes (B60) and soaking for 72 hours (S72) as well as boiling for 90 minutes (B90) and soaking for 72 hours (S72) lead to significant increase in the fat content. BS30/72 hours significantly increased the ash content compared with the raw seed and of all other treatments. Soaking for 8 and 16 hours (S8 and S16) as well as boiling for 90 minutes (B90) and soaking for 72 hours (S72) showed no significant difference ($p>0.05$) in the ash content of the treated seed. All other treatments significantly reduced the ash contents compared with the raw seed. Crude fibre was significantly higher ($p<0.05$) in samples subjected to BS90/72 hours than the raw seed and was significantly different from the values obtained with B60min/S72 hours and B90 minutes. The three toasting treatments (T10, T20 and T30) and B30min/S72 hours recorded the lowest moisture content. NFE was significantly higher with all the boiling, combination of boiling and soaking (B30min/S72 hours and B90min/S72 hours) and T10 minutes treatments and lower values were recorded for T20 and T30 minutes and S24 hours.

Table 1: Proximate composition of raw and processed *Moringa oleifera* seed

Treatment	Moisture	Crude Protein	Fat	Ash	Crude Fibre	NFE
Raw	3.63±0.26^{a}	34.28±0.65^{b}	35.27±0.35^{d}	3.74±0.32^{bc}	3.93±0.15^{bcd}	22.78±0.65^{f}
T10min	1.75±0.09^{f}	35.25±0.16^{a}	32.66±0.52^{ef}	2.81±0.15^{fg}	3.98±0.04^{abcd}	25.30±0.75^{e}
T20min	1.74±0.05^{f}	35.72±0.37^{a}	37.97±0.56^{ab}	3.12±0.11^{ef}	3.60±0.08^{e}	19.59±1.08^{h}
T30min	1.46±0.04^{g}	35.87±0.26^{a}	38.29±0.51^{a}	3.38±0.11^{de}	3.80±0.05^{cde}	18.68±0.82^{h}
B30min	2.58±0.17^{c}	29.46±0.50^{e}	25.04±0.19^{h}	2.93±0.07^{fg}	3.76±0.05^{cde}	38.80±0.57^{a}
B60min	2.79±0.06^{b}	32.52±0.52^{c}	25.37±1.01^{h}	2.74±0.05^{g}	4.04±0.16^{abc}	35.33±1.43^{b}
B90min	2.93±0.08^{b}	33.72±0.76^{b}	29.87±0.75^{g}	2.66±0.06^{g}	4.12±0.18^{ab}	29.64±0.76^{c}
S8hrs	2.31±0.11^{e}	31.77±0.42^{c}	33.40±0.49^{e}	4.06±0.18^{ab}	3.58±0.07^{e}	27.19±0.31^{d}
S16hrs	2.43±0.05^{cde}	33.79±0.25^{b}	35.77±0.33^{d}	3.74±0.18^{bc}	3.72±0.05^{de}	22.98±0.54^{f}
S24hrs	2.48±0.07^{cde}	34.44±0.51^{b}	36.99±0.18^{c}	3.65±0.06^{cd}	3.94±0.07^{bcd}	20.97±0.61^{g}
B30min/S72hrs	1.89±0.15^{f}	31.77±0.33^{c}	32.38±0.72^{f}	4.28±0.39^{a}	3.28±0.27^{f}	28.33±1.17^{cd}
B60min/S72hrs	2.36±0.05^{de}	30.79±0.30^{d}	37.30±0.38^{bc}	3.69±0.17^{cd}	4.21±0.21^{ab}	24.01±0.78^{ef}
B90min/S72hrs	2.55±0.08^{cd}	29.84±0.40^{e}	38.18±0.33^{ab}	2.85±0.17^{fg}	4.25±0.31^{a}	24.87±0.18^{e}
SEM	0.065	0.258	0.309	0.105	0.090	0.467

Mean with same letter in column are not significantly different ($p>0.05$). B = Boiling, T = Toasting, S= Soaking, BS = Boiling and soaking, SEM = Standard error of means

Proximate Composition of *Moringa oleifera* SEED after Oil Extraction

Table 2 presents the proximate composition of the processed (B90mins/S72hrs) and raw seed meal after oil extraction. The results revealed a significant ($p<0.05$) increase in the crude protein (55.05±0.17) and ash (6.01±0.10) contents of the processed seed meal and significantly ($p<0.05$) lower fat (9.39±0.11), moisture (1.50±0.07) and crude fibre (3.93±0.05) contents after oil extraction.

Table 2: Proximate composition of raw and processed (B90mins/S72hrs) *Moringa oleifera* seed after oil extraction

Composition	Treatments	
	Raw	Processed
Moisture	2.65±0.67[a]	1.50±0.06[b]
Crude protein	37.63±0.17[b]	55.05±0.16[a]
Fat	18.78±0.11[a]	9.39±0.11[b]
Ash	4.22±0.10[b]	6.01±0.10[a]
Crude fibre	4.05±0.05[a]	3.93±0.07[b]
NFE	32.68±0.25[a]	24.13±0.25[b]

Mean with same letter in row are not significantly different ($p>0.05$).

Effects of Processing on Anti-nutrients in *Moringa oleifera* Seed Meal

The anti-nutritional factors detected in the raw moringa seeds were (Oxalate, saponins, alkaloids, phytic acid, tannin, cyanide and phytate) with the following contents; 0.86±0.13, 1.50±0.06, 2.32±0.06, 269.84±1.62, 1.21±0.14, 0.56±0.04 and 69.82±0.86, respectively (Table 3). The anti-nutritional factors after processing (B90min/S72hrs) and oil extraction are also presented in Table 4.

The three toasting treatments (T10, T20, T30 minutes) significantly ($p<0.05$) reduced all the anti-nutrients in the processed moringa seeds though phytic acid and phytate levels remained high but significantly ($p<0.05$) lower than in the raw sample.

Boiling treatments (B30, B60, B90 minutes) also had a significant ($p<0.05$) reduction effect on all the anti-nutritional factors. Boiling for 90 minutes (B90) was successful in lowering the levels of oxalate, saponins, tannin, cyanide and phytate to zero.

Similarly, soaking treatments (S8, S16, S24 hours) was able to significantly ($p<0.05$) reduce all the anti-nutritional factors with the exception of (S8 hours) which significantly ($p<0.05$) increased the phytate level compared to what was obtained in the raw sample.

Combination of boiling and soaking treatments (B30mins/S72hrs, B60mins/S72 hrs, B90mins/S72hrs) significantly ($p<0.05$) reduced the anti-nutritional factors. Processing, B60mins/S72hrs and B90mins/S72hrs were able to reduce all the anti-nutritional factors to acceptable levels with the exception of phytic acid which remained significantly ($p<0.05$) higher.

Table 3: Anti-nutritional factors of raw and processed (B90mins/S72 hours) *Moringa oleifera* seed before oil extraction

	Anti-nutrients						
Treatment	Oxalate mg/100g	Saponins mg/100g	Alkaloids mg/100g	Phytic acid (mg/100g)	Tannins mg/100g	Cyanide (mg/100g)	Phytate (mg/100g)
Raw	0.86±0.13^{a}	1.50±0.06^{a}	2.32±0.06^{a}	269.84±1.62^{a}	1.21±0.14^{a}	0.56±0.04^{a}	69.82±0.86^{b}
T10min	0.42±0.01^{d}	0.21±0.01^{ef}	1.44±0.05^{e}	120.37±1.14^{d}	0.16±0.01^{b}	0.36±0.02^{b}	52.33±0.50^{c}
T20min	0.38±0.02^{d}	0.17±0.02^{fg}	1.31±0.06^{f}	108.58±0.46^{e}	0.12±0.01^{bc}	0.29±0.02^{c}	47.07±0.30^{d}
T30min	0.18±0.02^{e}	0.06±0.02^{i}	1.30±0.01^{f}	100.13±0.48^{f}	0.06±0.01^{cd}	0.16±0.01^{e}	22.05±0.32^{e}
B30min	0.75±0.02^{b}	0.25±0.02^{e}	1.66±0.06^{d}	80.12±0.48^{g}	0.06±0.01^{cd}	0.23±0.02^{d}	7.36±0.40^{h}
B60min	0.54±0.02^{c}	0.22±0.02^{ef}	0.38±0.02^{j}	21.86±0.85^{j}	0.04±0.01^{d}	0.15±0.02^{e}	0.72±0.13^{k}
B90min	0.20±0.02^{e}	0.12±0.02^{g}	1.11±0.04^{g}	9.99±0.33^{l}	0.03±0.00^{d}	0.09±0.01^{f}	0.00±0.00^{l}
S8hrs	0.77±0.02^{b}	0.66±0.02^{b}	2.09±0.06^{b}	180.17±0.30^{b}	0.04±0.01^{d}	0.27±0.01^{c}	78.01±0.31^{a}
S16hrs	0.59±0.03^{c}	0.52±0.02^{c}	1.87±0.08^{c}	67.1±0.33^{h}	0.02±0.00^{d}	0.07±0.02^{f}	12.54±0.25^{f}
S24hrs	0.16±0.01^{e}	0.38±0.02^{d}	0.99±0.06^{h}	15.60±0.23^{k}	0.01±0.00^{d}	0.02±0.01^{g}	6.17±0.23^{j}
BS30/72hrs	0.22±0.03^{e}	0.07±0.02^{gh}	0.77±0.02^{i}	134.65±0.53^{c}	0.02±0.01^{d}	0.34±0.02^{b}	9.31±1.30^{g}
BS60/72hrs	0.06±0.01^{f}	0.05±0.02^{hi}	0.32±0.03^{j}	25.00±0.74^{i}	0.01±0.00^{d}	0.00±0.00^{g}	0.00±0.00^{l}
BS90/72hrs	0.03±0.01^{i}	0.02±0.02^{i}	0.04±0.01^{k}	0.00±0.00^{m}	0.00±0.00^{d}	0.00±0.00^{g}	0.00±0.00^{i}
SEM	0.023	0.017	0.027	0.409	0.022	0.010	0.202
FAO/WHO	<5	<1	< 60	≤5	<20	<50	------

Mean with same letter in column are not significant (p>0.05). B = Boiling, S = Soaking, T = Toasting, SEM = Standard error of means`

Table 4: Anti-nutritional factors of processed (B90min/S72 hours) *Moringa oleifera* seed after oil extraction

Treatment	Anti-nutritional factors						
	Oxalate %	Saponins %	Alkaloids %	Phytic acid (mg/100g)	Tannins %	Cyanide (mg/100g)	Phytate (mg/100g)
B90mins/S72hrs	0.18±0.01	0.20±0.02	0.52±0.05	0.01±0.01	0.06±0.01	0.17±0.01	0.00±0.00
SEM	0.004	0.008	0.020	0.003	0.004	0.005	0.000

Values are presented as means SD of triplicate samples. B90/S72h = Boiling for 90 minutes and soaking for 72 hours; SEM = Standard error of means

DISCUSSION

Proximate Compositions of Raw and Processed (B90min/S72hrs) *Moringa oleifera* Seed before Oil Extraction

The findings in this study indicated that the different processing methods had different effects on the proximate composition of the moringa seed. The results for boiling (30, 60 and 90mins) and soaking (8, 16 and 24hrs) did not have positive effect on the crude protein contents of the processed seed. This is contrary to the findings of Mbah *et al.* (2012) who reported boiling of *M. oleifera* seed to have increased the availability of protein, fibre, iron and zinc. This result also contradicted a rise in crude protein reported by Wang *et al.* (2010) after cooking *Phaseolus vulgaris*. However, results in the present study are consistent with the finding of Seena *et al.* (2005) who reported a lower crude protein in cooked *Canavalia cathartica* seeds when compared with the roasted *Canavalia cathartica*.

The raw moringa seed meal had fat content of 35.27±0.35 which is lower than the value (42%) reported by Ogunsina *et al.* (2011) and higher than 30.36-35.20% observed by Anwar *et al.* (2006) for moringa seed meal. The fat content was also higher than the values reported for other oil seeds such as melon seeds (17.36-25.06%) by Ebuchi *et al.* (2006), and 24.8 and 30.0% for *Citrillus lanatus* and *C. colocynth* species respectively (Mabalaha *et al.*, 2007). These variations could be attributed to the different processing methods employed or the type of oil extraction process applied (Compaore, 2011). The fat content for both raw and defatted samples were also higher when compared with the fat content of 23.55% reported for soybean seeds (23.55%) (Paul and Southgate, 1985).

The moisture content recorded in this study, 3.63±0.26 for raw and defatted seed and 1.50±0.25 for treated and defatted samples, were lower than the values of 4.70 - 5.03% reported for raw moringa flour (Abiodun *et al*., 2012),4.20% for baobab seed flour (Adubiaro *et al*., 2011) and10.54% for bambara nut flour (Abiodun and Adepeju, 2011). The toasting of *M. oleifera* seeds suggests that processing successfully lower the moisture content. This might prolong the shelf life of *M. oleifera* seed. This is similar to the observation of Seena *et al.* (2005) who discovered a reduction in the moisture content of cooked *Canavalia catharticas* eeds.

There was a progressive reduction in the ash contents with increasing processing periods in all the treatments involving soaking and boiling and this could be attributed to leaching during the treatment into the media. Cooking in water reduces the ash and fat content of seed as a result of diffusion of minerals into the cooking water (Seena *et al*., 2005; Wang *et al.*, 2009). Toasting for 10, 20 and 30mins however, indicates a progressive increase of 2.81±0.15, 3.12±0.11 and 3.38±0.11 respectively, in the ash content.

Boiling for 90 minutes and soaking for 72 hours (B90mins/S72hrs) was the only treatment that led to significant ($p<0.05$) increase in the fibre content, unlike the other treatments. The fat level of the undefatted moringa samples was higher, although it was lower than the 42 percent reported by Ogunsina *et al*. (2011) and higher than the raw moringa seed flour fat content of 30.36-35.20 percent reported by Anwar *et al.* (2006). These variations in crude fat content can be attributed to the region where moringa is planted and the growing conditions of the plant.

Proximate Composition of Processed (B90min/S72hours) *Moringa oleifera* Seed Meal after Oil Extraction

The increase in the protein content of *M. oleifera* seed meal in the present study could be attributed to the displacement of oil from the seed thereby increasing other parameters. Robinson *et al*. (2001) reported that feed ingredients with crude protein greater than 20% are considered as protein source which qualifies *M. oleifera* meal as an alternative protein source. This result further indicated that oil extraction had significant influence on crude protein yield of *M. oleifera* seed meal.

The result obtained for the crude protein content was relatively higher than the findings of Olaofe *et al*. (2008) that seed of *Luffa cylindrical* after oil extraction contained 43.1% crude protein, but close to 54.4% recorded by Govardhan *et al*. (2013) for moringa cakes after oil extraction. The result of the presentstudy is similar to the report of Ndabigengeser and Narasiah (1998), Folid *et al.* (2001) and Compaoré *et al*. (2011) who reported that moringa seeds are a good source of fats, proteins, and crude fibers.

There was a significant reduction in the fat content of seed meal after oil extraction. The result of this study is in tandem with the finding of Govardhan *et al*. (2013) who observed that fat constituted substantial portion of the kernel weight (39.36g of fat/100g) of moringa seed sample. Earlier reports by Alobo *et al*. (2009) showed similar fat reduction in soybeans, beniseed and cashew nut after oil extraction. The higher protein, carbohydrate and ash contents (Table 4.2) recorded in this study agreed with the finding of Abiodun and Adepeju (2011) who reported higher values in the ash, crude fibre, protein, and carbohydrate contents of moringa seed cake after oil extraction. The higher values were as a result of the displacement of oil from the defatted samples thereby increasing other parameters.

The low moisture content found in the defatted moringa samples in this study could indicate that the microorganisms' activity is likely going to be reduced thereby increasing the shelf life of the defatted samples as observed by Adeyeye and Adejuyo (1994). The result also indicate that the crude fibre content of the raw and treated *M. oleifera*

seeds after oil extraction were within the crude fibre requirements (3-6%) necessary for growth of African catfish growth (Robinson *et al.*, 2001).

CONCLUSION

The nutrient compositions of the raw *M. oleifera* seed indicated its potential dietary ingredient as fish feed. However, the anti-nutritional factors analysed in the raw seed which composed of phytic acid >phytate> alkaloids >saponin>tannin >oxalate > cyanide, are known to be harmful and need to be treated before use in fish feed. The four processing methods employed: toasting (T) (for 10, 20 and 30 minutes); boiling (B) (for 30, 60 and 90 minutes); soaking (S) (for 8, 16, and 24 hours) and combination of boiling for 90 minutes and soaking for 72 hours (BS) had varying effects on the nutrients and anti-nutritional factors in the seed meal.

Protein is one of the important nutrients of interest in the moringa seed, and the processing methods had different effects on the protein in the seed. All the toasting treatments (T10, T20 and T30min) significantly increased the crude protein content while boiling (B30, B60 and B90mins) and soaking (8, 16 and 24hrs) did not. However, displacement of oil after extraction greatly enhanced the crude protein level of the treated (B90mins/S72hrs) seed. It could be concluded that this processing method coupled with oil extraction could effectively improve on the crude protein content in the seed.

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