Original Research Article

Comparative study of the nutritive and bioactive compounds of 3 cucurbits species grown in 2 regions of Côte d'Ivoire

ABSTRACT

Aims: The objective of this work was to contribute to the valorization of 3 local cucurbits oilseeds species grown in 2 producing regions of Côte d'Ivoire namely the Kabadougou and the Moronou. Thus, a comparative study on the main nutritive and bioactive compounds of these cucurbits was conducted.

Methodology: Mature dried seeds of *Citrullus lanatus* sp, *Lagenaria siceraria* Molina Standl*, and Cucumeropsis mannii* Naudin, locally named respectively *Wlewle, Bebou* and *Nviele,* were collected in the 2 regions, dehulled and processed for analyses. Standard procedures of AOAC, AFNOR and FAO were used for the determination of the nutritive and bioactive compounds.

Results: Wlewle species exhibited the highest content of lipid (51.07±1.32 %), energy caloric value (584.05±4.13kcal/), polyphenols (141±23.97) and flavonoids (0.19±0.02) content. *Nviele* species had the highest protein (38.90±0.93 %) and reducing sugar (70.62±1.03 mg/100g) contents. *Bebou* species exhibited the highest ash content (3.91±0.38 %) and total soluble sugars (3.42±0.19 %). Flavonoids content was similar for each species regardless of the region. Pearson's correlation analysis revealed (p< 0.01) that energy caloric and lipid were directly correlated but conversely with protein. Direct correlation was also observed between polyphenols and lipid content in *Wlewle* and *Nviele* varieties, while in the *Bebou*, this trend was observed with protein. Despite their small shape, *Wlewle* species provide higher caloric energy upon consumption.

Keywords: *Cucurbitaceae, Nviele, Bebou, Wlewle, nutritive compounds, bioactive substances.*

INTRODUCTION

Malnutrition is an important phenomenon affecting the whole world. In developing countries, about 1/3 of children are stunted and 5 to 15% are starving. Malnutrition is therefore a cause of disability, illness and mental and physical developmental delay, thus negatively impacting the socio-economic growth of countries [1]. The world's leading economists stated that one of the best investments countries could make would be investment in strategies to reduce malnutrition [2]. In 2000, the World Health Organization (WHO) set a goal to significantly reduce global food insecurity by 2015 [3]. Great progress was seen in Africa with a 30% drop in hunger from 1990 to 2015. However, this goal is far from being achieved due to many scourges [4]. Also, although food availability has increased, energy and protein intake are still below the levels recommended by FAO and WHO. This is partly due not only to poverty but also to lack of informations on nutritional and economic importance of many local food products as well as their technological properties and their possible utilization in fortification programs. The 29th FAO Regional Conference for Africa, held on April 7, 2016 in Abidjan, called for quick actions by member countries to end food insecurity by 2025 [5].

Cucurbits are among the most economically important vegetables in the world. In West Africa, there are at least five Cucurbitaceae species regularly cultivated by the populations [6]. These plant species are of significant socio-economic importance in the life of sub-Saharan people, especially in Côte d'Ivoire where they are source of small-scale livelihood for female farmers. Locally spelled ''*pistaches''*, the cucurbits kernels are usually processed into soups; it is very prized for home consumption and during festivities [7]. Previous studies have shown the nutritional interests of some cucurbits kernels [8, 9, 6]. They are rich source of protein and lipid, and could therefore be an asset in fortification programs, therefore increasing their use and valorization.

Legumes seeds, which constitute an essential part of human diet, are good source of protein, healthy lipid and bioactive phenolic compounds [10, 11]. Knowing that the biochemical composition of foods is greatly affected by cultural practices and environmental conditions [12], the previous data on nutritional values and bioactive compounds of *"pistaches"* seeds grown in Côte d'Ivoire should be updated. Data should be collected base on producing areas for a good screening and thus a better valorization.

This paper reports a comparative study on the main nutritive and bioactive contents of 3 local cucurbits species, namely *Citrullus lanatus, Lagenaria siceraria,* and *Cucumeropsis manii* produced in Northern (Kabadougou) and Eastern (Moronou) regions of Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1. Plant material

Mature and dried seeds of 3 cucurbit species *Citrullus lanatus* sp, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* Molina Standl; locally called *Wlewle*, *Nviele* and *Bebou* respectively; were used in this study

2.1.1. <u>Sampling</u>

Seeds were collected from two sites of Côte d'Ivoire: in the Moronou region located in Eastern part and in the Kabadougou region in the North. Three (3) villages per region were considered. Per village, 3 kg of each seeds type were bought from 3 different female farmers, thus, 9 samples per village and 27 per region. In total, 54 samples weighing 162 kg were collected in both regions. Upon arrival in the laboratory, cucurbits seeds were dehulled and a pool of 2 kg sample was dried and ground into powder. The resulting cucurbits flours were kept in airtight containers until use.

2.2. Determination of the nutritive compounds

Nutritive values were assessed by the determination of moisture, protein, lipid, carbohydrate and ash contents of all the samples using analytical grade chemicals and reagents from Sigma Chemical Co (St Louis, MO). Flours caloric energy values were also calculated. For each analysis, triplicate measurements were done.

2.2.1. <u>Moisture content of seeds</u>

Moisture content of the collected samples was determined according to AOAC [13]. Briefly, 5g of seeds were dried to a constant weight at 105 °C into a laboratory oven (type the Mark) and moisture content was calculated as follows:

Moisture content (%) = (mass of evaporated water/ mass of sample) * 100

2.2.2. <u>Lipid content</u>

Fat was quantified by solvent extraction in a Soxhlet apparatus using hexane as solvent for 7 h [13]. After oil extraction, solvent was evaporated from flour samples, and the percentage of fat was calculated as follows:

$$\textbf{Fat}\ (\%) = \frac{(W2 - W1) * 100}{W0}$$

With W0: the sample's weight; W1: the weight of empty flask; W2: the weight of the flask with the extracted fat.

2.2.3. <u>Total Protein</u>

Total protein content was determined according to AOAC [13], using Kjeldhal method. One (1) g of flour was mineralized at 400 °C for 2 h, with concentrated sulfuric acid (H_2SO_4) in presence of potassium sulfate catalyst. The solution was cooled down and diluted with distilled water before distillation. A volume of sodium hydroxide (NaOH) was added to an aliquot of the diluted solution and the mixture was distillated. The distillate was then collected in a flask containing boric acid and methylen bromocresol reagents and titrated for the total nitrogen with 0.1 N chlorine acid (HCl) till appearance of a light pink color. A factor of 6.25 was used to convert the total nitrogen into protein content according to the equation hereafter:

$$\textbf{Protein content } (\boldsymbol{g}/\mathbf{100}\ \boldsymbol{g}) = Total\ nitrogen\ content\ (g/100\ g) * 6.25$$

2.2.4. Ash content

Ash content was determined according to AOAC [14]. About 5 g of each flour was weighed into clean pre-weighed porcelain crucibles. The samples were transferred into a pre-heated muffle furnace and incinerated at 550°C for 24 hours. Total ash content was calculated as follows:

$$\textbf{Ash content } (\%) = \frac{(W2 - W1) * 100}{W0}$$

With *W0: the sample's weight; W1: the weight of the empty crucible; W2: the weight of the crucible with the ash*

2.2.5. Total carbohydrates, total soluble and reducing sugars

Total carbohydrates were deduced from the contents of protein, moisture, lipid and ash as indicated by FAO (FAO, 2002) in the following formula:

TC (%) = 100 – [Prot + Lip + Moi + Ash]

With TC, Prot, Lip, and Moi standing for total carbohydrate, protein, lipid and moisture contents

Ethanol- soluble sugar was extracted from 1 g of flour with 20 mL of 80% (v/v) ethanol, 2 mL of 10% (m/v) zinc acetate and 2 mL of 10% (m/v) oxalic acid. The extract was centrifuged at 3,000 rpm for 10 min. The ethanol residue was evaporated from the extract upon a hot sand bath. The spectrophotometric method of Dubois *et al*. [15] was used to determine the total soluble sugars of flour samples. It consisted in adding 0.9 mL of distilled water, 1 mL of 5% (m/v) phenol and 5 mL of 96% sulfuric acid into 100 µL of extract and then measuring the absorbance at 490 nm with a spectrophotometer (PG instruments). Reducing sugars of the samples were determined using the method of Bernfeld [16]. Briefly, 1 mL of extract was processed with 0.5 mL of distilled water and 0.5 mL of 3, 5- dinitrosalycilic acid. Absorbance of the final solution was read at 540 nm using spectrophotometer (PG instruments). Calibrations curves were constructed using standard solutions of glucose and sucrose.

2.2.6. Caloric energy value

Caloric energy value of each flour was calculated using Atwater specific factors for legumes and nuts, related to the main macronutrients, especially protein, lipid and carbohydrate [17] as stated below:

CE (kcal/100 g) = (3.47xProt) + (8.37xLip) + (4.07xCarb)

With CE, Prot, Lip, Carb standing for Caloric Energy and content of protein, lipid, and total carbohydrate

2.2.7. Evaluation of the nutritive contribution

The contribution of the nutritive compounds was estimated according to the Codex Alimentarius method that takes into account the concentration in nutritive compounds recovered from the food and the daily consumption of that food by an adult [18].

Estimated Daily Intake (EDI) = C × Q

With: C, nutritive compound concentration measured;
Q, food daily consumption [19].

2.3. Determination of bioactive compounds

2.3.1. Preparation of the methanolic extracts

A gram (1) g of dried and ground seeds was homogenized in 10 ml of 70% methanol. The mixture was centrifuged at 1000 trs/min for 10 min and the supernatant collected. The pellet was re-suspended in another 10 ml of 70% methanol and centrifuged once again as previously done. Collected supernatants were pooled and the volume made up to 50 ml with deionized water. This methanolic extract was stored in an amber bottle and used for analysis.

2.3.2. Total phenolic content (TPC)

Total phenolic content was determined according to Folin–Ciocalteu spectrophotometric method described by Singleton et al. [20]. In a test tube, a volume of 0.5 ml of the methanolic extract was added to 0.5 ml freshly prepared Folin-Ciocalteu reagent. The mixture was allowed to equilibrate for 3 min and then mixed with 0.5 ml of 20% sodium carbonate, and the volume made up to 5 ml with deionized water. The tube was incubated in the dark for 30 min and the absorbance read at 725 nm against a reagent blank.

Total phenolic content was determined using a standard curve of gallic acid, and the results was expressed as mg of gallic acid equivalents (GAE)/100g dry matter.

2.3.3. <u>Total flavonoids content</u>

Total flavonoids content was determined as previously described by Jia et al. [21]. In a test tube, 0.5 mL of the methanolic extract was diluted with 0.5 ml deionized water. A volume of 0.5 ml of aluminium chloride (10% p/v) and 0.5 ml of 1 M sodium acetate were successively added to the tube. The mixture was completed with 2 ml of deionized water and then allowed to stand for 30 min at room temperature. The absorbance was quickly measured at 415 nm against the blank. Catechin was used as standard and the results were reported as mg of catechin equivalents (CE)/100g of dry matter.

2.4. Statistical analysis

All experiments were conducted in triplicates. Data were submitted to analysis of variance (ANOVA) using SPSS software (SPSS 22.0, USA). Means were expressed with their Standard Deviation (SD) and compared using Student-Newman-Keuls and Least Significant Difference post-hoc tests at 5% significance. In addition, Multivariate Statistical Analysis (MSA) was performed through Principal Components Analysis (PCA) using STATISTICA software (version 7.1) for correlation between cucurbits varieties and their nutritive compounds.

3. RESULTS AND DISCUSSION

3.1. Moisture content and Nutritive compounds

Results for nutritive composition of the 3 cucurbits species collected from the Moronou and the Kabadougou are summarized in Tables 1, 2 and 3.

3.1.1. <u>Proximate Analysis of the Nutritive traits from cucurbits species</u>

The overall comparison of the nutritive values of the 3 cucurbits species analyzed is summarized in Table 1. No significant difference ($p \geq 0.05$) was observed in the moisture (2.68±0.53% to 3.04±0.13%) and the ash contents (3.28%±0.62 to 3.91%±0.38) of the 3 cucurbits species

However, significant differences ($p < 0.001$) in the lipid, protein, total carbohydrates, reducing sugars, total soluble sugars and total caloric energy contents of the 3 species were observed. Indeed, *Wlewle* species exhibited the highest lipid content (51.07±1.32%) but the lowest protein content (29.01±1.83%), while *Nviele* species showed the lowest lipid content (41.49±1.06%) but the highest protein content (38.90±0.93%). Both *Wlewle* and *Nviele* species recorded higher total carbohydrates content, with respective averages of 13.74±0.35% and 13.3±0.41%. But, only *Nviele* species exhibited the highest reducing sugars content (70.62±1.03 mg/100g), whereas *Bebou* species was the most provided in total soluble sugars (3.42±0.19%). When consumed, *Wlewle* species provide higher Caloric Energy (584.05±4.13 kcal/100g) ($p < 0.001$) than *Bebou* (553.51±8.44 kcal/100g) and *Nviele* (536.37±6.7 kcal/100g) species.

3.1.2. <u>Nutritive trend of cucurbits species by investigated region</u>

The nutritive compounds of the 3 cucurbits consumed in both investigated regions are presented in Table 2. In each region, moisture content among species is significantly different ($p < 0.001$). In the Moronou region, it varied from 2.47 ± 0.12% (*Wlewle*) to 3.13 ± 0.11% (*Nviele*) and in the Kabadougou, it went from 2.29 ± 0.75 % (*Nviele*) to 3.02% ±0.13 (Wlewle).

Ash content oscillated between 2.89% ± 0.38 % and 3.00±0.33 % in the Moronou and from 3.66% ± 0.31 to 3.93% ± 0.31 in the Kabadougou. But no significant difference ($p \geq 0.05$) was observed between the three species in each region, although a greater value was observed for *Bebou* in both regions.

Lipid content was statistically different for cucurbits species ($P < 0.001$). In both sites *Wlewle* species exhibited the highest lipid content and *Nviele* species the lowest. Lipid content in *Wlewle* species was

49.88% ± 0.21 from Moronou and 52.25% ± 0.31 from Kabadougou, against respective values of 42.42% ± 0.17 and 40.56% ± 0.45 for *Nviele* species.

Differences in protein content was also observed (*p*<0.001). In Moronou and Kabadougou, *Wlewle* had the lowest protein content (30.65%±0.42 and 27.38%±0.36) and Nviele the highest (39.71 and 38.09%±0.3).

Significant difference in carbohydrate contents was observed between species collected in the Moronou (*P*=0.04), with values varying from 12.30%±0.95 (*Bebou*) and 14%±0.27 (*Wlewle*). However, no obvious difference (*P*= 0.22) in carbohydrate contents of the samples was observed in the Kabadougou where values ranged from 12.45%±1.07 (*Bebou*) to 13.48±0.21 (*Wlewle*).

In both regions, reducing sugar content of species was statistically different. The highest reducing sugars content was obtained from *Nviele* (71.54 mg/100g and 69.71mg/100g), and the lowest from *Bebou* (51.70 and 49.87 mg/100g) as shown in table 2.

Total soluble sugars content was also different (*p*<0.001) in Moronou and Kabadougou where the highest total soluble sugars contents was observed for *Bebou* species (3.60±0.0% and 3.24±0.01%) and the lowest for *Wlewle* species (2.01±0.0 % and 2.06±0.0 %).

Total caloric energy (EC) values calculated from the main caloric nutrients were significantly different from one species to another. In Moronou and Kabadougou, the highest EC value was noticed for *Wlewle* species (580.85±0.35 and 588.60±3.45kcal/100g) and the lowest for *Nviele* species (542.04±0.98 and 530.69±3.89kcal/100g).

Table 1: General Values of the Nutritive compounds from cucurbits species

Species	Moi (%)	Ash (%)	Lip (%)	Prot (%)	Carb (%)	RS (mg/100 g)	TSS (%)	EC (kcal/100g)
Nviele	3.04±0.13 ^{a}	3.28±0.62 ^{a}	41.49±1.06 ^{c}	38.90±0.93 ^{a}	13.30±0.41 ^{a}	70.62±1.03 ^{a}	2.37±0.03 ^{b}	536.37±6.7 ^{c}
Bebou	2.68±0.53 ^{a}	3.91±0.38 ^{a}	45.35±1.47 ^{b}	35.62±1.23 ^{b}	12.37±0.91 ^{b}	50.79±1.03 ^{c}	3.42±0.19 ^{a}	553.51±8.44 ^{b}
Wlewle	2.75±0.33 ^{a}	3.33±0.46 ^{a}	51.07±1.32 ^{a}	29.01±1.83 ^{c}	13.74±0.35 ^{a}	64.96±0.67 ^{b}	2.03±0.03 ^{c}	584.05±4.13 ^{a}
F_{-value}	2.080	3.004	83.081	79.805	7.833	730.916	241.595	78.743
P_{-value}	0.159	0.080	< 0.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001

Table 2. Nutritive compounds of 3 cucurbits consumed in 2 regions of Côte d'Ivoire

Regions	Species	Moi (%)	Ash (%)	Lip (%)	Prot (%)	Carb (%)	RS (mg/100 g)	TSS (%)	EC (kcal/100g)
Moronou	***Nviele***	3.13±0.1 ^{a}	2.89±0.3 ^{a}	42.42±0.17 ^{c}	**38.09±0.3** ^{a}	13.47±0.48 ^{ab}	**71.54±0.21** ^{a}	2.39±0.0 ^{b}	542.04±0.98 ^{c}
	Bebou	3.06±0.05 ^{a}	3.89±0.5 ^{a}	44.02±0.19 ^{b}	36.73±0.22 ^{b}	12.30±0.95 ^{b}	51.70±0.25 ^{c}	**3.60±0.0** ^{a}	545.93±1.79 ^{b}
	Wlewle	2.47±0.1 ^{b}	3.00±0.3 ^{a}	**49.88±0.21** ^{a}	30.65±0.42 ^{c}	**14.00±0.27** ^{a}	64.37±0.24 ^{b}	2.01±0.0 ^{c}	**580.85±0.35** ^{a}
	F_{-value}	37.008	5.214	1302.372	454.871	5.645	5585.572	$1,35*10^{8}$	956.569
	P_{-value}	< 0.001	0.049	< 0.001	< 0.001	0.042	< 0.001	< 0.001	< 0.001
Kabadougou	***Nviele***	2.94±0.05 ^{a}	3.66±0.61 ^{a}	40.56±0.45 ^{c}	**39.71±0.3** ^{a}	13.12±0.32 ^{a}	**69.71±0.31** ^{a}	2.34±0.0 ^{b}	530.69±3.89 ^{c}
	Bebou	2.29±0.75 ^{b}	3.93±.0.31 ^{a}	46.67±0.29 ^{b}	34.52±0.29 ^{b}	12.45±1.07 ^{a}	49.87±0.27 ^{c}	**3.24±0.01** ^{a}	561.09±1.61 ^{b}
	Wlewle	3.02±0.13 ^{a}	3.66±0.31 ^{a}	**52.25±0.31** ^{a}	27.38±0.36 ^{c}	13.48±0.21 ^{a}	65.55±0.14 ^{b}	2.06±0.0 ^{c}	**588.60±3.45** ^{a}
	F_{-value}	28.229	0.394	796.614	1146.062	1.939	5174.570	102611.487	242.161
	P_{-value}	0.001	0.690	< 0.001	< 0.001	0.224	< 0.001	< 0.001	< 0.001

Means±SD with the same superscripts are not different at 5% significance for each nutritive trait (table 1) or per region and nutritive trait (table 2). ***Moi****: moisture content;* ***Ash****: ash content;* ***Lip****: lipid content;* ***Prot****: protein content;* ***Carb****: total carbohydrates content;* ***RS****: reducing sugars content;* ***TSS****: total soluble sugars content;* ***EC****: total caloric energy.* ***Wlewle****: Citrullus lanatus* sp; ***Nviele****: Cucumeropsis mannii* Naudin; ***Bebou****: Lagenaria siceraria* Molina Standl; F_{-value}*: value of the statistical Ficher test;* P_{-value}*: value of the statistical probability.*

3.1.3. Effect of the selected regions on the nutritive compounds of the 3 cucurbits species

Data showing the impact of the geographical location on the nutritive values of the investigated cucurbits species are recorded in Table 3.

***Nviele* species (*Cucumeropsis mannii* Naudin)**
Moisture, ash and total carbohydrate contents of *Nviele* species collected from Moronou were not different ($p \geq 0.05$) from that obtained from Kabadougou. But, samples from the Moronou region exhibited statistically higher lipid, soluble sugars, reducing sugars and caloric energy contents than that from the Kabadougou region. Oppositely, lower protein content was found in Nviele from Moronou compared to Kabadougou.

***Bebou* species *(Lagenaria siceraria* Molina Standl)**
Species from Moronou contained higher moisture content ($p < 0.05$) and were statistically richer in reducing sugar and total soluble sugars than species collected from Kabadougou. However, lipid, protein, and caloric energy contents were statistically higher for species collected in Kabadougou. Ash and Carbohydrate contents of species from both regions were statistically equivalent.

***Wlewle* species (*Citrullus lanatus* sp)**
Compared to the Moronou region, species from Kabadougou exhibited higher contents of moisture, lipid, reducing sugars, total soluble sugars and caloric energy ($p < 0.05$). But the samples collected from Moronou were, in the contrary, much richer in protein. Ash and carbohydrate contents of samples collected from both regions were not different ($p > 0.05$).

Table 3: Impact of the region on the Nutritive compounds of cucurbits species

Species	Regions	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Carbohyd. (%)	Red Sugars (mg/100 g)	TSS (%)	EC (kcal/100g)
Nviele	**Moronou**	**3.13±0.11^{a}**	**2.89±0.38^{a}**	**42.42±0.17 ^{a}**	38.09±0.3 ^{b}	**13.47±0.48^{a}**	**71.54±0.21^{a}**	**2.39±0.0 ^{a}**	**542.04±0.98^{a}**
	Kabadougou	**2.94±0.05^{a}**	**3.66±0.61^{a}**	40.56±0.45^{b}	**39.71±0.3^{a}**	**13.12±0.32^{a}**	69.71±0.31^{b}	2.34±0.0 ^{b}	530.69±3.89^{b}
	F_{-value}	6.850	3.469	44.550	44.433	1.100	720058	32793.185	23.881
	P_{-value}	0.059	0.136	0.003	0.003	0.353	0.001	< 0.001	0.008
Bebou	**Moronou**	**3.06±0.08^{a}**	3.89±0.51^{a}	44.02±0.19 ^{b}	36.73±0.22^{b}	**12.30±0.95^{a}**	**51.70±0.25^{a}**	**3.60±0.0 ^{a}**	545.93±1.79^{b}
	Kabadougou	2.29±0.7^{b}	**3.93±.0.31^{a}**	**46.67±0.29 ^{a}**	**34.52±0.29^{a}**	**12.45±1.07^{a}**	49.87±0.27^{b}	3.24±0.01^{b}	**561.09±1.61^{a}**
	F_{-value}	48.027	0.017	174.173	112.159	0.032	74.864	11137.604	118.290
	P_{-value}	0.002	0.903	< 0.001	< 0.001	0.866	0.001	< 0.001	< 0.001
Wlewle	**Moronou**	2.47±0.12^{b}	**3.00±0.33^{a}**	49.88±0.21 ^{b}	**30.65±0.42^{a}**	**14.00±0.27^{a}**	64.37±0.24^{b}	2.01±0.0 ^{b}	580.85±0.35^{b}
	Kabadougou	**3.02±0.13^{a}**	**3.66±0.31^{a}**	**52.25±0.31 ^{a}**	27.38±0.36^{b}	**13.48±0.21^{a}**	**65.55±0.14^{a}**	**2.06±0.0 ^{a}**	**588.60±3.45^{a}**
	F_{-value}	29.686	6.420	120.736	106.153	6.903	52.965	40029.185	10.111
	P_{-value}	0.006	0.064	<0.001	0.001	0.058	0.002	<0.001	0.034

For each cucurbit species, means±SD with the same superscripts are not different at 5% significance per nutritive parameter. ***Moi****: moisture content;* ***Ash****: ash content;* ***Lip****: lipid content;* ***Prot****: protein content;* ***Carb****: total carbohydrates content;* ***RS****: reducing sugars content;* ***TSS****: total soluble sugars content;* ***EC****: total caloric energy.* ***Wlewle****: Citrullus lanatus* sp; ***Nviele****: Cucumeropsis mannii* Naudin; ***Bebou****: Lagenaria siceraria* Molina Standl; F_{-value}*: value of the statistical Ficher test;* P_{-value}*: value of the statistical probability.*

3.2. Bioactive compounds in cucurbits species

3.2.1. Trend of the polyphenol compounds in cucurbits species

The overall comparison of the polyphenols parameters is summarized in the 1st part of Table 4. *Wlewle* species were statistically richer (P<0.01) in total polyphenols content (141.56±23.97 mg GAE/100 g DM) and flavonoids content (0.19±0.02 mg CE/100 g DM) than *Nviele* and *Bebou* species.

3.2.2. Total polyphenols and flavonoids contents of cucurbits species per region

Total polyphenols and total flavonoids contents of the cucurbit samples collected from the Moronou and the Kabadougou were statistically different (P<0.05) as shown in Table 4.
Polyphenols content of samples collected from Moronou varied from 106.5±1.07 (*Bebou*) to 124.16±1.44 mg GAE/100g DM (*Nviele*). Flavonoids content was between 0.10±0.01 mg CE/100g (*Bebou*) and 0.19±0.03 mg CE/100g (*Wlewle*).
In the Kabadougou region, the lowest polyphenols content was found in *Bebou* seeds (79.3 mg GAE/100g extract), whereas the highest content was obtained in *Wlewle* seeds (163.4 mg GAE/100g) (P<0.001). Flavonoids values varied (P= 0.006) from 0.10±0.01 mg CE/100g (*Bebou*) to 0.19±0.03 mg CE/100g DM (*Wlewle*).

Table 4. Total polyphenols and flavonoids contents of cucurbits species

Regions	Species	Total Polyphenols (mg GAE /100 g DM)	Total Flavonoids (mg CE /100 g DM)
General values	***Nviele***	110.52±14.99 ^{b}	0.14±0.02 ^{b}
	Bebou	92.92±1.07 ^{b}	0.10±0.01 ^{c}
	Wlewle	**141.56±23.97** ^{a}	**0.19±0.02** ^{a}
	F_{-value}	10.69	32.76
	P_{-value}	0.001	< 0.001
Moronou	***Nviele***	**124.16±1.44** ^{a}	0.14±0.03 ^{b}
	Bebou	106.5±1.07 ^{c}	0.10±0.01 ^{c}
	Wlewle	119.72±1.44 ^{b}	**0.19±0.01** ^{a}
	F_{-value}	143.157	17.528
	P_{-value}	< 0.001	0.003
Kabadougou	***Nviele***	96.88±1.12 ^{b}	0.12±0.01 ^{b}
	Bebou	79.34±0.99 ^{c}	0.11±0 ^{b}
	Wlewle	**163.4±1.81** ^{a}	**0.19±0.03** ^{a}
	F_{-value}	3215.017	13.719
	P_{-value}	< 0.001	0.006

Values with different superscript letters (a, b, c) within the same column are statistically different at 5% significance. GAE: Gallic acid equivalent; CE: Cathecin equivalent; DM: dry matter; ***Wlewle****: Citrullus lanatus sp;* ***Nviele****: Cucumeropsis mannii Naudin;* ***Bebou****: Lagenaria siceraria Molina Standl;* $\mathbf{F_{-value}}$*: value of the statistical Ficher test;* $\mathbf{P_{-value}}$*: value of the statistical probability.*

3.2.3. Effect of the selected regions on the bioactive compounds of cucurbits species

Impacts of the geographical region on the bioactive compounds of the 3 curcubits species is presented in Table 5. Data revealed higher total polyphenols for *Nviele* (124.16±1.44 mg GAE /100g DM) and *Bebou* (106.5±1.07 mg GAE /100g DM) collected in the Moronou region whereas the Kabadougou region recorded the greatest total polyphenols value in *Wlewle* samples (163.4±1.81 mg GAE /100g DM).

Table 5. Trend in total polyphenols and flavonoids contents of the 3 cucurbits species from investigated regions.

Species	Regions	Total polyphenols (mg GAE /100 g DM)	Total flavonoids (mg CE /100 g DM)
Nviele	Moronou	**124.16±1.44** ^{a}	**0.14±0.03** ^{a}
	Kabadougou	96.88±1.12 ^{b}	0.12±0.01 ^{a}
	F_{-value}	669.196	0.781
	P_{-value}	<0.001	0.427
Bebou	Moronou	**106.5±1.07** ^{a}	**0.10±0.01** ^{a}
	Kabadougou	79.34±0.99 ^{b}	**0.11±0.01** ^{a}
	F_{-value}	1041.972	4.000
	P_{-value}	<0.001	0.116
Wlewle	Moronou	119.72±1.44 ^{a}	**0.19±0.01** ^{a}
	Kabadougou	**163.4±1.81** ^{a}	**0.19±0.03** ^{a}
	F_{-value}	1069.481	0.125
	P_{-value}	<0.001	0.742

For each cucurbit species, values with different superscript letters (a, b, c) within the same column are statistically different at 5% significance. GAE: Gallic acid equivalent; CE: Catechin equivalent; DM: dry matter; ***Wlewle****: Citrullus lanatus sp;* ***Nviele****: Cucumeropsis mannii Naudin;* ***Bebou****: Lagenaria siceraria Molina Standl;* F_{-value}*: value of the statistical Ficher test;* P_{-value}*: value of the statistical probability*

3.3. Multivariate description of the cucurbit samples

Figure 1 shows the main correlations of the cucurbits samples (A) and the nutritive compounds (B) investigated within the F1-F2 factorial design of the principal components analysis (PCA); both factors (components) assuming 84.85% of the total variability.

Component F1 displayed an eigen-value of 5.62 and expresses 56.25% of total variance, while component F2 recorded eigen-value of 2.86 for 28.60% of total variance. Four correlations types were observed between samples and nutritive traits. Indeed, higher protein and total soluble sugars contents were provided by *Bebou* species from Moronou region, whereas the ash content was higher in *Bebou* species from Kabadougou region. In addition, the lipid, carbohydrate, caloric energy, total polyphenols and flavonoids contents were greater in *Wlewle* species from both regions (Wk and Wm), while the moisture and reducing sugars were higher in *Nviele* species (Nk and Nm).

3.4. Correlations between the studied variables

Correlations between the biochemical characteristics of the cucurbits samples have also been evaluated by the Pearson r index. Table 6 provides a general trend for all the samples analyzed. Significant r values, with *p*-values of 0.01, were considered over |±0.60|. Thus, the caloric energy was directly correlated with lipid content (r= 0.992) but conversely with the protein content (r= -0.981), both macromolecules running in opposite trend (r = - 0.991). This means that cucurbit poor in protein will contain higher amount of lipid and provide high energy calorie upon consumption.

Also, a positive correlation (r=0.68) was observed between flavonoid content and both lipid and carbohydrate meaning that a cucurbit rich in lipid and carbohydrate will contain high amount of flavonoids. Total polyphenols and flavonoids contents record similar trend (r= 0.676), and both were negatively correlated to the protein and total soluble sugars contents (-0.82 < r < -0.60).

Pearson's correlation of *Nviele* and *Wlewle* followed the general trend except *Bebou* where a negative correlation was observed between lipid and polyphenol content.

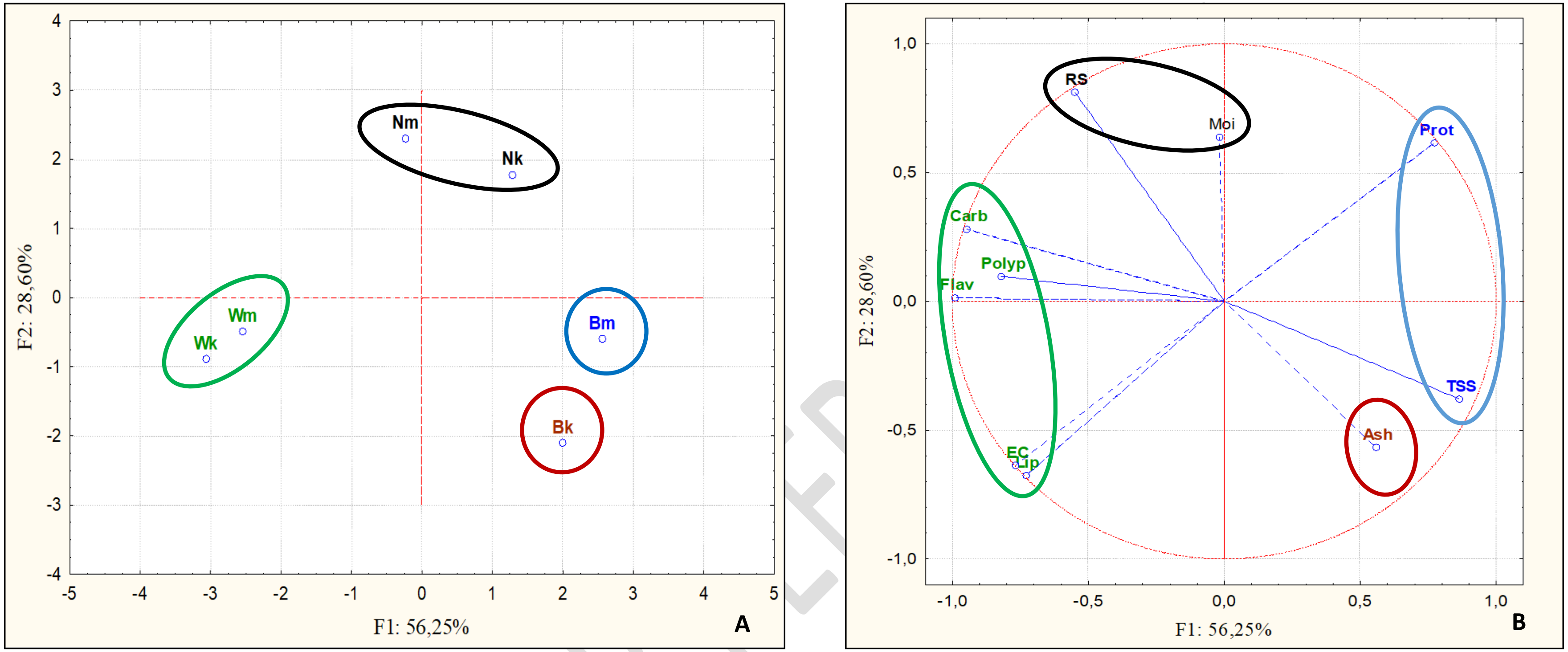

Figure 1: Correlations between the F1-F2 factorial design from the principal components analysis (PCA) of the samples (A) and main nutritive components (B) of the studied cucurbits species

Wk: *Wlewle of Kabadougou region,* ***Wm***: *Wlewle from Moronou region;* ***Nm***: *Nviele from Moronou region;* ***Nk***: *Nviele from Kabadougou region;* ***Bm***: *Bebou from Moronou region;* ***Bk***: *Bebou from Kabadougou region;* ***Moi***: *moisture content;* ***Ash***: *ash content;* ***Lip***: *lipid content;* ***Prot***: *protein content;* ***Carb***: *total carbohydrates content;* ***Flav***: *total flavonoids content;* ***Polyp***: *total polyphenols content;* ***RS***: *reducing sugars content;* ***TSS***: *total soluble sugars content;* ***EC***: *total caloric energy.*

Table 6: Matrix of Pearson correlation r of biochemical parameters of the 3 studied cucurbits

	Moi	Ash	Lip	Prot	Carb	RS	TSS	EC	Polyph	Flav
Mois	1									
Ash	-0.108	1								
Lip	-0.330	-0.004	1							
Prot	0.265	0.011	**-0.991**	1						
Carb	-0.056	**-0.732**	0.265	-0.336	1					
RS	0.454	-0.532	-0.157	0.077	0.588	1				
TSS	-0.076	0.495	-0.344	0.406	**-0.711**	**-0.840**	1			
EC	-0.367	-0.114	**0.992**	**-0.981**	0.356	-0.114	-0.392	1		
Polyph	0.484	-0.256	0.586	**-0.656**	0.452	0.494	**-0.610**	0.572	1	
Flav	-0.056	-0.384	**0.631**	**-0.679**	**0.677**	0.516	**-0.817**	**0.667**	**0.677**	1

Moi: *moisture content;* ***Ash***: *ash content;* ***Lip***: *lipid content;* ***Prot***: *protein content;* ***Carb***: *total carbohydrates content;* ***Flav***: *total flavonoids content;* ***Polyp***: *total polyphenols content;* ***RS***: *reducing sugars content;* ***TSS***: *total soluble sugars content;* ***EC***: *total caloric energy.*

3.5. Estimated Intakes

Based on their nutritive values, an estimated daily intake of cucurbit seeds was calculated based on a 2000-calorie diet for adult female and on a 2500-calorie diet for adult male (Table 7). Valued contributions were obtained for a daily consumption of a cup (~150g) of cucurbits seeds.

The studied cucurbits are rich in lipid and protein, but poor in carbohydrate. For a female, the consumption of a cup (~150g) of each species will cover at least 94% of the lipid requirement; more than 100% for protein, and 40% of the total energy requirement. For a male, at least 76% of lipid requirement, 83% of protein requirement and 32% of energy calorie requirement will be covered.

3.6. Discussion

Three (3) cucurbits consumed in Côte d'Ivoire, *Nviele, Bebou* and *Wlewle*, were collected from 2 producing regions, the Moronou and the Kabadougou. Nutritive and bioactive compounds were analyzed, and comparison was made between species collected from each region as well as between species collected from the two regions. Statistical analysis showed significant difference in the nutritive values and bioactive components of the cucurbit species.

Irrespective of the region and species, moisture content of the 3 cucurbits was around 3%. Differences observed in each region could be ascribed to morphological variations of the seeds, and also to seed maturity stage, drying methods and climatic conditions [12]. Cucurbit moisture content was below the standard (8%) established by FAO [22] for moisture content of grains and seeds. Since moisture content is an index of storage stability, it could be inferred a great stability of cucurbit samples during storage. Indeed, the lower the moisture contents of the seeds, the better their stability and therefore their quality. This low moisture content demonstrated the use of good post-harvest treatments (drying) by the farmers. Despite the existing difference in the climate of the two regions (the Moronou and Kabadougou regions), the final moisture content of the cucurbit samples was not quite different. These results show that women farmers in both regions were able to overcome the impact of climate variability on the moisture content of the samples. Moisture contents varying from 3 to 8% have been reported [6, 8, 23].

Table 7: Estimated daily intake of nutritive and bioactive compounds from the consumption of 150 g (1 cup) of *Nviele, Bebou* and *Wlewle* for adults.

		Lipid			Protein			Carbohydrate			Ash		Polyphenols		Energy value		
Women (2000 kcal diet based)	**Species**	**DRI**	**VC**	**%C**	**DRI**	**VC**	**%C**	**DRI**	**VC**	**%C**	**DRI**	**VC**	**DRI**	**VC**	**DRI**	**VC**	**%C**
		g/day			**g/day**			**g/day**			**g/day**		**mg/day**		**Kcal/day**		
	Nviele	65	61.5	94.62	42	**57**	**135.71**	300	**19.5**	6.5	-	4.92	-	165	2000	804	40.2
	Bebou		67.5	103.85		52.5	125		18	6		5.87		138		829.5	41.47
	Wlewle		**76.5**	**116.92**		43.5	103.57		**19.5**	6.5		5		211.5		**876**	**43.8**
Men (2500 kcal diet based)	**Species**	**DRI**	**VC**		**DRI**	**VC**		**DRI**	**VC**		**DRI**	**VC**	**DRI**	**VC**	**DRI**	**VC**	
		g/day			**g/day**			**g/day**			**g/day**		**mg/day**		**Kcal/day**		
	Nviele	80	61.5	76.87	52	**57**	**109.61**	375	**19.5**	**5.2**	-	4.92	-	165	2500	804	32.16
	Bebou		67.5	84.38		52.5	100.96		18	4.8		5.87		138		829.5	33.18
	Wlewle		**76.5**	**95.63**		43.5	83.65		**19.5**	**5.2**		5		211.5		**876**	**35.04**

DRI: Dietary Recommended Intake; VC: Valued contribution: requirement for a sedentary female or male; %C: percentage of valued contribution

The nature of the species and the geographical location did not affect (p≥0.05) the ash content of cucurbit species. However, highest numerical value was consistently found with the Bebou species. Ash values between 1.5 and 4% for the 3 studied species here have been reported [8, 24, 25]. These values were similar to those reported for peanut [25], but a little lower than that reported for soybean [26]. The cucurbits lipid content is quite high (40-52%) and are reported as oleaginous seeds. When compared to conventional oilseeds, cucurbit lipid content is 2 times higher than that of soy (18–20%) and 13 times higher than that of corn (3.1–5.7%) [27]. Regardless of the region, Wlewle species exhibited higher lipid content than the others and Nviele species the lowest. This result confirmed statement about species and variety effect on the oil content of seeds. Environmental effect on oil content of seeds was also observed by Achu et al [23]. They reported highest oil content for seeds collected from the high savanna and swamp forest. This was in a close agreement with results reported here for species collected from Kabadougou region (high savanna). However, [8] stated that oil content could also be affected by the varieties. Indeed, they pointed out different oil content for different varieties of *Wlewle*. Oil content between 30 and 60% was found during researches conducted on African cucurbits seeds. In this study, direct correlation (r = 0.992) was observed between the oil content of the seeds and their energy calorie value.
Protein content of seeds was between 27 and 38%. Such seeds are characterized as proteagineous. In this study, protein content was lower in *Wlewle* and higher in *Nviele*. Significant difference was also observed for the same species between regions.
Like for lipid, effects of variety and stage of maturity on protein content of *Lagenaria siceraria* (Bebou) have also been reported [6]. This could be the reason of the difference observed between the samples. The fact that lipid and protein contents were oppositely correlated (r = - 0.991) may explain why the *Wlewle* species had the lowest protein content. Generally, in protein-storing seeds, there is a negative correlation between protein and oil accumulation [28] (Borek et al, 2009). This fact has been well documented in soybean [29, 30, 31, 32].

Because of the high protein content of the analyzed cucurbits samples, these seeds could be an alternative source of dietary protein. They could then be used, like peanut, as protein/aminoacids supplements in therapeutic foods in areas facing wars and/or calamities, or in African countries like Côte d'Ivoire where much of the populations live on starchy foods.
Carbohydrate content of the cucurbit samples was not affected by the nature of the species and by the area of the collect. There seems to be a compensatory effect between protein and lipid, which does not have much influence on the total carbohydrates pool.
Polyphenols content of the cucurbit was affected by the species types and le area of collect.
The overall analysis pointed out higher polyphenols content in *Wlewle* and the lowest one in *Bebou*. Moderate direct correlation was noticed between polyphenols and lipid content (r = 0.586) while an opposite correlation was found between polyphenols and protein content (r = -0.656).
Kamda et al. [33] found lower phenolics in *L. siceraria* than in *C. mannii* and *C. lanatus*, species grown in Cameroon. This agrees with results reported in like species in this study. Several parameters could influence the level of secondary metabolics in plants. Studies showed that, not only extrinsic factors (geographic and climatic conditions), and intrinsic factors (genetics), but also the degree of maturity and the post-harvest processes (drying conditions and storage period of seeds) can have great impact on total phenolics contents, even in the same plant species [34, 35].
Flavonoids are the most common and abundantly distributed group of plant phenolic compounds, which usually are very effective antioxidants [36, 37]. Flavonoids contents varied from 0.10±0.01 to 0.19±0.03 mg CE/100g of sample. A similarity (*p*≥0.05) in the flavonoids contents was remarked for each species independently of the region (*p*≤0.05). In both regions, *Wlewle* species had the highest values (0.19±0.03mg CE/100g of matter) and *Bebou* specie the lowest. However equivalent content was observed for *Bebou* and *NViele*. These results suggested that flavonoids contents were more dependent on intrinsic or genetic factors (species) than on environmental or extrinsic factors. Lipid (r = 0.631) and carbohydrate contents (r = 0.677) were directly correlated with flavonoids contents.

4. CONCLUSION

This study assessed the nutritive and bioactive composition of cucurbits from 2 producing regions of Côte d'Ivoire. Based on their lipid and protein contents, cucurbits oilseeds can be classified as oleo-proteaginous seeds. Their biochemical compositions were influenced by the geographic location, the maturity stage and the seed variety. Based on our results and data from previous studies, a daily consumption of a cup of these seeds could cover at least 50% of lipid and 40% of protein requirements and energy calorie. These seeds are an asset in the fight against protein-energy malnutrition and/or in protein fortification programs.

But, before cucurbits seeds can be used as an ingredient for the fortification of several local products or in the industry, a good knowledge of the effect of some treatments (germination, fermentation, roasting, etc.) on their physico-chemical, functional and techno-functional properties is required.

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