

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - <a href="http://www.journalijar.com">www.journalijar.com</a></p> <h2 style="text-align: center;">INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p style="text-align: center;">Article DOI: 10.21474/IJAR01/10093 DOI URL: <a href="http://dx.doi.org/10.21474/IJAR01/10093">http://dx.doi.org/10.21474/IJAR01/10093</a></p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal Homepage: <a href="http://www.journalijar.com">http://www.journalijar.com</a> Journal DOI: 10.21474/IJAR01</p>
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### RESEARCH ARTICLE

#### DIETARY FIBRE AND VITAMIN E CONTENTS OF FIVE CUCURBITACEAE OILSEEDS FROM DIFFERENT REGIONS IN CAMEROON.

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#### Manuscript Info

##### Manuscript History

Received: 14 September 2019

Final Accepted: 16 October 2019

Published: November 2019

##### Key words:-

Dietary fibre, tocopherols, Cucurbit seeds.

#### Abstract

Dietary fibre and vitamin E levels were evaluated in five species of Cucurbit oilseeds (*Cucurbitopsis mannii*, *Cucurbita maxima*, *Cucurbita moschata*, *Lagenaria siceraria* and *Cucumis sativus*) from different agro-ecological zones in Cameroon, to supplement the available food composition data on these seeds. The results showed that the dietary fibre and vitamin E levels did not depend on the region of origin but on the species. The seeds were rich in dietary fibre especially insoluble dietary fibre, which varied from  $15.70 \pm 1.61$  (C. sativus) to  $19.21 \pm 1.29$  g/100 Edible Portion (EP) (C. mannii), making up 74 - 81 % of the total dietary fibre. Soluble dietary fibre ranged from  $4.35 \pm 1.18$  (C. maxima) to  $6.60 \pm 0.86$  g /100g EP (L. siceraria) and total dietary fibre was from  $20.19 \pm 1.81$  (C. sativus) to  $25.13 \pm 1.68$  g /100 g EP (L. siceraria). Alpha ( $\alpha$ ), gamma ( $\gamma$ ) and delta ( $\delta$ ) -tocopherols were present in all the samples. The  $\delta$  - tocopherol level was from  $0.23 \pm 0.32$  (C. moschata) to  $8.52 \pm 1.64$  (C. sativus),  $\alpha$ -tocopherol from  $3.87 \pm 3.14$  (C. maxima) to  $9.84 \pm 6.00$  (L. siceraria) and gamma ( $\gamma$ ) tocopherol from  $87.12 \pm 39.42$  (C. moschata) to  $644.86 \pm 130.50$   $\mu$ g/g of lipid (L. siceraria), making up 94-99% of total tocopherols in these seed oils. Total tocopherols were from  $92.72 \pm 42.19$  (C. moschata) to  $662.03 \pm 134.33$   $\mu$ g /g of lipid (L. siceraria). Consumption of 100 g of these seeds (especially L. siceraria), can cover the dietary fibre needs per day for children of 1 - 8 years old, women and 70% in men and the vitamin E needs per day for all age groups. The dietary fibre and vitamin E (antioxidants) levels in these seeds suggest their potential to fight against overweight, obesity and oxidative stress, preventing cancer. L. siceraria oil could also be added to palm oil during hot treatment, as antioxidant, to increase its stability by preventing free fatty acids from heat oxidation.

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#### Introduction:-

In many countries, economic development is closely linked to the health status of the population. The main challenge is to improve the global human health status without increasing expenditure on health. Non-communicable

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illnesses like type II diabetes mellitus, cancers and cardiovascular diseases, partly linked to inappropriate diet, are increasing in developing countries. In Cameroon, between 2000 and 2012, the percentages of total death linked to these illnesses were 2 %, 3 % and 11 % respectively (WHO, 2014). Insufficient knowledge on the nutritional composition of available foodstuffs and their health benefits, alongside low income, are some of the causes of low consumption of nutrient-rich foods and of malnutrition. Scientific research is therefore needed to discover, based on reliable analytical data, richer and locally available nutritional food sources, which could contribute to improve the health of the population.

Cucurbit seeds are largely cultivated in Nigeria and in *Cote d'Ivoire*, but only on a small scale in Cameroon (Mabaleha *et al.*, 2007). In these countries, the dried decorticated seeds are usually ground into a paste and used to thicken soups, or directly added in vegetable dishes. The Akan people in *Cote d'Ivoire* also use Cucurbit seed meals during the yam festival (Zoro Bi *et al.*, 2003). In Cameroon Cucurbit seeds are the main foods or ingredients during traditional and public ceremonies in some tribes (Fomekong *et al.*, 2008) such as *nguon* (Bamoun people), *ngodo* (Bassa people), birth celebrations (Bamileke people) and traditional marriages in most tribes in Cameroon. In some parts of Cameroon, some of the species are cultivated mostly for their leaves and fruits as vegetables, while the seeds are discarded because they are smaller in size and are very time-consuming to decorticate in great quantities.

Cucurbit (*egusi*) seeds are also considered as important sources of lipids (42.9 – 57.3%) (Achu *et al.*, 2005). Some of these seeds are also used for oil extraction (Schippers, 1997). Their oils contain 18.62 - 20.11% of saturated fatty acids, 14.90 - 32.40% of monounsaturated fatty acids and 35.72 - 56.84% of polyunsaturated acids. They have high levels of linoleic acid (49 – 69%) but very little linolenic acid (0.1 - 0.2%) (Fokou *et al.*, 2009; Anwar *et al.*, 2011 and Kim *et al.*, 2012). These seeds have low levels of carbohydrates (7.11 - 10.01%) (Achu *et al.*, 2005). They also contain minerals like phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese and especially calcium (129.7 - 269.7 mg/100g) in abundance (Nkanyiso *et al.*, 2015). These seeds have high antioxidant capacities (especially *C. sativus*) and the ability of their oils to trap the DPPH<sup>•</sup> radical, is similar to that of olive oil (especially *L. siceraria* oil) (Achu *et al.*, 2016).

Cucurbit seeds also have therapeutic properties (Nmila *et al.*, 2002). In traditional medicine, Cucurbit seed kernels are commonly used in renal disorders such as kidney and bladder stones, painful and burning micturition, ulcers in the urinary tract, suppression of urine, jaundice, chronic fevers, inflammation of the liver and kidney (Ullah *et al.*, 2015). Other studies have shown that Cucurbit oilseeds are therapeutic foods due to their high contents in  $\alpha$ -galactosidase, dihydroxytriterpenes, sitosterol, carotenes, triterpenoids and flavonoids (Akihisa *et al.*, 1997). The winter melon (*Benincasa hispida*) is rich in  $\alpha$ -tocopherol (31.1-207.6 mg/kg) and  $\delta$ -tocopherol (60.4-146.0 mg/kg). It also has high levels of  $\beta$ -sitosterol (54.62– 60.50%), campesterol (15.10–18.50%), stigmasterol (11.00–14.30%) and  $\Delta^5$ -avenasterol (6.40–8.14 %) (Anwar *et al.*, 2011). The tocopherols have the capacity to prevent the oxidation of unsaturated fatty acids in cell membranes and phytosterols help to prevent chronic diseases (Phillips *et al.*, 2005; Ryan *et al.*, 2007). Other studies have reported that Cucurbit seeds could be used to treat benign prostatic hyperplasia, to reduce blood cholesterol and to decrease the risk of certain types of cancers because of its high  $\beta$ -sitosterol content (Tsai *et al.*, 2006). In addition, a review of scientific studies from 1966 to 2005 revealed that  $\alpha$ -tocopherol might reduce the risk of Parkinson's disease (Etminan *et al.*, 2005). Similarly, a positive correlation has been shown between tocopherol intake and prevention of Alzheimer's disease (Li *et al.*, 2012). Cucurbit seeds also contain high levels of dietary fibre, which contribute in weight loss, hence preventing or reducing cardiovascular illnesses and type 2 diabetes (Liu *et al.*, 2003 and Galisteo *et al.*, 2008).

In view of all these nutritional and health benefits of Cucurbit (*egusi*) seeds, very little data is available on the dietary fibre and vitamin contents of these seeds especially from Cameroon. This study therefore analysed the contents of insoluble, soluble and total dietary fibre and a natural antioxidant (vitamin E) in the seeds of *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata*, *Lagenaria siceraria* and *Cucumis sativus* from different agro-ecological zones in Cameroon.

## Materials and Methods:-

### Collection and identification of samples

The seeds, dried and decorticated under local conditions by the farmers, were collected from three different agro-ecological zones in Cameroon, from September to November 2014. They comprised 29 samples of 5 species, identified at the National Herbarium in Yaoundé (Table 1).

**Table 1:-**Identification and geographical origin of the Cucurbit seeds sampled from different regions of Cameroon

Botanical identification	Identification Code at the National Herbarium of Cameroon	Agro-ecological Zone	Region	Locality
<i>Lagenaria siceraria</i> (Molina) Standl.	Jacques Felix's collection n°: 8540 registered under the n°: 24388 / FRSC.	High Plateaus of the West	North West	Mankon Ndop Wum
		High Plateaus of the West	West	Bafoussam Dschang Bamougoum
<i>Cucurbita moschata</i> (Lam.) Poir.	Jacques Felix's collection no: 8539 registered under the no: 24387 / FRSC.	High Plateaus of the West	North West	Mankon Zhoa Wum
		High Plateaus of the West	West	Dschang Foumbot Bamougoum
<i>Cucumeropsis mannii</i> Naudin	Westphal's collection no: 9389 registered under the no: 42485 / NHC.	High Plateaus of the West	North West	Bangolan Wum Essimbi
		High Plateaus of the West	West	Baloum Bamougoum Galim
<i>Cucumis sativus</i> L.	Westphal's collection no: 9062 registered under the no: 42457 / HNC.	Humid Forest	Centre	Bafia Mbalmayo Obala
		Swamp Forest	South West	Buea Mamfe Mundemba
<i>Cucurbita maxima</i> Duch.	Compared to the botanical collection of Westphal no: 9564 registered under the no: 42514 / NHC.	High Plateaus of the West	West	Galim Bangangte Foumbot
		High Plateaus of the West	North West	Kom Bangolan

FRSC: Forest Research Section of Cameroon, NHC: National Herbarium of Cameroon

#### Determination of water content

The water content of the seeds was determined according to the method of **AOAC (1980)**. About 10 g of seeds were dried in an air-ventilated oven (Mermmert /Beschickung-Loading, Modell 100 - 800) at 105 °C until constant weight. The water content was expressed in g / 100g of fresh weight (FW).

#### Treatment of samples

The decorticated seeds were dried in an air-ventilated oven (Mermmert /Beschickung-Loading, Modell 100 - 800) at 50 °C for 48 hours and ground with an electric grinder (Blender BI-500, Sinsung, Singapore). The seed flours were put in air-tight bottles and stored at 4 °C prior to analyses.

#### Lipid extraction

The total lipids of the seeds were extracted at room temperature with the solvent mixture extraction method described by **Folch et al. (1957)** with slight modifications. Dichloromethane which is more volatile than chloroform was used instead chloroform during the extraction step. 10 g of oven-dried seed powder were mixed with 50 mL of dichloromethane/methanol (2/1 v/v) solvent in a 100 mL beaker. The content was stirred for 20 minutes and filtered through a filter paper (Whatman N° 2). The operation was repeated on the residue. 20 mL of 0.9% NaCl was added to the filtrates. The mixture was poured into a separatory funnel, vigorously stirred and allowed to stand for 24 hours at room temperature for phase separation to take place. The lower phase that was made up of dichloromethane and dissolved lipids was collected into dark bottles and covered with a thin paper for 48 hours at room temperature, in the dark, for the solvent to evaporate. The extracted oil was stored at 4 °C and later used for the analysis of vitamin

E. The extraction residues, representing the defatted samples were dried at room temperature (by allowing the traces of solvent to evaporate) for 48 h and weighed. They were later ground and used for the analysis of dietary fibre.

#### **Analysis of dietary fibre: soluble, insoluble and total dietary fibre**

Dietary fibre was analysed using the enzymatic method described by **Prosky *et al.* (1992): AACC method 32-05, AOAC 985. 29a**. The defatted sample was sequentially digested using the Megazyme kits containing three thermostable enzymes:  $\alpha$ -amylase (Megazyme cat. no. E-BLAAM), protease (Megazyme cat. no. E-BSPRT) and amyloglucosidase (Megazyme cat. no. E-AMGDF). The fibre content was corrected using the blank, residual protein and ash contents as described below.

#### **Enzymatic digestion**

Exactly 1 g of defatted sample was weighed in duplicate into two 400 mL beakers and mixed with 50 mL of 0.08 M phosphate buffer (pH 6.0). The mixtures were well homogenized. 50  $\mu$ L of heat-stable  $\alpha$ -amylase (Megazyme cat. no. E-BLAAM) were added into each beaker. The beakers were covered with aluminium foil and incubated in a water bath at 97 °C for 35 minutes with stirring every 5 minutes. The mixtures were removed from the water bath, allowed to cool to 60 °C and the pH was adjusted to 7.5 by adding 1.5 mL of NaOH, 0.275 N. 100  $\mu$ L of protease (Megazyme cat. no. E-BSPRT) were added. The temperature of the water bath was adjusted to 60 °C and the beakers incubated at this temperature for 30 minutes with shaking every 5 minutes. After cooling, the pH was adjusted to 4.5 with 5 mL of 0.325 N HCl. 200  $\mu$ L of amyloglucosidase (Megazyme cat. no. E-AMGDF) were then added and the flasks were again incubated at 60 °C for 30 minutes.

#### **Assay of insoluble fibre**

The mixtures were filtered on previously weighed ( $P_1$ , g) sintered glass N° 1 and the residues rinsed with two portions of 10 mL of preheated distilled water at 70 °C. The filtrates, containing the soluble fibre were collected into 250 mL beakers for the assay of soluble dietary fibre. The residues were washed again two times with 10 mL ethanol 95% and 10 mL acetone, and finally dried in an oven at 105 °C for 12 hours. The sintered glasses with the residues were then cooled in a desiccator for 1 hour and weighed ( $P_2$ , g). The difference in weight gave the mass of the insoluble fibre. One residue was analysed for protein, determined by the Kjeldahl method and the second residue was incinerated for ash content determination (**AOAC, 1980**). The content of the uncorrected insoluble fibre, reported to 100 g of defatted sample, was corrected with its protein and ash contents, in order to obtain the insoluble fibre content (g /100g) of the oven-dried, ground and defatted seeds.

#### **Assay of Soluble fibre**

The soluble fibre contained in the filtrate was precipitated for 1 hour with 320 mL of hot ethanol 95%. The resulting mixture was filtered through a weighed ( $P'_1$ , g) sintered glass N° 1. The residue was washed two times with 10 mL of distilled water, two times with 15 mL of 78% ethanol (v/v), two times with 15 mL of 95% ethanol, and two times with 15 mL of pure acetone. The residue was then dried in an oven at 105 °C for 12 hours, the glass was cooled in a desiccator for 1 hour and weighed ( $P'_2$  g). The difference in weight gave the mass of the soluble fibre. The residual protein content was determined by the Kjeldahl method in one of the residues. The factor 6.25 was used as conversion factor of nitrogen to protein. The residual ash in the other residue was determined by weighing after incineration at 525 °C for 5 hours (**AOAC, 1980**). Protein and ash contents were subtracted from the weight of the dry residue to correct the soluble fibre content.

#### **Calculation of total dietary fibre**

The total dietary fibre was defined as the sum of the soluble fibre and insoluble fibre.

Another flask, the blank containing only enzyme, reagents and solvent, underwent the same procedure as the trials. The insoluble, soluble and total dietary fibre contents were expressed in g /100g Edible Portion (EP).

#### **Analysis of vitamin E**

Vitamin E was analysed directly from the lipid extracts using Ultra-High Performance Liquid Chromatography (UHPL) paired with fluorescence detection according to Buttriss and Diplock (**1984**) with the method described in **Awada *et al.* (2013)**. Briefly, the separation, detection and quantification of tocopherol isomers was performed with an HPLC apparatus (RS-LC Ultimate 3000, DIONEX) equipped with a fluorescence detector ( $\lambda_{ex}$  = 295 nm,  $\lambda_{em}$  = 330 nm) and fitted with a silica column (Uptisphere strategy SI, L x d: 150x4.6 mm, granulometry: 2.2  $\mu$ m, INTERCHIM).

After injection of the oil samples (40  $\mu$ L) dissolved in n-hexane at 20 mg/mL (sample temperature, 10°C), the separation of isomers was achieved in isocratic mode with n-hexane /terbutylmethyl ether (90/10, v/v) at a flow rate of 1.0 mL/min and a pressure of 80 - 90 bars. The temperature of the column oven was regulated at 30°C. The identification of the isomers was performed by comparing the peak retention times with that of a standard mixture of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -tocopherols (tocopherol set Calbiochem Cat : 613424-1SET) and  $\gamma$ -tocotrienol standard (Cayman Chemical Company, ref: 10008494). All determinations were performed in triplicate. The quantification was made with external calibration curves prepared from a range of calibration solutions made with the same standards. The results were expressed as  $\mu$ g/g of lipid and  $\mu$ g /g EP of seed.

### Statistical Analyses:-

Analysis of variance, ANOVA was done (at a probability level of 5% with the software STATGRAPHICS plus 5.0), to evaluate the interaction between the species and their ecological region of cultivation and between the various species of seeds without considering the region of cultivation. Where the ANOVA test indicated significant differences, the Least Significance Difference, LSD test (at the same probability level) was used to locate these differences.

### Results and Discussion:-

#### Water content

The water contents of the Cucurbit oilseeds are presented on Table 2. They ranged from  $3.94 \pm 0.40$  (*C. maxima*) to  $5.69 \pm 0.73$  g / 100g fresh weight, FW (*C. sativus*) and did not depend on the region of cultivation ( $p > 0.05$ ) but on the specie. There was no significant difference between the water contents of *L. siceraria* ( $4.85 \pm 0.17$ ), *C. moschata* ( $5.30 \pm 0.32$ ) and *C. mannii* ( $5.32 \pm 0.39$ ). These values were similar to those found for Cucurbit seeds from Niger, *Luffa aegyptiaca* ( $5.25 \pm 0.35$ ) and *Luffa cylindrica* ( $5.05 \pm 0.34$ ) (Sadou et al., 2005) and *C. sativus* ( $5.65 \pm 0.97$ ) from Cameroon. They were however lower than water contents from previous studies on *C. moschata* ( $8.21 \pm 1.47$ ), *C. maxima* ( $6.94 \pm 1.92$ ), *C. mannii* ( $6.49 \pm 0.62$ ) and *L. siceraria* ( $6.09 \pm 0.20$ ) (Achu et al., 2005). These different values especially from Cameroon seeds may be due to the state of maturity of the seeds, the season of harvest, the conditions and duration of drying and storage of the seeds by the farmers before they were bought and taken to the laboratory for analyses. These values are also similar to those obtained by Kershaw and Hackett (1987) for other edible oilseeds such as cottonseeds (6.46%), peanuts (4.58%), palm kernel (5.31%), sesame (4.60%) and sunflower seeds (6.58%). The seeds were bought already dried locally by the farmers. The water content in these seeds was generally lower than 6%. The low water content in these Cucurbit seeds allows them to be free from microbial growth, hence enabling preservation for a long time (closed to a year and even more) even at room temperature, if they are well dried under local conditions.

#### Insoluble, soluble and total dietary fibre contents

The dietary fibre contents of the seeds are shown on Table 2.

**The insoluble dietary fibre (IDF)** content varied from  $15.70 \pm 1.61$  (*C. sativus*) to  $19.21 \pm 1.29$  g /100 g EP (*C. mannii*). It did not depend on the region of cultivation ( $p > 0.05$ ) but on the specie. *C. mannii* ( $19.21 \pm 1.29$ ) and *L. siceraria* ( $18.53 \pm 1.67$ ) had similar IDF contents, significantly higher than the rest of the seeds. The seeds analysed in the present study had lower IDF content than bambara groundnuts ( $20.68 \pm 0.6$ ), black beans ( $21.61 \pm 0.7$ ), white beans ( $21.69 \pm 0.4$ ) and peas ( $22.21 \pm 0.9$  g /100g EP) (Tanya et al., 1997). Insoluble fibre is insoluble in water. It is made up of hemicellulose, cellulose and lignin (Howarth et al., 2001). It increases the duration of chyme in the stomach, increases satiety and eases faecal excretion by improving hydration (Chaplin, 2004). The insoluble dietary fibre made up the bulk of dietary fibre in these seeds (74 - 81 % of the total dietary fibre). These seeds therefore have potential for easing faecal excretion and preventing constipation (Pereira et al., 2001).

**The soluble dietary fibre (SDF)** levels varied from  $4.35 \pm 1.18$  (*C. maxima*) to  $6.60 \pm 0.86$  g /100 g EP (*L. siceraria*). They were independent of the region of cultivation ( $p > 0.05$ ) but significantly depended on the Cucurbit specie ( $p < 0.05$ ). The SDF content for *L. siceraria* ( $6.60 \pm 0.86$ ) was similar to that of *C. mannii* ( $5.87 \pm 0.53$ ), but higher than those of *C. sativus* ( $4.49 \pm 0.45$ ) and *C. maxima* ( $4.35 \pm 1.18$  g /100 g EP) (LSD test,  $p < 0.05$ ). The content for *C. moschata* seeds ( $5.32 \pm 0.62$  g /100 g EP) was not significantly different from those of the other species. The content of SDF makes up 19 - 26 % of the total dietary fibre in these Cucurbit seeds. These seeds are richer in SDF than black beans ( $3.85 \pm 0.3$ ), bambara groundnut ( $3.15 \pm 0.4$ ) and peas ( $2.44 \pm 0.1$  g /100 g EP) (Tanya et al., 1997). Soluble fibre are components of fibre that easily dissolve in water. They are made up of pectin,

gums and mucilage (Hanai *et al.*, 1997). Soluble fibre can decrease low-density lipoprotein (LDL) cholesterol levels and contribute to regulate blood glucose and insulin levels (Ripsin *et al.*, 1992; Brown *et al.*, 1999).

The total dietary fibre (TDF) levels ranged between  $20.19 \pm 1.81$  (*C. sativus*) and  $25.13 \pm 1.68$  g /100g EP (*L. siceraria*). The TDF content of *C. moschata* ( $21.30 \pm 2.34$ ) was similar to those of *C. sativus* ( $20.19 \pm 1.81$ ) and *C. maxima* ( $22.75 \pm 0.24$ ), while that of *C. mannii* ( $25.08 \pm 1.31$ ) was similar (LSD test,  $p > 0.05$ ) to that of *L. siceraria* ( $25.13 \pm 1.68$ ) with higher values. The variation in dietary fibre levels of these seeds could be due to their genetic diversity and the state of maturity of the seeds. The values of *L. siceraria* and *C. mannii* were closer to those of black beans (25.46) and peas (24.65) whereas those of the other species were similar to that of bambara groundnut (23.82 g /100 g EP) (Tanya *et al.*, 1997). Following the recommendations of the American Diabetes Association, 100g of these Cucurbit oilseeds (15.70 - 19.21 g /100 g EP) therefore contain the required insoluble dietary fibre (16g/day) whereas, their soluble fibre is lower than 8g/day. The recommended allowances for dietary fibre from 1 - 8 years is 19 - 25g/day, from 9 - 13 years is 26 - 38g/day, from 19 - 50 years is 25 - 38 g/day and more than 50 years is 21 - 30g/day in women and men of the same age respectively. (Institute of Medicine, 2005). 100g of these Cucurbit seeds contained 20.19 – 25.13g EP of TDF. This means that consumption of 100 g of these seeds especially *L. siceraria*, can cover the dietary fibre needs per day for children of 1 - 8 years old and women, and also cover about 70% of fibre needs in men.

The high contents of dietary fibre in these seeds are mostly due to the high levels of insoluble dietary fibre, IDF. The similarity of the values of insoluble and soluble dietary fibre in *C. mannii*, *C. maxima* and *C. moschata* could be due to their vegetal matrix comparable to those of *L. siceraria* and *C. sativus*. These Cucurbit seeds would therefore contain more cellulose, hemicellulose and lignin (IDF fraction) than pectin, gums and mucilage, which are components of soluble fibre. IDF could help facilitate digestion and improve the functioning of the digestive system. The SDF (pectin, gum and mucilage), in these seeds present in lower levels than IDF could also be useful in regulating LDL cholesterol, blood glucose and insulin levels (Meyer *et al.*, 2000).

**Table 2:-**Water and dietary fibre (Insoluble, IDF; Soluble, SDF and Total, TDF) contents of Cucurbit oilseeds from Cameroon

Species	Region	Locality	Water (g /100g FW)	IDF (g /100g EP)	SDF (g /100g EP)	TDF (g /100g EP)
<i>L. siceraria</i>	West North	Mankon	4.66	19.82	5.90	25.73
		Ndop	5.01	18.61	8.16	26.78
		Wum	4.63	15.24	6.70	21.94
			<b>4.77 ± 0.21</b>	<b>17.89 ± 2.37</b>	<b>6.92 ± 1.14</b>	<b>24.82 ± 2.54</b>
	West	Bafoussam	4.95	19.35	5.75	25.10
		Dschang	5.02	19.40	6.64	26.04
		Bamougoum	4.84	18.73	6.45	25.18
			<b>4.94 ± 0.10</b>	<b>19.16 ± 0.38</b>	<b>6.28 ± 0.47</b>	<b>25.44 ± 0.52</b>
		<b>Mean</b>	<b>4.85 ± 0.17<sup>a</sup></b>	<b>18.53 ± 1.67<sup>b</sup></b>	<b>6.60 ± 0.86<sup>c</sup></b>	<b>25.13 ± 1.68<sup>c</sup></b>
<i>C. moschata</i>	North West	Mankon	4.73	11.17	6.29	17.45
		Zhoa	5.55	15.05	5.19	20.23
		Wum	5.21	18.65	5.67	24.32
			<b>5.16 ± 0.41</b>	<b>14.96 ± 3.64</b>	<b>5.71 ± 0.55</b>	<b>20.67 ± 3.46</b>
	West	Dschang	5.36	16.73	6.00	22.73
		Foumbot	5.34	15.83	5.41	21.24
		Bamougoum	5.62	17.27	4.57	21.84
			<b>5.44 ± 0.16</b>	<b>16.61 ± 0.73</b>	<b>5.63 ± 0.72</b>	<b>21.94 ± 0.75</b>
		<b>Mean</b>	<b>5.30 ± 0.32<sup>bc</sup></b>	<b>15.78 ± 2.58<sup>a</sup></b>	<b>5.32 ± 0.61<sup>abc</sup></b>	<b>21.30 ± 2.34<sup>a</sup></b>
<i>C. mannii</i>	North West	Bangolan	5.16	20.10	5.43	25.53
		Wum	5.44	19.98	6.26	26.24
		Essimbi	5.18	18.63	5.33	23.96
			<b>5.26 ± 0.15</b>	<b>19.57 ± 0.81</b>	<b>5.67 ± 0.51</b>	<b>25.24 ± 1.17</b>
	West	Baloum	4.91	19.83	6.53	26.36
		Bamougoum	5.20	19.89	5.44	25.33

		Galim	6.05	16.82	6.25	23.06
			<b>5.38 ± 0.59</b>	<b>18.84 ± 1.76</b>	<b>6.07 ± 0.57</b>	<b>24.92 ± 1.69</b>
		<b>Mean</b>	<b>5.32 ± 0.39<sup>bc</sup></b>	<b>19.21 ± 1.29<sup>b</sup></b>	<b>5.87 ± 0.53<sup>c</sup></b>	<b>25.08 ± 1.31<sup>c</sup></b>
<i>C. sativus</i>	Centre	Bafia	5.36	17.90	4.70	22.61
		Mbalmayo	5.70	13.02	4.29	17.30
		Obala	4.86	15.43	3.75	19.18
			<b>5.31 ± 0.42</b>	<b>15.45 ± 2.44</b>	<b>4.25 ± 0.48</b>	<b>19.70 ± 2.69</b>
	South West	Buea	5.92	15.93	4.47	20.39
		Mamfe	6.98	16.58	4.70	21.27
		Mundemba	5.28	15.33	5.05	20.37
			<b>6.06 ± 0.86</b>	<b>15.94 ± 0.63</b>	<b>4.74 ± 0.29</b>	<b>20.68 ± 0.52</b>
<i>C. maxima</i>	West		<b>Mean</b>	<b>5.69 ± 0.73<sup>c</sup></b>	<b>15.70 ± 1.61<sup>a</sup></b>	<b>4.49 ± 0.45<sup>a</sup></b>
						<b>20.19 ± 1.81<sup>a</sup></b>
		Galim	4.15	17.52	5.38	22.89
		Bangangte	3.73	18.70	4.13	22.83
	North West	Foumbot	4.33	17.58	5.02	22.61
			<b>4.07 ± 0.31</b>	<b>17.93 ± 0.66</b>	<b>4.84 ± 0.64</b>	<b>22.77 ± 0.15</b>
		Kom	3.34	17.59	4.82	22.42
		Bangolan	4.16	20.60	2.42	23.02
			<b>3.75 ± 0.58</b>	<b>19.09 ± 2.12</b>	<b>3.62 ± 1.70</b>	<b>22.72 ± 0.43</b>
		<b>Mean</b>	<b>3.94 ± 0.40<sup>b</sup></b>	<b>18.40 ± 1.32<sup>b</sup></b>	<b>4.35 ± 1.18<sup>a</sup></b>	<b>22.75 ± 0.24<sup>ab</sup></b>

EP = Edible portion of seeds, FW = Fresh Weight

#### Anova:

There was no significant difference ( $p > 0.05$ ;  $n = 3 \times 2$ ) between the mean values of the parameters studied (water, IDF, SDF, TDF) for the same specie from the two regions of cultivation.

In the same column, mean values with different letter superscripts in the same column indicate significant differences between given Cucurbit species ( $p < 0.05$ ;  $n = 6$ ).

#### Vitamin E content

Tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ - tocopherol) and tocotrienols were analysed in the oils extracted from the seeds. The results are shown on Table 3. These results showed the absence of all forms of tocotrienols.  $\beta$ -tocopherol was also absent while the other three forms of tocopherols were present in all the samples. The tocopherol contents did not depend on the region of origin ( $p > 0.05$ ) but on the specie ( $p < 0.05$ ).

**Alpha ( $\alpha$ )-tocopherol** was absent in *C. mannii* oils. It ranged from  $3.87 \pm 3.14$  ( $1.42 \pm 1.16$   $\mu\text{g}$  /g EP of seed) (*C. maxima*) to  $9.84 \pm 6.00$   $\mu\text{g}$  /g of lipid ( $3.12 \pm 1.96$   $\mu\text{g}$  /g EP of seed) (*L. siceraria*).  $\alpha$ -tocopherol makes up **0.9 - 5.8%** of the total tocopherols in these oils. The value for *L. siceraria* was significantly higher than ( $p < 0.05$ ) those of the other species. These values were higher than those of *Luffa* (Cucurbit seeds from Niger) ( $4.20 \pm 2.38$   $\mu\text{g}$  /g of lipid) (**Sadou et al., 2005**) except *C. maxima* and *C. sativus* ( $4.21 \pm 4.41$   $\mu\text{g}$  /g of lipid). The  $\alpha$ -tocopherol levels of the oils in the seeds studied were much lower than those of *C. maxima* ( $27.1 - 75.1$   $\mu\text{g}$  /g of lipid) from New-Zealand and Africa, cultivated in an experimental farm in Iowa, United States of America (**Stevenson et al., 2007**), *C. maxima* ( $128 \pm 14.42$   $\mu\text{g}$  /g of lipid) from Tunisia (**Rezig et al., 2012**) and *C. maxima* ( $203 - 353$   $\mu\text{g}$  /g of lipid) from Serbia (**Naziri et al., 2016**). This large difference in values may be due to the differences in the areas and methods of cultivation of the seeds, treatment of the seeds after harvest and the methods of extraction of the oils for vitamin E analysis.  $\alpha$ - tocopherol decreases lipid peroxidation in food oils during processing and storage. It Inhibits protein kinase C- mediated monocyte superoxide production, smooth muscle cell proliferation, platelet aggregation and adhesion. Alpha tocopherol modulates inflammatory response by inhibiting 5-lipoxygenase, which in turn decreases monocyte IL-1 $\beta$  release (**Mathur et al., 2015**).

The oils contained mostly **gamma ( $\gamma$ )-tocopherol**, which varied from  $87.12 \pm 39.42$  ( $39.34 \pm 12.17$   $\mu\text{g}$  /g EP of seed) (*C. moschata*) to  $644.86 \pm 130.50$   $\mu\text{g}$  /g of lipid ( $208.61 \pm 49.09$   $\mu\text{g}$  /g EP of seed) (*L. siceraria*). The high value in *L. siceraria* was followed by that of *C. sativus* ( $453.45 \pm 61.79$   $\mu\text{g}$  /g of lipid) ( $190.12 \pm 30.56$   $\mu\text{g}$  /g EP of seed) which were significantly higher ( $p < 0.05$ ) than those of *C. mannii* ( $363.33 \pm 111.60$   $\mu\text{g}$  /g of lipid) ( $79.44 \pm$

19.15  $\mu\text{g/g}$  EP of seed) and *C. maxima* ( $206.28 \pm 136.17 \mu\text{g/g}$  of lipid) ( $76.21 \pm 43.30 \mu\text{g/g}$  EP of seed).  $\gamma$ -tocopherol makes up **94 - 99 %** of the total tocopherols in these oils. *C. moschata* and *C. maxima*, which had lower levels of  $\gamma$ -tocopherol, were however richer in this compound than those of pumpkin cultivars from New-Zealand and Africa, cultivated in an experimental farm in Iowa, United States of America such as chacha (74.9), hyvita (77.1) and sweet mama ( $75.8 \mu\text{g/g}$  of lipid), while the value in *L. siceraria* was higher than that of big max ( $492.8 \mu\text{g/g}$  of lipid) (Stevenson *et al.*, 2007) and Luffa ( $472.18 \pm 186.70 \mu\text{g/g}$  of lipid) from Niger (Sadou *et al.*, 2005). The  $\gamma$ -tocopherol content of *C. maxima* was also higher than that of this specie from Tunisia ( $113.66 \pm 1.52 \mu\text{g/g}$  of lipid) (Rezig *et al.*, 2012). *C. mannii* oil (363.33) showed similar values to that of other edible oils such as groundnut oil ( $360 \mu\text{g/g}$  of lipid), while all the samples studied contained lower  $\gamma$ -tocopherol levels than soybean oil ( $700 \mu\text{g/g}$  of lipid) (Luciani *et al.*, 2001). The content of *L. siceraria* was similar to that of *C. maxima* (647 - 775  $\mu\text{g/g}$  of lipid) from Serbia (Naziri *et al.*, 2016).  $\gamma$ -tocopherol has most of the effects of  $\alpha$ -tocopherol and is more potent. It removes peroxynitrite-derived reactive nitrogen species. It traps electrophilic nitrogen oxide in cell membranes during inflammation via formation of 5-nitro- $\gamma$ -tocopherol. It inhibits smooth muscle cell proliferation, decreases platelet aggregation and delays intra-arterial thrombus formation (Mathur *et al.*, 2015).

**Delta ( $\delta$ )-tocopherol** content varied from  $0.23 \pm 0.32$  ( $0.12 \pm 0.22 \mu\text{g/g}$  EP of seed) (*C. moschata*) to  $8.52 \pm 1.64 \mu\text{g/g}$  of lipid ( $3.53 \pm 0.44 \mu\text{g/g}$  EP of seed) (*C. sativus*). The value for *C. sativus* was significantly higher than ( $p < 0.05$ ) those of the other species.  $\delta$ -tocopherol makes up **0.2 - 2.4 %** of the total tocopherols in these oils. These values were lower than those of pumpkin cultivars ( $35.3 - 1109.7 \mu\text{g/g}$  of lipid) from New-Zealand and Africa, cultivated in an experimental farm in Iowa, United States of America (Stevenson *et al.*, 2007) and *C. maxima* from Tunisia ( $177 \pm 14.17 \mu\text{g/g}$  of lipid) (Rezig *et al.*, 2012).  $\delta$ -tocopherol has potent anti-inflammatory and antioxidant properties by inhibiting cyclooxygenase and 5-lipoxygenase activities. Like other tocopherols,  $\delta$ -tocopherol contributes to inhibit smooth muscle cell proliferation (Mathur *et al.*, 2015).

**The total tocopherol** levels ranged from  $92.72 \pm 42.19$  ( $61.34 \pm 51.16 \mu\text{g/g}$  EP of seed) (*C. moschata*) to  $662.03 \pm 134.33 \mu\text{g/g}$  of lipid ( $214.17 \pm 50.47 \mu\text{g/g}$  EP of seed) (*L. siceraria*). The high value of *L. siceraria* was followed by that of *C. sativus* ( $466.18 \pm 66.88 \mu\text{g/g}$  of lipid) ( $195.30 \pm 31.28 \mu\text{g/g}$  EP of seed) which were significantly higher than those of the other seeds. The tocopherol content of the lipids from *C. maxima* ( $215.38 \pm 142.72$ ) was lower than that of this specie from Tunisia ( $418.66 \pm 33.36 \mu\text{g/g}$  of lipid) (Rezig *et al.*, 2012). Apart from *L. siceraria*, the tocopherol values of the sample in this study were lower than those of *C. maxima* seeds ( $589.4 - 1234.2 \mu\text{g/g}$  of lipid) from New-Zealand and Africa, cultivated in an experimental farm in Iowa, United States of America (Stevenson *et al.*, 2007).

Generally, the results of this study showed low levels of  $\delta$ -tocopherol (0.2 - 2.4%) followed by  $\alpha$ -tocopherol (0.9 - 5.8%) and high levels of  $\gamma$ -tocopherol (94 - 99%).  $\alpha$ -tocopherol was absent in *C. mannii*. These Cucurbit seed oils had good vitamin E levels (total tocopherols) ( $92.72 - 662.03 \mu\text{g/g}$  of lipid) ( $79.59 - 214.17 \mu\text{g/g}$  of seed), with *L. siceraria* having the highest levels. The recommended allowances for vitamin E (as alpha tocopherol equivalence) from 1-13 years is 6 -11 mg/day, above 14 years is 15 mg/day and in pregnancy and lactation is 15-19 mg/day (Institute of Medicine, 2000). 100 g of these Cucurbit seeds contained 7.9 - 21.4 mg of vitamin E. This means that consumption of 100 g of these seeds especially *L. siceraria*, can cover the vitamin E needs per day for all age groups. Among the different forms of tocopherol,  $\alpha$ -tocopherol is the most biologically active form, while  $\gamma$ -tocopherol is the most abundant form in foods of plant origin. Vitamin E is a natural antioxidant (Jordan and Pattison, 1996).  $\alpha$ -tocopherol and  $\gamma$ -tocopherol are the most widespread forms in nature. Although  $\alpha$ -tocopherol is the natural form that presents most vitamin or biological activities,  $\gamma$ -tocopherol has 10 - 30 % and  $\delta$ -tocopherol has 1 % of  $\alpha$ -tocopherol activity (Tafazoli *et al.*, 2005). These seeds therefore have antioxidant properties. Their oils could have potential for preventing oxidative stress, tiredness and ageing of cells (Martin, 2001), hence could play a beneficial role in preventing degenerative pathologies such as cardiovascular illnesses, ocular and neurodegenerative pathologies. They can also regulate some gene expressions like those involved in endogenous cholesterol synthesis (Landrier *et al.*, 2010). In addition, tocopherols are among the most important lipid-soluble antioxidants in food as well as in human and animal tissues. Vitamin E is a potent chain-breaking antioxidant that inhibits the production of reactive oxygen species during lipid oxidation. Alpha tocopherols mainly inhibit the production of new free radicals while  $\gamma$ -tocopherol traps and neutralises the existing free radicals. This helps prevent chronic diseases associated with reactive oxygen specie molecules such as cancer (Rizvi *et al.*, 2014). Potential health benefits of tocols include prevention of certain types of cancer, heart disease, and other chronic ailments (Shahidi and Camargo, 2016). Technologically, the tocopherol-rich oils may be mixed with some polyunsaturated fatty acids-rich oils to protect



them against oxidation (Shahidi and Zhong, 2010). During hot treatment of palm oil for example, the addition of tocopherol as antioxidant increased its stability by preventing free fatty acids from heat oxidation (Supriyono *et al.*, 2016). It assures the stability of colour and structure and improves the taste and odour of food (Bourgeois, 2003). In the Cosmetic industry, the tocopherol compounds are used as antioxidant, light stabilizer, hair and skin conditioning and emulsifying agents (Gottschalck and Breslawec, 2012).

**Table 3:-Vitamin E (tocopherol) contents of Cucurbit oils ( $\mu\text{g/g}$  of lipid)**

Species	Regions	Localities	Tocopherols					
			$\alpha$	$\beta$	$\gamma$	$\delta$	Total	
<i>L. siceraria</i>	North West	Mankon	15.07	nd	737.90	6.99	759.96	
		Ndop	nd	nd	546.69	5.64	552.33	
		Wum	14.92	nd	829.33	9.26	853.52	
			<b>10.00 ±8.66*</b>		<b>704.64 ± 144.20*</b>	<b>7.30 ± 1.83*</b>	<b>721.93±154.15*</b>	
	West	Bafoussam	13.62	nd	465.98	6.11	485.71	
		Dschang	5.90	nd	624.09	9.75	639.74	
		Bamougoum	9.52	nd	665.17	6.22	680.91	
			<b>9.68 ± 3.87*</b>		<b>585.08 ±105.17*</b>	<b>7.36 ± 2.07*</b>	<b>602.12±102.89*</b>	
		Mean	<b>9.84± 6.00<sup>a</sup></b>		<b>644.86 ±130.50<sup>a</sup></b>	<b>7.33 ± 1.75<sup>a</sup></b>	<b>662.03 ±134.33<sup>a</sup></b>	
	<i>C. moschata</i>	North West	Mankon	8.70	nd	108.29	nd	116.99
Wum			4.10	nd	123.64	0.58	128.32	
			<b>6.40± 3.26*</b>		<b>115.96 ± 10.86*</b>	<b>0.29 ± 0.41*</b>	<b>122.65 ± 8.01*</b>	
West		Dschang	9.26	nd	114.46	0.57	124.29	
		Foumbot	3.06	nd	50.63	nd	53.69	
		Bamougoum	1.75	nd	38.56	nd	40.31	
			<b>4.69± 4.01*</b>		<b>67.88 ± 40.78*</b>	<b>0.19 ± 0.33*</b>	<b>72.76 ± 45.12*</b>	
		Mean	<b>5.37 ± 3.40<sup>b</sup></b>		<b>87.12 ± 39.42<sup>b</sup></b>	<b>0.23 ± 0.32<sup>b</sup></b>	<b>92.72 ± 42.19<sup>b</sup></b>	
<i>C. mannii</i>		North West	Bangolan	nd	nd	241.29	1.38	242.67
			Wum	nd	nd	349.49	4.11	353.60
	Essimbi		nd	nd	431.30	3.88	435.18	
					<b>340.70 ± 95.31*</b>	<b>3.12 ± 1.52*</b>	<b>343.82 ± 96.63*</b>	
	West	Baloum	nd	nd	239.30	1.81	241.11	
		Bamougoum	nd	nd	525.46	2.90	528.35	
		Galim	nd	nd	393.14	2.13	395.27	
					<b>385.97 ± 143.21*</b>	<b>2.28 ± 0.56*</b>	<b>388.25±143.75*</b>	
		Mean			<b>363.33 ±111.60<sup>c</sup></b>	<b>2.70 ± 1.12<sup>c</sup></b>	<b>366.03 ±112.21<sup>c</sup></b>	
	<i>C. sativus</i>	Centre	Bafia	8.06	nd	512.55	9.36	529.97
Mbalmayo			1.32	nd	360.91	6.39	368.63	
Obala			0.96	nd	401.42	6.86	409.24	
			<b>3.45± 3.10*</b>		<b>424.96 ±78.51*</b>	<b>7.54 ± 1.6*</b>	<b>435.95 ± 83.92*</b>	
South West		Buea	1.21	nd	458.38	8.42	468.01	
		Mamfé	2.36	nd	470.71	9.41	482.47	
		Mundemba	11.37	nd	516.72	10.68	538.76	
			<b>4.98 ± 5.56*</b>		<b>481.93 ± 30.75*</b>	<b>9.50 ± 1.13*</b>	<b>496.42 ± 37.38*</b>	

		Mean	4.21± 4.41b		453.45 ± 61.79 <sup>c</sup>	8.52 ± 1.64 <sup>a</sup>	466.18 ± 66.88 <sup>c</sup>
<b>C. maxima</b>	West	Galim	5.98	nd	280.51	7.75	294.24
		Bangangté	6.58	nd	320.81	7.76	335.15
		Foumbot	2.93	nd	154.84	3.44	161.20
			5.16 ± 1.96		252.05± 86.57*	6.32 ± 2.49*	263.53 ± 90.95*
	North West	Kom	nd	nd	68.95	1.98	70.93
					68.95 *	1.98 *	70.93*
		Mean	3.87 ± 3.14 <sup>b</sup>		206.28 ± 136.17 <sup>d</sup>	5.23 ± 3.48 <sup>a</sup>	215.38±142.72 <sup>d</sup>

\* = no significant difference between the parameters analysed for the same specie from different regions. Detection limit: 1 ng/g; quantification: 10 ng/g

Means with different letter superscripts in the same column indicate a significant difference ( $p < 0.05$ ) between the parameters analysed for the various species of seeds.

$\alpha$  = Alpha;  $\beta$  = Beta;  $\gamma$  = Gamma;  $\delta$  = Delta ; EP = Edible portion, nd = not detected

### Conclusion:-

This study, which was aimed at determining the levels of dietary fibre and vitamin E in Cucurbit seeds from different regions in Cameroon, revealed that the levels of these parameters varied according to the specie, and not according to the region of cultivation of the seeds. The seeds were rich in dietary fibre especially insoluble dietary fibre. This could help facilitate digestion, improve the functioning of the digestive system. The vitamin E composition showed that the seeds had high levels of vitamin E (tocopherols), especially *L. siceraria*. There was the absence of  $\gamma$ -tocotrienol and  $\beta$ -tocopherol in all the samples and  $\alpha$ -tocopherol in *C. mannii*. These oils contained  $\alpha$ ,  $\gamma$  and  $\delta$ - tocopherols, with  $\gamma$ - tocopherol having the highest levels in these oils. Although  $\alpha$ -tocopherol is the primary form that presents most biological activities,  $\gamma$ -tocopherol has 10 to 30 % equivalence of  $\alpha$ -tocopherol activity. These seeds therefore have antioxidant properties. Their oils could have potential for preventing oxidative stress, tiredness and ageing of cells, hence could play a beneficial role in preventing cancer and degenerative pathologies such as cardiovascular illnesses. These oils (*L. siceraria*) could be added to palm oil during hot treatment, as antioxidant, to increase its stability by preventing free fatty acids from heat oxidation.

### Acknowledgement:-

This research was supported by the International Foundation for Science, Stockholm, Sweden through a grant to Dr. ACHU Mercy BIH épouse LOH. We are also very grateful to the Institute of Medical Research and Medicinal Plant Studies (IMPM) through the Centre for Research in Food and Nutrition (CRAN) for helping in the analyses of dietary fibre.

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