

## Physico-chemical properties of leaves of *Gnetum africanum* (L.) and *Gnetum buchholzianum* (L.) (*Gnetaceae*) from Cameroon

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### Abstract

A comparative study was carried out on physico-chemical properties of leaves of *Gnetum africanum* and *Gnetum buchholzianum* L. from Sa'a and Limbe neighbourhood, the two main harvesting areas of these leafing vegetables in Cameroun. Mean protein (N x 6.25) value was 17.11% and did not show significant difference between species from the two areas. Water contents ( $37.91 \pm 1.59$  %) and carbohydrates ( $41.73 \pm 2.13$  % DW) were higher in sample of *G. buchholzianum* from Sa'a. For both species, lipids content was significantly ( $P < 0.05$ ) greater in Limbe than in Sa'a samples. Potassium was the most abundant mineral with values ranging from  $1.90 \pm 0.05$  mg/100g (*G. africanum*) to  $2.49 \pm 0.26$  mg/100 g DW (*G. buchholzianum*) and was more concentrated in *G. buchholzianum* from Limbe. In this area, Mg ( $0.28 \pm 0.03$ ), Na ( $0.16 \pm 0.01$ ) and Fe ( $0.59 \pm 0.004$  mg/100g DW) contents were significantly ( $P < 0.05$ ) higher in *G. africanum* sample. Essential aminoacids especially threonine, isoleucine and leucine were found in greater amount ( $P < 0.05$ ) in Limbe samples compared to those from Sa'a. Sulfur amino acids were the most limiting but chemical balance was more than 33%. Limbe samples were rich in unsaturated fatty acids. Linolenic and oleic acids were the most abundant unsaturated fatty acids. Based on the above results, *Gnetum* spp leafing vegetables could be recommended as inexpensive sources of protein, essentials fatty acids and potassium for human and nutrition.

**Key words:** *Gnetum* spp, leaves, physico-chemical properties

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## Introduction

Availability of basic foodstuffs is limited in many countries because of low food production, soils poverty and unbalance between consumption and production of many crops. In some developing countries, this crucial problem is accentuated by limited resources, wars and conflicts which hinder access to food resources (FAO, 2008). To solve this alarming issue, people of many developing countries especially from rural areas, include wild products in their daily diets. In Central Africa for example, consumption and use of significant amounts of wild products are of current practices (Mialoundama and Loubelo, 2007). These products of gathering known as Non Timber Forest Products (NTFP) have solved some hunger problems in the Guinea Gulf countries where they somehow replaced food crops in rural regions, as well as urban centers (Mialoundama, 2007). These locally exploited products include various fruits, vegetables, tubers and miscellaneous products (Mbolo *et al.*, 2002). The importance of NTFP in food safety has strongly increased in latest years and their sustainable management is a priority defined in a joint plan of the Central Africa Forests Commission (COMIFAC) (Sven, 2001).

In Central Africa and in Cameroon in particular, leaves of *G. africanum* and *G. buchholzianum* are important NTFP used as foods. These vegetables belong to the Gnetaceae family which grows spontaneously in the forests (Schippers, 2000). Some researchers are investigating on its domestication. Their leaves are consumed as vegetables in Cameroon and Central African Countries including Angola. Locally known under the name of *okok* or *eru* in Cameroon, *fumbwa* in Democratic Republic of Congo or *afang* in Nigeria, these vegetables play a significant socio-economic role and contribute to food safety and generation of incomes for people and urban actors implied in these activities (Kanmegne *et al.*, 2007). They are important source of fibers, essential amino acids, vitamins and minerals (Fadi *et al.*, 2011).

Differences have been found in the contents of amino acids and mineral in leaves of

the two african species of *Gnetum* in the Republic of Congo (Mialoundama, 1988; 2007). It would be interesting to appreciate the extent of these differences according to ecological areas in order to make possible recommendations for dietary uses of these leafy vegetables.

The aim of this study was to provide some data on physico-chemical properties of leaves of *Gnetum* spp from two main production areas in Cameroon (Sa'a and Limbe). Those datas could contribute to improve the nutritional knowledge of the vegetable and possible integration in the agroforestry policies.

## Materials

Leaves of *G. africanum* and *G. buchholzianum* were harvested during drying season (November – February 2012) in Limbe botany garden (South-West Cameroon; longitude : 4°0'46" North, latitude : 9°13'13" East) and in a forest Sa'a (Monatele; longitude: 4°16'0" North, latitude: 11°12'0" East). Leaves were abundantly washed with tap water, rinsed thoroughly with deionized water, dried at ambient temperature (approximately 28°C) for two weeks and then reduced in powder using a stainless steel crusher (Moilnex, France). The resulting powder were preserved in a desiccator for subsequent analyses.

## Methods

### Chemical analysis

AOAC (1999) methods were used to perform all the analyses. Moisture was determined on fresh leaves. Fat was extracted by Soxhlet method. Ash, crude proteins (N x 6.25) and crude fibers were analyzed on the defatted powders. Carbohydrate contents were obtained by the method of difference. From the defatted acid-digested samples, Ca, Mg, Na, P, K and Fe were determined using Perkin Elmer atomic absorption spectrophotometer (Model 1100, Perkin Elmer, USA) equipped with a Perkin Elmer microprocessor HGA 700 (AOAC 1999). Each mineral content was calculated from known standards. The results were expressed in mg/100 g of dried sample (Benton and Vernon, 1990).

### Fatty acids composition

Fatty acids composition was determined by gas chromatography AOAC (1990). Peaks were recorded and treated in a computer with a Word Processing Winilab Perichrom 1998 (Paris, France). Identification of peaks was based on the retention times of unknown

compounds and their comparison with those of known chromatograms (Bornaz *et al.*, 1992).

### Amino acids composition

Amino acids of the defatted powder were determined in a Pico-Tag Station (Waters, Milford, MA, USA) equipped with an automatic analyzer model 420A Perkin Elmer PE Biosystems (Foster City, CA, USA) according to the prescribed procedure. Results were recorded and calculated using a software Model 600 Data Analysis System (ABI, Perkin Elmer). For the determination of cysteine and methionine, the samples were previously oxidized with performic acid according to the method described by Spindler *et al.* (1984). Content of various amino acids recovered were calculated as g/100 g of proteins and compared to the FAO (2013) reference pattern. The chemical essential amino acids (EAA) score was calculated as follows:

$$EAA = \frac{gofEAAin\ 100gof\ test\ protein}{gofEAAin\ 100\ gof\ FAO/WHO\ reference} \times 100$$

### Calorific value

The calorific value of the defatted powders was calculated by multiplying the mean values of crude proteins, fat and carbohydrate by factors of 4, 9, and 4, respectively taking the sum of the products and expressing the results in Kcal according to Edem *et al.* (1990).

### Statistical analysis

All the measurements were made in triplicate. Analysis of variance was used to compare means. Significant differences were defined at  $P < 0.05$ . Multiple comparisons were made using the Duncan test.

## Results and discussion

### Chemical composition

Table 1 shows proximate composition of *Gnetum* spp. Water content varied significantly ( $P < 0.05$ ) according to plant species harvesting area. However, it was higher in *G. bucholzianum* from Sa'a (37.91%). There was no significant difference ( $P < 0.05$ ) in the ash contents between the two plants species (7.74 – 8.25% DW). Proteins contents of *G. africanum* and *G. bucholzianum* from Sa'a and Limbe (16.47 – 17.55% DW) were not

statistically different ( $P < 0.05$ ). These contents were similar to that of *Gnetum* seeds (Ekop, 2007) and approximately 1.3 times higher than those found in leaves of *Kigelia africana*, a plant used for nutritional and pharmacological purposes in West and South Africa (Glew *et al.*, 2010). Protein contents of samples were comparable to data obtained by Mialoundama (2007) who indicated the value between 16.5 - 18.2% in the *Gnetum* leaves from Democratic Republic of Congo.

Lipids contents of both species ranged from 6.28% to 8.91% (DW) in Limbe and differed significantly ( $P < 0.001$ ) from the 4.37 to 5.47% (DW) values from Sa'a. These contents were closed to 5.9 to 6.2% found by Mialoundama (2007) in Democratic Republic of Congo. Crude fibers were more than 26% (DW) with a scored value of 37.80 % (DW) in *G. africanum* leaves from Sa'a. Carbohydrates and crude fibers were the major constituents in both species. They were high in contents in *G. africanum* compared to *G. buchholzianum*. Results obtained with *G. africanum* and *G. buchholzianum* from both area justify the high calorific value of those samples (241.25 – 310.23 Kcal/100g).

For the proximate composition, the differences between the two species with respect to the area are based on the lipids, carbohydrates and crude fibers contents.

Table 2 shows some mineral contents of the two species with respect to the area. In both harvesting areas, potassium was the most abundant mineral with values ranging from 1.48 to 2.49 mg/100 g (DW). It was significantly ( $P < 0.001$ ) higher in *G. buchholzianum* leaves than in *G. africanum*. This value was closed to 2.15% (DW) found by Tchientcheu-Kamga *et al.* (2013) in *Solanum aethiopicum* which is another common edible vegetable in Cameroon. These values were also closed to 2.5 mg/100 g (DW) found in *Solanum aethiopicum* but were less than 54.20 mg/100 g and 42.89 mg/100g (DW), respectively found in *Amaranthus hybridus* and *Solanum nigrum* (Akubugwo *et al.*, 2007a, 2007b)

As the main intracellular cation in mammals, K is involved in maintaining cell volume, enzyme activity, transmission of nerve impulses, and regulation of blood pH (Pasch, 2006). Including *Gnetum* spp leaves in a diet could therefore contribute to maintain and to promote those physiological activities. Mg, Na, Ca, Fe and P represented less than 1 mg % of total minerals of both plants. Na was the less abundant mineral and together with Mg and Ca they were more concentrated in *G. africanum* than in *G. buchholzianum*. Whatever the area, these mineral contents were generally low compared to those of some local vegetables such as *Solanum nigrum* (Akubugwo *et al.*, 2007). The mineral contents could vary with respect to the ecological area.

According to their physico-chemical composition, *G. africanum* and *G. buchholzianum*

leaves show higher values as nutritious green vegetables. Their average protein content (17.11%) did not vary with location. Some significant differences perhaps due to environmental factors were found in contents of nutrients of leaves from the two areas. Situated at about 50 Km from Yaounde, Sa'a village has a typical equatorial climate with four distinct seasons including two raining and two drying seasons. Soil is a ferruginous type. Limbe is bordered by a wetted forest which stretched on a slope of Mount Cameroon in the South West Region of the Cameroon. It has a long lasting raining season interrupted by two or three months of dry season. Soil is covered by volcanic rocks (Sieffermann, 1973). As it generated from a rich rock, soil of this region has significant reserve in readily available mineral substances (Laplante and Bachelier, 1954). Indeed, soil and climate related factors could explain some chemical changes found between the two locations and plants. However *G. africanum* and *G. buchholzianum* leaves showed high nutritional value.

### **Fatty acids composition**

Four main fatty acids accounted for more than 70 % of the total fatty acids in oil extracted from leaves of *Gnetum* spp (table 3): palmitic acid (C16:0) (28.67 to 33.63%), linolenic acid (C18:3) (10.04 to 35.13 %), oleic acid (C18:1) (10.61 to 20.39 %) and linoleic acid (C18:2) (10.42 to 18.38 %). Fatty acids content varied with the two plant species and harvesting area. From each sample, the sum of saturated fatty (SFA) and unsaturated fatty acids (UFA) was calculated. Ratios of the sum of UFA varied with respect to the specie from 31.07 % to 60.03% and were in general elevated in samples from Limbe samples. Oils from Sa'a with respect to the specie contained saturated fatty acids 54 and 68.93% respectively for *G. africanum* and *G. buchholzianum*. Palmitic acid was the most abundant fatty acid excepting *G. buchholzianum* from Limbe. In this later, Linoleic acid was the most abundant (35.15 %) of total fatty acids. Samlpe from Lombe, according to the specie, contained more PUFA respectively 36.94 and 43.55 % for for *G. africanum* and *G. buchholzianum*. These values were only between 20 and 27.16% for samples from Sa'a. Ecological area could explain these highest differences. PUFA are especially important as constituents of phospholipids where they appear to confer distinctive properties to membranes, modulating their structure and function. They are precursors of eicosanoids, such as prostaglandins, thromboxanes, leukotrienes, and lipoxins, and docosanoids, including resolvins and protectins, which have important biological properties (Gil *et al.*, 2003).

**Table 1: Proximate composition of *Gnetum* spp.leaves (in % DW) from Limbe (L) and Sa'a (S) and calorific values**

Species	Water	Ash	Proteins (Nx6.25)	Lipids	Carbo- hydrates	Crude fibers	Calorific values (Kcal/100g)
<i>G. africanum</i> (L)	29.11 ± 0.57 <sup>a</sup>	8.25 ± 0.16 <sup>a</sup>	16.47± 0.02 <sup>a</sup>	6.28± 0.03 <sup>b</sup>	41.04± 0.56 <sup>a</sup>	28.05± 0.81 <sup>a</sup>	289.56
<i>G. africanum</i> (S)	33.57± 0.64 <sup>b</sup>	7.89 ± 0.16 <sup>a</sup>	17.51± 1.44 <sup>a</sup>	4.37± 0.19 <sup>a</sup>	33.22± 2.96 <sup>b</sup>	37.80± 1.34 <sup>b</sup>	242.25
<i>G. buchholzianum</i> (L)	33.57± 1.50 <sup>b</sup>	8.38 ± 0.30 <sup>a</sup>	16.94± 0.92 <sup>a</sup>	8.91± 0.59 <sup>c</sup>	40.57± 1.92 <sup>a</sup>	26.30± 0.97 <sup>a</sup>	310.23
<i>G. buchholzianum</i> (S)	37.91± 1.59 <sup>c</sup>	7.74 ± 0.26 <sup>a</sup>	17.55± 0.58 <sup>a</sup>	5.47± 0.78 <sup>a</sup>	41.73± 2.13 <sup>a</sup>	26.97± 1.19 <sup>a</sup>	286.35

Column values with the same letter are not significantly different at 5% threshold.

Water content is expressed as % of wet weight while ash, proteins, lipid and carbohydrate are expressed as % of dry weight. (L): Limbe; (S): Sa'a

**Table 2: Mineral contents (mg/100 dry weight) of *G. africanum* and *G. buchholzianum* leaves from Limbe (L) and Sa'a (S)**

Species	Fe	K	Mg	Na	Ca	P
<i>G. africanum</i> (L)	0.59±0.004 <sup>a</sup>	1.90 ± 0.05 <sup>a</sup>	0.28 ± 0.03 <sup>a</sup>	0.16 ± 0.0 <sup>a</sup>	0.88 ± 0.05 <sup>a</sup>	0.18 ± 0.00 <sup>a</sup>
<i>G. buchholzianum</i> (L)	0.53±0.005 <sup>bc</sup>	2.49 ± 0.26 <sup>b</sup>	0.17 ± 0.01 <sup>b</sup>	0.07 ± 0.00 <sup>b</sup>	0.47 ± 0.03 <sup>b</sup>	0.15 ± 0.00 <sup>a</sup>
<i>G. africanum</i> (S)	0.49±0.007 <sup>c</sup>	1.48 ± 0.14 <sup>c</sup>	0.22 ± 0.04 <sup>a</sup>	0.15 ± 0.01 <sup>a</sup>	0.85 ± 0.04 <sup>a</sup>	0.15 ± 0.03 <sup>a</sup>
<i>G. buchholzianum</i> (S)	0.50±0.005 <sup>c</sup>	1.67 ± 0.07 <sup>d</sup>	0.17 ± 0.01 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.39 ± 0.03 <sup>c</sup>	0.13 ± 0.01 <sup>b</sup>

Column values with the same letter are not significantly different at 5% threshold.

**Table 3: Fatty acids composition (%) of *Gnetum* species**

Fattyacids	<i>Gnetum africanum</i>		<i>Gnetum buchholzianum</i>	
	Limbe	Sa'a	Limbe	Sa'a
C6 :0	0.34±0.04 <sup>a</sup>	0.31±0.01 <sup>a</sup>	-	4.38±0.11
C8 :0	0.47±0.03 <sup>d</sup>	0.31±0.04 <sup>e</sup>	-	1.74±0.09 <sup>f</sup>
C10:0	0.96±0.01 <sup>a</sup>	0.84±0.03 <sup>b</sup>	1.07±0.40 <sup>c</sup>	6.69±0.31 <sup>d</sup>
C13:0	0.41±0.30 <sup>b</sup>	0.51±0.04 <sup>c</sup>	0.42±0.11 <sup>b</sup>	0.63±0.05 <sup>d</sup>
C14:0	0.51±0.30 <sup>a</sup>	2.41±0.04 <sup>c</sup>	0.36±0.05 <sup>d</sup>	2.37±0.14 <sup>c</sup>
C15 :0	1.73±0.01 <sup>a</sup>	1.81±0.09 <sup>a</sup>	2.19±0.05 <sup>b</sup>	1.06±0.02 <sup>c</sup>
C16 :0	28.67±0.30 <sup>d</sup>	33.63±0.17 <sup>c</sup>	32.48±0.16 <sup>a</sup>	33.58±0.64 <sup>c</sup>
C17 :0	0.40±0.01 <sup>a</sup>	0.45±0.01 <sup>b</sup>	1.03±0.09 <sup>c</sup>	1.60±0.06 <sup>d</sup>
C18 :0	6.89±0.03 <sup>c</sup>	12.13±0.12 <sup>d</sup>	6.46±0.17 <sup>b</sup>	14.79±0.29 <sup>a</sup>
C18 :1	20.39±0.66 <sup>d</sup>	18.83±0.43 <sup>c</sup>	12.40±0.29 <sup>a</sup>	10.61±0.43 <sup>b</sup>
C18 :2	18.38±0.48 <sup>c</sup>	13.04±0.22 <sup>a</sup>	8.42±0.49 <sup>b</sup>	10.42±0.54 <sup>a</sup>
C18 :3	21.26±0.22 <sup>a</sup>	14.12±0.22 <sup>b</sup>	35.13±0.04 <sup>c</sup>	10.04±0.15 <sup>d</sup>
C20:0	-	1.57±0.03 <sup>a</sup>	-	1.41±0.04 <sup>b</sup>
SFA	39.97	54.01	44.05	68.93
UFA	60.03	45.99	55.95	31.07
PUFA	39.64	27.16	43.55	20.46

Column values with the same letter are not significantly different at 5% threshold.

SFA : saturated fatty acids ; UFA : unsaturated fatty acids



**Table 4: Amino acids composition of *Gnetum* spp (% DW)**

Aminoacids	<i>G. africanum</i>		<i>G. buchholzianum</i>		Standard * (FAO, 2013)
	Limbe	Sa'a	Limbe	Sa'a	
Tyr	6.13±0.24 <sup>a</sup>	4.45±0.74 <sup>b</sup>	6.90±1.65 <sup>a</sup>	6.90±1.65 <sup>a</sup>	
Phe	28.84±3.52 <sup>a</sup>	21.64±3.31 <sup>b</sup>	27.92±4.01 <sup>a</sup>	27.92±4.01 <sup>a</sup>	
Tyr+ Phe	34.97	26.09	34.82	34.82	52
Thr	55.61±0.06 <sup>a</sup>	51.62±2.05 <sup>b</sup>	54.30±2.12 <sup>a</sup>	51.85±1.13 <sup>b</sup>	31
Ile	42.50±4.06 <sup>a</sup>	38.03±2.79 <sup>b</sup>	44.33±2.89 <sup>a</sup>	40.39±1.48 <sup>b</sup>	32
Leu	45.71± 3.15 <sup>a</sup>	41.34±5.99 <sup>b</sup>	44.27±0.59 <sup>a</sup>	42.84±0.79 <sup>b</sup>	66
Lys	40.07±3.94 <sup>a</sup>	39.00±3.08 <sup>a</sup>	39.37±1.71 <sup>a</sup>	40.45±1.65 <sup>a</sup>	57
Val	32.78±3.21 <sup>a</sup>	32.38±0.79 <sup>a</sup>	32,58±1,23 <sup>a</sup>	30.94±0.34 <sup>a</sup>	43
His	16.33±0.24 <sup>a</sup>	15.01±3.42 <sup>c</sup>	18.47±4.07 <sup>b</sup>	15.38±1.42 <sup>c</sup>	20
Met+Cys	4.8±0.13 <sup>a</sup>	4.6±0.23 <sup>a</sup>	5.20±0.17 <sup>b</sup>	5.40±0.06 <sup>b</sup>	27
Trp	26.60±0.15 <sup>a</sup>	25.40±0.15 <sup>a</sup>	24.31±0.15 <sup>a</sup>	25.00±0.14 <sup>a</sup>	8.5
<b>EAA</b>	<b>299.37</b>	<b>273.47</b>	<b>297.65</b>	<b>286.49</b>	<b>316.5</b>
Asn+Asp	61.08±6.37 <sup>a</sup>	59.62±3.25 <sup>a</sup>	58.44±6.02 <sup>a</sup>	58,51±1,08 <sup>a</sup>	
Gln+Glu	61.50±0.36 <sup>a</sup>	59.73±3.99 <sup>a</sup>	60.56±1.08 <sup>a</sup>	57,94±3,36 <sup>b</sup>	
Pro	56.28±1.03 <sup>a</sup>	55.39±2.22 <sup>a</sup>	54.25±0.70 <sup>a</sup>	52,53±0,22 <sup>b</sup>	
Ser	39.10±2.42 <sup>a</sup>	35.52±2.96 <sup>b</sup>	38.60±1.41 <sup>a</sup>	38,51±1,99 <sup>a</sup>	
Gly	67.33±3.40 <sup>a</sup>	64.82±3.88 <sup>c</sup>	60.33±1.29 <sup>b</sup>	57,77± 3,41 <sup>d</sup>	
Ala	28.41±0.60 <sup>a</sup>	28.55±0.74 <sup>a</sup>	28.15±3.42 <sup>a</sup>	25,64±5,92 <sup>b</sup>	
Arg	25.86±1.76 <sup>a</sup>	21.98±1.08 <sup>b</sup>	24.20±1.71 <sup>a</sup>	24,21±4,55 <sup>a</sup>	
<b>NEAA</b>	<b>339.56</b>	<b>325.61</b>	<b>343.53</b>	<b>315.11</b>	
<b>AAE/TAA (%)</b>	<b>46.85</b>	<b>45.64</b>	<b>47.83</b>	<b>47.62</b>	

Column values with the same letter are not significantly different at 5% threshold. (N = 3)  
 EAA= essential amino acids; NEAA= non essential amino acids ; TAA =total amino acids  
 \* (FAO, 2013)

### Amino acids composition

The results shown in table 4 indicated that leaves of *G. africanum* from Limbe were rich in essential amino acids than those of Sa'a samples. Differences between the two zones were significant (P <0.05) for aromatic amino acids, threonine, isoleucine and leucine.

Isoleucine (38.03 - 44.33 %), threonine (51.62-55.61 %) and tryptophan 24.31-26.60 %) contents were greater than that of the reference protein (FAO, 2013). Sulfur amino acids were the most limiting. Due to the fact that these *Gnetum* leaves are prepared with meat and fish and other ingredients the deficiency of these amino acids could be filled in. Ratio of essential amino acids to total sum of amino acids (AAE/TAA) was more than 45% for all samples, indicating a good equilibrium between amino acids of proteins (Blankeship and Alford 1983).

## Conclusion

This study showed that *Gnetum africanum* and *Gnetum buchholzianum* leaves from Limbe and Sa'a have appreciable amounts of protein, fibers and potassium. Their minerals and organic compound contents varied between Limbe and Sa'a. The study further revealed that they are good source of essential fatty acids and essential amino acids. All essentials amino acids were present and this vegetables could therefore be used to enrich low protein diets. In case of a good bioavailability of these substances in foods, *Gnetum* spp leaves have high nutritional value and could be recommended as cheap sources of plant protein, essential amino acids, essentials fatty acids, energy and potassium.

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