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IMPACT OF PROCESSING METHODS ON CHEMICAL AND PHYTOCHEMICALS OF *Moringa oleifera* SEED KERNEL MEAL

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ABSTRACT

The high cost of protein sources in the fish feed industry is a challenge to tackle, and this leads to a study of alternative non-conventional plant protein sources (*Moringa oleifera* seed kernels meals) MSKM. The moringa seed kernel was treated using four methods namely: ethanol soaking, boiling, toasting, autoclaving, and raw. The conventional method of treatment (toasting) for soybean meal in fish feed was adopted. After the treatments, the treated seeds were analyzed for nutrient contents and phytochemical screening. Anti-nutritional factors such as phytates and flavonoids were negative among all the treatments. Alkaloids were significantly ($P < 0.05$) higher (4.35 ± 0.00) in raw moringa seed kernels but lower (2.12 ± 0.00) in ethanol-soaked moringa seed kernels. Tannins were detected in all the treatment methods except in boiled moringa seed kernels which were not detected. Saponins were not detected in toasted moringa seed kernel but detected in all other treatments, which was significantly ($P < 0.05$) higher (5.91 ± 0.00) in raw and lower (5.16 ± 0.00) in boiled moringa seed kernels. The proximate compositions of differently treated moringa seed kernels meals and soybean meal recorded higher crude protein (CP) content in toasted moringa seed kernel meals but lower (24.58 ± 0.00) in raw moringa seed kernel meals. The lipid content (27.74 ± 0.00) was significantly ($P < 0.05$) higher in autoclaved moringa seed kernel meals and significantly ($P < 0.05$) lower (20.33 ± 0.00) in soybean meal. Ash contents was significantly ($P < 0.05$) higher (7.13 ± 0.00) in soybean meal and significantly ($P < 0.05$) lower (2.90 ± 0.00) in boiled moringa seed kernel meals. The results indicated a significant decrease in the anti-nutritional contents among the treatment methods and improved the nutritional composition of moringa seed kernel meals. This has indicated the impact of the treatment methods used in the research and also the potential of moringa seed kernels has in substituting the conventional plant protein sources in animal diets. The treatment methods employed in the present study are recommended for other non-conventional plant protein sources as well as other treatment methods on moringa seed kernels that are not used in this research for further studies.

Key words: Anti nutrients, meal, moringa, treatments, kernels, proximate, protein and seeds

INTRODUCTION

The basic concern in aquaculture is providing a diet with all required nutrients in suitable proportions to optimize the growth of fish and reduce the cost of production. The high cost of fish meal, unreliable supply, and demand made it necessary to test alternative non-conventional sources of protein from plant origin in fish feeds either for partial or total replacement of fishmeal [1]. The presence of phytochemicals in plant products was hindering the utilization of feed by fish and therefore limiting their use in fish feed [2]. There is a changing perception concerning the potential of many plant secondary metabolites and several treatment systems attempted to eliminate the anti-metabolic factors and toxic principles of plant protein sources in fish feed which recorded a partial success. Nutrition is the most expensive aspect of the intensive aquaculture industry; it represents the highest operating costs [3]. The quality of nutrients in feed ingredients is among the prerequisites after it is available for the production of high-quality feeds. Protein is the main nutrient that cannot be compromised in the choice of ingredients for fish feed formulation and preparation [4]. Moreover, the cost of protein itself accounts for about 50% or more of the total feed in an intensive system of culture.

The *Moringa oleifera* tree is a fast growing and aesthetically pleasing tree. The species of *M. oleifera* is characterized by its length, pods of drumstick shaped that develop its seeds within the first year of planting. *M. oleifera* grows to four (4) meters and can bear its fruit within the same year of planting [5]. *M. oleifera* tree is also called Miracle tree, Mother's best friend, never die, and Benzolive tree [6]. It is widely known as the horseradish or drumstick tree and is a native of the sub-Himalayan region of northwest India. The tree ranges in height from 5–12 m and the fruits (pods) are around 50 cm long. *M. oleifera* is esteemed as a versatile plant as it has multiple uses. The leaves, fruits, flowers, and immature pods of the tree are edible and they are part of the traditional diets in many countries of the tropics and sub-tropics [7]. The leaves of the tree are used to make soup in northern Nigeria while the seeds are left unused. This study investigated the impacts of treatment techniques on the proximate and phytochemical contents of moringa kernel seed meal.

MATERIALS AND METHODS

Sources of plant materials and treatments

The *Moringa oleifera* seed kernels were purchased from the moringa plantation garden in Guringawa quarters, Kumbotso L.G.A. Kano, Nigeria.

The moringa seeds were obtained from matured dried pods. The collected seeds were shelled manually to obtain the cream-coloured kernels. The seeds of moringa

were dehulled clean of its dirt using hand picking and winnowing. The seeds were grounded using pestle and mortar first, before being milled to give fine powder and sieves using a sieving material to keep in a polythene bag until the analysis. The following treatments were employed in this study: raw, toasting, boiling, autoclaving, and ethanol soaking.

Preparation of moringa seed kernel

Raw moringa seed kernel

The moringa seed kernels were collected from matured dried pods and shelled manually to obtain the cream-coloured seeds, the raw seeds were cracked using pestle and mortar, and the meals were packaged in cellophane bags before use. The samples were collected for proximate analysis and phytochemical screening [8].

Toasted moringa seed kernel

Clean kernels were toasted using a gas cylinder until it turned brownish before the cracking points. The toasted seed kernels were milled and the samples were analyzed for proximate composition and anti-nutrient content [9].

Boiled moringa seed kernel

Four (4) liters of clean water were heated to the boiling point at 100°C, while the water was boiling, moringa seed kernels were poured into it and allowed for 5 minutes before the seed kernels were removed with a colander (sieve) to drain the water. The drained seed kernels were air-dried to constant weight. Dried seed kernels were milled and packed in cellophane bags before the samples were analyzed for proximate composition and anti-nutrient content [10] modified.

Autoclaved moringa seed kernel

The moringa seed kernels were autoclaved using recommended fifteen (15) minutes Sulehria [11] at 121-124°C (200 kPa) for sterilization. To control and monitor the process, temperature was used, to obtain the required steam temperature while the pressure was mainly used as described by the World Health Assembly Resolution WHAR.10.

Ethanol soaked moringa seed kernel

A 500ml of absolute ethanol was used to soak a powdered sample of fifty grams (50g) for twelve (12) hours. It was stirred at different intervals of time. After twelve (12) hours, the samples were double-filtered with a muslin cloth and obtained in conical flasks. The filtrate was dried at a temperature of 45 °C inside a hot-air oven as described by Handa [12].

Phytochemical analysis of moringa seed kernel meal

Phytochemical screening of raw and treated moringa seed kernel meal was conducted using standard methods according to Yankanchi [13] to identify the active constituents of moringa seed kernel meal.

Tannins analysis

The sample amounting to 0.5 g of seed kernels was boiled in water (20 ml) inside a test tube and later filtered. Drops of 0.1% ferric chloride were added to it for brownish-green or blue-black coloration to be observed.

Test for saponins

A powdered sample of 2g quantity was boiled using 20 ml of distilled water inside a water bath and later filtered. A distilled water of 5 ml volume was mixed with the filtrate of 10 ml and shaken to observe persistent froth (stable). The frothing was mixed with three (3) drops of olive oil was and shaken vigorously for the formation of emulsion.

Test for alkaloids

Two hundred and fifty (250mg) of the powdered extract of the moringa seed sample plus 1% HCl was added in steam, and the mixture with a drop of dragon droffs reagent was shaken vigorously, and allowed to settle for about ten (10) minutes. Formations of the orange precipitate indicate the presence of alkaloids.

Test for flavonoids

The 0.30g sample was weighed into a beaker and extracted using 30cm³ of water (distilled) for about 2 hours and later filtered using Whatman filter paper number 42 (125 mm). Ten 10cm³ of aqueous filtrate of extracted seeds were added to 5cm³ of the 1.0 M dilute ammonia solution and then an additional 5 cm³ of concentrated tetraoxosulphate (VI) acid. Yellow coloration appearance with disappearance on standing indicated the presence of flavonoids [14].

Test for phytate

Phytate (inositol hexaphosphate) contents of the samples were measured according to the modified Uppstrom *et al.*, (1980). An aliquot powdered sample of 10g gram quantity was extracted at room temperature overnight with vigorous shaking in an MSE orbital shaker at 200rpm for an hour to ensure homogeneity; it was later neutralized by adding 20ml of 20% sodium hydroxide: a 50ml of 1% ferric chloride in 1N hydrochloric acid and the mixture brought just to boil. This resultant mixture was filtered through an ashless Whatman No. 0 filter paper. The resultant residue was dissolved in 10ml of 2% sodium hydroxide. This procedure dissolves phytic acid while precipitating ferric hydroxide which was removed through filtration. The digestion of the filtrate (containing phytic acid) was carried out with a mixture of concentrated sulphuric acid and perchloric acid in the ratio 5:1 (v/v) in a Kjeldahl

flask. The pH of 4.5 of the digests was adjusted and phosphate determined colorimetrically at 710nm. A phytic phosphate concentration was computed using the standard inorganic phosphate curve.

Chemical composition of treated moringa seed kernel meal and soybean

Chemical composition

The nutritional analysis of untreated (raw) and treated *M. oleifera* seed kernel meals was determined, the parameters include moisture, protein (CP), lipid, fibre, ash, and carbohydrate contents according to the methods of Association of Analytical Chemistry [8].

Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using the statistical package (SPSS) software (Version 20.0). Tukey test was used to separate the means at 5% significant level.

RESULTS AND DISCUSSION

Proximate compositions of processed *Moringa oleifera* seed kernel meals (MSKM) and (soybean) SBM

The mean proximate compositions of different treatment methods are presented in Table 1. The crude protein of toasted moringa seed kernels (41.12 ± 0.00) was significantly ($p < 0.05$) higher than other treatment methods employed, followed by toasted soybean meal (39.27 ± 0.00) but significantly ($p < 0.05$) lower in raw moringa seed kernel meal (24.58 ± 0.00). Crude fibre content was significantly ($p < 0.05$) low in an autoclaved (5.65 ± 0.00) seed kernel meal and higher in toasted seed kernel meal (8.40 ± 0.00). However, lipid content was significantly ($p < 0.05$) higher in autoclaved seed kernel meal (27.74 ± 0.00) but lower in toasted soybean meal (20.33 ± 0.00). Moisture content was significantly ($p < 0.05$) lower in toasted (2.37 ± 0.00) SBM and higher in ethanol-soaked (9.22 ± 0.00) moringa seed kernel meal. NFE was significantly higher ($p < 0.05$) in raw moringa seed kernel meal (34.85 ± 0.00) but lower in ethanol (18.26 ± 0.00) and toasted (18.68 ± 0.00) moringa seed kernel meal, respectively.

Phytochemical compositions of processed *Moringa oleifera* seed kernels meals (MSKM)

The results for phytochemical compositions of processed *Moringa oleifera* seed kernels meals (MSKM) are presented in Table 2. The non-detectable anti-nutrients in all the treatment methods were phytate (0%) and flavonoid (0%). Alkaloids were significantly ($p < 0.05$) higher (4.35 ± 0.00) in raw but not detected in toasted MSKM. Tannins were also detected in all the treatment methods except boiled MSKM. It was significantly ($p < 0.05$) higher (0.58 ± 0.00) in raw but significantly ($p < 0.05$) lower

(0.46 ± 0.0) in ethanol-soaked MSKM. Saponins were not detected in toasted MSKM but detected in all other treatment methods which were significantly ($p < 0.05$) higher (5.91 ± 0.00) in raw but lower (5.16 ± 0.00) in boiled MSKM.

The high price of animal protein sources is directing the interest of researchers toward several leguminous seeds that can serve as potential sources of vegetable protein and other nutrients for human foods and livestock feeds [15]. Phytochemical factors can cause poor availability or utilization of nutrients, producing effects that include reduction in feed intake, neurological effects, and even death [16]. From the results of the present study, moringa seed kernels were positively affected by the treatment methods as it enhances the nutritional contents of the treated seeds, generally when compared to untreated seed kernels (raw seed). The treatment methods also express their effects differently as toasted appeared to be the best among all treatments in terms of crude protein value. This is not in line with the findings of Orire [17], who presented a decrease in performance of toasted locust bean meal-based diet which could be attributed to the effect of temperature on the seed that probably denatured the protein content and therefore rendered it less digestible, unavailable and less soluble. High levels of crude protein and fat recorded in the present research agree with the reports of Foild [18] and Muhd [16] where they observed high contents of proteins and lipids in moringa and Bambara nut seeds, respectively. The protein value (27.44%) recorded by Olugbemi [19] was only higher than untreated seeds in the present study but lower than all treated seeds. Another study reported (30.65%) crude protein in *Moringa oleifera* leaves [20]. The low moisture content observed in all treatment methods employed in the present study indicated that microorganism activity would be reduced and, therefore, extended the shelf life of the formulated diets [21]; [22]. The percentage (%) of dry matter (DM) recorded in this study was high generally and is in line with the findings of Olugbemi [19]; Mutayoba [20] recorded dry matter values of 93.7% and 87.20 %, respectively. The crude fibre and ash recorded by them were slightly lower than the values obtained in the present study. The difference might be due to the variations in geographical location of growth and development or stage of maturity of the moringa plants.

A study by Krishnaiah [23] reported a range of 0.24-0.52% alkaloids, 1.1-2.3% saponins, and 0.32 - 0.62% flavonoids in various Malaysian herbs. This revealed that plants have different concentrations of compositions. Generally, all treatment methods in the present study lower the anti-nutritional contents of moringa seed kernels compared to (raw) untreated seeds. Phytates and flavonoids were not present in an untreated seed. Hot water boiling was reported to reduce anti-nutrients of plant products, [24], which agrees with the findings of the present study. A significantly lower phytic acid in the diet with toasted ingredients (5.65-5.76%)

compared to the diet with untoasted ingredients (5.85-5.92%) was recorded by Kang'ombe [25]. Ndidi [26] observed a similar change after toasting Bambara nuts (*Vigna subterranean*). Tannins can form complexes with metal ions as well as macromolecules such as proteins and polysaccharides as they are plant polyphenols. Dei [27] observed reduced feed efficiency and weight gain in animals as a result of dietary tannins. Tannins were reduced in all treated seeds except in boiling which it was not present. Saponins were also lowered in all treated seeds but absent in toasted seeds. Saponins are glycosides and include steroid saponins and triterpenoid saponins [27]. High levels of saponins in feed affect feed intake and growth rate in poultry [27]. Reduction in feed intake has been ascribed to the bitter taste of saponins and due to the irritating taste [28]. Saponins (in excess), cause hypocholesterolaemia because they bind cholesterol, making them unavailable for absorption [29]. Saponins also have haemolytic activity against red blood cells [30]. Saponin-protein complex formation can reduce protein digestibility [31]. Oxidative phosphorylations are known to be inhibited by alkaloids with subsequent impairment of oxygen consumption in fish exposed [32]. The treatments in the present study appeared to be effective in drastically lowering the alkaloid contents in the seeds and even eliminating it in toasted seed kernels. This agrees with the findings of Bamgbose, [33], who reported a more effective wet heat processing of plant protein in reducing or eliminating various phytochemicals contained in them.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The phytochemicals present in the moringa seed kernels could be reduced through adequate treatment methods using boiling, toasting, autoclaving, and ethanol-soaking respectively, which in turn improve the nutrient content of the seed. Therefore, other treatment methods are recommended for further studies as well as experimental trials in fish feed should be conducted using the above treatment methods in moringa seed kernels.

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Conflict of interest

Authors declare that there is no conflict of interest, and, therefore, give consent for the publication of the article.



Table 1: Proximate composition of processed *Moringa oleifera* seed kernel meals (MSKM) and SBM

Differently Proximate compositions (%)	Processed RMSKM	Moringa TMSKM	seed kernels BMSKM	And AMSKM	SBM EMSKM	Meals TSBM
Moisture	5.48±0.00 ^d	3.05±0.00 ^e	8.85±0.00 ^b	7.31±0.00 ^c	9.22±0.00 ^a	2.37±0.00 ^f
Ash	3.80±0.00 ^c	4.14±0.00 ^b	2.90±0.00 ^d	3.39±0.00 ^c	4.88±0.00 ^b	7.13±0.00 ^a
Fat	25.12±0.00 ^c	24.61±0.00 ^d	22.07±0.00 ^e	27.74±0.00 ^a	26.27±0.00 ^b	20.33±0.00 ^f
Protein (CP)	24.58±0.00 ^f	41.12±0.00 ^a	30.65±0.00 ^e	35.25±0.00 ^c	33.07±0.00 ^d	39.27±0.00 ^b
Fibre (CF)	6.17±0.00 ^c	8.40±0.00 ^a	6.60±0.00 ^c	5.65±0.00 ^d	8.30±0.00 ^a	7.13±0.00 ^b
N.F.E	34.85±0.00 ^a	18.68±0.00 ^e	28.93±0.00 ^b	20.66±0.00 ^d	18.26±0.00 ^e	22.35±0.00 ^c

Mean values in the same row with different superscripts are significantly different (P < 0.05)

Key: RMSK= raw moringa seed kernel meal, TMSKM= toasted moringa seed kernel meal
BMSKM= boiled moringa seed kernel meal, AMSKM= autoclaved moringa seed kernel meal
EMSKM= ethanol soaked moringa seed kernel meal and TSBM= toasted soybean meal

Table 2: Phytochemical compositions of processed *Moringa oleifera* seed kernels meals (MSKM)

Different Parameters	Processing Raw	methods of Toasted	moringa Boiled	kernels Autoclaved	meals Ethanol soak
Alkaloid (%)	4.35±0.00 ^a	0.00±0.00 ^c	2.23±0.00 ^b	2.20±0.00 ^b	2.12±0.00 ^b
Tannins (%)	0.58±0.00 ^a	0.50±0.00 ^b	0.00±0.00 ^c	0.50±0.00 ^b	0.46±0.00 ^b
Saponins (%)	5.91±0.00 ^a	0.00±0.00 ^d	5.16±0.00 ^c	5.70±0.00 ^b	5.60±0.00 ^b
Phytate (%)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Flavonoid (%)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Mean values in the same row with different superscripts are significantly different (P < 0.05)

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