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CHEMICAL CONTENT OF THE SEEDS AND PHYSICO-CHEMICAL CHARACTERISTIC OF THE SEED OILS FROM *CITRULLUS COLOCYNTHIS*, *COCCINIA GRANDIS*, *CUCUMIS METULIFERUS* AND *CUCUMIS PROPHETARUM* OF NIGER

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ABSTRACT. The aim of this study was to determine the chemical composition of the seeds of *Citrullus colocynthis,Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* belonging to the family of Cucurbitaceae. For each variety, the ash, protein, fat, calcium, copper, iron, phosphorus, magnesium, potassium, sodium and zinc were determined. The oils were characterized by the determination of the acid (I_A), iodine (I_I), saponification (I_S), refraction (I_R) numbers as well as the fatty acids and anti-oxidants composition and the non-saponification percentage. Our results showed: 13.19-26.86 % protein content, 14.48-24.62 % fat content, and 2.00-4.46 % % ash content. Respectively the mineral matter was as follow: calcium (246.90-569.47 mg/100 g); copper (5.10-5.79 mg/100 g); iron (10.95-15.57 mg/100 g); magnesium (209.83-289.07 mg/100 g); potassium (465.27-1205.12 mg/100 g); phosphorus (30.03-49.13 mg/100 g); sodium (11.89-16.67 mg/100 g) and zinc (1.05-2.92 mg/100 g). The physicochemical characterization of the oil revealed that: I_A was within 2.90-6.31 mg of KOH/g, I_S within 171.67-208.90 mg of KOH/g, I_I within 138.10-146.74 g of iodine for 100 g of oils and I_R within 1.4607-1.4620. The non-saponification percentage was within 1.07-3.39 % and the predominant fatty acids were palmitic acid (15.12-17.29 %), stearic acid (7.65-9.04 %), oleic acid (10.77 %-18.57 %) and linoleic acid (56.21-64.188 %). The study of the anti-oxidants fraction showed: 8.04-80 mg/kg α-tocopherol content, 83.14-619.37 mg/kg γ-tocopherol content and 0.05-0.23 mg/kg β-carotene content.

KEY WORDS: *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus*, *Cucumis prophetarum*, Protein, Oil, Fatty acids, Antioxidants, Minerals

INTRODUCTION

In sub-Saharan Africa and particularly in Niger, the four great nutritional diseases identified by WHO: proteino energetic malnutrition, ferriprive anaemia, troubles due to the deficiency of iodine and vitamin A deficiency constituted major public health. The children and the women in child bearing age are the principal victims [1]. The population growth, urbanization, the economic crisis and climatic disparity namely desertification constituted many other worsening factors. The mobilization of the resources available in the natural environment of the malnourished populations may be taken as an alternative way to solve the problem. The seeds of Cucurbitaceous constituted one of these natural resources which could be used as potential sources of nutrient and micro nutriments whose deficiency is of acuity [2]. Indeed the Cucurbitaceous existed in abundance on all the extent of the territory either in state cultivated or in a wild state and were rooted perfectly in the culinary practices of the populations [3-5]. Nevertheless, the cultivated species are planted less often with nutritional goal than for their multiple uses as containers, kitchen utensils and musical instruments. The seeds constituted consequently under products which it is advisable to develop. Unfortunately, the chemical

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profile of these seeds is little or badly known. However such knowledge could make it possible to make adapted recommendations for the rebalancing of the food intake of these malnourished populations. In a previous study we have established the chemical profile of the seeds of *Lagenaria siceraria* [6] and *Luffa aegyptiaca* and *Luffa cylindrical* [7] of Niger.

The aim of this work was to establish the chemical profile *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* of Niger belonging to the cucurbitaceous family. We were particularly interested to these seeds, as they aroused little scientific interest up to now. There exist two varieties of *Citrullus colocyntis* known by their pulps taste. A sweet one much more studied and a bitter one that is less studied. The bitter pulp can not be meant for consumption because of its tenor in cucurbitacins. The seeds on the other hand can be removed and roasted as an edible commodity [8]. The seeds can also be ground into a powder and used as a soup thickener or flavouring agent [9, 10]. In Niger, the seeds are collected, dried, the kernels grinded and consumed by the prospected populations [4]. In pharmacology, the seeds have a drastic and tonic laxative property [9]. They also have anti-diabetic activity [11]. In Niger bitter fruits are used as laxatif. Young sprouts and mature red fruits of *Coccinia grandis* are used as food [9]. In pharmacology, the entire plant is used for the treatment of scabies (9). Its fresh leaves are consumed raw in case of snake bite [12]. In the prospected zone the populations used the fruit juice for tattoo. The ripe fruit of *Cucumis metuliferus* are currently eaten raw or boiled in the prospected zone. Harvested young and green fruits are used as seasoning in Senegal [9]. In pharmacology, the dried fruit is incinerated and meant to treat sore throat [12]. In the prospected zone, the fruit is used in the treatment of certain birds' diseases and in coetaneous infections. The fruits of *Cucumis prophetarum* are consumed mostly in Chad, a country East of Niger [9]. In pharmacology, leaves infusion is used to treat dysentery and diarrhoeic diseases [9]. In Niger, the populations in the prospected zones used the fruits in the treatment of measles.

EXPERIMENTAL

The fruits were cut into two and seeds incrusted in the pulp were removed. The seeds were dried on the straw mattresses and crushed using a crusher Retsch type (strainer diameter, 1 mm). The moisture content was determined by drying in a drying oven (Memmerl mark) according to the AOAC method [13]. Ash was obtained after mineralization by drying the grinded sample previously dried [14]. Protein content was determined using the recommended method of Kjeldahl [15], which consist of digestion, distillation and titration. The nitrogen content was converted to protein by multiplying by 6.25. Crude lipids were extracted with Soxhlet by percolation of hexane according to the method of IUPAC [16]. The solvents and reagents are from Prolabo (France) quality for analyses. Ash was solubilized in the hydrochloric acid (Prolabo, France). The contents of copper, zinc, calcium, magnesium and iron were established by the atomic absorption spectroscopy; those of sodium and potassium by emission, and then those of phosphorus by colorimetric [17, 18]. The acid, saponification, iodine and refraction numbers were determined according to the recommended methods of IUPAC [19-22]. The unsaponifiable was determined according to the French standard T60205 per extraction with the ethyl ether [15]. The solvents and reagents are from Prolabo (France) quality for analyses. Lipids were extracted according to the methods of Folch *et al.* [23]. The transesterification of fatty acids was carried out with 30-min incubation in methanol sulfuric acid (Merck) (19:1, v/v) at 80 °C with 19:0 and 21:0 (Sigma Chemical Co. St Louis) as internal standards and the methyl esters were extracted by hexane (Merck) and analyzed by gas chromatography with a Fractovap (Erba Science, Massy, France chromatograph) and a WCOT fused-silica capillary column (50 m x 0.32 mm internal diameter coated with 100 % cyanopropyl-siloxane 88 phase (Chrompack. Les Ulis, France). The conditions were as follows: ionization detector, 250 °C; injector, 230 °C

and oven program 10 °C/min from 100 to 170 °C then 2 °C/min from 170 to 200 °C; hydrogen was used as the carrier gas with a gas flow rate of 1 mL/min. Quantitative analysis was achieved with reference to the internal standards by using an ENICA 10 integrator (Delsi Nemarg, Argenteuil, France). Results are expressed as mean ± SEM [24].

RESULTS AND DISCUSSION

Chemical composition of the seeds and mineral composition of the ash

The chemical composition of the seeds is given in Table 1. The moisture content ranged from 3.68 % (*Cucumis metuliferus*) to 4.91 % (*Citrullus colocynthis*). The moisture content was 4.19 % in *Coccinia grandis* and 4.46 % in *Cucumis metuliferus*. The low level of ash was found in *Citrullus colocynthis* (2.00 %) and the high level in *Coccinia grandis* (4.19 %). The ash content of *Cucumis prophetarum* and *Cucumis metuliferus* were respectively 3.22 % and 3.26 %. The lowest crude protein content (13.19 %) was found in *Citrullus colocynthis* while the highest (26.86 %) was found in *Cucumis metuliferus*. The intermediary crude proteins content were found in *Coccinia grandis* (19.45 %) and in *Cucumis metuliferus* (23.19 %). The fat content ranged from 14.48 (*Coccinia grandis*) to 24.62 % (*Cucumis metuliferus*). The intermediary fat contents were found in *Citrullus colocynthis* (18.59 %) and in *Cucumis metuliferus* (23.79 %).

The mineral composition, expressed in mg per 100 g, of the ash of the seeds of the four species of cucurbitaceous was reported in Table 2. Calcium ranged from 247 mg/100 g (*Cucumis metuliferus*) to 569 mg/100 g (*Citrullus colocynthis*). Copper ranged from 5.1 mg/100g (*Citrullus colocynthis*) to 5.8 mg/100 g (*Cucumis prophetarum*). Iron ranged from 10.9 mg/100 g (*Cucumis metuliferus*) to 15.6 mg/100 g (*Cucumis prophetarum*). Magnesium ranged from 210 mg/100 g (*Citrullus colocynthis*) to 289 mg/100 g (*Cucumis metuliferus*). Phosphorus ranged from 30.0 mg/100 g (*Citrullus colocynthis*) to 49.1 mg/100 g (*Coccinia grandis*). Potassium ranged from 465 mg/100 g (*Citrullus colocynthis*) to 1205 mg/100 g (*Coccinia grandis*). Sodium ranged from 11.9 mg/100 g (*Citrullus colocynthis*) to 16.7 mg/100 g (*Cucumis metuliferus*). Zinc ranged from 1.1 mg/100g (*Citrullus colocynthis*) to 2.9 mg/100 g (*Cucumis prophetarum*).

Table 1. Moisture content, ash, protein and fat (g/100 g) of the seeds of *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum*.

Plant source	Moisture	Ash	Protein	Fat	R_1
Citrullus colocynthis N = 21	4.91 ± 1.66	2.00 ± 0.58	13.19 ± 1.31	18.59 ± 1.30	1.5
Coccinia grandis N = 3	4.19 ± 0.01	4.19 ± 0.33	19.45 ± 0.40	14.48 ± 0.20	0.75
Cucumis metuliferus N = 15	4.46 ± 0.91	3.26 ± 1.36	23.19 ± 2.32	23.79 ± 2.46	1
Cucumis prophetarum N = 6	3.68 ± 0.64	3.22 ± 0.58	26.86 ± 2.26	24.62 ± 3.12	0.9

Table 2. Mineral composition (mg/100g) of the seeds of *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum*.

Mineral	*Citrullus colocynthis* N = 21	*Coccinia grandis* N = 3	*Cucumis metuliferus* N = 15	*Cucumis prophetarum* N = 6
Calcium	569 ± 196	344 ± 22	247 ± 80	260 ± 28
Copper	5.1 ± 1.7	5.5 ± 0.1	5.4 ± 3.1	5.8 ± 0.2
Iron	11.6 ± 8.4	14.5 ± 3.9	10.9 ± 1.1	15.6 ± 4.6
Magnesium	210 ± 47	269 ± 0.5	289 ± 50	239 ± 18
Phosphorus	30.0 ± 4.2	49.1 ± 1.6	44.7 ± 11.1	46.2 ± 3.3
Potassium	465 ± 225	1205 ± 4.6	1174 ± 370	1137 ± 589
Sodium	11.9 ± 5.3	16.5 ± 2.3	16.7 ± 7.1	12.8 ± 5.0
Zinc	1.1 ± 0.1	1.7 ± 0.1	1.7 ± 0.3	2.9 ± 1.9

Physicochemical characteristics of the fatty substances

The acid, iodine, saponification value and refraction index as well as the percentage of the unsaponifiable were expressed in Table 3. The acid number ranged from 2.90 (*Coccinia grandis*) to 6.31 (*Cucumis metuliferus*). The saponification number ranged from 172 (*Coccinia grandis*) to 209 (*Cucumis metuliferus*). The refraction index was 1.46. Finally the percentage of the unsaponifiable ranged from 1.07 (*Citrullus colocynthis*) to 3.39 % (*Coccinia grandis*). The iodine number ranged from 138 (*Citrullus colocynthis*) to 147 (*Cucumis metuliferus*).

Table 3. Acid (I_A), iodine (I_I), refraction (I_R), and saponification (I_S) numbers and percentage of the unsaponifiable of the oils of the seeds of *Citrullus colocynthis, Coccinia grandis,Cucumis metuliferus* and *Cucumis prophetarum*.

Plant source	I_A	I_I	I_R	I_S	% of unsaponifiable
Citrullus colocynthis N = 21	4.06 ± 4.74	138 ± 9	1.4658 ± 0.0020	205 ± 1	1.07 ± 0.36
Coccinia grandis N = 3	4.86 ± 0.58	145 ± 1	1.4664 ± 0.0003	172 ± 2	3.39 ± 0.33
Cucumis metuliferus N = 15	2.90 ± 2.28	147 ± 3	1.4671 ± 0.0006	209 ± 1	1.29 ± 0.32
Cucumis prophetarum N = 6	6.31 ± 0.29	141 ± 2	1.4674 ± 0.0005	185 ± 7	1.51 ± 0.20

Composition in fatty acids and antioxidants

The Table 4 showed the major fatty acids of the seed oils of the four especies. The palmitic acid and the stearic acid constituted the two principal saturated fatty acids. The palmitic acid ranged from 15.12 % (*Coccinia grandis*) to 17.29 % (*Citrullus colocynthis*) and the stearic acid from 7.65 % (*Cucumis metuliferus*) to 11.40 % (*Coccinia grandis*). The oleic acid was the principal mono-unsaturated fatty acid. It ranged from 10.77 % (*Cucumis prophetarum*) to 18.57 % (*Cucumis metuliferus*). It was significant to note the particularly high content of linoleic acid, which was an essential fatty acid. The linoleic acid ranged from 56.21 % (*Cucumis metuliferus*) to 64.188 % (*Cucumis prophetarum*). On the other hand linolenic acid content, another essential fatty acid, was very low (traces). The unsaturated fatty acids represent from 73 % (*Citrullus colocynthis*) to 76 % (*Cucumis prophetarum*) of the total fatty acids. Finally the ratio R_2 (saturated fatty acids / unsaturated fatty acids) ranged from 0.317 (*Cucumis metuliferus*) to 0.37 (*Coccinia grandis*). They were essentially much unsaturated oils.

Table 4. Composition in fatty acids expressed as percentage by weight (g/100 g) and in antioxidants of the seed oils of *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum*.

Fatty acids	*Citrullus colocynthis* N = 21	*Coccinia grandis* N = 3	*Cucumis metuliferus* N =15	*Cucumis prophetarum* N = 6
C14:0	0.15 ± 0.12	0.16 ± 0.02	0.14 ± 0.08	0.08 ± 0.01
C16:0	17.3 ± 9.5	15.1 ± 0.6	16.2 ± 4.3	15.9 ± 1.3
C18:0	9.04 ± 2.18	11.40 ± 1.16	7.65 ± 1.56	7.83 ± 0.51
C20:0	0.28 ± 0.09	0.33 ± 0.02	0.29 ± 0.14	0.27 ± 0.06
C16:1n-9	0.14 ± 0.13	0.13 ± 0.10	0.16 ± 0.11	0.11 ± 0.01
C18:1n-9	12.3 ± 4.2	11.7 ± 0.3	18.6 ± 12.8	10.8 ± 0.7
C18:1n-7	0.48 ± 0.06	0.71 ± 0.01	0.54 ± 0.03	0.61 ± 0.04
C8:2n-6	60.1 ± 12.1	60.0 ± 2.2	56.2 ± 15.9	64.2 ± 1.1
C18:3n-3	0.27 ± 0.21	0.47 ± 0.11	0.26 ± 0.25	0.26 ± 0.04
R_2 (sat/insat)	0.365	0.370	0.320	0.317
α-tocopherol	45.1 ± 64.9	8.04 ± 0.37	79.9 ± 104.1	20. 4 ± 7.1
γ-tocopherol	435 ± 178	83.1 ± 2.2	517 ± 110	619 ± 29
β-carotene	0.18 ± 0.14	0.05 ± 0.00	0.13 ± 0.06	0.23 ± 0.01

From Table 4, the α-tocopherol ranged 8.04 mg/kg (*Coccinia grandis*) to 80 mg/kg (*Cucumis metuliferus*), the γ-tocopherol ranged from 83.1 mg/kg (*Coccinia grandis*) to 619 mg/kg (*Cucumis prophetarum*) and the β-carotene 0.05 mg/kg (*Coccinia grandis*) to 0.23 mg/kg (*Cucumis prophetarum*).

Chemical composition of the seeds and mineral composition of the ash

It is significant to note that the chemical profile of the seeds, the mineral content of the ashes, the physico-chemical characteristic, the fatty acids and antioxidants composition of the oils of the seeds of *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* have caused up till now little scientific interest. Indeed until now we have not seen any report concerning the chemical profile, the mineral content of the ashes, the physico-chemical characteristic, the fatty acids and antioxidants composition of the oils of these seeds. The results reported in this paper, therefore, constituted an original work. The seeds of the variety of *Citrullus colocynthis* with sweet pulp on the other hand, caused much scientific interest in various countries. The variety with bitter pulp studied in the present work was, therefore, lesser known. Nevertheless, the results previously reported for the sweet pulp variety showed an ash, proteins and fat [10, 25, 26] content and fatty acids [27-31] profile closer to these of the seeds of the better pulp. The proteins and fat contents of the seeds of the four species of Cucurbitaceae were lower than the one we previously reported for the seeds of *Lagenaria siceraria* [6], *Luffa* aegyptiaca and *Luffa cylindrical* [7] of Niger. Nevertheless the proteins content was close if not higher than the conventional oleaginous seeds: copra, (20-22 %); palm-tree (15-20 %); shea tree (15-16 %) [15]; the groundnut (29.5-30.1 %) [32] as well as sesame (21.10-22.50 %) [33]. Table 1 showed that the ratio R_1 = (crude lipids content/crude proteins content) ranged from 0.75 (*Coccinia grandis*) to 1.5 (*Citrullus colochinths*). The ideal oleo-proteaginious seeds would be the one containing as much oils as protein, which would correspond to ratio $R_1 = 1$. This ideal value of R_1 was found in *Cucumis metilerus* ($R_1 = 1$). It would be interesting to establish the amino acids profile of the proteins of the studied seeds in order to appreciate their possible nutritional interest.

Table 2 showed that the seeds of the studied varieties would represent potential sources in mineral and notably in calcium and iron, the scarcity of which constitute a problem in public health. The calcium and iron content were similar to the one we previously reported but the zinc content was lower [6, 7]. It is interesting to note that the level in calcium was relatively much higher than those reported in other varieties of cucurbitaceous in Cameroon [34].

Physicochemical characteristics of the fatty substances

The measurement of the free acidity of a fatty substance is one of the best means to establish its deterioration by hydrolysis of its triglycerides. It seems that the seeds of cucurbitaceous would be very rich in lipases and in lipoxygenases [35]. Table 3 showed that the acid numbers ranged from 2.90 to 6.31. This values were similar to the ones we previouly reported for the *Luffa* and *Lagenaria* varieties [6, 7]. These acid numbers were in between those of *C. lanathus* and of *C. pepo* [35]. Saponification numbers of C*occinia grandis* and C*ucumis prophetarum* seeds'oils were similar to those we reported in the *Luffa* [7] whereas; those of the *Citrullus colocynthis* and *Cucumis metuliferus* were rather similar to that of *Lagenaria* [7]. Theses saponification numbers were near to conventional oils, i.e. groungnut (186-196), palm oil (195-205) currently used in Niger but lower than that of palmist oil (246-254). The refraction number was characteristic of the group to which belongs the fatty substance. The refraction numbers were very homogeneous (1.466) and were well compared to those previously reported [35]. Unsaponifiable fractions were relatively higher than that previously reported [35] but similar to those of *Luffa* [5] except

in *Coccinia grandis* that had a relatively high content (3.39). The iodine number accounts for the degree of unsaturation of a fatty substance. The high iodine numbers reported in Table 3 revealed that the oils of the seeds of *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* were essentially unsaturated. They were much more unsaturated than conventional ground nut oils (85-98), cotton seeds oil (101-115), sunflower (103-116) and palmist oil (12-19) [15] currently consumed in Niger.

Composition in fatty acids and antioxidants

The high iodine numbers observed in Table 3 were confirmed in Table 4 by the high linoleic acid content, i.e. 60 % of the total fatty acids content. The amount of linoleic acid observed was similar to that we previously reported for the seeds of *Lagenaria siceraria* [5]. This high content in linoleic acid seems to be a characteristic of cucurbitaceous seeds oils [35, 36]. This could confer a particular nutritional interest knowing that the linoleic acid is an essentially fatty acid. Results previously reported confirmed the low linolenic content we have observed in this study [35, 36]

One of the major roles of antioxidants was to protect the double bonds of the unsaturated fatty acids against oxidation. Antioxidants also exercised essential physiological effects in human. Thus, cardiovascular risk would inversely be correlated with the consumption of α and γ tocopherols [37, 38]. These two molecules would be also determinant in the prevention of cancer [39, 40] in blocking the inflammatory process [41]. In general β-carotene also exercise a beneficial effect against atherosclerosis and vascular diseases. In fact, as tocopherols, the β-carotene would increase the low density lipoproteins (LDL) resistance against oxidation [42]. The profile in antioxidants of the oils expressed in Table 4 constituted another original work. The seeds' oils of the four varieties were potential sources of α-tocopherol (8.04 to 80 mg/kg), and γ-tocopherol (83.14 to 619.37 mg/kg). The content in α-tocopherol was however lower than in groundnut (80 to 176 mg/kg) [15]. The comparison of conventional oils showed that the oils of the studied varieties were more or less rich in γ-tocopherol, i.e. groundnut (360 mg/kg), maize (1200 mg/kg), olive (26 mg/kg), soybean (700 mg/kg), sunflower (93 mg/kg) mg/kg) [15, 43]. The low β-carotene content observed (0.05 to 0.23 mg/kg) was also found in currently consumed oils: groundnut (0.13 mg/kg), soybean (0.28 mg/kg), maize (1.2 mg/kg), sunflower (2.9 mg/kg) [15, 43].

CONCLUSIONS

The chemical content of the seeds of *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* was up to now lesser known. The experimental results reported in this paper revealed that these seeds could be potential sources of nutrient mainly of proteins, essential fatty acids (60 % of the total fatty acids), calcium and iron. It might be important in an up coming study to establish the bioavailability of the iron. For, in the sub-Saharan Africa, the ferriprive anaemia represented another major public health for children and women in age of giving birth.

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