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Comparative effect of processing methods on the nutrient and anti-nutrient composition of *Moringa oleifera* leaf

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Abstract

This study investigated the comparative effect of different processing methods on the nutrient and antinutrient profiles of *Moringa oleifera.* Fresh *Moringa oleifera* leaves were harvested from Abeokuta (South West Nigeria), and were either air dried until crispy, then milled to consistency and packed in air tight container (moringa leaf meal MLM) or, processed into Moringa leaf protein concentrate (MLPC) using village level, low cost fractionation scheme. The proximate analysis, vitamin, mineral and antinutrient profile of MLM and MLPC were carried out. The result showed that MLPC has a higher proximate content (%DM ± SD) of crude protein, ash, ether extract of 45.38 ± 2.17, 9.52 ± 0.95 and 20.00 ± 0.65 compared to 27.50 ± 1.30, 6.71 ± 0.50 and 5.50 ± 0.17 respectively in MLM, but MLPC has a lower crude fibre content of 1.50 ± 0.07 compared to 14.50 ± 0.95 in MLM. The MLPC also contained higher concentrations of the essential minerals of sodium, potassium, calcium, magnesium, zinc and iron at 230.27 ± 14.16, 275.77 ± 10.36, 4000.86 ± 236.47, 640.39 ± 23.07, 5.22 ± 0.39 and 65.47 ± 2.59 mg/100g DM respectively. In MLPC, vitamin A, B2, B3, B1and C concentrations are 44.23, 30.08, 12.00, 5.10 and 7.80 mg/100gDM respectively and, were all higher than the values for MLM except Manganese and Vitamin C, where the concentration was higher in MLM at 62.73 ± 0.36 mg/100g DM and 16.90 ± 0.61 mg/100g DM compared to 3.33 ± 0.19 mg/100gDM and 7.80 ± 0.45 mg/100g DM, respectively in MLPC. The MLPC also contain lower concentration of phytate, oxalate and saponin except tannin. Thus, fractionation of moringa leaf produces higher essential nutrient and lower antinutrient compositions of *Moringa oleifera* protein concentrate (MLPC) compared to the commonly produced air dried Moringa leaf meal. MLPC could be a better substitute compared to MLM and can be used as food supplement to treat infant malnutrition, improve growth, nutrition and health status of the human populace especially in developing countries of the world.

Keywords: moringa leaf meal, moringa leaf protein concentrate, nutrient and antinutrient profiles

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Introduction

Moringa oleifera Lam (syn. *M. pterygosperma*; commonly known as "The Miracle Tree," "Horseradish tree," or "Ben oil tree") is a common and most widely distributed species of *Moringa*ceae family, having an extensive range of medicinal uses with high nutritional value throughout the world. It is native to Western and sub-Himalayan tracts, India, Pakistan, Asia, and Africa and is well distributed in America, Philippines, Cambodia, and the Caribbean Islands, (Somali *et al*, 1984; Morton, 1991; Mughal *et al*, 1999). Almost every part of this highly valuable tree have long been consumed by humans and used for various domestic and small scale industrial purposes (Suaib Luqman *et al*, 2012). World organizations such as Trees for Life, Church World Service, and Educational Concerns for Hunger Organization have advocated *Moringa* as "Natural Nutrition for the Tropics" in various parts of the world (Souza and Kulkarni, 1993; Palada, 1996; Fuglie, 1999).

In addition, the leaf of *M. oleifera* has been reported to be a valuable source of both macro- and micro nutrients, which include *β*-carotene, protein, vitamin C, calcium, and potassium and acts as a good source of natural antioxidants (Dillard and BruceGerman, 2000; Siddhuraju and Becker, 2003). Moringa leaves have been used to treat malnutrition, especially among infants and nursing mothers for enhancing milk production, (Price, 1985; Dillard and BruceGerman, 2000; Estrella *et al*, 2000). The leaves are commonly dried and crushed into powder, and used in sauce and soups. One tablespoon of leaf powder provides 14% of the protein, 40% of the calcium, 23% of the iron and most of the vitamin needs of a child aged one to three. Six tablespoons of the leaf powder will provide nearly all of a woman's daily iron and calcium needs during pregnancy and breast feeding. Moringa leaves contain a wealth of essential, disease preventing nutrients including all the essential amino acids. The dried leaves contain higher amounts of these nutrients except vitamin C (Fuglie, 2005). It does possess nutritional, prophylactic and therapeutic properties (Fahey, 2005). Moringa leaves can be eaten fresh, cooked, or stored as dried powder for many months without losing much of its nutritional value. The tree is in full leaf at the end of the dry season when other foods are typically scarce.

Leaf concentrate is an extremely nutritious food made by mechanically separating indigestible fiber and soluble antinutrients from much of the protein, vitamins and minerals in certain fresh green plant leaves using a low cost simple village fractionation scheme (Fellows, 1987). The leaf concentrate technology is a simple, cheapest dietary source of essential nutrients, easier to preserve than green vegetable and viable option in reducing the endemic problem of protein undernutrition. David (1993) reported that it is an excellently acceptable food by children, extremely nutritious, richer in vitamin A and Iron than any other commonly available food. It is environmentally friendly yet easy to make and cheap compared to other green vegetables. The by-product makes an excellent nutrient dense feed for farm animals, a good source of biogas, fertilizer and ethanol production.

This study aims at producing air dried moringa leaf meal MLM and moringa leaf protein concentrate, MLPC, using village level low cost fractionation scheme, evaluate and compare the nutritional and antinutritional composition of the two products, with the objective of revealing the potential of these products to alleviate malnutrition and in ethno-medicine.

Materials and methods

Collection and identification of sample

Moringa oleifera fresh leaves were collected from the school farm plot of Egba High School, Abeokuta, Ogun state, South West Nigeria. The sample was identified by the Consulting Botanist in the Botany Department, University of Lagos, Akoka, Nigeria, with identification number LUH 1296A.

Preparation of moringa leaf meal (MLM)

The fresh moringa leaves were simply air dried until crispy, ground to consistency and bottled in an air tight dark container at room temperature until needed for analysis.

Preparation of moringa leaf protein concentrate (MLPC):

Fresh moringa leaves were plucked and washed thoroughly to remove sand and stones. It was then pulped and processed as described by Fellows (1987). The pulping was done using laboratory pestle and mortar and, kitchen scale blender. The pulped leaves were squeezed tightly through a muslin cloth to drain the juice out of the pulped leaves. The juice collected was boiled evenly on a low furnace at 80-90ºC for 10 min. This procedure produced a curd (concentrate) separated from the whey. After cooling, the liquid was filtered using a semi permeable cloth, and pressed to dryness. The residue was evenly spread on a tray and allowed to air dry. The air dried residue is the leaf concentrate, which was washed with water, repressed and air dried. It was stored in an air tight bottle container until analysis.

Chemical analysis

Proximate composition

The proximate composition for MLM and MLPC were determined for moisture, ash, crude fat, nitrogen free extract and crude fibre as described by AOAC, (1990). The nitrogen and crude protein (N x 6.25) were as described by Kjeldhal method (AOAC, 1990).

Mineral analysis

The mineral composition was determined in duplicates using the Atomic absorption spectrophotometer (AAS) as described by AOAC (1990). The model of AAS used was Perkin Elmer A analyst 200, after ashing and HNO_3 and HCl digestion.

Vitamin analysis

The vitamins composition was determined using High Performance liquid chromatography.

Antinutritional factors analysis

Phytate was determined using the method of Sudarmadji and Markakis (1977), by titration with ferric (III) chloride solution.

Oxalate was determined by extracting the sample with water for 3hours and the absorbance read on spectrophotometer at 420nm. Standard solutions of Oxalic acid were prepared and their absorbance read on the spectrophotometer at 420nm. The content of oxalate was determined from the standard curve.

Saponin content was determined using the method of Makkara and Becker (1996) by comparing the absorbance of the sample extracts with standard saponin at 380nm.

Tannin content was also determined using the method of Griffiths and June (1977). The sample was extracted with a mixture of acetone and glacial acetic acid for 5hours and the absorbance read on the spectrophotometer at 500nm. The tannin content was read from the standard curve of standard tannic acid.

Statistical analysis

All the data generated were subjected to descriptive statistic including mean and standard deviation (Olawuyi, 1996). Student's t-test was used to compare the means and significant difference was set at $p < 0.05$

Results

The results of the proximate analysis of MLM and MLPC are shown in Table 1. MLM had on dry matter basis, crude protein, crude fibre, ash, ether extract and nitrogen free extract (NFE) contents of 27.50 ± 1.30%, 14.50 ± 0.95%, 6.71 ± 0.50%, 5.50 ± 0.17% and 34.79 ± 1.90%, respectively. MLPC had significantly higher ($p<0.05$) crude protein, ash and ether extract content of 45.38 ± 2.17%, 9.52 ± 0.95% and 20.00 ± 0.65%, respectively, but significantly ($p<0.05$) lesser crude fibre and NFE content of 1.50 ± 0.07% and 17.45 ± 1.18%, respectively.

The results of the mineral analysis are shown in Table 2, the concentrations of sodium, potassium, calcium, magnesium, zinc and iron (mg/100g DM) in MLM were 160.11 ± 4.85, 225.52 ± 5.66, 1980.18 ± 118.82, 120.21 ± 3.45, 2.94 ± 0.24, 41.07 ± 2.14, respectively, while MLPC had significantly ($p<0.05$) higher values of 230.27 ± 14.16, 275.77 ± 10.36, 4000.86 ± 236.47, 640.39 ± 23.07, 5.22 ± 0.39, 65.47 ± 2.59 mg/100g DM.

The results of vitamin analysis are shown in Table 3. MLPC had significantly ($p<0.05$) higher concentration (mg/100g DM) of water soluble vitamins A, B2, B3, and B1 at 44.23 ± 1.81, 30.08 ± 1.02, 12.10 ± 0.44 and 5.10 ± 0.24 respectively compared to the values of 17.60 ± 0.78, 21.30 ± 0.95, 7.90 ± 0.31 and 2.30 ± 0.11 in MLM. However the Vitamin C concentration was significantly ($p<0.05$) lower in MLPC at 7.8 ± 0.45 mg/100g DM compared to 16.90 ± 0.61 mg/100g DM in MLM.

The result of anti-nutritional composition of MLM and MLPC is shown in Table 4. There was significant ($p<0.05$) reduction in the concentrations (mg/100g DM) of phytate, oxalate and saponins at 7.80 ± 0.45, 10.56 ± 0.63 and 1.50 ± 0.12 respectively in MLPC compared to 9.89 ± 0.66, 90.56 ± 0.75 and 2.69 ± 0.19 respectively in MLM. The concentration of tannin was not significantly ($p>0.05$) higher in MLPC at 0.44 ± 0.03 than in MLM at 0.25 ± 0.06mg/100g DM.

Discussion

The proximate values obtained for MLPC are close to the mean values reported by Fasuyi and Aletor (2005) for cassava leaf meal protein concentrate which contained crude protein 47%, crude fibre 2%, Ether extract 21.6%, ash 7.8%, NFE 15.9%. The MLPC values are however higher than the mean values of 29.9% CP, 6.51% ash, 5.7% CF, 19.9% ether extract and 38.5 % NFE reported by Agbede (2005) for seven common tropical leguminous leaves protein concentrates. Sodamade *et al.* (2013) reported 39.13% CP, 5.43% CF, 2.43% ether extract and 6.00% ash for moringa leaf protein concentrate. This proximate result shows that MLPC is a potential alternative food/feed protein source, because the mean crude protein content of 45.13 ± 2.17% DM is comparable to those reported for most commonly used tropical legumes (FAO, 1973; Aletor and Aladejimi, 1989). The ash content of 9.52±0.95% DM is higher than values obtained for vegetable species (Sodamade *et al*, 2013), some tropical leguminous leaves protein concentrates (Aletor and Adeogun, 1995; Oke *et al.,* 1995; Agbede, 2005), cassava leaf protein concentrate (Fasuyi and Aletor, 2005), but at variance with values reported for MLPC by Sodamade *et al.* (2013). The lower crude fibre content of the MLPC (1.50 ± 0.07% DM) compared to the MLM (14.50± 0.95% DM) showed that the MLPC could be ideal for infants, human and non-ruminants consumption, because higher crude fibre content (>5% for infants and preschool children, Codex, 1985) can cause increase bulk, lower calorie value, intestinal irritation, lower digestibility and overall decreased nutrient utilization in human and monogastric animals (Johnson, 1987). The high fat content could implicate higher energy content and fat soluble vitamins.

There is a relative abundance of the macro and micro minerals, especially Calcium, Iron and Zinc in MLM and MLPC. Sodium and Potassium are good sources of electrolyte for the body. Calcium is required by infants, pregnant and lactating mothers for good bones, teeth maintenance and development thus, preventing osteoporosis, (FAO, 2001). It is also required for blood clotting and nervous function. Iron is an essential trace mineral required for the formation of haemoglobin and myoglobin for oxygen transport thus preventing anemia, (FAO, 2001) It is also required for cell growth and division, functioning of the central nervous system, oxidation of carbohydrates, proteins and fats, transfer of electrons in the electron transport chain for the formation of adenosine trisphosphate (ATP) in energy metabolism (Kozart, 2007; Umar *et al.,* 2007). Zinc is required for the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and insulin. It is also essential to the structure and function of several enzymes, reproduction and growth of sperm cells. It is reported to have anti-viral, anti-bacteria, anti-fungal and anti-cancer properties (Brisbe *et al.*, 2009). It also exhibits antioxidant activity in combination with Copper (Guo *et al.*, 2010). Thus, a good mineral content spectrum of MLM and MLPC will play a major role in their nutritional, medicinal and therapeutic properties (Alkharusi *et al.*, 2009).

The water soluble vitamins values obtained for MLM are close to values reported by Fuglie (1999), Moyo *et al* (2011), and Mensah (2012). Beta carotene is the main safe source of Vitamin A, required in physiological functions such as vision, bone growth, immunity, maintenance of epithelial tissue, metabolism of blood iron status and thus reduce anemia (Thurber and Fahey, 2009; Babu, 2000). β-carotene has antioxidant property, (FAO, 2001). Vitamin C plays physiological functions in immune stimulation, anti-allergic, antioxidant, synthesis of collagen for connective tissue, wound healing, teeth and gum development and iron absorption (Oguntibeju, 2008). The β-vitamins are involved as coenzymes in energy metabolism and redox reactions of the cells, nerve impulse conduction and muscle action, cell growth and reproduction (FAO, 2001).

Moringa leaf meal has high concentrations of phytate, oxalate, saponin but, low tannin content. These are plant secondary metabolites which are recently been studied for their medicinal activities and health promoting effects. Fractionation reduces the content of phytate, oxalate and saponin. Antinutritional factors (ANF) affect the overall nutritional value of foods by reducing food digestion, absorption and digestive enzyme activities. They could also sometimes when taken together have serious and fatal effects (Osagie, 1998; Ojiako, 2008). Generally, ANF has biochemical and toxicological adverse effects (Soetan and Oyewole, 2009).

The values for proximate, mineral and vitamin contents of MLM reported in this study are at variance with various values in literature (Sanchez-Machado *et al.*, 2009; Moyo *et al.*, 2011; Madukwe *et al.*, 2013; Ogbe and Affiku, 2012). The nutritional variations observed among the studies could be due to factors like genetic background of the plants such as cultivar, environmental factors such as soil and climate, cultivation method used which includes frequency of harvesting, age of plant or leaves, method of storage (drying, refrigeration, freezing) between collection and analysis (Barminas *et al.*, 1998; Broin, 2006; Sanchez-Machado *et al.*, 2009; Moyo *et al.*, 2011).

Conclusion

In conclusion, the study showed that moringa leaf is rich in nutrient such as protein, minerals and vitamins. Fractionation to produce protein concentrate is a better way to concentrate the nutrients, and reduce the crude fibre, ANF, compared to air drying that produced MLM. The lower water content of MLPC would ensure proper conservation of nutrient for nutritional, therapeutic and curative functions. It will also ensure all year round availability. Further studies would be required to compare the protein quality of the two products.

Table 1: Proximate composition of MLM and MLPC (%DM ± SD)

Proximate Parameters	Moringa Leaf Meal (MLM)	Moringa Leaf Protein Concentrate (MLPC)
Crude Protein	27.50 ± 1.30	45.38 ± 2.17*
Ether Extract	5.50 ± 0.17	20.00 ± 0.65*
Crude Fibre	14.50 ± 0.95*	1.50 ± 0. 07
Ash	6.71 ± 0.50	9.52 ± 0.95*
Moisture	11.00 ± 0.50*	6.15 ± 0.45
Nitrogen Free Extract	34.79 ± 1.90*	17.45 ± 1.18

(Mean, n=3), * Mean value significantly different at $p < 0.05$

Table 2: Mineral composition of MLM and MLPC (mg/100g DM ± SD)

Minerals	Moringa Leaf Meal (MLM)	Moringa Leaf Protein Concentrate (MLPC)
Sodium	160.11 ± 4.85	230.27 ± 14.16*
Potassium	225.52 ± 5.66	275.77 ± 10.36*
Calcium	1980.18 ± 118.82	4000.86 ± 236.47*
Magnesium	120.21 ± 3.45	640.39 ± 23.07*
Zinc	2.95 ± 0.24	5.22 ± 0.39*
Iron	41.07 ± 2.14	65.47 ± 2.59*
Manganese	6.73 ± 0.36*	3.33 ± 0.19

(Mean, n=3), * Mean value significantly different at $p < 0.05$

Table 3: Vitamin composition OF MLM and MLPC (± SD mg/100g DM)

Vitamins	Moringa leaf meal (MLM)	Moringa leaf protein concentrate (MLPC)
Vitamin A	17.60 ± 0.78	44.23 ± 1.81*
Vitamin B2	21.30 ± 0.95	30.08 ± 1.02*
Vitamin B3	7.90 ± 0.31	12.10 ± 0.44 *
Vitamin B1	2.30 ± 0.11	5.10 ± 0.24*
Vitamin C	16.90 ± 0.61*	7.80 ± 0.45

(Mean, n=3), * Mean value significantly different at $p < 0.05$

Table 4: Antinutrient composition of MLM and MLPC (mg/100g DM ± SD)

ANF	MLM	MLPC
Phytate	9.89 ± 0.66*	7.80 ± 0.45
Oxalate	90.56 ± 0.75*	10.56 ±0.63
Tannin	0.25 ± 0.09	0.44 ± 0.03
Saponin	2.69 ± 0.19*	1.50 ± 0.12

(Mean, n=3), * Mean value significantly different at $p < 0.05$

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