

# Determination Of Free Fatty Acids And Some Minerals Element Of Lyophilized Milk And Seed From Two Species Of African Gourd Seeds: *Cucumeropsis mannii* (Naud.) and *Citrullus lanatus* (Thunb.)

Josiane Enzonga<sup>1,2\*</sup>, Jean Paul Latran Ossoko<sup>1,2</sup>, Yves Okandza<sup>1</sup>, Michel Didace MvoulaTsieri<sup>1</sup>, Jalloul Bouajila<sup>2</sup>

<sup>1</sup>Laboratoire de Contrôle et Qualité des aliments de l'Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien Ngouabi, BP 69 Brazzaville (Congo); <sup>2</sup>Laboratoire des Interactions, Moléculaires et Réactivité Chimique et Photochimique, UMR CNRS5623, Université de Toulouse, Paul Sabatier, Toulouse, France

## ABSTRACT

The *Cucumeropsis mannii* (Cm) and *Citrullus lanatus* (Cl) seeds of the cucurbitaceae family are natural matrices rich in biological active substances, which give them a great advantage in terms of valorization. The objective of this work was to search for free fatty acids and some minerals in the seeds and lyophilized milk extracted from these seeds. The free fatty acids and some minerals were investigated using analytical tools such as gas chromatography coupled with mass spectroscopy (GC-MS), Atomic Absorption Spectrometry (AAS) and flame emission. From the chromatographic profiles and the mass spectra, four major fatty acids have been identified, two saturated fatty acids (palmitic and stearic acids), a monounsaturated fatty acid (oleic acid) and a polyunsaturated fatty acid of the n-6 series (linoleic acid). However, linolenic acid of the n-3 series was not detected, indicating that this very essential fatty acid is missing in seeds and squash gourd milk. Our quantitative analyzes have shown that linoleic acid (the essential fatty acid) predominates in two types of lyophilized milks, while saturated fatty acids have low levels. The study has measured seven (7) minerals, phosphorus, magnesium and potassium predominate in seeds and the two types of milk; on the contrary calcium, iron, manganese and sodium have demonstrated very low contents. These results show that gourd milk is a food of particular interest, both in terms of health and nutrition. So, it should supplement or replace very expensive animal milk or less-appreciated soymilk.

Keywords: Fatty acids; Cucumeropsis mannii; Citrullus lanatus; Chromatographic profiles; Minerals

### INTRODUCTION

Milk is an important conventional source of balanced lipids and fatty acids (FA). It also provides five dietary minerals (magnesium, sodium, calcium, phosphorus and potassium) needed in children's diets [1]. Milk is considered as a food of inestimable nutritional value because it is left or used almost in its natural state. In sub-Saharan Africa, particularly in Congo-Brazzaville, cow's milk production is non-existent and therefore all production is imported. Excessive costs of imported milk make it inaccessible to low-income households living below the poverty line [2]. This situation, particularly among children and pregnant women, causes the phenomenon of malnutrition and for the country the massive flight of foreign exchange [2]. However, even if milk is a food of animal origin, it also contains bad cholesterol (LDL) and a significant amount of saturated fatty acids which according to the epidemiological data contribute to the development of cardiovascular diseases, several forms of cancer and atherosclerosis in humans [3]. Maintaining a healthy diet, on the one hand, low in saturated fatty acids and bad cholesterol and rich in monounsaturated and polyunsaturated fatty acids (PUFA), and on the other hand rich in micronutrients offers the greatest potential for reducing risk of cardiovascular disease in all known approaches [4,5]. The primary role of PUFAs is structural components. They are the basis of the lipid bilayer which constitutes all cell membranes, which implicates them in membrane fluidity and permeability as well as in many intracellular signaling pathways. The multiplicity of their roles in many physiological processes gives them a major impact on health and chronic diseases, such as regulation of plasma lipid levels, immune functions and the risk

**Correspondence to:** Josiane Enzonga, Laboratoire de Contrôle et Qualité des aliments de l'Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien Ngouabi, BP 69 Brazzaville (Congo); Laboratoire des Interactions, Moléculaires et Réactivité Chimique et Photochimique, UMR CNRS5623, Université de Toulouse, Paul Sabatier, Toulouse, France. Tel: + 22679047527; E-mail: josiane0204@yahoo.fr

Received: May 20, 2019, Accepted: December 11, 2019, Published: December 18, 2019

**Citation:** Enzonga J, Ossoko JP, Okandza Y, Tsieri M, Bouajila J (2019) Determination Of Free Fatty Acids And Some Minerals Element Of Lyophilized Milk And Seed From Two Species Of African Gourd Seeds: Cucumeropsis mannii (Naud.) and Citrullus lanatus (Thunb.). J Nutr Food Sci 9:765. doi: 10.4172/2155-9600.1000765

**Copyright:** © 2019 Enzonga J et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Enzonga J, et al.

of diabetes or obesity, but also visual and cognitive functions [6]. Omega 6 and omega 3 PUFAs are able to interfere on the etiology of a large number of pathologies (cardiovascular and inflammatory diseases, cancers, neuropathies, etc) [7]. Concerning omega-3, it has been shown that they exert antioxidant, anti-inflammatory and anti-thrombotic actions and delay the development of atheroma plaques [8]. In addition, there is evidence that minerals play a role in the structural, biochemical and nutritional functions necessary for mental and physical human health [1]. For example, calcium and phosphorus are a larger fraction of the milk needed for bone growth and appropriate development of newborn babies.

The Second International Conference on Nutrition (ICN2), held in Rome from 19 to 21 November 2014 [9], drew global attention to malnutrition in all its forms. For instance, in sub-Saharan Africa, one of the ways to improve the nutritional and health status of populations is to supplement animal milk with vegetable drinks rich in polyunsaturated fatty acids (PUFAs) and micronutrients. Thus, researchers must redouble their efforts to identify underutilized vegetable sources rich in polyunsaturated fatty acids, necessary for the improvement of human health because the use of synthetic substances can be dangerous for human health. This approach, for example, is aimed at producing naturally occurring plant milk or vegetable beverages of inestimable nutritional value. So far, most work in this field has been consisted in producing milk from legume seeds, particularly soybeans, but the taste of this milk is not very popular in Africa. In contrast, African cucurbit seeds such as Cucumeropsis mannii (CM) Naudin and Citrullus lanatus (CL), generally processed into pastas for concocting popular sauces from traditional and urban societies, are rich in lipids and essential fatty acids [10]. They can therefore be valorized and thus constitute a solution to health problems and child malnutrition and pregnant women. However, very few investigations on their valorization have been carried out up to now. Meité et al. have demonstrated their contribution to the fortification of bread [11].

The valorization of these two species of Curcubitaceae seeds by the production of a vegetable milk, which could supplement or replace cow's milk or soya milk, requires a complete knowledge of the nutritional composition of the raw material and the vegetal milk. Our previous work has shown the richness of seeds and milk in proteins, amino acids (Phe, Thr, Val, Trp, Ile, and Leu), lipids and non-negligible ash contents [12,13].

The objective of this study is to determine, on the one hand, the fatty acid profile of the lipid fraction and, on the other hand, the mineral salts of seeds and milk.

### MATERIAL AND METHODS

### Plant material

CM and CL seeds were purchased in local markets. CM and CL are plants of the cucurbitaceae family of tropical and subtropical Africa used only for their seeds. Specimens were identified by Dr AP Mbembé at the Department "Center for Research on Plant Genetic Improvement "(CERAG) Brazzaville, Congo" and voucher specimens were deposited in the Department of Botany in the above-mentioned institute. In this study, the lyophilizates and the seeds of these species were used.

### Reagents

The standard fatty acids and internal standard, namely palmitic, stearic, oleic, linoleic and myristic acid were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

### Preparation of gourd milk

CM and CL milks were obtained after maceration of ground samples in water, then these mixtures were filtered. The milk obtained was then freeze-dried. Using a benchtop lyophilizer, all water contained in the milk was sublimated under vacuum and the product was thus dehydrated for 72 hours. The powder obtained was stored at -20°C in freezer until use.

### Fatty acids analysis

The analysis of the fatty acids was carried out according to the method proposed by Hubert [14] with some modifications.

### Preparation of the fat fraction

The fat fraction was isolated from 5 g of lyophilisate and crushed seeds; by addition of cyclohexane after a cold maceration under magnetic stirring for 16 H. The solvent is eliminated after filtration by a rotary evaporator at 39°C. The oil extracted was stored at 20°C.

### Preparation of FAME

Each 0.3 g of oil sample is weighed into the 50 mL groundnecked flasks and then diluted in a solution of methanolic soda (10 mL, 0.5 M). The internal standard used was Tetradecanoic acid or myristic acid (C14: 0, 10 mg/mL, V=1 mL cyclohexane). After homogenization, the flask is surmounted by a refrigerating rod and then placed in a water bath at 65°C for 30 minutes. The methylation of the fatty acids is then carried out by the addition of 2.5 mL of a solution of concentrated commercial hydrochloric acid 35% (9.7 mL) diluted with 41.5 mL of methanol (85%/15, v/v, methanol/water) [15]. This Mixture is stirred for 15 seconds and then brought to 65°C for 10 minutes to obtain the methyl esters (FAME). After cooling in water bath cold, 5 mL of distilled water and 10 mL of cyclohexane are added. This mixture is transferred to a separatory funnel, after a brief stirring which allows migration of the methyl esters to the organic phase. After settling, the organic phase is recovered in the 10 mL vial for chromatographic analyses in gas phase coupled to mass spectrometry (GC-MS). The analyses are performed in triplicate.

### GC-MS analysis

The gas chromatography analyses were carried out on a Varian Star 3400 chromatograph Cx (Les Ulis, France) fitted with a fused-silica capillary column DB-5MS (5% phenylmethyl polysyloxane) of dimensions 30 m × 0.25 mm, 0.25  $\mu$ m film thicknesses). The GC was operated under temperature conditions programmed from 60°C to 260°C, with temperature rise of 5°C/min and 15 minutes for isotherm at 260°C. A second gradient of 40°C/min was applied to increase the temperature to 340 °C. The total time of analysis was 57 minutes. The samples were dissolved in petroleum ether, 10  $\mu$ L were injected using a split mode of ratio 1:10. The carrier gas was helium. The mass spectrometer operating parameters were: electron multiplier between 1400 and 1500 V, transfer line 200°C.

Mass spectra were analyzed in the range of 40-650 atom mass units (amu). The fatty acid esters were then identified by their mass spectrum compared to those of the database thanks to Xcalibur software.

### Determination of some minerals

The determination of the minerals was carried out employing the ORSTOM guide. It took place in two stages: mineralization and dosage.

### Mineralization

The mineralization was performed by putting in a furnace 2 g of sample placed in a porcelain dish and an empty capsule to make the white at the temperature of 450° C for 2 H. The ash obtained was cooled and then moistened with 2 to 3 mL of water and 1 mL of concentrated hydrochloric acid. The mixture thus obtained was heated on a plate until the first vapors started appearing. It was filtered and then transferred to a 100 ml flask and rinsed 3 times with hot water.

The filter paper and its contents were placed in a porcelain dish and incinerated for 30 minutes at a temperature of 550°C. The ashes were transferred to a Teflon capsule, added with 5 mL of hydrofluoric acid. This mixture was heated in a bain-marie at a temperature below 100°C and added with 1 ml of concentrated hydrochloric acid. After washing with lukewarm water, the mixture was filtered in a 100-ml volumetric flask and then adjusted to the mark after cooling.

### Dosage

Phosphorus, calcium, sodium, potassium, magnesium, manganese and iron were measured using the cold colorimetric method, atomic absorption spectrometry (AAS) and flame emission.

### Determination of phosphorus

Phosphorus was dosed by the cold colorimetric method using Murphy and Riley's method [16]. Murphy and Riley's reagent was obtained by adding solution A and B. Solution A was prepared by mixing 50 mL of water and 10 mL of concentrated sulfuric acid in a 100 mL vial. After cooling, 0.6 g of ammonium molybdate and 0.014 g of potassium antimonyl tartrate were added and the flask was then rounded up to the mark. As for solution B, 2 g of ascorbic acid, 50 ml of distilled water and 5 ml of concentrated hydrochloric acid were mixed, and the mixture was supplemented up to the mark of gauge. In a 200 mL vial, A and B solutions were mixed to obtain Murphy and Riley's reagent. In a plastic pill box, 0.5 mL of mineralized sample, 10 mL of distilled water and 3 mL of Riley reagent were mixed. After 30 minutes of incubation, the absorbance of the mixture was read at the wavelength (II) of 660 nm. The tests were carried out in triplicate.

### Determination of iron

The iron was dosed after mixing in a plastic pill box 5 mL of mineralized sample, 5 mL of 1% hydroxylamine chloride, 2 mL of 3% sodium citrate, 2 mL of sodium acetate buffer solution, at pH 3.5 and 2 mL of 0.2% orthophenanthroline solution. Under the same conditions, a standard range of iron was prepared. After

30 minutes of incubation at room temperature, the absorbance of the reaction medium was measured using the spectrophotometer at 490 nm.

# Determination of calcium, magnesium, potassium, sodium and manganese

The samples to be dosed were prepared by putting in a 50 mL flask 1 mL of mineralized sample adjusted to 50 mL with the lanthanum solution. Calcium, magnesium and manganese were determined using atomic absorption with the following spectral conditions: calcium (I=5 A,  $\Lambda$ =422.7 nm), magnesium (I=3 A,  $\Lambda$ =285.2 nm), manganese (I=5 mA,  $\Lambda$ =279.5 nm). Potassium and sodium were measured in flame emissions with these spectral conditions: sodium (I=5A,  $\Lambda$ =589.0 nm), Potassium (I=3A,  $\Lambda$ =766.5 nm). After plotting the calibration lines for each element, the concentrations read for the sample and blank were determined.

### Statistical analysis

Statistical analysis of the data was carried out, with the aid of the R. 3.3.3 statistical software. A Shapiro-Wilk test was used to check for normality of the data which proved the data not to be normally distributed therefore non-parametric tests were used in the analysis of data.

### **RESULTS AND DISCUSSION**

### Analysis of samples of milk lyophilized and seeds

The chromatograms obtained from extracts of lyophilized milk and seeds of CL and CM are presented in Figures 1-4. The qualitative profiles of detected and separated fatty acids are similar. Four major free fatty acids, especially palmitic, stearic, oleic and linoleic acids were identified thanks to their mass spectrum compared to those of the database using the Xcalibur software. The different profiles show that linoleic acid represents the highest fatty acid. It should be noted that these major fatty acids were also identified in the seeds of CM and CL [17-20]. The works of these authors [21-23] showed that the four fatty acids identified are also in the majority in cow, goat and sheep milks.

The fatty acids identified in the lyophilized milk and seeds from CM and CL were quantified employing calibration curves of standards fatty acids, namely palmitic, oleic, stearic and linoleic. The peak areas being plotted against the spiked concentration of four corresponding standard FA (palmitic acid:  $R^2$ =0.997, linoleic acid:  $R^2$ =0.991, oleic acid:  $R^2$ =0.992, stearic acid:  $R^2$ =0.990).

The concentrations of obtained fatty acids and the yield of lipid fraction in the gourd milks and seeds are presented in Figures 5 and 6. The yields of the lipid fraction from the seeds of CM and CL being respectively 52.91% and 53.14%, these values are similar to those obtained in the literature [10,18,20]. As for the lyophilized milk from CM and CL, the yields of the lipid fraction being respectively 32.73% and 37.92%, these amounts are higher than those found by Park [24] in the powdered goat milk (28.2%) and by Mazumder [25] in the soymilk powder (27,60%). From these results, it is clear that both the seeds and the lyophilized milk have high contents in lipid fraction.

Linoleic acid is the major fatty acid in CM ( $30.77 \pm 0.57\%$ ) and CL ( $35.19 \pm 1.12\%$ ) seeds and in the relative milks with respective

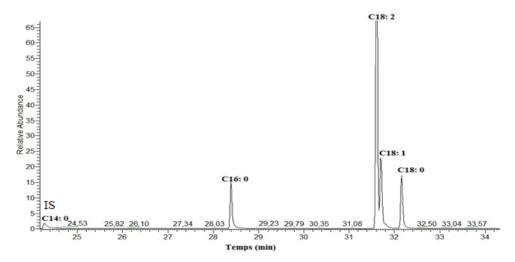


Figure 1: Separation of fatty acids of the lyophilized milk sample from CM extract using GC-MS. C16:0: Palmitic acid, C18:2: Linoleic acid, C18:1: Oleic acid, C18:0: Stearic acid. IS (C14:0): Internal standard; CM: Cucumeropsis mannii.

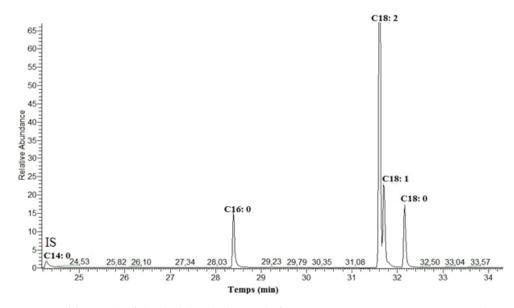


Figure 2: Separation of fatty acids of the lyophilized milk sample from CL extract using GC-MS .C16:0: Palmitic acid, C18:2: Linoleic acid, C18:1: Oleic acid, C18:0: Stearic acid. IS (C14:0): Internal standard; CL: Citrullus lanatus

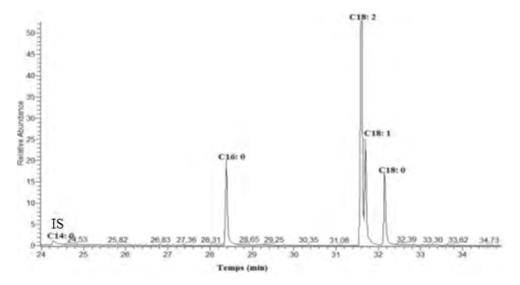


Figure 3: Separation of fatty acids of the seeds from CM using GC-MS .C16:0: Palmitic acid, C18:2: Linoleic acid, C18:1: Oleic acid, C18:0: Stearic acid. IS: Internal standard; CM: Cucumeropsis mannii

values of 24.37  $\pm$  0.95% and 30.35  $\pm$  0.95%. Furthermore, the oleic fatty acid contents in the milk from CL (10.60  $\pm$  0.52%), the

seeds from CL (14,09  $\pm$  0.39%) and CM (13.51  $\pm$  0.59%) are not negligible.

### OPEN OACCESS Freely available online

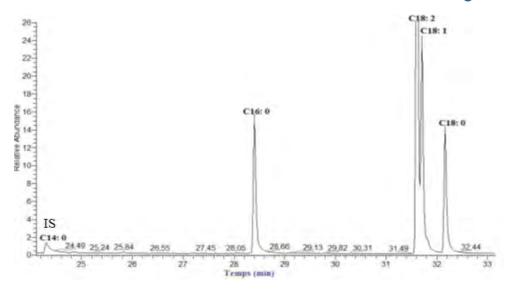
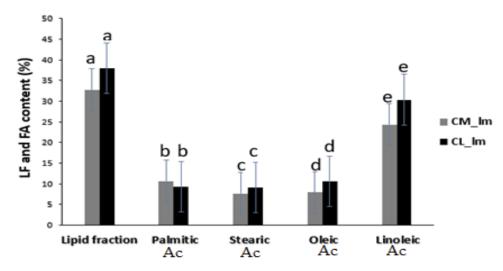
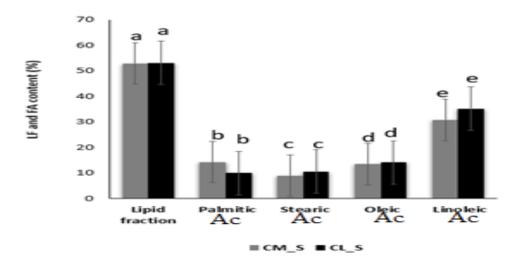


Figure 4: Separation of fatty acids of the seeds from CL using GC-MS .C16:0: palmitic acid, C18:2: Linoleic acid, C18:1: Oleic acid, C18:0: Stearic acid. IS: Internal standard; CL: *Citrullus lanatus*.



Note: For the same parameter represented by the grouped bars, when the letters above are identical, there is no significant differences between the two samples (P>0.05).

**Figure 5:** Content (%) of lipid fraction (LF) and fatty acids (FA) in lyophilized milk from *Cucumeropsis mannii* and *Citrullus lanatus*. CM\_lm: Milk lyophilized *Cucumeropsis mannii*, CL\_lm: milk lyophilized *Citrullus lanatus*. Ac: Acid



Note: For the same parameter represented by the grouped bars, when the letters above are identical, there is no significant differences between the two samples (P>0.05).

Figure 6: Content (%) of lipid fraction (LF) and fatty acids (FA) in seeds from *Cucumeropsis mannii* and *Citrullus lanatus* CM\_S: seeds *Cucumeropsis mannii*; CL\_S: seeds *Citrullus lanatus*; Ac: Acid.

### Enzonga J, et al.

These results show that lyophilized milk and seeds from CL are characterized by high levels of linoleic, oleic and stearic acids, while the lyophilized milk and seeds from CM are characterized by high levels of palmitic acid. However, the differences observed about average contents of palmitic, linoleic, stearic and oleic acid in seeds one the other hand, and the two milks on the other hand of CM and CL are no statistically different (P >0.05).

This study revealed the presence of Linoleic acid (C 18:2), of the omega-6 group, in the seeds and lyophilized milk from CM and CL. It is considered as an essential fatty acid because the animal and human organisms are unable to synthesize these compounds [26]. Several studies have reported that this essential fatty acid (EFA) serves as an *in vivo* precursor of arachidonic acid. A main function of this fatty acid is the production of prostaglandins which regulate body functions such as heart rate, blood pressure, blood clotting, and this EFA also plays a role in immune function by regulating inflammation thus encouraging the body to fight infections [27]. Linoleic acid: omega 6 is also necessary for a good growth in children, especially for neural tube development and the maturation of sensory systems. Research with humans has indicated a role for linoleic acid as a precursor of prostaglandin E, in the prevention of gastric ulcers [28].

The fatty acid contents of gourd milk are compared with those from soya, cow, sheep and goat milks (Table 1). In comparison with the soymilk [29], the palmitic acid is in the same range of concentration; solely, stearic acid is higher in our samples. The oleic and linoleic acids are much higher in the soymilk. The linolenic acid was not detected in the gourd milks.

Comparing with the cow milk [22,30], we observe that linoleic acid is more concentrated in our samples; the other fatty acids (stearic, palmitic and oleic acids) are more concentrated in cow milk.

### OPEN OACCESS Freely available online

Comparing with the sheep, goat and human milks [23,30], we notice the linoleic acid is more concentrated in our sample, stearic acid is in the same range of concentration; the others acids (oleic and palmitic acids) are higher in these animal and human milks.

### Mineral composition

Two microelements and five macroelements were determined in the present study. Table 2 shows the contents of Ca, Mg, K, Na, Mn, Fe and P in the ash of gourd seeds from CM and CL, while Table 3 shows the same elements in two type of lyophilized milk from CM and CL. These results indicate high content of phosphorus, magnesium and potassium. There are also non-negligible content of calcium and sodium while manganese and iron are in trace form.

It is noted that lyophilized milk of gourd seeds is a non-negligible source of calcium; however, the levels obtained are much lower than the recommended calcium intake dietary. Indeed, recommended calcium dietary intake per day [31] is 800 mg for children 1 to 10 years and 800-1200 mg for children adults. However, in the present study, 100 g of CM and CL milk give 116.67 and 101.67 mg of calcium respectively, i.e., 14.6% and 12.7% of intake recommended calcium in children, 9.7-14.6% and 8.5-12.7% in the adults. These intakes being low, the fortification of the lyophilized milk of CM and CL in calcium could be considered. The differences observed in the average contents of seven minerals measured in the two Cucurbitaceae species (CM and CL) are not statistically significant.

Table 4 comparing our results and those obtained by Ghada [32] and Mazumder [25] on the elements mineral composition of camel, cow, goat, human milks and soymilk powder. This table shows calcium content of lyophilized milk almost similar to that of camel, cow and goat milks, but than that lesser powder soymilk. This table also shows that with the exception of the Na content which is high

Table 1: Fatty acid composition in (g/100 g) the lyophilized milk of two species from seed gourd in comparison with values of cow, goat, sheep and human milks.

Fatty acids	<sup>a</sup> CM_lm	<sup>a</sup> CL_lm	Soya Milk <sup>b</sup>	Cow 1	Milk <sup>c,d</sup>	Sheep Milk <sup>e</sup>	Goat Milk <sup>e</sup>	Human <sup>d</sup>
Palmitic: C16:0	10.650± 0.35	9.24± 0.09	10.7	26.5	28.5	22.5-28.2	23.2-34.8	28.1
Stearic: C18:0	7.65 ± 0.36	9.07 ± 0.75	3.79	14.6	13.3	8.51-11.0	5.77-13.2	6.9
Oleic: C18:1	7.87 ± 0.25	$10.60 \pm 0.52$	20.4	29.8	26	17.8-23.0	15.4-27.7	33.6
Linoleic: C18:2	24.37 ± 0.95	30.35 ± 0.95	54.8	2.5	2.9	2.89-3.57	2.49-4.34	6.4
Linolenic: C18:3	Ndf	Ndf	7.53	Ndf	1.7	0.52-1.04	0.19-0.87	1.1

<sup>a</sup>This work: (CM\_lm Peñalo [29]; <sup>b</sup>Jandal JM [22]; <sup>c</sup>Malacarne [30]; <sup>d</sup>Park [23]; <sup>c</sup>Nd: Stands for not detected.

Seeds					
Minerals	СМ	CL	P-value		
Ca(mg/100 g)	$101.67 \pm 1.73^{a}$	$103.33 \pm 5.77^{a}$	0.9		
Mg(g/100 g)	$7.55 \pm 0.15^{a}$	$8.72 \pm 0.55^{a}$	0.77		
K(g/100 g)	$11.75 \pm 0.01^{a}$	$15.77 \pm 0.23^{a}$	0.44		
Na(mg/100 g)	123.33 ± 5.77 <sup>a</sup>	$120.33 \pm 1.52^{a}$	0.51		
Mn(mg/100 g)	$11.33 \pm 1.15^{a}$	$10.33 \pm 0.55^{a}$	0.82		
Fe(mg/100 g)	$8.93 \pm 0.05^{a}$	$6.2 \pm 0.1^{a}$	0.48		
P(g/100 g)	$16.17 \pm 0.07^{a}$	$19.48 \pm 0.40^{a}$	0.57		

 Table 3: Minerals composition of the lyophilized milks of two species gourd (CM and CL).

Lyophilized Milks						
Minerals	СМ	CL	P-value			
Ca(mg/100 g)	$116.67 \pm 28.8^{a}$	$101.67 \pm 2.88^{a}$	0.31			
Mg(mg/100 g)	$473.33 \pm 20.8^{a}$	$506.67 \pm 5.77^{a}$	0.28			
K(mg/100 g)	$760 \pm 70^{a}$	$900 \pm 15.27^{b}$	0.00059			
Mn(mg/100 g)	$11.67 \pm 0.57^{a}$	$12.33 \pm 1.57^{a}$	0.57			
Fe(mg/100 g)	$1.4 \pm 0.43^{a}$	$8.75 \pm 1.51^{b}$	0.021			
P(g/100 g)	$4.78 \pm 0.24^{a}$	$5.26 \pm 0.09^{a}$	0.87			

CM: Cucumeropsis mannii; CL: Citrullus lanatus

When for the same line, the superscript letters are identical the averages are identical to the 5% threshold. Otherwise, the means are significantly different at the threshold of 5%.

CM: Cucumeropsis mannii; CL: Citrullus lanatus

When for the same line, the superscript letters are identical; the averages are identical to the 5% threshold. Otherwise, the means are significantly different at the threshold of 5%.

### OPEN OACCESS Freely available online

Table 4: Minerals composition in the lyophilized milk of two species gourd in comparison with camel, cow, goat and human milk (mg/100 g).

Milks							
Minerals	CML <sup>a</sup>	CLL <sup>a</sup>	Soyamilk powder <sup>c</sup>	Camel <sup>b</sup>	Cow <sup>b</sup>	Goat <sup>b</sup>	Human <sup>b</sup>
Ca	$116.67 \pm 28.8$	$101.67 \pm 2.88$	206	111.36	119.90	130.28	32.36
Mg	473.33 ± 20.8	506.67 ± 5.77	429	6.70	13.42	13.87	3.43
K	760 ± 70	900 ± 15.27	2515	156.32	13.42	13.87	3.43
Mn	11.67 ± 0.57	12.33 ± 1.57	2.28	0.013	0.0037	0.0138	0.09
Fe	1.4 ± 0.43	8.75 ± 1.51	6,37	0.23	0.07	0.06	0.053
Р	4780 ± 240	5260 ± 90	494	81.17	95.03	110.16	13.97
`his work; <sup>b</sup> Gl	nada [32]; <sup>c</sup> Mazumder	[25]					

in animal and human milks, the Mg, K, Mn, Fe and P contents are higher in lyophilized milk of gourd seeds. This table also shows that, except the K content which is high in powder soymilk, the Mg, Mn, Fe and P contents are higher in lyophilized milk of gourd seeds.

The lyophilized milk and the gourd seeds are rich in mineral substances essential to the body and beneficial to health. Notably phosphorus which participates with calcium in the fortification of adult bones plays a role in bioactivity and osmotic balance in cellular metabolism. Phosphorus is a major element with many important biological functions in the human body. It occurs as organic or inorganic phosphate in all body tissues and fluids, and in the main component of many biological compounds, including lipids, proteins, carbohydrates and nucleic acids [33]. Calcium is responsible for many regulatory functions, such as normal cardiac rhythm maintenance, blood clotting, hormone secretion, muscle contraction and enzyme activation [34]. Magnesium participates in the contraction of the heart muscle, and plays various roles in the metabolism of Proteins, carbohydrates and in the synthesis of DNA. Magnesium also forms a complex with ATP (Mg-ATP) which serves as the true substrate. Potassium is the major cation in the intracellular fluid. Nerve impulses are transmitted by electrical currents associated with momentary exchange of potassium and sodium ions [35]. There is also evidence that this element plays a role in the regulation of osmotic pressure, blood pressure and acid-base balance [36]. Potassium is also an enzyme activator. Manganese is an essential trace mineral that plays an important role in reproductive hormone production, urea excretion, immunity, and joint/leg development [37].

### CONCLUSION

Our study showed similar chromatographic profiles of lyophilized milks and seeds from gourd (*Cucumeropsis mannii* and *Citrullus lanatus*). All the fatty acids detected are well separated except linoleic and oleic acids which are co-eluted. Four fatty acids were identified and quantified (linoleic, oleic, stearic and palmitic acids). Quantitative analysis of fatty acids in both lyophilized milk and seeds revealed the predominance of linoleic acid, an essential fatty acid. The gourd milk and seeds are therefore undeniable sources of polyunsaturated fatty acids. Statistical analysis showed that the seeds and milk of *Citrullus lanatus* are characterized by high levels of linoleic, oleic and stearic acids, while the seeds and milk of *Cucumeropsis mannii* are characterized by high contents of palmitic acid. The minerals essential to the body and beneficial for health such as phosphorus, magnesium and potassium are majority in the gourd milk and seeds. However, the calcium, iron and manganese contents in the lyophilized milk of two species of Cucurbitaceae (CM and CL) in comparison with other types of milk (cows, goat, camel, human and soy milks) are not so negligible. As a result, gourd seeds should arouse interest as raw materials to produce vegetable milk and the lyophilized milk from these seeds could complement cow, goat, camel, human and soya milks.

### ACKNOWLEDGEMENT

J Enzonga would like to thank Agence Universitaire de la Francophonie and International Foundation of Science for their financial support.

### REFERENCES

- Sumya K, Masum AKM, Islam MA, Harun-ur Md. Mineral profiles of powdered milk, yoghurt, ice cream and raw milk. Asian J Med Biol. 2017;3(2):294-297.
- UNDP. Rapport national sur le développement humain: Développement humain en situation post conflit, Brazzaville. 2003.
- Corral AR, Somers VK, Korinek J, Johnson JS, Thomas RJ, Allison TG, et al. Update in prevention of atherosclerotic heart disease: Management of major cardiovascular risk factors. Rev Invest Clin. 2006;58:237-244.
- Laidlaw M, Holub BJ. Effects of supplementation with fish oil-derived n\_3 fatty acids and\_linolenic acid on circulating plasma lipids and fatty acid profiles in women. Am J Clin Nutr. 2003;77(1):37-42.
- Okuda N, Ueshima H, Okayama A, Saitoh S, Nakagawa H, Rodriguez BL, et al. Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese–American men in Hawaii: the INTERLIPID study. Atherosclerosis. 2005;178(2):371-379.
- Blondeau N, Stéphane M, Schneider. Les acides gras essentiels de la famille des oméga-3 et la santé de la mère et de l'enfant Omega-3 fatty acids for mother and child health. Nutrition Clinique Et Métabolisme. 2006 ;20(2): 68-72.
- Guesnet P, Alessandri JM, Astrorg P, Pifferi F, Lavialle M. Epidémiologie nutritionnelle", ISTNA/CNAM, 2005;12:5-6.
- 8. Colussi G, Catena C, Novello M, Bertin N, Sechi LA. Impact of omega-3 polyunsaturated fatty acids on vascular function and blood pressure: relevance for cardiovascular outcomes. Nutr Metab Cardiovas. 2016.
- FAO. la situation mondiale de l'alimentation et de l'agriculture. Protection sociale et agriculture: Briser le cercle vicieux de la pauvreté rurale. 2015.
- Loukou AL, Gnakri D, Djè Y, Kippré AV, Malice M, Baudoin JP, et al. Macronutrient composition of three cucurbit species cultivated for seed consumption in Côte d'Ivoire. Afr J Biotechnol. 2007;6:529-533.

### OPEN OACCESS Freely available online

#### Enzonga J, et al.

- Meité A, Kouamé KG, Coulibaly KS, Offoumou AM. Étude de la valeur nutritionnelle du pain normal et des pains composites contenant de la farine de graines délipidées de *Citrullus lanatus* (cucurbitacées). Bull Soc R Sci Liege. 2008;77:80-103.
- Yoca EJ, Nitou JG, Kippré AV, Niamayoua RK, Tsieri MM, Silou T. Caractérisation chimique et évaluation de la température de conservation du lait des graines de cucurbitacées : *Cucumeropsis mannii* et *Citrullus lanatus*. J Anim Plant Sci. 2011;10(1):1232-1238.
- Enzonga J, Varravaddheay OM, Couderc F, Boutonnet A, Poinsot V, Tsieri MM, et al. Determination of free amino acids in Africa gourd seed milks by capillary electrophoresis with light-emitting diode induced fluorescence and laser-induced fluorescence detection. Electrophoresis. 2013;34(18):2632-2638.
- Hubert J. Caractérisation biochimique et propriètés biologiques des micronutriments du germe de soja : Étude des voies de sa valorisation en nutrition et santé humaines. Thèse de doctorat. Institut National Polytechnique, Toulouse, (France). 2006.
- 15. Ichihara K, Fukubayashi Y. Preparation of fatty acid methyl esters for gas liquid chromatography. J Lipid Res. 2010;51(3):635-640.
- Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31.
- Achu MB, Fokou E, Tchiégang C, Fotso M, Tchouanguep MF. Chemical characteristics and fatty acid composition of cucurbitaceae oils from Cameroon. IUFoST. 2006;10:1249-1259.
- Besong SA, Ezewe MO, Fosung CN, Senwo ZN. Evaluation of nutrient composition of African melon oilseed (*cucumeropsis mannii* naudin) for human nutrition. Int J Nutr Metab. 2011;3(8):103-118.
- Conto LC, Gragnani MA, Maus D, Ambiel HC, Chiu MC, Grimaldi R, et al. Characterization of crude watermelon seed oil by two different extractions methods. J Am Oil Chem Soc. 2011;88(11):1709-1714.
- Fokou E, Achu MB, Kansci G, Ponka R, Fotso M, Tchiégang C, et al. Chemical properties of some cucurbitaceae oils from Cameroon), Pak J Nutr. 2009;8(9):1325-1334.
- Ceballos LS, Morales ER, Adarve GT, Castro JD, Martinez LP, Sanz Sampelayo M R (2009) Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. J Food Compos Anal. 22(4):322-329.
- 22. Jandal JM. Comparative aspects of goat and sheep milk. Small Ruminant Research. 1996;22(2):177-185.

- 23. Park YW, Juarez M, Ramosc M, Haenlein GF. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research. 2007;68(2):88-113.
- 24. Park YW. Goat milk products: Quality, composition, processing, and marketing. Encyclopedia of Animal Science. 2010.
- Mazumder MA, Hongsprabhas P. A review on nutrient quality of soymilk powder for malnourished population. Pak J Nutr. 2016;15(6):600-606.
- 26. Svahn JC, Feldl F, Raiha NC, Koletzko B, Axelsson IE. Different quantities and quality of fat in milk products given to young children: Effects on long chain polyunsaturated fatty acids and trans fatty acids in plasma. Acta Pediatrica, 2002;91(1):20-29.
- Nakamura MT. The return of 3 fatty acids into the food supply. Landbased animal food products and their health effects. Am J Clin Nutr. 2000;71(5):1215-1216.
- Grant HW, Palmer KR, Kelly RW, Wilson NH, Misiewicz JJ. Dietary linoleic acid, gastric acid, and prostagland in secretion. Gastroenterology. 1988;94(4):955-959.
- Peñalvo JL, Castilho MC, Matallana MC, Irene M, Silveira N, Torija ME. Fatty acid profile of traditional soymilk. Eur Food Res Technol. 2004;219(3):251-253.
- 30. Malacarne M, Martuzzi F, Summer A, Mariani P. Protein and fat composition of mare's milk: Some nutritional remarks with reference to human and cow's milk. Int Dairy J. 2002;12(11):869-877.
- FNB Recommended Dietary Allowances, National Research Council National Academy of Science, USA. 1989.
- 32. Soliman GZ. Comparison of chemical and mineral content of milk from human, cow, buffalo, camel and goat in Egypt. Egyptian J Hosp Med. 2005;21:116-130.
- Cashman KD. Macroelements, nutritional significance. In: Roginski, H., Fuquay, J.W., Fox, P.F. Encyclopedia of Dairy Sciences, London: Academic Press, UK. 2002;2051-2058.
- Smun Z, Nerver A, Jasmina H, Dubravka S. Mineral elements in milk and dairy products. Mijekarstvo. 2012:62(2):111-125.
- 35. Stryer L. Biochemistry. W.H. Freeman and Co. New York, USA. 2000.
- Yellen G. The voltage gated potassium channels and their relatives. Nature. 2002;419:35-42.
- WHO/FAO Report: Trace elements in Human nutrition and health. 1996.