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Proximal and Elemental Composition of *Moringa oleifera* (Lam) Leaves from Three Regions of Chad

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ABSTRACT

In Chad, like in many countries around the world, *Moringa oleifera* leaves are eaten by some people in the regions where this plant grows. No research has been done on chemical and the elemental composition of the leaves from this country. In this study, total proteins, aminoacids profile, carbohydrates, total lipids, crude fibbers, chlorophyll A and B, vitamin C and minerals were measured. The results reveal that leaves of *Moringa oleifera* are a rich source of proteins (31.5%) but contain less carbohydrates (13.5%) and lipids (2.5%). The amino acid profile shows a well balance composition. The highest amino acid percent is that of Glycin (9%), the lowest is that of sulfur amino acids, Methionine and Cystein (average 0.4%). The leaves of *Moringa* contain more ascorbic acid (average 250 mg/100 g fresh matter) than orange and lemon. Compared to the daily requirement of elementals, *Moringa* leaves are a good dietary source for 2 macro elements, calcium and magnesium and for 2 micro elements, manganese and copper. *Moringa* leaves, thus, constitute a good source of protein, vitamin C and some elements of the human diet.

Key words: Proximal, elemental, *Moringa oleifera*, leaves, composition, Chad

INTRODUCTION

Developing countries are characterized by increased of population growth rate compared to agricultural low production. It's necessary and urgent looking to alternative sources of food led by indigenous knowledge. Parts of numerous wild plants are used by populations as food. Among them, parts of the genus *Moringa* are used. About 14 species of this genus are known, almost from India origin. The most known is *Moringa oleifera* (Lam) syn. *Moringa pterygosperma* which parts are used as food and drug (Lalas and Tsaknis, 2002). Leaves, flowers and immature pods are edible and they form a part of traditional diets in many countries (Anwar and Rashid, 2007). Some parts of *Moringa* are used for treatment of diseases (Caceres *et al*., 1992) and seeds powder is used for water purification because of its bioactive and sedimentation properties (Kalogo *et al*., 2000). Others parts like roots, bark and seeds are also used for the diseases treatment.

Many researches have been conducted on nutritional value of *Moringa* leaves in several countries. Aslam *et al*. (2005) report the mineral composition of *Moringa* leaves and pods from different regions of India. Yang *et al*. (2006) have done a comparison of *Moringa* leaves from four species of Taiwan including *Moringa oleifera*, *Moringa stenopetala*, *Moringa peregrina* and *Moringa drouhardii*. Elkhalifa *et al*. (2007) have evaluated the nutritional value of *Moringa* leaves

from Sudan. Oduro *et al.* (2008) have reported the nutritional composition of *Moringa oleifera* leaves compared to that of seven varieties of *Ipomoea batatas* from Ghana. Magat *et al.* (2009) reported the nutrients content and other elements of different samples of *Moringa oleifera* from The Philippines. Saidu and Jideobi (2009) have analyzed the proximate and elemental of leafy vegetables grown in Minna and environs including *Moringa oleifera*.

Suchada *et al.* (2010) have evaluated the nutrients and minerals content of samples of *Moringa oleifera* from Thailand while Yameogo *et al.* (2011) have reported the chemical composition and nutritional values of *Moringa oleifera* leaves from Burkina Faso. Kasolo *et al.* (2010) reported the phytochemicals and used of *Moringa oleifera* leaves in Uganda. Mahmood *et al.* (2010), in a literature review reported the uses and benefits of *Moringa* from Pakistan and south Asia, this included the nutritional value, the therapeutic use and water purification properties. Joshi and Metha (2010) reported the effect of dehydratation methods on the nutritive value of *Moringa oleifera* leaves from India. In this study, they have used three methods (Sun, shadow and oven) of drying and their effect on the chemicals composition. Anjorin *et al.* (2010) reported the mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions of Nigeria. Moyo *et al.* (2011) characterize the *Moringa oleifera* leaves from South Africa. In this study, they have determinate the nutritional composition, condensed tannins and phenolics, fatty acids and amino acids profiles, β-carotene and vitamin E. Ayssiwede *et al.* (2011) have also determinate the chemical and macro minerals composition of ten unconventional species leaves from Senegal including *Moringa oleifera* with the aim to feed Chikens and other animals. All these authors found that *Moringa oleifera* leaves contain an acceptable quantity of total protein, lipids, carbohydrates and all needed macro and micronutrients. Recently, Mensah *et al.* (2012) have determinate the phytochemical, nutritional and antibacterial properties of dried Moringa leaves from central province of Nigeria. They found dried leaves of Moringa a good source of nutrients for human with well balance essential amino acids. The antibacterial analysis reveal that only the alcoholic extract inhibited the growth of *Staphylococcus aureus*, one of the four bacteria tested.

Khan *et al.* (2006) have determinate the vitamin C content of various fruits and vegetables from Bengladesh including leaves of *Moringa oleifera*. They found that from 11 fruits and leaves, the *Moringa* leaves had the highest Vitamin C content. Iqbal and Bhanger (2006) have determinate the antioxidant compounds from samples of *Moringa oleifera* leaves grown in five regions of Pakistan included ascorbic acid. They found that all samples contain high quantity of vitamin C with a slight difference among locations.

In Chad, *Moringa oleifera* grows generally around the houses and are used for enclosure.

Young *Moringa* leaves are cooked with spinach and eaten with "êche or Muru", a traditional dough prepared using cereals' flour. Nothing is known about the nutritional value of *Moringa oleifera* leaves consumed in Chad. The aim of the present research is to evaluate the nutritional composition of the *Moringa oleifera* leaves from three regions of Chad including total protein, amino acids profile, lipids, carbohydrates, fibers, chlorophyll A and B, total carotenoids, vitamin C and mineral composition.

MATERIALS AND METHODS

Samples: Leaves of *Moringa oleifera* where collected randomly in three regions of Chad at july 2012, one sample in the vicinity of Faculty of Sciences (N'Djaména, central region of Chad), the second at Moundou (Southern) and the third one at Sarh (Southern east). In the former region

(N'Djamena) the mean annual rainfall is 800 mm. Moundou and Sarh receive more rain, more than 1200 mm year^{-1}. In southern and south east regions, the *Moringa* trees are more vigorous than those of N'Djaména region probably due to the difference of rainfall per year.

The young green leaves were collected. One part is placed in plastic bags in coolers, covered with ice and transported to the "Laboratoire de Recherche sur les Substances naturelles" for the determination of vitamin C and pigments. A second part is air dried and 1kg of each are placed in polyethylene bags and transported to laboratory for other analysis.

Dry matter determination: Samples, 5 g of air dried *Moringa* leaves from N'Djamena (Ndj), Moundou (Mou) and Sarh (Sar) are weighted and placed in an drying oven at 110°C until there is no variation of mass. The dry sample is weighted and the dry matter calculated (AOAC, 1990).

Total protein: Total protein was determinate by the micro method of Kjeldahl through nitrogen content determination followed by its conversion by a factor of 6.25.

For amino acids determination, powder of dry leaves were hydrolysed, under nitrogen, in HCl vapour at 120°C for 24 h using a Pico-Tag work station (Waters). Along with 2-β-mercaptoethanol (4%) to preserve sulphur containing amino acids, 200 µL of 6N HCl were placed in the hydrolsysis tank. After hydrolysis, 10mmol of glucosamic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a Speedvac apparatus (Savant Instruments Inc., Farmingdale) and taken up with 0,05M lithium citrate buffer, pH 2.2. The samples were submitted to ion exchange chromatography in an automatic amino acids analyser (Beckman 3600). Amino acids were detected by the ninhydrin reaction, identified by their retention time and wavelength ratio and quantified by their absorption at 750 nm (440 nm for proline).

Total carbohydrates: Total carbohydrates were determinate by the phenol-sulphuric method (Saha and Brewer, 1994; Ausubel *et al.*, 1995) after extraction with ethanol 80%.

Total lipids: Total lipids were determined by Soxlhet method using Soxtherm 2000 apparatus with n-hexane as solvent.

Crude fibbers: They were determinate by the insoluble method of Deymie *et al.* (1981) with a slight modification. In this method, sample of leaves powder is digested by formic acid 80% (V/V) in boiling water. The insoluble phase is recovered and submitted to NaOH digestion. The remaining insoluble phase was submitted to incineration. The crude fibbers are calculated as the difference of the mass between insoluble phase recovered after alkaline digestion and ash obtained after incineration.

Vitamin C: The total vitamin C content was estimated using the UV spectrometric method described by Khan *et al.* (2006). In this method, vitamin C is extracted with metaphosphoric-glacial acetic acid solution. The extracted vitamin C is converted to dehydroascorbic acid by a bromine solution. The dehydroascorbic acid reacts with 2, 4 dinitrophenylhydrazine in presence of H_2SO_4. The absorbance of the coloured solution is measured at 521 nm. A calibration curve is used to determine the vitamin C content.

Minerals determination: Potassium was determined using spectrometric method (AFNOR, 1982), Calcium, Magnesium, Copper, Iron and Sodium were determined using AOAC (2005). Manganese and Phosphorus were determined by the method approved by USEPA (1974). Zinc was determined using AOAC method (AOAC, 1990).

Pigments determination: Chlorophyll A, B and total carotenoid content of *Moringa* leaves were determinate using spectrometric method described by Dere *et al.* (1998). The samples are treated with methanol (96%). The absorbance of the supernatant obtained after centrifugation is measured at 470, 653 and 666 nm and the following formula are used to calculate the level of the pigments:

- Chlorophyll A = 15.65 A666-7.340 A653
- Chlorophyll B = 27.05 A653-11.21 A666
- Total carotenoids = 1000 A 470-2.860 ChA-129.2 ChB/245

Statistical analysis: All essays were done in triplicate. The means and standard deviation are calculated using SPSS for windows 13. Student's t-test was used for differences of mean performance of samples from the different regions implying significance at $p<0.05$.

RESULTS AND DISCUSSIONS

Proximate composition: Table 1 shows that all the 3 *Moringa* samples contain total protein average 31.5%. There is no significant difference of protein content of the 3 samples. These results are the same with those of some researchers but vary slightly from those of others. Moyo *et al.* (2011), in their nutritional characterization of *Moringa* leaves from Limpopo province of South Africa, found that samples contain 30% of protein. Oduro *et al.* (2008) found *Moringa* leaves from Kumasi (Ghana) contain average 27.5% of crude protein while Ayssiwede *et al.* (2011) found a crude protein content of 28.5 for *Moringa* leaves from Senegal. Joshi and Metha (2010) obtain less crude protein value of 23.32% for sun dried leaves of *Moringa* from Jaipur, India. Campaore *et al.* (2011) found a greatest protein content of 35.56% of *Moringa* leaves from Burkina Faso. In the same country, Yameogo *et al.* (2011) found smaller value of 27% of protein from samples at Ouagadougou. Suchada *et al.* (2010) found the crude protein content ranging from 19 to 27% from dried leaves of *Moringa* of eleven regions of Thailand. Mensah *et al.* (2012) in the study of nutritional value of Moringa leaves from central Nigeria found only 6.8% of total protein content.

Except the result of Mensah *et al.* (2012) all these research results show the protein content not less than 20%. The *Moringa* leaves constitute a good source of protein for poor people of less developed country which cannot find animal protein all days.

Table 1: Chemical composition of *Moringa oleifera* leaves from the 3 regions (N'Djaména = Ndj, Moundou = Mou and Sarh = Sar)

Analysis	Ndj	Mou	Sar
Dry matter (g/100 g fresh leaves)	20.92±0.22	21.83±0.14	22.08±0.21
Ash (g/100 g DM)	6.73±0.74	7.22±0.54	6.94±0.61
Protein (g/100 g DM)	32.06±0.12	31.78±0.17	31.53±0.22
Lipids (g/100 g DM)	2.34±0.42	2.78±0.53	2.94±0.88
Carbohydrates (g/100 g DM)	13.41±0.43	14.22±0.24	14.06±0.12
Fibbers (g/100 g DM)	8.07±0.93	7.94±0.72	7.85±0.86

The same table shows an average 13.5% of carbohydrates, 2.5% of lipids. Moyo *et al.* (2011) found a greater value (6.5%) for fat but they haven't determinate carbohydrates. Oduro *et al.* (2008) found the same value (2.23%) for crude fat but much greater content of carbohydrates (43.88%). On *Moringa* news link, Mélanie Broin mentioned 40% for carbohydrates and 8% for crude fat of dry matter. Yameogo *et al.* (2011) found similar amont of carbohydrates ranging from 35.7% to 43.3% but a higher content of crude fat from 12.5 to 21.6% of dry matter. Suchada *et al.* (2010) found crude fat similar to the present results (average 2%) and they also reported other authors' results which are not higher than 2%. Talreja (2011) obtain a similar among of crude fat (2.3%) and a higher content of carhydrates (38.2%) for dry matter. Mensah *et al.* (2012) found only 0.041% of carbohydrates and 0.5% of fat.

These results show that leaves of *Moringa* contain only a small quantity of carbohydrates and lipids and they cannot provide energy to human organism.

Using the coefficients of Merrill and Watt (1955) adopted the energy value of dry *Moringa* leaves is 206,75 Kcal per 100 g of dry matter. This result is lower than that found by Yameogo *et al.* (2011) for dry leaves from Burkina faso (average 339.1 Kacl).

In the culinary processing, oil is added and the sauce is eaten with dough of cereals flour which is carbohydrates rich which provide an additional energy to organism. The estimated dry matter, ash and crude fibber of this study are not significantly different of those of the authors mentioned above.

Amino acids profile: Table 2 presents the dry *Moringa* leaves amino acids profile. This table shows 17 of the 20 natural amino acids. Asparagine and glutamine have been converted to aspartic acid and glutamic acid while tryptophane has been destroyed by the hydrolysis. *Moringa* leaves also exhibit all essential amino acids (those with asterisk) making them suitable for infant and sick persons nutrition. The highest amino acid percent is that of glycine (average 9%) and the lowest those of cysteine and methionine (average 0.4%). These two amino acids are those which contain

Table 2: Amino acids profile of *Moringa oleifera* samples from the 3 regions (N'Djaména = Ndj, Moundou = Mou and Sarh = Sar)

Amino acid (% of DM)	Ndj	Mou	Sar
Gly	9.40	8.74	9.12
Ala	1.09	0.98	1.04
Val*	1.32	1.44	1.23
Leu*	1.94	1.78	1.69
Ile*	1.22	1.12	1.30
Pro	1.19	1.04	1.13
Arg	1.55	1.43	1.47
His*	0.62	0.56	0.53
Lys*	1.24	1.39	1.33
Met*	0.42	0.39	0.33
Cys	0.37	0.44	0.37
Phe*	1.43	1.50	1.41
Ser	0.92	0.88	0.79
Tyr*	0.88	1.02	0.97
Asp	1.46	1.38	1.29
Glu	2.33	2.45	2.37
Thr*	1.42	1.37	1.33

*: Essential amino acids

Table 3: Pigments and vitamin C content of *Moringa oleifera* of the 3 regions (N'Djaména = Ndj, Moundou = Mou and Sarh = Sar)

Pigment	Ndj	Mou	Sar
Chlorophyll A (µg g^{-1})	100.23	97.88	99.12
Chlorophyll B (µg g^{-1})	43.65	44.29	44.53
Total carotenoid (µg g^{-1})	49.19	50.08	50.77
Vitamin C (mg/100 g)	254±2.13	249±3.18	257±3.13

sulphur. Moyo *et al.* (2011) have detected 19 of 20 natural amino acids using an amino acids detector after hydrolysis. They have also detected all the 10 essential amino acids like in this study. They found that the highest amino acid percent is that of alanine (3.03%) and the lowest that of cysteine, only as trace (0.01%). Talreja (2011), in a study of dry leaves from India, presents only the 10 essential amino acids. The highest content is that of leucine (1.95%), different to Moyo *et al.* (2011) and this study findings but the lowest is the same amino acid, methionine (0.35%). Moringanews website shows that the lowest amino acids in dry *Moringa* leaves are the sulphur amino acids with the same percent like these findings, 0.37% for methionine and 0.36% for cysteine. Although Mensah *et al.* (2012) found less total protein content, their study gave the same percents of almost all essential amino-acids.

All these results show that the greatest amino acid content of Moringa leaves is a neutral amino acid, glycine for this study, alanine or leucine for cited findings. The lowest amino acid percents are those of sulphur amino acids.

Pigments and vitamin C: All three samples of Moringa exhibit the same levels of chlophyll A, chlorophyll B, total carotenoid and vitamin C content (Table 3). No bibliographic data was found about pigments from *Moringa oleifera*. Pigments (Chlorophyll A, Chlorophyll B and carotenoids) are pigments which permit plant to realize photosynthesis, the processus that cause light energy to turn into chemical energy in organic compounds. Dere *et al.* (1998) have used three solvents (Diethy ether, methanol and acetone) for the determination of chlorophyll A, B and total carotenoid from four algae species. They found the highest content of chlorophyll A in *Cladophora glomerata* species but they found no significant difference in the chlorophyll B content with the three others species (*Ulva rigita*, *Codium tomentosum* and *Cladosthephus verticilatus*). The β-carotene, a carotenoid, is also known as provitamin A because it is converted to form vitamin A which protect human eyes.

There is also no significant difference of vitamin C content of the three location samples of *Moringa*. This mean value is average 250 mg/100 g of fresh matter. Vitamin C plays many functions in the human body including antioxidant agent, cofactor for enzymes. The daily requirement of vitamin C for adults range from 75 to 125 mg depending of activities. The value obtain in this study shows that only 50 g or less powder of *Moringa* is enough to achieve the required value.

Macro and micro-elements: Table 4 shows the minerals contents in *Moringa* leaves. Human body needs macro and micro elements for his development and metabolism. Calcium is the major constituent of bone and teeth and needed for the regulation of nerve and muscle function. Sodium and potassium are principal cations in extracellular fluids, they are involved in the regulation of plasma volume and acid-base balance. Phosphorus is a constituent of adenosine triphosphate, the energy compound of the body and is involved in many metabolism procedures. Iron is a constituent

Table 4: Macro-elements (g/100 g DM) and micro-elements (ppm) in *Moringa oleifera* leaves from the 3 regions (N'Djaména = Ndj, Moundou = Mou and Sarh = Sar)

Element	Ndj	Mou	Sar
Macro-elements			
Sodium (g/100 g DM)	0.08	0.06	0.09
Potassium (g/100 g DM)	1.73	1.62	1.59
Calcium (g/100 g DM)	1.23	1.12	1.09
Magnesium (g/100 g DM)	0.39	0.32	0.40
Phosphorus (g/100 g DM)	0.32	0.33	0.29
Micro-elements			
Iron (ppm)	97.12	99.21	100.02
Manganese (ppm)	29.33	27.43	28.11
Zinc (ppm)	19.14	17.17	18.02
Copper (ppm)	9.07	10.22	9.34

Table 5: Mean content of minerals, daily requirement for adults and quantity needed day^{-1}

Element	Mean content	Daily requirement (mg day^{-1})	Quantity needed (g day^{-1})
Macro-elements			
Sodium (g/100 g DM)	0.08	1200-1500	1500-1875
Potassium (g/100 g DM)	1.65	4500-4700	272-289
Calcium (g/100 g DM)	1.15	1000-1200	87-104
Magnesium (g/100 g DM)	0.37	320-420	86-113
Phosphorus (g/100 g DM)	0.31	580-1055	187-340
Micro-elements			
Iron (ppm)	98.78	18-27	182-273
Manganese (ppm)	28.29	1.8-2.3	64-81
Zinc (ppm)	18.11	8-11	442-607
Copper (ppm)	9.54	900	9.43

of haemoglogin, the oxygen carrier in cellular respiration. Magnesium is a component in many metabolism systems. Copper is a constituent of enzymes and is necessary for the growth and bone formation (Soetan *et al*., 2010). There is no significant difference for the elemental composition of the samples from the three regions.

Table 5 shows the content of the 5 macro-elements and 4 micro-elements determinate, the daily requirement and the quantity a man will eat to achieve the quantity needed. As shown on Table 5, *Moringa* dry leaves are a good dietary source for only 2 macro-elements, calcium and magnesium. For the 3 others tested, potassium, sodium and phosphorus, they are poor source. Human need to eat more than 100 g to achieve the quantity needed of these minerals per day. For micro-elements, only the manganese and copper requirements per day can be achieve with less than 100 g of dry leaves.

CONCLUSION

There are no differences in chemical composition of *Moringa oleifera* leaves from the three regions of Chad. *Moringa* leaves are rich source of protein with well balance of essential aminoacids. They contain more vitamin C than lemon and orange which are considered the main source of this compound. Calcium, magnesium, copper and manganese could be provided to human health by the consumption of these leaves. They can thus constitute a good source of many

nutrients for people of less developed country which cannot find meat sufficiently every day. They don't contain enough lipids and carbohydrates but they are consumed with oil and dough prepared using cereals' flour.

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