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FATTY ACIDS, TOCOPHEROLS AND STEROLS PROFILES OF *CUCUMEROPSIS EDULIS* UNCONVENTIONAL OIL SEEDS

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ABSTRACT

The physicochemical properties, fatty acids, tocopherols, tocotrienols and phytosterols profiles of *Cucumeropsis edulis* oil seeds harvested in Benin have been undertaken in order to know its potential uses. The results revealed that this oil contained unsaturated fatty acids such as linoleic (63.35%) and oleic (18.81%) acids. Unsaponifiables (1.85%) of the oil of *Cucumeropsis edulis* contained 141 mg/100g of phytosterols (of which 82.60% consisted of β-sitosterol), 86.9 mg/100g of tocopherols which are major with the alpha form (76.9%) and minors with tocotrienols. 0.35g/100g of phospholipids have been also quantified on the basis of the content in phosphor. The study indicates that this oil could be used as biofuel and in the fields of cosmetic and nutrition regarding its contents.

KEYWORDS: unconventional oil seeds, *Cucumeropsis edulis*, fatty acids, tocopherols, phytosterols.

INTRODUCTION

Unconventional oilseeds, harvested in tropical forests or domesticated, are used for foods and cosmetics as unguents or medicinal purposes.[1,2] They have shown to be majors source of dietary ingredients related to their fatty acids composition. They also might provide nutraceuticals such as tocopherols.[3,4] Unfortunately, the chemical compositions of many unconventional oilseeds are still remaining unknown. So, recent studies focused on the

chemical compositions of them in the view to increase the supply of nutritional and functional products.[5,6]

Cucumeropsis edulis is an intercropping plant which can be grown with little water supply. It belongs to the Cucubirtaceae family and commonly named melon. The seeds are recognized rich in crude fat and protein and are used as soup thickeners that why it extracted by local methods for food purposes.[7] The different uses of this oil may depend on its physicochemical properties and its chemical compounds. For example, it's widely used in the preparation of artisanal soaps which means it has a high saponification value. Despite these considerations, few studies have attempted to characterize this unconventional oilseed. To our knowledge, as regards its composition in fatty acids and unsaponifiable compounds, only some Cucubirtaceae (excepted *Cucumeropsis edulis*) of Nigeria were studied.

Yet, more and more studies have shown that a change in sources of fatty acids were nutritionally beneficial to health. Specially, essential fatty acids which occur in unconventional oilseeds are not synthesized by human body.[8,9] For example the linoleic (Ω-6) acid, the more distributed essential fatty acid, can be found in conjugated linoleic acid (CLA) due to its origin.[10] Likewise, the literature review on tocopherols and tocotrienols released by unconventional oilseeds, indicating that these unsaponifiables matters are GRAS (Generally Recognized As Safe).

In Benin, the almonds extracted from *Cucumeropsis edulis* are used in the culinary art and the culture of *Cucumeropsis edulis* plants represents a potential source of supplementary incomes. [10] In spite of its largest uses and agronomic assessments, *Cucumeropsis edulis* was during a long time absent of the main programs of research and development. To our knowledgeno investigations have been conducted on the chemical composition (fatty acids, tocols and phytosterols) of *C. edulis* oil seeds. That why, we aim to complete scientific data on this oilseed by characterizing its fatty acids and unsaponifiables matters.

MATERIAL AND METHODS

Samples

The seeds have been harvested in Ketou, in the Southern Benin (West Africa). They have been dried in the sun and manually cleaned. Then, they are sorted out appropriately, ridded of all foulness and shelled ground finely. The obtained powder has been conditioned to 25°C, before the extraction of oil.

Extraction of oil

The extraction of oil has been made with the hexane by the Soxhlet method according to the norm of NF TV03-924. The oil and hexane mixture has been collected and the solvent has been eliminated by rotary evaporation. The extracted oil has been conditioned in dark bottles under inert atmosphere.

Chemical properties of studied oil

The moisture and volatile content were determined according to the NF T 60-201 norm. Acidity, peroxide and saponification values were determined respectively by NF T60-204S, NF T60-220, NF ISO 3657 norms. The iodine value was determined by using Wijs methods.

Dosage of the mineral elements of the defatted cake of *Cucumeropsis edulis* oilseeds

The mineral elements (N, P, Ca, Mg, Mn, Zn, Cu, Na) have been quantified by ICP (induced coupled plasma) after dry-ashing the samples. The device consisted of Varian Vista spectrophotometer equipped with a CCD detector (coupled charge device, JobinYvon) on the wave lengths: λ = 214.914 nm for the phosphor and λ = 589.592 nm for sodium. Dosages have been done using references samples as standards.

Determination of the fatty acids composition by CPG

It was done by preparing the fatty acid methylic esters (FAMEs). These last have been prepared according to the NF T60-233 norm. Then, 1µL of FAMEs dissolved in hexane have been injected in a CPG Agilent 6890 HP series (Agilent, USA), equipped with a INNOWAX (Agilent, USA) column type with 30m of long, 0,32mm of internal diameter and 0,25µm of film thickness as parameters. The injector was in 1/80 split ratio to the temperature of 250°C. The flow rate of carrier gas head pressure (helium) was 1,5mL/ min. The temperature of FID was 270°C. The programming of the temperature of the oven was 150°C during 3mn follow-up of an increase at 3°C/mn until 220°C (26.3mm) that was maintained constant until the end of the acquirement (35.3mn). FAMEs were identified by comparing with data obtained by running fatty acid standards sample obtained from Sigma-Aldrich. In order to verify the reproducibility of the results, every injection was taken three times in the same operative conditions.

Quantification of phytosterols composition

It was done after the determination of the unsaponifiables matters by the IUPAC methods.[11,12,13] The sterols fraction was isolated from the thin layer silicate chromatography

plate (Alltech, 20 x 10 cm, 250µm). The elution was made by chloroform and ethylic ether (90/10, %v/v) mixture. The sterol fraction was further analyzed in isotherm conditions (285°C) by FISON GC 8000 unit equipped witha GC8000 equipped with a SAC-5 (Sigma-Aldrich, USA)column (30m, 0,25mm and 0,25µm) type.). The temperature of the FID detector was set at 300°C and the split injector (split ratio 1/100) was at the same temperature. Sterols were quantified by internal standard method by using cholesterol as standard. The analysis was performed in triplicate.

Determination of the tocolscomposition by HPLC UV

The analysis of the tocols has been achieved by HPLC in normal phase. 20 mg of the oil was dissolved in hexane and isopropanol (99:1) mixture. After filtering, it was injected at a flow rate of 1 mL/mn in Agilent Series 1100 (France) device equipped with a quaternary pump, a manual injector with 20 µL buckle of injection. The apparatus was coupled with UV DAD (set in λ = 295 nm) detectors. The column was Luna Si 60Phenomenex (France) (5µm, 4.6x 250mm) type. The picks have been identified by injection of standards of tocols (products Sigma Aldrich). The curves of standardization were drawn while proceeding to a range of dilution of 0.3-8 µL/mL.

RESULTS AND DISCUSSION

Chemical properties of extracted oil

The physicochemical and chemical profiles of the clear yellow obtained oil are presented in Table 1. The yield (53%) is superior to the previous value related to the seed originated from Nigeria.[14] In the same way, oil extracted from *Cucumeropsis edulis* seed harvested in Benin has a weaker acidity (1.22±0,02%) compared those harvested in Nigeria.[11] That indicated that this oil can be used as food purposes. But, the peroxide value (0.06±032 meq O_2/Kg) is conform the value set by the Codex Alimentarius norms (<10meq O_2/Kg). [15] It can be explained by Appelbaum *et al*. (1989) hypothesis who found that the high presence of unsaturated compounds such as carotenoids, unsaturated fatty acids and vitamins would undergo oxidizations with formation of peroxides.[16]This hypothesis can be verified latter by analyzing the unsaturated fatty acids composition of the oil.

The high obtained saponification value (198±0.21 mg KOH/g) is consistent with the traditional use of this oil for making soap. It comparable with the saponification values of conventional oils such as peanuts (187-196 KOH/g), soy (189-195mgKOH/g) and cotton

(189-195 KOH/g). It also can indicate the preponderance of the fatty acids having C18 backbone.

Table 1 : Physicochemical and chemical profiles of *Cucumeropsis edulis* seeds oils	
Oilyield's (%-MS)	53.44±0.18
Acidity (% of oleicacids)	1.22±0.02
Saponification value (mg KOH/g)	198±0.21
Iodine value (g/100g)	114.80
Refractory index	1.46
Peroxid value (meq O_2/Kg)	0.06±032
Unsaponifiable (%)	1.85 ± 0.21

Mineral elements and content in phospholipids

The dosage of the phosphor permits to evaluate the presenceof phospholipids in oil. In the table 2, the mineral elements and the phospholipids contents of *Cucumeropsis edulis* harvested in Benin are reported. The contents of Ca and Mg (74 ppm and 62 ppm respectively) are higherthan in Na (17.9 ppm). It is superior to those cited by Dako *et al.* (2006).[12] This could indicate that the total phospholipids (0.35g/100g) are more in non-hydratableform. It shows that the cake of *C. edulis* could be used as a fertilizer, owingto its high phosphor content.

Table2 : Mineral elements and phospholipids content of *Cucumeropsis edulis* seeds oils	
P (ppm)	340
Phospholipides (g/100g)	0.35
K (ppm)	Nd
Na (ppm)	17.9
Ca (ppm)	74
Mg (ppm)	62
Fe (ppm)	0.7
Zn (ppm)	1.5
Mn (ppm)	0.34

Nd : No détected

Composition in fatty acids of *Cucumeropsis edulis* oil

The fatty acid composition of *Cucumeropsis edulis* oil is reported in the table 3. The values are comparable with those of edible oils.[17,18,19] This oil is rich in essential fatty acid such as linoleic (63.35%.), as we assumed above. This proportion is major than those quantified in Cucurbitacea oil seed of pumpkin (43.0±53.0%) but it is similar to that found for the European variety of *Cucurbita pepo*.[20] So, the oil of *Cucumeropsis edulis* is good source of

linoleic acid. It is increasingly recognized that an insufficient intake of omega-6 acid such as linoleic causes growth retardation in children, heart attack risk and skin ailments.[21,22] Study showed that monounsaturated fatty acid diets decrease HDL-cholesterol concentrations which affect positively cardiovascular disease risk.[23] The monounsaturated fatty acid of *C. edulis* oil's consisted of oleic (18.81 ± 0.35%). The proportion is lower than the one of Pumpkin seed oil but relatively closed with some unconventional oil seeds.[18,24] Few amount (0.90 ± 0.18%) of Ω-3 (linolenic) fatty acid was detected and the proportion of saturated fatty acids of the oil, palmitic (9.49 ± 1.23%) and stearic (7.45 ± 0.18%),are less than the one of some edible oils. [25]

Table 3 : Fatty acids compositions (%) of *Cucumeropsis edulis* seed oils	
Myristicacid (C14:0)	Nd
Palmiticacid (C16:0)	9.49 ± 1.23
Stearicacid (C18:0)	7.45 ± 0.18
Oleicacid (C18:1, n-9)	18.81 ± 0.35
Linoleicacid (C18:2, n-9,12)	63.35 ± 0.20
Linolenicacid	0.90 ± 0.18

Nd : No detected

Composition of the oil of *C. edulis* in phytosterols and lupeol

The composition of the unsaponifiable matters of *C. edulis* oil in phytosterols and lupeol is presented in table 4. The results were in agreement with that recounted for many vegetable oils where β-sitosterol (82.60%) constitute the major phytosterol follow-up by stigmasterol (13.05%).[23,26] In the same way, the total phytosterols (141 mg/100g) is similarto those of other edible oils.[14,15] Plant phytosterols have been described as anti-inflammatory and anti-cancer compounds.[27] The triterpen lupeol is also quantified in relatively high proportion (4.14%) compared to previous values.[5]

Table 4 : Phytosterols and triterpen content of *Cucumeropsis edulis* seed oils		
Constituants	**Retention time (min)**	**Teneur (%p)**
7-dehydrocholesterol	9.66	0,02 ± 0,01
Lupeol	10.15	4,14 ± 0,02
Lanosterol	10.68	0,020± 1,05
Cholesterol	13.34	0,01± 0,06
Stigmasterol	14.80	13,05 ± 0,00
β- sitosterol	16.04	82,60 ± 0,15

Composition of the oil of *C. edulis* in tocols

Tocopherols and tocotrienols compounds of *C. edulis* oils are quantified and presented in table 5. The total quantity is 86.90mg/100g. These chemical compounds which constitute vitamin E have antioxidant properties. [28, 29, 30] α-tocopherol is the most important tocol quantified (76.90%). It has been shown that α-tocopherol derived from natural vegetable oil had a superior power to that of synthetic antioxidant tocopherols. [31, 32]

Table 5 : Tocols content of *Cucumeropsisedulis seed oils*

Constituants		**Retention time (min)**	**Teneur (%)**
Tocotrienols	δ-tocotrienol	5 .67	0,01±0,34
	(β+γ)- tocotrienol	7.14	0,55±0,05
	α- tocotrienol	10.50	0,22±1,12
Tocopherols	δ-tocopherol	11.14	0,17±0,00
	(β+γ)-tocopherol	17.40	22,14±0,22
	α-tocopherol	21.16	76,90±0,34

CONCLUSION

The present work has focused on the chemical composition of *Cucumeropsis edulis* oilseed harvested in Benin. This investigating reveals that the oil contains high proportion of unsaturated fatty acids and significant contents in tocols and phytosterols. The viscosity of the studied oil is weaker than the one of the cotton and the soy oils what offers possibilities of its use as biofuel. In addition to its content in nutraceutical compounds (sterols and tocopherols), it could serve oil of table. In sum, this oil could be used in feeding, in cosmetics and as a biofuel.

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