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Comparative study of proximate, amino acids and fatty acids of *Moringa* oleifera tree

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ABSTRACT

Proximate, amino acids and fatty acids compositions of the leaves, stem and root were determined in *Moringa oleifera*. The proximate composition was determined in fresh samples while other parameters were determined on dry weight basis. Both crude protein and total ash followed the trend of (g/100 g): leaves > root > stem but the soluble carbohydrate had a trend of root > stem > leaves. Leaves had the highest level of total amino acids (76.4 g/100 g) and followed by the root (70.9 g/100 g) while stem had 65.4 g/100 g. For the EAA, it was 35.4 g/100 g (leaves) > 28.4 g/100 g (root) > 26.3 g/100 g (stem). The total sulphur amino acid was generally low at 2.81-3.06 g/100 g but the % Cys in TSAA was slightly high at 48.5-51.6 %. In the amino acids scores, the following amino acids had scores greater than 1.0 in comparison with whole hen's egg: Gly (1.56-1.97), Glu (1.15-1.28), and Cys (1.14-1.16) whereas Met was the limiting amino acid in each of the three samples with values of 0.31 (leaves), 0.23 (stem) and 0.30 (root); in the pre-school children requirements, these amino acids had scores greater than 1.0: Met + Cys (1.12-1.22), Phe + Tyr (1.04-1.11) and His (1.02-1.08) whereas Lys was the limiting amino acid with values of 0.57 (leaves), 0.64 (stem) and 0.63 (root); on the provisional amino acid scoring pattern, only Phe + Tyr had scores greater than 1.0: Phe + Tyr (1.17, leaves), 1.09 (stem) and 1.10 (root), Val was the limiting amino acid here: 0.63 (leaves), 0.62 (stem) and 0.69 (root). P-PER had values of 1.60-1.72 and EAAI had values of 0.86-0.93. The samples were basically acidic with pI values of 5.4 to 5.8. The most concentrated fatty acid was SFA with values of (%): 97.5 (stem) > 58.0 (leaves) > 53.8 (root). Predominant MUFA was in root with a trend of (%): 38.7 (root) > 4.61 (leaves) > 1.00 (stem) and in PUFA, trend was 37.4 (leaves) > 7.54 (root) > 1.20 (stem). Under the fatty acid parameters, leaves were best in 4/6 (66.7 %) in EPSI, PUFA, PUFA/SFA and MUFA + PUFA whereas root was best in 2/6 (33.3 %) in LA/ALA and MUFA/SFA.

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Introduction

Moringa oleifera Lamarck belongs to the Moringaceae family which has 14 species of deciduous trees classified in a single genus, namely Moringa Adans[1]. It is a deciduous tree or shrub, fast-growing drought resistant, average height of 12 metres at maturity. Other 12 varieties of Moringa species are: M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. ovalifolia, M. peregrina, M. pygmaea, M. rivae, M. ruspoliana, M. stenpetala. M. oleifera (synonym M. pterygosperma Gaertn.) is the most widely known species and is planted in the whole tropical belt[2]. It is a multipurpose tree widely distributed in India, the Philippines, Sri-Lanka, Thailand, Malaysia, Burma, Pakistan, Singapore, the West Indies, Cuba, Jamaica and Nigeria[3]. Commonly known as the horse-radish' tree, arising from the use of the root by Europeans in India as a substitute for horse- radish, Cochlearia armoracia (synonym Armoracia rusticana). Common name of M. oleifera in Malabar is Moringo and this is the origin of the generic name[4]. Other common names are Benzolive, Drumstick tree, Kelor, Marango, Mlonge, Mulangay, Saijhan and Sajna. In Nigeria, the Yoruba call it ewé ìgbálè[5]. It is cultivated to use as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs)[3, 6-10]. It has also been reported that the seeds are sometimes eaten without any heat treatment[3]. Like C. armoracia, the roots of Moringa are pungent and were commonly used as a condiment or garnish. Such a practice would not now be recommended as the root has been shown to contain 0.105 % alkaloids, especially moringinine and a bacteriocide, spirochine, both of which can prove fatal following ingestion[11-12]. The other widely used common name is 'drumstick' tree, arising from the shape of the pods, resembling the slender and curved stick used for beating the drum.

For centuries, people in many countries have used Moringa leaves as traditional medicine for common ailments. Clinical studies have begun to suggest that at least some of these claims are valid. Some country traditional uses are[13]:

India: Traditionally used for anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, glandular swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis.

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Guatemala: Traditionally used for skin infections and sores.
Malaysia: Traditionally used for intestinal worms.
Puerto Rico: Traditionally used for anemia, glandular swelling and lactating.

On modern uses, over the past two decades, many reports have appeared in mainstream scientific journals describing its nutritional and medicinal properties. Its utility as a non-food product has also been extensively described. Every part of Moringa tree is said to have beneficial properties that can serve humanity. People in societies around the world have made use of these properties. For example, the multiple uses of M. oleifera in rural and/or urban areas in Zimbabwe are shown in Figure I[14].

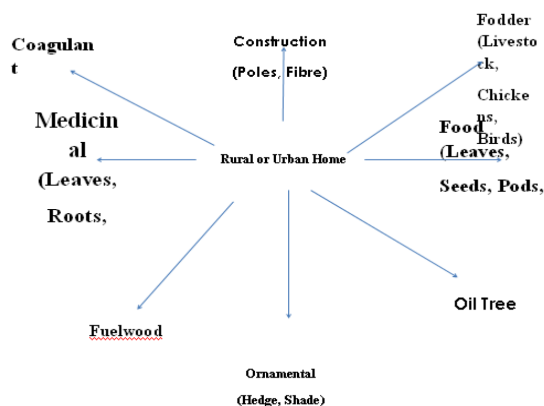

Figure I. Multiple uses of Moringa oleifera in rural and/or urban areas in Zimbabwe

Some reports had been published on Moringa oleifera. They included work of Oliveira et al.[15] on compositional and nutritional attributes of seeds from the multiple purpose tree Moring oleifera Lamark; Dalen et al. [16] on synergy between Moringa oleifera seed powder and alum in the purification of domestic water; work on Moringa oleifera[13] giving the nutritional values of Moringa leaves (fresh ram and dried leaf powder) and the pods; work of Anhwange et al. [17] on the amino acid composition of the seeds of Moringa oleifera (Lam), Detarium microcarpum (Guill and Sperr). The present work is the Bauhinia monandra (Linn.). proximate, amino acids and fatty acids analyses of Moringa Oleifera leaves, stem and root.

Materials and methods

Sample collection

The Moringa leaves were obtained from a local farm in Afao-Ekiti, Ekiti State, Nigeria. Also a matured disease free M. oleifera stem of about 1.5 ft was obtained also from Afao- Ekiti farm. The root of the plant was also obtained from Afao-Ekiti.

Sample treatment

The leaves were air dried and later pulverised into powder and then stored in plaster container for the various analyses. The stem was reduced to smaller sizes using a knife. The air dried stem was then milled into flour using a mechanical blender; flour was stored in plastic container and placed in a cool, dry place prior to analyses. The freshly obtained root was washed to remove sand and drained; it was then oven dried at low temperature and milled into flour. The flour was kept in a well tight sample bottle and kept in a cool place (laboratory refrigerator,-4 °C).

Proximate analyses

The proximate analyses of the samples for moisture, total ash and crude fibre were carried out in triplicate using the methods described in AOAC[18]. The nitrogen was determined by the micro-Kjeldahl method described by Pearson[19] and the nitrogen content was converted to protein by multiplying by 6.25. Carbohydrate was determined by difference. All the proximate values were reported in g/100 g sample.

Determination of amino acid profiles

(i) Defatting: 2.0 g of each sample were weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 v/v mixture) using Soxhlet Extraction apparatus as described by AOAC[18]. The extraction lasted for 5 h.

(ii) Hydrolysis of the samples: About 30 mg of the defatted samples was weighed into glass ampoules. Seven milliliters of 6MHCl were added and oxygen was expelled by passing nitrogen into the ampoule. (This is to avoid possible oxidation of some amino acids during hydrolysis.) The glass ampoule was then sealed with Bunsen burner flame and put in an oven present at 105±5°C for 22 h. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in the freezer.

(iii) Loading of the hydrolysate into the TSM analyser/amino acid analyses: The amount loaded was between 5 to 10 microlitres. This was dispensed into the cartridge of the analyser. The amino acid analysis was done by ion-exchange chromagraphy[20] using a Technicon Sequential Multisample Amino Acid Analyser (Technicon Instruments Corporation, New York, USA). Tryptophan was not determined due to cost. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for 76 min. The gas flow rate was 0.50 ml/min at 60 °C with reproducibility consistent within ± 3 %.

(iv) Method of calculating amino acid values from the chromatogram peaks: The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width of half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

NE =Area of Norleucine Peak/Area of each amino acid. A constant S was calculated for each amino acid in the standard mixture:

$S_{std} = NE_{std}$ x mol. Weight x μMAA_{std}

Finally the amount of each amino acid present in the sample was calculated in g/100 g protein using the following formula:

Concentration (g/100 g protein) = NH x W@NH/2 xS_{std} x C

Where:

C = Dilution x 16/Sample wt (g) x N %x 10 vol. loaded ÷ NH x W (nleu)

Where: NH = net height
W = width@half height
Nleu = norleucine

The amino acid values reported were the averages of two determinations. Norleucine was the internal standard.

Estimation of isoelectric point (pI)

The theoretical estimation of isoelectric point (pI) was determined using the equation of Olaofe and Akintayo[21] and information provided by Finar [22].

$$IP_m = \sum_{i=1}^{n} IP_i X_i$$

where IPm is the isoelectric point of the ith amino acid in the mixture and Xi is the mass or mole fraction of the ith amino acid in the mixture.

Estimation of predicted protein efficiency ratio (P-PER)

The predicted protein efficiency ratio (P-PER) was estimated by using the equation given by Alsmeyer et al.[23].

P-PER = -0.468 +0.454 (Leu) -0.105 (Tyr).

Estimation of dietary protein quality

The amino acid scores were calculated using three different procedures:

- The total amino acids scores were calculated based on the whole hen's egg amino acid profiles[24].
- The essential amino acids scores were calculated using the formula (provisional amino acid scoring pattern)[25]:

Amino acid score = Amount of amino acid per test protein [mg/g]/Amount of amino acid per protein in reference [mg/g].

- The essential amino acids scores (including His) based on pre-school child suggested requirement[26].

Essential amino acid index (EAAI)

The essential amino acid index (EAAI) was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus histidine in the equation[27]:

$$\text{Essential amino acid index} = \sqrt[9]{\frac{\text{mg Lysine in 1 g test protein}}{\text{mg Lysine in 1 g reference protein}} \times \text{etc. for all 8 essential amino acids + His}}$$

Leu/isoleucine ratio

The leucine/isoleucine ratios, their differences and their percentage differences were also calculated.

Calculation of other protein quality parameters

Determination of the ratio of total essential amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulphur amino acids (TSAA), percentage cystine in TSAA (% Cys/TSAA), total aromatic amino acids (TArAA), total neutral amino acids (TNAA), total acidic amino acids (TAAA) and total basic amino acids (TBAA) were estimated from the results obtained for amino acids profiles.

Extraction of lipid for fatty acid analyses

About 0.25 g of each sample was weighed into the extraction thimble. 200 ml of petroleum other (40-60 ^{o}C boiling range) was measured and then added to the dried 250 ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled[18]. The lipid was extracted for 5 h. The extraction flask with the oil was oven dried at 105 ^{o}C for 1 h. The flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask with the dried oil was measured.

Preparation of methyl esters and analyses

50 mg of the extracted oil was saponified for 5 min at 95 ^{o}C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralised by 0.7 MHCl. 3 ml of 14 % boron triflouride in methanol was added[18]. The mixture was heated for 5 min at 90 ^{o}C to achieve complete methylation process. The fatty methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for analysis and 1µ l was injected into the injection pot of the GC. The fatty acid methyl esters were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. A09.01) [1206] software [GMI, Inc, Minnesota, USA]) fitted with a flame ionisation detector. Nitrogen was the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 60 ^{o}C, first ramping at 10 ^{o}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{o}C/min for 4 min and maintained for 10 min. The injection temperature was 250 ^{o}C whilst the detector temperature was 320 ^{o}C. A capillary column (30m x 0.25 mm) packed with a polar compound (HP INNOWAX) with a diameter (0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard fatty acid methyl esters.

Other fatty acid parameters calculated were total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), PUFA/SFA, n-6/n-3, LA/ALA, MUFA/SFA and Essential PUFA Status Index (EPSI).

Quality assurance

Standard chromatograms were prepared for fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient determined for each fatty acid parameter. Correlation coefficient should be > 0.95 for the result to be acceptable. It was performed with Hewlett Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blvd Ramsey, Minnesota, 55303, USA).

Statistical analysis

Statistical analysis was carried out to determine coefficient of variation in per cent (CV %), mean and standard deviation for the parameters[28].

Results and discussion

Table I depicts the proximate composition of Moringa oleifera tree parts on wet weight basis. The leaves were highest in moisture, crude protein, total ash and crude fibre. It is interesting to note that the stem had the highest level of available carbohydrate and the root had the highest level of total energy; the root also had the highest level of calculated total fatty acids. The various parameters determined were variously distributed in the samples, this could be seen in the coefficient of variation per cent (CV %) with values of 11.2-124 being highest in total fatty acids and crude fat (124 % in each case) and lowest in the moisture content (11.2 %). Literature value of fresh leaves in moisture was 75 %, 6.70 g/100 g (protein), 1.7 g/100 g (crude fat), 13.4 g/100 g (carbohydrate), 0.9 g/100 g (crude fibre)[13].

Table II shows the amino acid (AA) composition for each sample. Glumatic acid had the highest concentration among their groups and it is an acidic AA. On the other hand, whilst aspartic acid was the second highest concentrated AA in stem and root, arginine was the second highest AA in the leaves. Aspartic acid is a non-essential and acidic AA, arginine is an essential AA and is a basic AA. The highest essential amino acid EAA in each sample was arginine (8.22 g/100 g,) leaves, leucine (5.12 g/100 g, stem) and leucine (5.33 g/100 g, root). The CV % of the AA values were generally low with the exception of isoleucine and arginin5.33 with respective CV % values of 46.8 and 92.7 whilst rest CV % values ranged from 0.06-16.9 showing the closeness of the AA values in the various samples to each other. Table III shows the concentrations of total AA (TAA), total essential AA (TEAA), total acidic AA (TAAA), total neutral AA (TNAA), total sulphur AA (TSAA), total aromatic AA (TArAA) and their percentage levels.

Arginine (1.77-8.22 g/100 g crude protein, cp) is essential for children and reasonable levels were present in the leaves samples: the lysine contents of the samples (3.31-3.69 g/100 g cp) were about one half to the content of the reference egg protein (6.3 g/100 g), and any of the samples will therefore serve as an average source for the AA. The present contents of TEAA in the leaves are comparable to some literature values of non-conventional meat sources (g/100 g): 35.1 (Zonocerus variagatus) [29]; 35.0 (Macrotermes bellicosus)[30]; 42.8 (Limicolaria sp.), 36.1 (Archatina archatina), 45.0 (Archachatina marginata)[31]; 38.6 (heart) and 42.2 (liver) of African giant pouch rat (Cricetomys gambianus)[32]. The contents of TSAA were generally lower than the 5.8 g/100 g cp recommended for infants[33]. The ArAA range suggested for ideal protein (6.8-11.8 g/100 g)[33] has present values slightly greater than the minimum and close to the maximum, i.e. 6.54-7.00 g/100 g cp. The ArAA are precursors of epinephrine and thyroxin [34]. The percentage ratios of TEAA to the TAA in the samples were 46.4 % (leaves), 40.2 % (stem) and 40.1 % (root) which were strongly comparable to that of egg (50 %)[35], 43.6 % reported for pigeon pea flour[36], 43.8-44.4 % (beach pea protein isolate)[37], 46.2 % (liver) and 46.3 % (heart) reported for African giant pouch rat (Cricetomys gambianus)[32]. The percentage ratios of TEAA to the TAA in the samples were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults[33].

Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. For examples, (Cys/TSAA) % were 36.3 in M. bellicosus[30]; 25.6 in Z. variegatus[29]; 35.5 in A. marginata, 38.8 in A. archatina and 21.0 in Limicolaria sp., respectively[31]; 23.8-30.1 % in three fresh fish consumed in Nigeria [38] and 29.8 % in Gymnarchus niloticus (Trunk fish)[39]. In contrast, many vegetable proteins contain substantially more Cys than Met, for examples, 62.9 % in coconut endosperm [40, 41]; its range is 58.9-72.0 in guinea corn [42]; it is 50.5 % in cashew nut [43]; it is 40.7 % in Triticum durum[44] and 44.4 % in Parkia biglobosa seeds[45]. In our present samples % (Cys/TSAA) values ranged from 48.5-51.6 which were much closer to the usual plant values. Thus, for animal protein, Cys is unlikely to contribute up to 50 % of the TSAA[46]. The % Cys/TSAA had been set at 50 % in rat, chick and pig diets[46] but not in man. Cys can spare with Met in improving the protein quality and has positive effects on mineral absorption, particularly zinc[47].

Table IV shows methionine to be the limiting amino acid of all the samples on comparison with whole hen's egg; the samples values ranged 0.23-0.31. However, the correction factors are not similar; this is because the limiting methionine value in leaves was 0.31, 0.23 in stem and 0.30 in root. In order to fulfil the daily need for the entire AA in the samples, it would require 100/31 or 3.23 times as much leaves protein and 100/30 or 3.33 times as much root protein to be eaten when they serve as the sole protein in the diet. In Table V lysine was the limiting amino acid among all the samples with values of 0.57 (leaves), 0.64 (stem) and 0.63 (root). This value would require a correction factor of 100/57 or 1.75 (leaves), 100/64 or 1.56 (stem) and 100/63 or 1.59 (root). In Table VI, lysine was the limiting amino acid in leaves with a value of 0.60, in stem and root the limiting amino acid in each case was isoleucine with respective values of 0.47 and 0.60. Hence the correction factors in leaves would be 100/60 or 1.67, in stem correction would be 100/47 or 2.13 and in root correction would be 100/60 or 1.67.

The experimentally determined PER usually ranged from 0.0 for a very poor protein to a maximum possible of just over 4[48]. The P-PER (Table VII) values were 1.72 (leaves and root in each case) and 1.60 in the stem. Values in fish sources: 2.22, 1.92, 1.89 in three fresh water fishes[38], liver (2.62) and heart (2.32) in African giant pouch rat[7]. The P-PER of the whole hen's egg is 2.88 which is higher than Moringa oleifera tree parts. The present result indicated that the physiological utility in the body of leaves and root would be much better than the stem. A common feature of sorghum and maize is that the proteins of these grains contain a relatively high proportion of leucine. It was therefore suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra[49]. It has been shown that high Leu in the diet impairs the metabolism for Try and niacin and is responsible for niacin deficiency in sorghum esters[50]. High Leu is also a factor contributing to the pellagragenic properties of maize[51]. Further studies have shown that the biochemical and clinical manifestations of dietary excess of Leu could be counteracted not only by increasing the intake of niacin or tryptophan but also by supplementation with isoleucine[52, 53]. These studies suggested that the leucine/isoleucine balance is more important than dietary excess of Leu alone in regulating the metabolism of Try and niacin hence the disease process. The present Leu/Ile ratios were low in value at 1.21-2.72 but the P-PER values in stem (2.72) and root (2.24) were higher than in three fresh water fishes of 2.0-2.6 [38]. The present report showed Leu to range from 5.12-5.38 g/100 g cp which were about one half of 11.0 g/100 g cp, therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas[54]. The calculated isoelectric point (pI) ranged from 5.4-5.8. The information on pI is a good starting point in predicting the pI for proteins in order to enhance a quick precipitation of protein isolate from biological samples[55]. The relatively low values of pI could be a function of the TAAA (19.9-22.4 g/100 g cp) or 27.7-31.6 % which were much higher than the TBAA (7.41-13.6 g/100 g cp or 10.6-17.8 %) (Table IV). The essential amino acid index (EAAI) ranged from 0.86-0.93 (Table VII) which were lower than the value of 1.26 in defatted soy flour[56] and lower than 1.55 in whole hen's egg. It should be noted that the absence of Try in the present report bore no significance in the EAAI; for example EAAI without Try in soy flour remained 1.26 while it reduced to 1.54 in the whole hen's egg, i.e., a reduction of 0.01 or 0.645 %. The EAAI method can be useful as a rapid tool to evaluate food formulation for protein quality[57].

The fatty acids composition of M. oleifera tree parts was depicted in Table VIII. In all the samples, the following fatty acids were not detected in the samples: C8:0, C10:0, C12:0, C17:0, C20:0 and C16:1. Saturated fatty acids are the main fatty acids found in meat and dairy products. Unsaturated fats are most commonly found in the oils of vegetables, such as avocado and also in nuts and fish. Unsaturated fats are considered the healthiest dietary fats. Another classification of fats is the essential fatty acids. Adequate intakes of essential fatty acids may protect against heart diseases and diabetes [58]. It has been estimated that as much as 50 % of the American population may consume insufficient quantities of essential fatty acids[59].

Palmitic acid (C16:0) is a saturated fatty acid (SFA) accounting for about 27 % of the fatty acids in beef. The C16:0 were high in all the samples with following trend in the samples: 49.7 % (root) < 54.3 % (leaves) < 96.0 % (stem) of total fat in each case. There is strong evidence that C16:0 raises serum

cholesterol levels[60] and that this occurs predominantly by increasing bad cholesterol (LDL) levels. This fatty acid accounts for most of the cholesterol- raising activity from beef, thereby increasing the risk of atherosclerosis, cardiovascular disease and stroke[61], this meant that the risk factor of C16:0 would be as: stem > leaves > root. Stearic acid (C18:0) is a SFA accounting for about 18 % of fatty acid (FA) in beef. The levels of C18:0 in the samples varied from 0.90-2.96 % of total fat. Several studies have shown that the C18:0 effects on total cholesterol is minimal and not detrimental to human health [62, 63, 64, 65]. For practical purposes, C18:0 is essentially neutral in its effects on serum total cholesterol, similar to oleic acid [60]. It is not clear why C18:0 does not raise cholesterol level as do other SFAs. A possible reason could be that it is rapidly absorbed into the body tissue compared with other SFAs[60]. However, it has been observed in dogs, rats and hamsters that C18:0 or stearic acid-rich glycerides are absorbed less efficiently than SFAs of shorter chain length or their glycerides [66]. Some investigators have speculated that C18:0 may be thrombogenic (causes blood clotting). This effect has not been proven[60]. Also, the effects of C18:0 on hypertension, cancer, obesity and other illnesses are unknown[59]. Both lauric acid (C12:0) and myristic acid (C14:0) are related to human health issues. Lauric acid was not detected in any samples. Lauric and myristic FAs are responsible for raising bad cholesterol levels in blood serum[60] and have been shown to be strongly correlated with early heart attack[67]. However, the percentages of myristic acid (less than 1 %, actually 0.20-0.46 %) in the samples were small in quantity. Accumulation of certain long-chain fatty acids is associated with degenerative diseases of the central nervous system, such as behenic acid (C22:0; about 1 % in beef fat) and lignoceric acid (C24:0; about 1 %) as well as that of the unsaturated members of the C22 and C24 group[68]. The samples under discussion had levels of C22:0 as 0.15-0.87 % total fat and C24:0 as 0.10-0.35 % of total fat. These values are less than the values in beef[68]. Accumulation occurs because enzymes needed to maintain turnover of those fatty acids are lacking[69]. Behenic acid has been detected to be a cholesterol-raising SFAs factors in humans[70].

Oleic acid (C18:1) is the primary mono-unsaturated fatty acid in beef and accounts for about 33 % of FA in beef. It is also found in rich amount in olive, canola and peanut oils. Available evidence indicates that while most SFAs raise serum cholesterol concentrations the monounsaturated oleic acid does not[71]. For practical purposes, it is convenient to use the neutrality of oleic acid as a baseline with which to judge the responses of other FAs. The fact that the body synthesizes a large quantity of oleic acid suggests that it has a variety of biological uses, and to this extent the concept of the neutrality of oleic acid can be extended to imply safety[60]. The major source of oleic acid among the samples was the root with a level of 38.2 % of the total fat followed distantly by the leaves with 4.28 % and stem with 0.80 %. In several studies on the relative carcinogenicity of FAs or their ability to suppress the immune system, oleic acid was the FA with the least negative effect[60]. One reason why oleic acid may not raise serum cholesterol concentrations is because it is a favoured substrate for the liver enzyme that converts cholesterol to an inactive form (the Acyl CoA tranferase: cholesterol acyltransferase)[60]. Other MUFAs are palmitoleic acid (C16:1) (about 2-3 % in beef) and erucic acid (C22:1; about 1 % in beef fat). Palmitoleic acid (C16:1 cis) is a MUFA also found in rich amounts in macadamia nuts, olive, canola and peanut oils[68]. This MUFA is beneficial in reducing bad cholesterol (LDL) and it behaves like an unsaturated FA in its effect on HDL cholesterol[72]. It also reduces the fat deposition in blood vessels and blood clot formation[60]. Palmitoleic acid (C16:1) was detected at a level of 0.25 % in the leaves. The C18:1 FAs may be elongated and desaturated in adipose tissue to produce long chain fatty acids (C22 and C20), which are beneficial for human health[73]. Erucic acid (C22:1; about 1 % in beef fat) is a fatty acid that is apparently responsible for a favourable response of persons with nervous system disorders[74]. The administration of erucic acid in the diet will reduce the serum levels and brain accumulation of very long chain SFA (such as C26:0) responsible for demyelination [75, 76]. Erucic acid occupied levels of 0.08-0.46 % in the samples fats.

It has been found that stearic acid (C18:0) cannot be stored well in tissue. It is converted to oleic acid (cis-18:1) apparently so body fat can be maintained in a "liquid state" at body temperature[77]. The enzyme that adds a double bond to C18:0 to form C18:1 cis is SCD (stearoyl-CoA desaturase-or Δ^9-desaturase). Most tissue of ruminants, mice, rat and chicken, have SCD, especially in the intestines, liver, adipose tissue and mammary glands. This enzyme has also been detected in humans. However, the distribution of SCD in human is unknown. In humans, the liver is the principal tissue containing SCD and presumably also has the highest SCD activity[78].

The major polyunsaturated fatty acids found in beef fat are linoleic acid (LA) (C18:2 cis) (about 3.5 %), alpha-linolenic acid (ALA) (C18:3) (1.5 %), arachidonic acid (AA) (C20:4) (about 1 %), etc[79]. ALA is classified as a short-chain omega-3 FA and is also found in nuts and seeds. LA is also found in corn, sunflower oil, safflower oil and soybeans. AA is found in brain, liver, glandular and egg lipids. Both LA and AA FAs belong to the omega-6 family of fatty acids.

The omega-3 FAs present in pastures, like the ALA (C18:3), appear to have little direct value for human health. However, the human body can add 2 or 4 carbons to these 18-carbon chains fats to produce 20-or 22-carbon chain omega-3 fatty acid. Thus, ALA is a precursor for EPA (C20:5) and DHA (C22:6) FAs, which are important for human health. It has been suggested that ALA has a beneficial effect on cardiovascular heart deisease[80, 59]. However, other studies reported no evidence of ALA having a positive effect on cardiovascular heart disease [81, 82]. Although ALA supplementation causes an increase in the blood and plasma levels of ALA, EPA and DPA, no benefit has shown on either risk factors for cardiovascular diseases or on the secondary prevention of cardiovascular heart disease[82]. More studies have to be done to determine ALA beneficial effects on human health. ALA may help balance LA (C18:2) and be beneficial[68]. Alpha-linolenic acid in our samples was very much unevenly distributed having a CV % of 164. However, very high percentage level of ALA was recorded in leaves at 33.0 % whereas it is almost at trace levels in stem (0.30 %) and root (0.85 %).

For many years linoleic acid (C18:2; omega-6) was thought to be the preferable fatty acid for the diet because it was considered to be the most effective cholesterol-lowering fatty acid. However, despite an increase in linoleic acid intake (from about 4 % to 7 %), there has been a growing reservation about recommending its consumption, due to no proven long-term safety[60]. In humans high supplemental intakes of LA can lower good cholesterol concentration and may increase the risk for cholesterol gallstones.

Table I. Proximate composition of *Moringa oleifera* tree parts in g/100 g (wet weight samples)

Parameter	Leaves	Stem	Root	Mean	SD	CV %
Moisture content	75.8	67.2	60.7	67.9	7.57	11.2
Crude protein	3.00	1.05	1.62	1.89	1.00	53.1
Crude fat	0.40	0.15	2.28	0.94	1.16	124
Total ash	2.40	0.70	2.05	1.72	0.90	52.2
Crude fibre	1.10	0.42	0.84	0.79	0.34	43.4
Soluble carbohydrate	17.3	30.5	32.6	26.8	8.29	31.0
Total fatty acid^{++}	0.32	0.12	1.82	0.75	0.93	124
Total energy (kJ/100 g)	360	542	666	523	154	29.4

SD = standard deviation; CV % = coefficient of variation per cent; $^{++}$ = crude fat x 0.8.

Table II. Amino acid composition of *Moringa oleifera* tree parts in g/100 g (dry weight

Amino acid	Leaves	Stem	Root	Mean	SD	CV %
Glycine	5.09	4.69	5.25	5.01	0.29	5.76
Alanine	3.29	3.43	3.49	3.40	0.10	3.02
Serine	4.33	3.68	4.28	4.10	0.36	8.82
Proline	2.45	2.79	2.73	2.66	0.18	6.82
Valine^{+}	3.15	3.09	3.43	3.22	0.18	5.64
Threonine^{+}	3.28	4.02	4.47	3.92	0.60	15.3
Isoleucine^{+}	4.44	1.88	2.38	2.90	1.36	46.8
Leucine^{+}	5.38	5.12	5.33	5.28	0.14	2.61
Aspartic acid	6.27	6.13	7.00	6.47	0.47	7.22
Lysine^{+}	3.31	3.69	3.67	3.56	0.21	0.06
Glutamic acid	15.1	13.8	15.4	14.8	0.85	5.75
Methionine^{+}	0.99	0.72	0.97	0.89	0.15	16.9
Phenylalanine^{+}	4.62	4.06	4.34	4.34	0.28	6.45
Histidine^{+}	2.05	1.95	1.93	1.98	0.06	3.25
Arginine^{+}	8.22	1.77	1.92	3.97	3.68	92.7
Tyrosine	2.38	2.48	2.25	2.37	0.12	4.87
Cystine	2.06	2.09	2.09	2.21	0.23	10.3

$^{+}$ = essential amino acid.

Table III. Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic (g/100 g crude protein) of *M. oleifera* tree parts (dry weight samples)

Amino acid	Leaves	Stem	Root	Mean	SD	CV %
Total amino acid (TAA)	76.4	65.4	70.9	70.9	5.51	7.77
Total non-essential amino acid (TNEAA)	41.0	39.1	42.5	40.9	1.70	4.17
Total essential amino acid (TEAA)						
-with His	35.4	26.3	28.4	30.1	4.78	15.9
-no His	33.4	24.4	26.5	28.1	4.72	16.8
Total neutral amino acid (TNAA)	41.5	38.1	41.0	40.2	1.85	4.61
Total acidic amino acid (TAAA)	21.4	19.9	22.4	21.2	1.24	5.84
Total basic amino acid (TBAA)	13.6	7.41	7.52	9.50	3.53	37.2
Total sulphur amino acid (TSAA)	3.05	2.81	3.06	2.97	0.14	4.76
Total aromatic amino acid (TArAA)	7.00	6.54	6.59	6.71	0.25	3.76
% TNEAA	53.6	59.8	59.9	57.8	3.59	6.22
% TEAA						
-with His	46.4	40.2	40.1	42.2	3.59	8.50
-no His	43.7	37.2	37.4	39.4	3.69	9.36
% TNAA	54.3	58.2	57.8	56.8	2.17	3.82
% TAAA	27.7	30.5	31.6	29.9	2.02	6.74
% TBAA	17.8	11.3	10.6	13.2	3.95	29.8
% TSAA	3.99	4.30	4.31	4.20	0.18	4.33
% Cys in TSAA	51.6	48.6	48.5	49.6	1.78	3.59
% TArAA	9.16	10.0	9.29	9.48	0.45	4.77

Table IV. Amino acids scores with respect to whole hen's egg (amino acids value were in g/100 g)

					Amino acids scores		
Amino acid	Hen's egg	Leaves	Stem	Root	Leaves	Stem	Root
Glycine	3.00	5.90	4.69	5.25	1.97	1.56	1.75
Alanine	5.40	3.29	3.43	3.49	0.61	0.64	0.65
Serine	7.90	4.33	3.68	4.28	0.55	0.47	0.54
Proline	3.80	2.45	2.79	2.73	0.64	0.73	0.72
Valine	7.50	3.15	3.09	3.43	0.42	0.41	0.46
Threonine	5.10	3.28	4.02	4.47	0.64	0.79	0.88
Isoleucine	5.60	4.44	1.88	2.38	0.79	0.34	0.43
Leucine	8.30	5.38	5.12	5.33	0.65	0.62	0.64
Aspartic acid	10.7	6.27	6.13	7.00	0.59	0.57	0.65
Lysine	6.20	3.31	3.69	3.67	0.53	0.60	0.59
Glutamic acid	12.0	15.1	13.8	15.4	1.26	1.15	1.28
Methionine	3.20	0.99	0.72	0.97	0.31	0.23	0.30
Phenylalanine	5.10	4.62	4.06	4.34	0.91	0.80	0.85
Histidine	2.40	2.05	1.95	1.93	0.85	0.81	0.84
Arginine	6.10	8.22	1.77	1.92	1.35	2.90	0.31
Tyrosine	4.00	2.38	2.48	2.25	0.60	0.62	0.56
Cystine	1.80	2.06	2.09	2.09	1.14	1.16	1.16

Table V. Amino acids scores with respect to pre-school children requirements (amino acids values were in g/100 g)

					Amino acids scores		
Amino acid	Pre-school	Leaves	Stem	Root	Leaves	Stem	Root
Leucine	6.60	5.38	5.12	5.33	0.82	0.78	0.81
Isoleucine	2.80	4.44	1.88	2.38	1.59	0.67	0.85
Lysine	5.80	3.31	3.69	3.67	0.57	0.64	0.63
Met + Cys	2.50	3.05	2.81	3.06	1.22	1.12	1.22
Phe + Tyr	6.30	7.00	6.54	6.59	1.11	1.04	1.05
Tryptophan	1.10	-	-	-	-	-	-
Threonine	3.40	3.28	4.02	4.47	0.96	1.18	1.31
Valine	3.50	3.15	3.09	3.43	0.90	0.88	0.98
Histidine	1.90	2.05	1.95	1.93	1.08	1.03	1.02

- = not determined.

Table VI. Amino acids scores with respect to provisional amino acid scoring pattern of the FAO (amino acids values were in g/100 g)

					Amino acids scores		
Amino acid	Scoring values	Leaves	Stem	Root	Leaves	Stem	Root
Isoleucine	4.00	4.44	1.88	2.38	1.11	0.47	0.60
Leucine	7.00	5.38	5.12	5.33	0.77	0.73	0.76
Lysine	5.50	3.31	3.69	3.67	0.60	0.67	0.67
Met + Cys (TSAA)	3.50	3.05	2.81	3.06	0.87	0.80	0.87
Phe +Tyr	6.00	7.00	6.54	6.59	1.17	1.09	1.10
Threonine	4.00	3.28	4.02	4.47	0.82	1.01	1.12
Tryptophan	1.00	-	-	-	-	-	-
Valine	5.00	3.15	3.09	3.43	0.63	0.62	0.69

Table VII. Some special parameters from the amino acids values of *M. oleifera* tree parts

Parameter	Leaves	Stem	Root	Mean	SD	CV %
P-PER[a]	1.72	1.60	1.72	1.68	0.07	4.12
Leu/Ile	1.21	2.72	2.24	2.06	0.77	37.5
Leu/Ile (difference)	0.94	3.24	2.95	2.38	1.25	52.7
% Leu-Ile (difference)	17.5	63.3	55.0	45.3	24.4	53.9
pI[b]	5.8	5.5	5.4	5.57	0.21	3.74
EAAI[c]	0.93	0.86	0.91	0.90	0.04	4.01

[a]Predicted protein efficiency ratio; [b]Isoelectric point; [c]Essential amino acid index.

Table VIII. Fatty acids composition of *Moringa oleifera* tree parts in percentage (%) of total fat

Fatty acid	Leaves	Stem	Root	Mean	SD	CV%
Saturated fatty acids						
Caprylic acid (C8:0)	ND	ND	ND	-	-	-
Capric acid (C10:0)	ND	ND	ND	-	-	-
Lauric acid (C12:0)	ND	ND	ND	-	-	-
Myristic acid (C14:0)	0.22	0.20	0.46	0.29	0.14	49.9
Palmitic acid (C16:0)	54.3	96.0	49.7	66.7	25.5	38.2
Margaric acid (C17:0)	ND	ND	ND	-	-	-
Stearic acid (C18:0)	2.96	0.90	2.42	2.09	1.07	51.1
Arachidic acid (C20:0)	ND	ND	ND	-	-	-
Behenic acid (C22:0)	0.15	0.30	0.87	0.44	0.38	86.3
Lignoceric acid (C24:0)	0.32	0.10	0.35	0.26	0.14	52.5
Total SFA	58.0	97.5	53.8	70.0	24.1	34.4
Monounsaturated fatty acid						
Palmitoleic acid (C16:1)	0.25	ND	ND	-	-	-
Oleic acid (C18:1)	4.28	0.80	38.2	14.4	20.7	143
Erucic acid (22:1)	0.08	0.20	0.46	0.25	0.19	77.7
Total MUFA	4.61	1.00	38.7	14.8	20.8	141
Diunsaturated fatty acid						
Linoleic acid (C18:2)	4.23	0.70	6.13	3.69	2.76	74.7
Triunsaturated fatty acid						
Alpha-linolenic acid (C18:3)	33.0	0.30	0.85	11.4	18.7	164
Tetraunsaturated fatty acid						
Arachidonic acid (C20:4)	0.20	0.20	0.56	0.32	0.21	65.0

Table IX. Some special parameters from the fatty acids values

Parameter	Leaves	Stem	Root	Mean	SD	CV %
EPSI[a]	8.12	1.20	0.19	3.17	4.32	136
LA/ALA	0.13	2.33	7.21	3.22	3.62	112
PUFA	37.4	1.20	7.54	15.4	19.3	126
PUFA/SFA	0.65	0.01	0.14	0.26	0.34	127
MUFA/SFA	0.08	0.01	0.72	0.27	0.39	145
MUFA + PUFA	42.0	2.20	22.3	22.2	19.9	89.8

[a]Essential PUFA Status Index.

In addition, the presence of linoleic acid in bad cholesterol lipids makes them more prone to oxidation, which could promote atherosclerosis. Because of these detrimental effects, current recommendations have been moderated and now caution that intakes of this fatty acid should not exceed current concentrations (about 7 % of total energy intake)[60]. Surprisingly, recent information from the American Heart Association indicates that LA has a noticeable effect on lowering cholesterol further than oleic and palmitic acids when plasma cholesterol levels are high (> 200 mg/dl). They suggest that at a 10 % calorie intake in the form of polyunsaturated fats, LA achieves a maximal effect on cholesterol lowering. It also has been suggested[83] that a higher intake of LA appear to protect against stroke, possibly through potential mechanism of decreased blood pressure, reduced platelet aggregation, and enhance deformability of erythrocyte cells. More studies need to be done to determine the effect of linoleic acid in human health. The LA values in the samples were 4.23 % (leaves), 0.70 % (stem) and 6.13 % (root).

It has been suggested that arachidonic acid (AA) (C20:4) is detrimental to human health[84]. However, it promotes inflammation that is an important protective response when one is injured. It also forms the basis of anti-inflammatory prostaglandins that the body uses, to reduces inflammation[85].

The amount of AA in beef is very low (less than 0.5 % of total fat); thus, great amounts of beef have to be consumed to detect any contradictory effect. The AA in M. oleifera samples ranged from 0.20-0.56 % of total fat.

In Table IX are shown some parameters calculated from the results in Table VIII. The relative values of PUFA in all the samples made them important in diet. The eicosanoids help regulate blood lipid clot formation, blood pressure, blood lipid (including cholesterol) concentration, the immune response, the inflammation response to injury and infection and many other body functions[86]. The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and polyunsaturated fats [87]. The present PUFA/SFA was just fair in leaves (0.65) but very low in stem (0.01) and root (0.14). The relative proportion of MUFA/SFA is an important aspect of phospholipid compositions and changes to this ratio have been claimed to have effects on such disease states as cardiovascular disease, obesity, diabetes, neuropathological conditions and cancer. The MUFA/SFA levels in the samples ranged from 0.01-0.72 which was almost at per with PUFA/SFA. For example, MUFA/SFA has been shown to have cytoprotective actions in pancreatic β-cells. cis-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. They are now recognised by nutritionists as being beneficial in the human diet. The n-6 and n-3 FAs have critical roles in the membrane structure[88] and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the n-6 and n-3 FAs in the diet can be of considerable importance[89]. The ratio of n-6 to n-3 or specifically LA to ALA in the diet should be between 5:1 and 10:1[89] or 4-10 of n-6 FAs to 1.0 g of n-3 FAs[90]. As LA is almost always present in foods, it tends to be relatively abundant in animal tissues. The LA/ALA values in the Moringa oleifera tree parts were leaves (0.13), stem (2.33) and root (7.21) which were mostly outside the above ratios except in the root. The best source of the unsaturated FAs was the leaves having MUFA + PUFA values of 42.0 % > 22.3 % (root) > 2.20 % (stem). The PUFA levels had values of 37.4 % (leaves) > 7.54 % (root) and 1.20 % (stem). A suitable indicator of essential PUFA status is the essential PUFA status index (EPSI), which is the ratio between all essential PUFA (the sum of all n-3 and n-6 FAs) and all non-essential unsaturated FAs (the sum of all n-7 and n-9 FAs). The higher the EPSI the better the essential PUFA status. The EPSI values in the samples ranged from 0.19-8.12.

Conclusions

This study showed that the three parts of the Moringa oleifera parts: leaves, stem and root had different levels of the parameters determined. This is demonstrated as follows: the following parameters were of highest values in leaves [crude protein, total ash, total amino acids, total EAA, P-PER, EAAI and C18:3], root was best in [P-PER (shared with the leaves), oleic acid, linoleic acid and C20:4] and stem was best only in SFA. On the whole the leaves were best in 7/10 (70.0 %) parameters, stem best in 1/10 (10.0 %) and root was best in 3/10 (30.0 %).

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