

Comparative Study of Nutritional Profiles and Phytochemical Components of Raw, Blanched and Fermented Flour from the Leaves of *Moringa oleifera lam*

Ijarotimi OS,* Fagbemi, TN & Osundahunsi OF

Department of Food Science and Technology, Federal University of Technology Akure, Nigeria

ABSTRACT

Background: The medicinal properties of *Moringa oleifera* plants have been extensively investigated but less is known of its nutrients and phytochemical components. This study evaluated the nutritional and phytochemical profiles of *Moringa* leaves. **Methods:** *Moringa* leaves were freshly harvested from Federal University of Technology community in Akure. The leaves were processed into flour and evaluated for nutritional qualities after being subjected to shade drying, blanching and fermentation techniques. **Results:** The moisture contents of flour from raw, blanched and fermented leaves ranged from 6.88±0.70g/100g to 7.19±0.64g/100g, while the protein contents were between 24.39±0.18g/100g and 29.93±2.77g/100g. Among the minerals present, potassium had the highest concentration, while copper the lowest value. The Ca/P and Na/K molar ratios of the samples ranged between 18.3 to 24.5 and 0.3 to 0.4 respectively. Total essential amino acids plus histidine and arginine ranged between 38.16g/100g and 42.24g/100g. The phytochemical/antinutrient concentrations in fermented leaf flour had significantly lower tannin, phytate, trypsin, oxalate, phenolic, alkaloid, flavonoid, saponin and terpenoid contents when compared with the flour of blanched and raw leaves. The predicted protein efficiency ratio, essential amino acid index and biological values were highest in raw leaf flour and lowest in blanched leaf flour. The digestible indispensable amino acid index ranged from 51.7% in raw leaf flour to 85.2% in fermented samples. **Conclusion:** Flour from fermented *Moringa oleifera* leaves had better nutrient composition, nutritional quality, and a reduction in some antinutrients/phytochemicals than flour from blanched leaves.

Keywords: Chemical composition, *Moringa oleifera* leaves, nutritional quality

INTRODUCTION

Plants are a primary source of medicines, food and shelters to animals and humans. Leafy vegetables are important items of medicine and diet in many homes, and are

valuable sources of nutrients like protein, carbohydrate, fat/oil, mineral, vitamins, fiber (Mosha & Gaga, 1999) and bioactive components (tannins, alkaloids, terpenoids, steroids and flavonoids). Phytochemicals are a group of non-nutrient bioactive

* Correspondence author: Oluwole Steve Ijarotimi; Email: soijarotimi@gmail.com or soijarotimi@futa.edu.ng

compounds found naturally in plants that work in conjunction with other plant components as a defensive mechanism for the plants against diseases and external attacks (Vasanthi, ShriShriMal & Das, 2012). In humans, many phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes like ageing (Burns *et al.*, 2001).

Moringa oleifera, commonly called the drum stick, is a tree native to India, but has been planted and domesticated in many other countries, including Nigeria. It is the most known and widely cultivated variety of the genus *Moringa*, family *Moringaceae*. *Moringa oleifera* is also known by other common names such as Mallungay (Philippines), Benzolive tree (Haiti), Horse raddish tree (Florida) and Nebeday (Senegal). In Nigeria, it is known as Zogale in Hausa, Okwe Oyibo in Igbo, Ewe Ile in Yoruba and Jeghel-agede in Tiv. The leaves, seeds and flowers have good nutritional and therapeutic values, and study show that the leaves have been used to prevent or treat protein-energy malnutrition and other nutritional related diseases (Tete-Benissan *et al.*, 2012). *Moringa oleifera* leaves are low in fat and carbohydrate and an excellent source of amino acids, particularly sulphur containing amino-acids, that is, methionine and cystine which are often in short supply in the plant kingdom.

METHODS

Materials and samples collection

Moringa oleifera leaves were collected in the month of January 2013 from Federal University of Technology, Akure, community of Nigeria. The leaves were identified and authenticated at the herbarium unit of the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria.

Preparation of moringa leaf flour samples

Moringa leaves were processed using home-based processing techniques i.e., shade drying, blanching and fermentation.

Raw Moringa oleifera leaf flour: Harvested fresh leaves of *Moringa oleifera* were divided into three portions. A portion (1kg) of the leaves was spread on a tray and shade dried at room temperature for 72 hours. The dried leaves was milled into powder with an electric blender (SMB 2898: Super master, Japan) (Figure 1).

Blanched Moringa oleifera leaf flour: The freshly harvested leaves were washed with distilled water, steam blanched, oven dried (Gellenhamp) at 40°C for 48 hours, milled into powder with an electric blender (SMB 2898: Super master, Japan) (Figure 1).

Fermented Moringa oleifera leaf flour: The third portion of the leaves (1kg) was subjected to fermentation. The freshly harvested leaves were washed and cut into pieces of 5mm and then steamed blanched and wrapped in plantain leaves. The leaves were allowed to ferment for 2-3 days. The fermented leaves were oven dried (Gellenhamp) at 40°C for 48 hours, milled into powder with an electric blender (SMB 2898: Super master, Japan) (Figure 1).

Chemical composition

Proximate Analysis: The nutrient composition of the flour samples was determined using the standard procedures of Association of Official Analytical Chemists (2005). The samples were determined for moisture content in a hot-air circulating oven (Galenkamp). Ash was determined by incineration (550°C) of known weights of the samples in a muffle furnace (Gallenkamp, size 3). Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40° to 60°C) in a soxhlet extractor. Protein (N × 6.25) was determined by the Kjeldahl method. Crude fibre was determined after

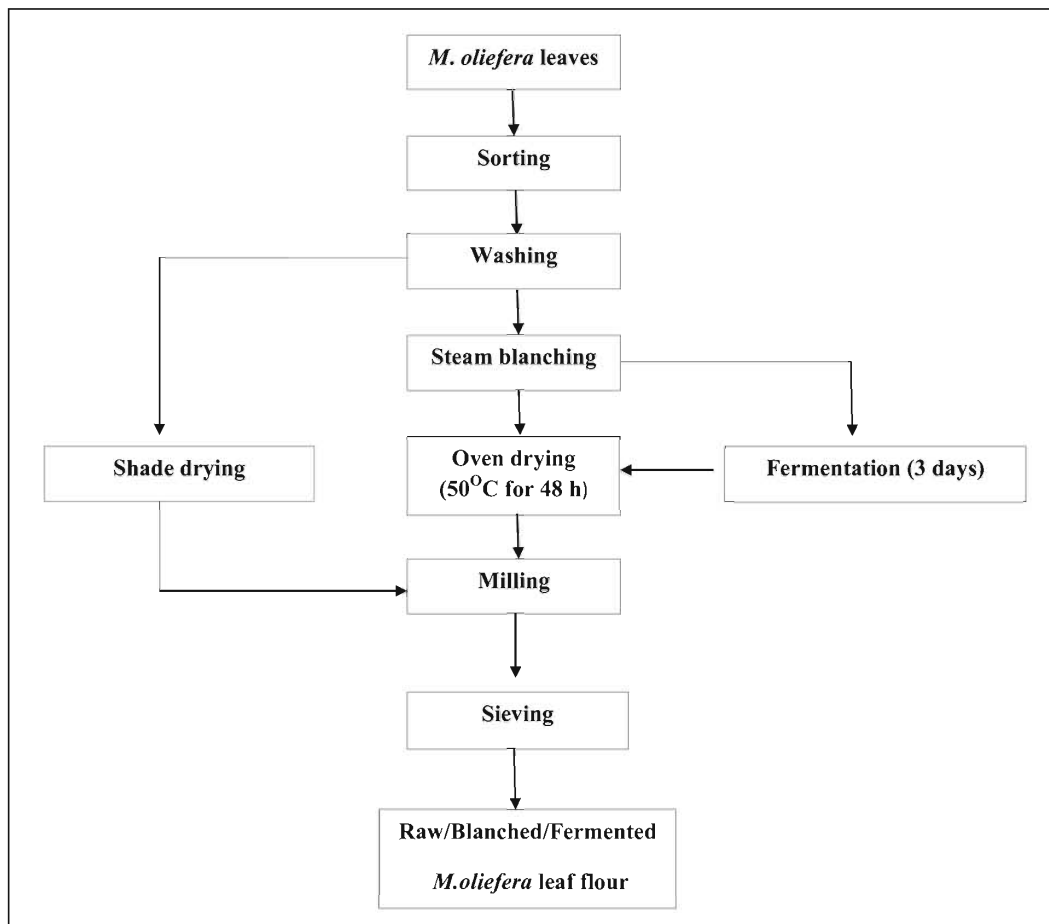


Figure 1. Processing of raw, blanched and fermented *Moringa oleifera* leaf flour

digesting a known weight of fat-free sample in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide. The carbohydrate content was determined by subtracting the total crude protein, crude fibre, ash, and fat from the total dry weight (100 g) of the food sample differences.

Energy value calculation (calorific value): Energy values of the samples were calculated by using specific values of Atwater factors for protein (4.0kcal.), fat (9.0kcal.) and total carbohydrate (4.0kcal.) (Iombor, Umoh & Olakumi, 2009).

Mineral determination: AOAC (2005) methods were used to determine mineral composition of the samples. One gram of

sample was digested with nitric/ perchloric/ sulphuric acids mixture in ratio 9:2:1 respectively and filtered. The filtrate was made up to 5 ml mark in a volumetric flask. Filtered solution was loaded to Atomic Absorption Spectrophotometer, (model703 Perkin Elmes, Norwalk, CT, USA). The standard curve for each mineral, that is, calcium, magnesium, iron, aluminium, lead, copper and zinc, was prepared from known standards and the mineral value of samples estimated against that of the standard curve. Sodium and potassium values were determined using Flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK),

while phosphorous was determined using the Vanado-molybdate method.

Determination of amino acid profile: The flour sample hydrolysates were prepared following the method of FAO/WHO (1991). Each of the defatted samples was weighed (200 mg) into a glass ampoule, 5ml of 6M HCl added and hydrolysed in an oven, preset at $105 \pm 5^\circ\text{C}$ for 22 h. Oxygen was expelled in the ampoule by passing nitrogen gas into it. Amino acid analysis was done by ion-exchange chromatography¹⁹⁷⁷⁷ using a Technicon Sequential Multisample Amino Acid Analyser (Technicon Instruments Corporation, New York, USA). The period of analysis was 76 min, with a gas flow rate of 0.50ml/min at 60°C , with reproducibility set at $\pm 3\%$. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein. Tryptophan was not determined. Norleucine was used as the internal standard

Determination of nutritional quality

Calculation of the Digestible Indispensable Amino Acid Scores (DIAAS): The DIAAS of the amino acids composition were computed according to the formula expressed in Equation 1 as described by FAO (2013). The ratio of indispensable amino acids in test proteins (IAA) to recommended amino acid scoring patterns for humans (6 months to 3 years) FAO (2013) was calculated using the following equation:

$$\text{DIAAS \%} = 100 \times \frac{\text{lowest value [(mg of digestible dietary indispensable amino acid in 1 g of the dietary protein) / (mg of the same dietary indispensable amino acid in 1g of the reference protein)]}}{1} \quad 1$$

Computed Protein Efficiency Ratio (C-PER): Protein efficiency ratio of the raw, blanched and fermented *M. oleifera* leaves flour samples were calculated according to the equations developed by Satterlee *et al.* (1982).

$$\text{PER} = 0.06320 [X_{10}] - 0.1539 \quad 2$$

where $X_{10} = \text{Thr} + \text{Val} + \text{Met} + \text{Ile} + \text{Leu} + \text{Phe} + \text{Lys} + \text{His} + \text{Arg} + \text{Tyr}$

Calculation of Essential Amino Acid Index (EAAI): Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the equation below (Mente *et al.*, 2002; Adeyeye & Aremu, 2010):

$$\text{EAAI} = (100a \times 100b \times \dots 100j / av \times bv \times \dots \times jv)^{1/n} \quad 3$$

where n = number of essential amino acids, a, bj = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine) in test sample and av, bv jv = content of the same amino acids in standard protein (%) (egg or casein) respectively.

Biological value (BV): The biological values (BV) of the leaf flour samples were computed using the regression equation below (Malomo, Alamu & Oluwajoba, 2013).

$$\text{BV} = 1.09 (\text{EAA Index}) - 11.7 \quad 4$$

Nutritional Index (NI): The nutritional index of the flour samples was calculated using the formula of Alipour *et al.* (2010).

$$\text{Nutritional Index (\%)} = \frac{[\text{EAAI} \times \text{protein (g/100g)}]}{100} \quad 5$$

Preparation of methyl esters of fatty acids: Oil was extracted from the food samples using the Goldfish apparatus, saponified and then esterified using 10% (v/v) boron trifluoridemethanol (Method No 969.33) as described by AOAC (2005) to fatty acid methyl esters (FAMES).

Analytical conditions: The FAMES were separated using an HP 6890 N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector and a Supelco SPB-PUFA capillary column (No: 24314; 30 m \times 0.25 mm \times 0.5 μm); the column temperature was kept at 210°C , injector temperature at 220°C

and the detector temperature at 280°C. The flow rate of the carrier gas (He, Istrabenz 99.9999%) was 1.0 ml/min. Reliability and accuracy of the analytical methods for the detection of fatty acids were ensured by the use of the certified reference matrix that consisted of a mixture of 37 FAME standards (Supelco 37 Component FAME mix, Sigma-Aldrich, St. Louis, MO, USA). The amounts of each of the fatty acids were calculated from the areas of the internal standards (heptadecanoic acid) (Porzucek & Raznikiewicz, 1990). The contents of the particular fatty acids were expressed as percentages of the sum of all of the fatty acids analysed.

Phytochemical/antinutrient determination

The methods of Harbone (1973) were used to determine quantitatively the concentration of saponin, alkaloid and flavonoid. Phytate and tannins contents of the samples were determined using the Latta & Eskin (1980) method while standard methods of AOAC (2005) were used to determine oxalate and total phenol. Trypsin inhibition activity was determined based on the method described by Lqari *et al.* (2002)

Statistical analysis

The data were analysed using SPSS version 16.0. The mean and standard error of means (SEM) of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means; the means were separated using the new Duncan multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Macronutrient and mineral composition

The results of macronutrient and mineral compositions of raw, blanched and fermented *Moringa oleifera* leaf flour samples are presented in Table 1. Moisture content of the flour samples ranged from $6.88 \pm 0.70\%$

in blanched leaf (BML) flour to $7.19 \pm 0.64\%$ in fermented leaf (FML) flour. There was no significant difference between the moisture content of the blanched and fermented Moringa leaf flour samples compared with that of raw leaf flour ($P > 0.05$). The moisture contents in moringa samples of the present study were lower than that reported by Busani *et al.* (2011).

The level of moisture content in flour samples is very important as it determines the activities of micro-organisms in the food sample. Several studies have reported that low moisture content in food samples increases the storage period of the food products while high moisture content in foods encourages microbial growth, hence, facilitating food spoilage (Alozie *et al.*, 2009). The protein content of raw Moringa leaf flour ($29.93 \pm 2.77\%$) was higher than blanched ($28.20 \pm 2.02\%$) and fermented samples ($24.39 \pm 0.18\%$). This observation could be attributed to the effects of heat during blanching or blanching prior to the fermentation process. Previous studies have reported that during blanching, the protein content of food products are usually decreased due to heat activity (Ilelaboye, Amoo & Pikuda, 2013). The protein content of Moringa leaf flour samples observed in this study were higher when compared with other vegetables like *T. triangulatae* (2.5g/100 g), *C. crepidioides* (3.4g/100 g), *S. nigrum* (2.9g/100g), *A. hybridus* (2.9 g/100g) and *T. occidentalis* (4.3g/100g) reported by Aletor & Adetogun (1995), but closer to the value reported by Busani *et al.* (2011) for dried Moringa leaves flour (30.29 %). The energy value of fermented leaf flour ($367.03 \pm 8.58\text{kcal}$) was higher than the blanched ($358.73 \pm 4.23\text{kcal}$) and raw samples ($351.0 \pm 3.90\text{kcal}$) respectively. This observation could be attributed to the hydrolysis of complex carbohydrates to smaller molecules thereby making them available as a source of energy.

The range of mineral contents of the raw, blanched and fermented flour samples

Table 1. Proximate and minerals composition of raw, blanched and fermented *Moringa oleifera* leaf powder

Nutrients	RML	BML	FML	*Acceptable level
<i>Macronutrients (g/100g)</i>				
Moisture	6.93 ^a ±0.98	6.88 ^a ±0.70	7.19 ^a ±0.64	<5
Protein	29.93 ^a ±2.77	28.20 ^a ±2.02	24.39 ^a ±0.18	>15
Fat	5.45 ^a ±1.38	5.47 ^a ±1.55	6.15 ^a ±1.87	10-25
Ash	10.16 ^a ±2.52	7.19 ^a ±1.01	6.91 ^a ±0.68	<3
Fiber	8.90 ^a ±1.00	10.09 ^a ±0.13	9.93 ^a ±0.48	<5
Carbohydrate	45.54 ^a ±5.67	49.04 ^a ±4.46	53.53 ^a ±2.15	64
Energy (kcal.)	351.0 ^a ±3.90	358.73 ^a ±4.23	367.03 ^a ±8.58	400-425
<i>Minerals (mg/100g)</i>				
Calcium	3853 ^a ± 4.24	2310 ^a ± 0.71	2779 ^a ± 1.41	*500
Magnesium	1862 ^a ± 1.41	1298 ^a ± 2.83	1427 ^a ± 2.83	*76
Sodium	3235 ^a ± 2.83	2282 ^a ±1.41	2998 ^a ± 2.12	*296
Potassium	9729 ^a ± 1.41	6185 ^a ± 2.83	8197 ^a ± 3.54	*516
Phosphorus	157.5 ^a ± 2.12	126.5 ^a ± 2.12	124.5 ^a ± 2.12	*456
Zinc	1.61 ^a ± 0.01	1.60 ^a ± 0.14	1.56 ^a ± 0.02	*3.2
Copper	0.72 ^a ± 0.01	0.60 ^a ± 0.01	0.68 ^a ± 0.03	*160
Iron	3.49 ^a ±0.01	3.35 ^a ± 0.21	3.10 ^a ± 0.01	*16
Na/K	0.3	0.4	0.4	**1.4-3.4
Ca/P	24.5	18.3	22.3	**1.6-3.6
Fe/Cu	4.8	5.6	4.6	**0.2-1.6
Zn/Cu	2.2	2.7	2.3	**2.0-4.0
Ca/K	0.4	0.4	0.3	**2.2-6.2

Means with different superscript in the same row are significantly different at $p < 0.05$.

KEY: FML: Fermented Moringa leaves, BML: Blanched Moringa leaves, RML: Raw (shade dried) Moringa leaves. *FAO/WHO (1991).

was 0.72±0.01 to 9729±1.41 mg/100g, 0.60±0.01 to 6185±2.83 mg/100g, and 0.68±0.03 to 8197±3.54 mg/100g, respectively. Potassium showed the highest concentration, while copper had the lowest value. This observation agreed with that of Olaofe & Sanni (1998) who reported that potassium is the most abundant mineral in agricultural products. Comparatively, the raw sample had the highest concentrations of calcium, magnesium, sodium, potassium, phosphorus, zinc, copper and iron when compared with the fermented and blanched samples.

The mineral contents of Moringa leaf samples were higher than other leafy vegetables reported by Nkafamiya *et al.* (2010). The high content of minerals in

Moringa leaves such as calcium and iron could offer nutritional and health benefits. The Ca/P and Na/K molar ratios of Moringa leaf flour samples ranged from 18.3 to 24.5 and 0.3 to 0.4 respectively. All the samples met the recommended values for Na/K (<0.1) and Ca/P (>2.0) indicating potential nutritional benefits of the Moringa leaves.

Amino acid profile, chemical score and nutritional quality

Table 2 shows the amino acid profile and nutritional quality of raw, blanched and fermented *Moringa* leaf flour respectively. Total amino acid of the Moringa leaf samples ranged from 76.42g/100g in blanched to 42.24g/100g in raw sample. A

Table 2. Amino acid profile (g/100g protein) and nutritional quality of raw, blanched and fermented *Moringa oleifera* leaf flour

Amino acids	RML	BML	FML
<i>Dispensable amino acids (DAA)</i>			
Alanine	4.14 ^a ±0.02	3.33 ^a ±0.01	4.85 ^a ±0.90
Aspartic acid	8.44 ^a ±0.63	9.54 ^a ±0.44	8.05 ^a ±0.02
Glutamic acid	12.84 ^a ±0.74	12.43 ^a ±0.91	12.96 ^a ±1.25
Serine	3.89 ^a ±0.02	3.59 ^a ±0.26	3.48 ^a ±0.23
ΣDAA	29.31	28.89	29.34
<i>Conditionally indispensable (CI)</i>			
Cysteine	1.25 ^a ±0.12	1.29 ^a ±0.21	0.88 ^a ±0.10
Glycine	3.51 ^a ±0.21	4.27 ^a ±0.58	3.79 ^a ±0.62
Proline	3.82 ^a ±0.38	3.21 ^a ±0.28	3.33 ^a ±0.54
Tyrosine	2.73 ^a ±0.14	2.58 ^a ±0.14	2.03 ^b ±0.17
ΣCI	11.31	11.35	10.03
<i>Indispensable amino acid (IAA)+Histidine+Arginine</i>			
Histidine	4.62 ^a ±1.17	4.65 ^a ±1.33	5.15 ^a ±1.65
Arginine	5.03 ^a ±0.09	4.94 ^a ±0.44	5.21 ^a ±0.66
Isoleucine	3.49 ^a ±0.57	3.79 ^a ±0.13	3.83 ^a ±0.14
Leucine	7.69 ^a ±0.44	7.70 ^a ±0.06	7.82 ^a ±0.31
Lysine	6.73 ^a ±0.85	6.12 ^a ±0.73	6.80 ^a ±1.35
Methionine	1.10 ^a ±0.01	0.53 ^b ±0.23	1.09 ^a ±0.12
Phenylalanine	4.61 ^a ±0.02	3.80 ^b ±0.12	4.26 ^{ab} ±0.27
Threonine	3.25 ^a ±0.08	2.86 ^a ±0.01	2.97 ^a ±0.21
Valine	5.72 ^a ±0.41	3.77 ^b ±0.08	4.92 ^a ±0.71
Tryptophan	-	-	-
ΣIAA	42.24	38.16	42.05
<i>Nutritional quality</i>			
Σ Amino acids	82.85	76.42	81.44
ΣIAA+His+Arg/ΣAA%	60.48	60.89	63.42
ΣIAA/ΣAA%	55.09	54.42	54.74
ΣIAA/ΣAA%	44.91	43.47	45.24
ΣSAA(Meth+Cys)	2.35	1.82	1.97
ΣArEAA (Phe+Tyr)	7.34	6.38	6.29
ΣIAA/ΣDAA	0.82	0.80	0.83
PER	2.69	2.42	2.63
EAAI (%)	76.79	63.87	75.29
Predicted BV (%)	71.99	57.92	70.38
Nutritional Index (%)	23.9	18.0	18.3

Means with different superscript in the same row are significantly different at $p < 0.05$.

KEY: FML: Fermented Moringa leaves, BML: Blanched Moringa leaves, RML: Raw Moringa leaves.

slight reduction in amino acid profile of the blanched and fermented *Moringa* sample was observed compared with that of the raw sample, which could be attributed to effects of heat during the blanching or to

biochemical activities of microorganisms during the fermentation process. This finding did not agree with other reports, which indicated increases in the amino acid profile of food products that were subjected

to either blanching or germination or fermentation processing techniques (Ochanda *et al.*, 2010).

Total indispensable amino acid plus (Histidine and arginine) were 38.16g/100g, 42.05g/100g and 42.24g/100g for blanched, fermented and raw samples respectively. Comparatively, the value of total indispensable amino acid (TIAA) including histidine and arginine of the *Moringa oleifera* samples were higher than FAO/WHO (1991) recommended value for children (33.9g/day). It is well known that histidine and arginine are essential for the growing infant, and both were reasonably present in the *Moringa oleifera* samples. The percentage ratios of TIAA including histidine and arginine to the total amino acids (TAA) in the samples were 60.48%, 60.89% and 63.42% for the raw, blanched and fermented samples respectively. These values were comparatively higher than those of pigeon pea flour (43.6%) reported by Oshodi, Olaofe & Hall (1993), egg (50%) and the recommended values for infants (39%), children (26%) and adults (11%) (FAO/WHO, 1991).

The predicted protein efficiency ratio (PER) of the *M. oleifera* leaf flour samples were 2.69, 2.42 and 2.63 for raw, blanched and fermented samples respectively. These

values were higher than that reported by Olaofe, Adeyeye & Ojugbo (2013) for *Moringa* leaves (1.72), but comparable to that of hen's egg (2.88). The high values of PER in these samples indicated that their protein content would be fully utilised when consumed. The essential amino acid index (EAAI) of the raw, blanched and fermented samples were 0.77, 0.64 and 0.75% respectively, and these values were comparable to the report of Olaofe *et al.* (2013) for *Moringa* leaves (0.93), stems (0.86) and roots (0.91). These values were lower than that for whole hen's egg (1.55) (FAO/WHO, 1991). The predicted biological values (BV) were 72.0%, 57.9% and 70.4% for the raw, blanched and fermented samples respectively. Nutritionally, a protein-based food material is said to be of good nutritional quality when its biological values is 70% and above. Similarly, a protein based food material is assumed to be good quality with an EAAI of 90% or greater, to be useful when it is approximately 80%, and to be inadequate when it is below 70% (Mente *et al.*, 2002; Adeyeye, 2010). In terms of BV and EAAI, the *Moringa* leaves are a good source of protein, and can be used to complement cereals, particular maize, which is notably low in lysine and tryptophan (Table 3).

Table 3. Chemical score of raw, blanched and fermented *Moringa oleifera* leaf flour

Amino acids	FAO/WHO REF	RML	BML	FML
Lysine	5.8	116.03	105.52	117.24
Threonine	3.4	95.59	84.12	87.35
Valine	3.5	163.43	107.71	140.57
Methionine	2.2	50.00	24.09	49.55
Isoleucine	2.8	124.64	135.36	136.79
Leucine	6.6	116.52	116.67	118.48
Phenylalanine	2.8	164.64	135.71	152.14
Histidine	1.9	243.16	244.74	271.05
Tryptophan	1.1	-	-	-
Arginine	2	251.50	247.00	260.50
1 st LAA		Methionine	Methionine	Methionine
2 nd LAA		Threonine	Threonine	Threonine

FAO/WHO (1991); LAA: Limiting amino acids.

The digestible indispensable amino acid scores (DIAAS) of raw, blanched and fermented *Moringa oleifera* leaf flour are presented in Figure 2. The fermented sample (85.2%) had the highest DIAAS value followed by blanched (73.3%) and raw samples (51.7%) respectively. The disparity observed between these values could be due to the effects of processing techniques that the leaf samples were subjected to. Processing methods like blanching or fermentation have been reported to improve the nutritional and functional properties of plant-based food samples (Yagoub & Abdalla, 2007).

Fatty acid profile

The fatty acid compositions of raw, blanched and fermented *Moringa* leaf flour are presented in Table 4. The predominant saturated fatty acids in *Moringa* leaf flour samples were myristic and lauric acid but concentrations were small with values ranging from 2.42 ± 0.02 – $4.21\pm 0.02\%$ and 2.71 ± 0.03 – $3.51\pm 0.02\%$ respectively. The predominant polyunsaturated fatty acid was linoleic with the values of 1.26 ± 0.01 , 1.17 ± 0.02 and $1.40\pm 0.02\%$ for raw, blanched and fermented samples respectively. Oleic acid was the predominant fatty acid in

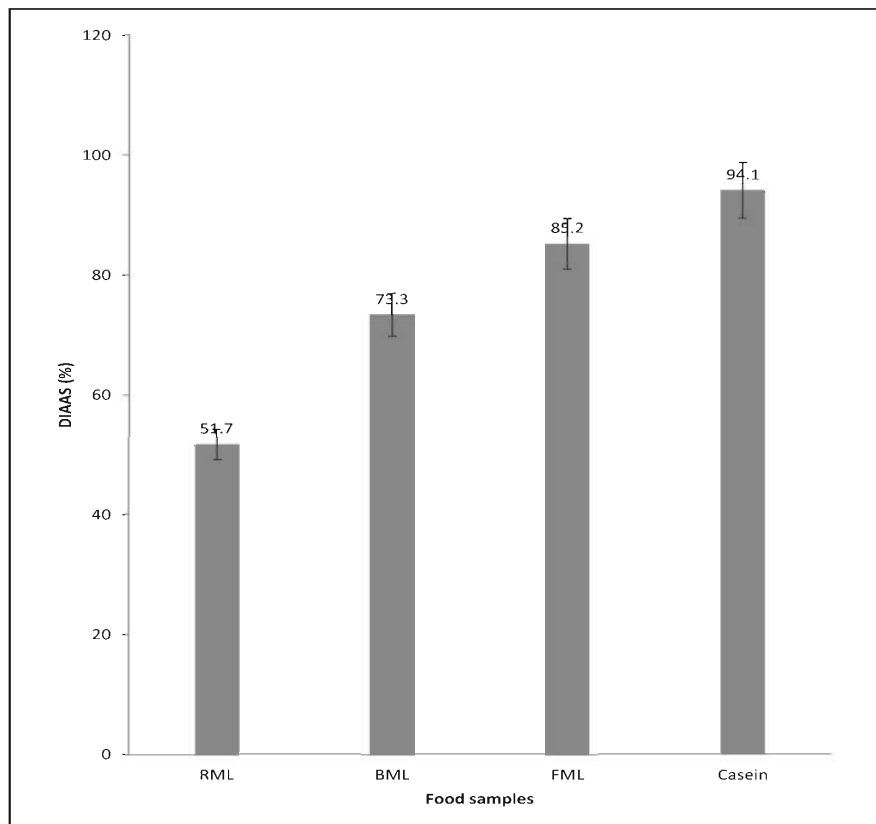


Figure 2. Digestible Indispensable Amino Acid scores (DIAAS) of the formulated food samples calculated using ileal indispensable amino acid digestibility values and reference amino acid pattern for a child (6 months to 3 years)

Table 4. Fatty acid profile (%) of raw, blanched and fermented *Moringa oleifera* leaf flour

Fatty acids	RML	BML	FML
<i>Saturated fatty acids (SFA)</i>			
Myristic acid	3.60 ^b ±0.01	2.42 ^c ±0.02	4.21 ^a ±0.02
Palmitic acid	1.27 ^b ±0.01	1.19 ^c ±0.01	1.36 ^a ±0.02
Stearic acid	0.10 ^b ±0.01	0.06 ^b ±0.01	0.17 ^a ±0.01
Arachidic acid	0.06 ^{ab} ±0.01	0.02 ^b ±0.01	0.10 ^a ±0.01
Behenic acid	-	-	-
Erucic	0.05 ^{ab} ±0.01	0.02 ^c ±0.00	0.08 ^a ±0.01
Lignocenic	0.10 ^b ±0.01	0.06 ^c ±0.01	0.14 ^a ±0.01
Margaric	0.22 ^{ab} ±0.01	0.16 ^b ±0.01	0.27 ^a ±0.05
Lauric	2.71 ^b ±0.03	2.58 ^c ±0.02	3.51 ^a ±0.02
ΣSFA	8.11	6.51	9.84
<i>Poly unsaturated fatty acids (PUFA)</i>			
Linolenic acid	0.06 ^{ab} ±0.01	0.04 ^b ±0.00	0.09 ^a ±0.01
Linoleic	1.26 ^b ±0.01	1.17 ^b ±0.02	1.40 ^a ±0.02
Arachidonic acids	-	-	-
Docohexanoic acid	-	-	-
ΣPUFA	1.32	1.21	1.49
<i>Mono unsaturated fatty acids (MUFA)</i>			
Palmitoleic acid	0.22 ^b ±0.01	0.15 ^c ±0.01	0.31 ^a ±0.02
Oleic acid	2.60 ^b ±0.03	2.19 ^c ±0.02	2.82 ^a ±0.03
ΣMUFA	2.82	2.34	3.13

Key: FML: Fermented Moringa leaves, BML: Blanched Moringa leaves, RML: Raw Moringa leaves.

monounsaturated fatty acid with values between 2.19±0.02% in blanched and 2.82±0.03% in fermented samples. Nutritionally, polyunsaturated fatty acids are common in vegetable oils and fish (Olaofe *et al.*, 2013), and are recommended for their nutritional benefits including disease prevention (Alfaia *et al.*, 2009).

Phytochemicals/antinutrient composition

The phytochemical/antinutritional factors of raw, blanched and fermented *Moringa oleifera* leaf flour samples are presented in Table 5. The phytochemicals/antinutrient concentration in the fermented moringa leaf flour samples had significantly lower tannin, phytate, trypsin, oxalate, phenolic, alkaloid, flavonoid, saponin and terpenoid contents, except in steroid when compared with the blanched and raw Moringa samples. It is

known that the concentration of antinutritional factors in food products are generally reduced after processing, particularly using fermentation and germination techniques (Nkafamiya *et al.*, 2010).

It is well recognised that foods of plant origin contain a wide range of non-nutrient phytochemicals that are synthesised by plants for their own defense and for other biological functions (Thurber & Fahey, 2009). Plant foods containing these non-nutrient phytochemicals have been reported to have multiple beneficial biological effects at low dosage, including antioxidant activity, anti-inflammatory action, and inhibition of platelet aggregation, antimicrobial activities and antitumor activities (Thurber & Fahey, 2009).

Table 5. Phytochemicals/antinutrient composition (mg/100g) of raw, blanched and fermented *Moringa oleifera* leaf flour

Antinutrients	RML	BML	FML
Tannin	347.67 ^a ±1.45	75.00 ^b ±0.04	49.33 ^c ±1.15
Phytate	103.67 ^a ±0.67	62.00 ^b ±0.57	44.0 ^c ±0.57
Trypsin inhibitor	1.76 ^a ±0.00	1.06 ^b ±0.0	0.24 ^c ±0.00
Oxalate	313.0 ^a ±1.02	139.33 ^c ±0.33	206.0 ^b ±0.57
Steroid	45.0 ^b ±0.0	36.67 ^c ±1.20	74.33 ^a ±0.67
Phenolic	38.57 ^b ±0.03	42.17 ^a ±0.33	12.50 ^c ±0.05
Alkaloid	358.33 ^c ±4.41	1868.3 ^a ±4.04	870.00 ^b ±5.10
Flavonoid	1250.0 ^a ±0.13	556.67 ^b ±3.33	28.87 ^c ±1.66
Saponin	768.33 ^b ±6.03	1450.0 ^a ±2.88	453.33 ^c ±1.67
Terpenoid	255.0 ^b ±2.88	323.33 ^a ±1.67	61.67 ^c ±1.67

Key: FML: Fermented Moringa leaves, BML: Blanched Moringa leaves, RML: Raw Moringa leaves

CONCLUSION

This study evaluated effects of blanching and fermentation processing methods on the nutrient and phytochemical composition of flour from *Moringa oleifera* leaves. The fermented leaf flour sample had better nutrient composition, nutritional quality, reduction in some antinutrients/phytochemicals and functional properties when compared with blanched leaf sample. This study indicated that flour made from *Moringa oleifera* leaves has potential nutritional quality.

REFERENCES

- Adeyeye EI (2010). Effect of cooking and roasting on the amino acid composition of raw groundnut (*arachis hypogaea*) seeds. *Acta Sci Pol Technol Aliment* 9(2): 201-216.
- Adeyeye EI & Aremu MO (2010). Comparative evaluation of the amino acid profile of the brain and eyes of guinea fowl (*Numida meleagris*) hen. *The Open Nutraceutical J* 3: 220-226.
- Aletor VA & Adetogun OA (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food Chem* 53(4): 375-379.
- Alfaia CPM, Alves SP, Martins SIV, Costa ASH, Fontes CMGA, Lemos JPC, Bessa JB & Prates JAM (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chem* 114: 939-946.
- Alipour HJ, Shabanpoor B, Shabani A & Mahoonak AS (2010). Effects of cooking methods on physico-chemical and nutritional properties of Persian sturgeon *Acipenser persicus* fillet. *Int Aquat Res* 2: 15-23
- Alozie YE, Iyam MA, Lawal O, Udofia U & Ani IF (2009). Utilisation of Bambara ground flour blends in bread production. *J Food Technol* 7(4): 111-114.
- Association of Analytical Chemists (AOAC)(2005). Official Methods of Analysis(17th ed.). W Horowitz L(ed). AOAC, Gaithersburg, MD.
- Burns JPT, Gardner D, Mathew GG, Duthie M, Lean E & Crozier A (2001). Extraction of phenolic and changes in antioxidant activity of red wine during vinification. *J Agric Food Chem* 49: 5797-5808.
- Busani M, Masika PJ, Hugo A & Muchenje V (2011). Nutritional characterisation of Moringa (*Moringa oleifera* Lam.) leaves. *African J Biotechnol* 10(60): 12925-12933.
- FAO/WHO (1991). Protein Quality Evaluation. Report of Joint FAO/WHO Expert

- Consultation. FAO Food and Nutrition Paper 51. FAO, Rome, Italy.
- FAO (2013). Dietary Protein Quality Evaluation in Human Nutrition: Report of an FAO Expert Consultation (pp. 1-79). Rome, Italy.
- Harbone JB (1973). Phyto Chemical Methods. London. Chapman and Hall, Ltd., 49-188.
- Ilelaboye NOA, Amoo IA & Pikuda OO (2013). Effect of cooking methods on proximate composition, gross energy and dietary fibre of some green leafy vegetables. *Eur J Applied Eng Sci Res* 2(2): 41-47
- Iombor TT, Umoh EJ & Olakumi E (2009). Proximate composition and organoleptic properties of complementary food formulated from millet (*Pennisetum polystachyllum*), soybeans (*Glycine max*) and crayfish (*Euastacus spp*). *Pakistan J Nutr* 8(10): 1676-1679.
- Latta, MC & Eskin M (1980). A simple and rapid colorimetric method for phytate determination. *J Agric Edu Chem* 8: 1313.
- Lqari, H., Vioque J, Pedroche J, & Millan F (2002). *Lupinus angustifolius* protein isolates: chemical composition, functional properties and protein characterisation. *Food Chem* 76: 349-356.
- Malomo O, Alamu EA & Oluwajoba SO (2013). Chemical evaluation of protein quality of sprouted maize and cowpea. *Inter J Nutr Food Sci* 2(5): 254-260.
- Mente E, Coutteau P, HoulihanD, DavidsonI & Sorgeloos P (2002). Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source. *J Experimental Biol* 205: 3107-3122.
- Mosha TC & Gaga HE (1999). Nutritive value and effect of blanching on trypsin and chymotrypsin inhibitor activities of selected leafy vegetables. *Plant Foods for Human Nutr* 54: 271-283.
- Nkafamiya II, Osemeahon SA, Modibbo UU, Aminu A (2010). Nutritional status of non-conventional leafy vegetables, *Ficus asperifolia* and *Ficus sycomorus*. *Afr J Food Sci* 4(3):104-108.
- Ochanda SO, Onyango CA, Mwasaru AM, Ochieng JK, & Mathooko FM (2010). Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya. *J Appl Biosci* 34: 2128 – 2134.
- Olaofe O, Adeyeye EI & Ojugbo S (2013). Comparative study of proximate, amino acids and fatty acids of the *Moringa oleifera* tree. *Elixir Appl Chem* 54: 12543-12554.
- Olaofe O & Sanni CO (1998). Mineral contents of agricultural products. *Food Chem* 30: 7377.
- Oshodi AA, Olaofe O & Hall GM (1993). Amino acid, fatty acid and mineral composition of pigeon pea (*Cajanus cajan*). *Inter J Food Sci Nutr* 43: 187- 191.
- Porzucek H & Raznikiewicz L (1990). Fatty acid composition and lipooxygenase activity of flours and protein isolates from leguminous plants. *Sweden Agric Res* 20: 31-34.
- Satterlee LD, Kendrick JG, Marshall HF, Jewell DK, Ali RA, Heckman MM, Steinke HF, Larson P, Phillips RD, Sarwar G & Slump P (1982). *In vitro* assay for predicting protein efficiency ratio as measured by rat bioassay: collaborative study. *J Assoc Off Anal Chem* 65(4): 798-809.
- Tete-Benissan A, Quashie MLA, Lawson-Evi K, Kokou K & Gbeassor M (2012). Nutritional recovery of HIV positive and HIV negative undernourished patients utilising *Moringa oleifera* leaves. *J Animal Plant Sci* 15(2): 2184-2199.
- Thurber MD & Fahey JW (2009). Adoption of *Moringa oleifera* to combat under-nutrition viewed through the lens of the "diffusion of innovations" theory. *Ecol Food Nutr* 48(3): 212 – 225.
- Vasanthi HR, ShriShriMal N & Das DK. (2012). Phytochemicals from plants to combat cardiovascular disease. *Curr Med Chem* 19(14): 2242-51.
- Yagoub AA & Abdalla AA (2007). Effect of domestic processing methods on chemical, *in vitro* digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranea*) seed. *Res J Agric Biol Sci* 3: 24-34.