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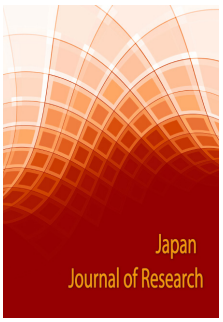

Biochemical Characterization of the Unsaponifiable Fraction of *Coula edulis* Oil Collected at Sembé

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Abstract

Coula edulis Baill., is a non-timber forest product which represents a considerable food, cultural and economic stake for the populations of Central Africa. It belongs to the order of Olacales and the family of Olacaceae.

The unsaponifiable fraction of the oil of Coula edulis Baill. from Sembé, extracted by Soxhlet (NF ISO 82 62-3) with n-hexane was the subject of this study. The extracted fat content is: 25.91 ± 0.16% per 100 g of almonds.

Conventional methods were used to characterize the unsaponifiables of this oil: Extraction with diethyl ether (NF EN ISO 3596) to determine the content of unsaponifiables; High Performance Liquid Chromatography (NF EN ISO 9936) for tocopherols and tocotrienols; determining the content of individual and total sterols (NF EN ISO 12228); total polyphenols by Folin's method and alkanes by HPLC-GC-FID coupling. We obtained: Tocopherols and Tocotrienols (α-tocopherol acetate <5 mg/Kg of fat, α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, Δ-tocotrienol <2 mg/Kg of fat for each molecule); Sterols: β-sitosterol (49.3%), Stigmasterol (41.3%), Δ5-avenasterol (3%), Campesterol (1.5%), Δ7-stigmastenol (1.2%), Clerosterol (1%), Sitostanol (0.9%), Cholesterol (0.8%), Δ5,24-Stigmastadienol (0.8%), Brassicasterol, 24-methyl-cholesterol, Campestanol, Δ5, 23-stigmastadienol and Δ7- avenasterol 0.1% for each of the molecules. The content of total sterols is: 959 mg/Kg, that of total polyphenols, 34 mg/Kg and that of alkanes, 98 mg/Kg. These molecules are of paramount importance in human nutrition and in medicine.

Introduction

In Africa, non-timber forest products (NTFPs) have, for centuries, played an important food and commercial role. They still contribute today to the reduction of poverty and the food security of forest and peri-forest populations in Central Africa [1]. In the Republic of Congo, there are non-wood forest products of plant origin that are well known to rural populations and have significant nutritional potential. Despite their capital importance in the diet of rural populations, several plant species still remain underexploited due to a lack of adequate scientific information. Among these plant species is *Coula edulis*, a plant belonging to the order *Olacales* and the family *Olacaceae*.

Coula edulis is a multi-purpose species whose nuts are consumed and marketed by African populations, but little known and underused. Despite its importance, no assessment of its economic potential has ever been undertaken [2]. Still qualified as a wild mango tree thanks to the shape of its fruits, *Coula edulis* Baill. is a medium-sized arborescent species (30 m in height) of the lower levels of dense humid evergreen forest and whose natural range extends from Sierra Leone to the Democratic Republic of Congo [3]. It prefers clayey soils. Its phenology is characterized by annual and regular flowering and fruiting which extend in Gabon over approximately four months, between January and April [2]. The stones of *Coula edulis* fruits play a significant role in the diet and the local African economy. *C. edulis* constitutes one of the examples of unconventional crops for the development of local populations and industrial applications. Some authors have reported that *Coula edulis* hazelnuts from the Ivory Coast constitute a source of proteins, lipids, minerals and oleic acid [4]. In the Republic of Congo, no studies on the biochemical characterization of *Coula edulis* hazelnut kernels have been carried out. Thus, we have set ourselves the scientific interest of characterizing the unsaponifiable fraction of the oil extracted from the hazelnut kernels of C. edulis fruits in order to clearly identify all the biomolecules of great interest for science that it has.

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Material and methods

Plant material

The plant material used consisted of almonds extracted from the nuts of ripe the *Coula edulis* fruits. The fruit collection took place in May 2022 in Zoulabouth, a locality in the sub-prefecture of Sembé in the department of Sangha in Congo Brazzaville.

Methods

Sample preparation

The hazelnuts from the *Coula edulis* fruits were transferred to the laboratory where they were mechanically shelled to separate the kernels and shells. The almonds obtained were placed in the oven set at a temperature of 65°C. The almonds were dried until their masses became constant. After drying, the almonds were ground using a porcelain mortar to obtain a powder.

Shelling and weighing

This operation consists of extracting the almonds from the fresh seeds of the *Coula edulis* fruits from the shells. These almonds are then weighed.

Drying

The weighed almonds are placed in the oven set at a temperature of 65°C. These almonds remain in the oven until their masses become constant.

Grinding

The almonds taken out of the oven are cooled in the open air for one hour and are then crushed.

Extraction of fat or lipids

The fat was extracted by the Soxhlet method (NF ISO 82 62-3) with n.-hexane. After the separation of the saponifiable and unsaponifiable fractions of the lipids, we retained the latter which constituted our study sample.

Classic methods have been used to characterize unsaponifiables: Extraction with diethyl oxide (NF EN ISO 3596) for the determination of unsaponifiables; high performance liquid chromatography (NF EN ISO 9936) for tocopherols and tocotrienols; individual and total sterol contents (NF EN ISO 12228); total polyphenols by the Folin method and alkanes by HPLC-GC-FID coupling.

Determination of the content of the unsaponifiable fraction

The unsaponifiable fraction of the fat extracted from the kernels of *Coula edulis* seeds was analyzed using Agilent type 7890 gas chromatography, coupled with type 5979 mass spectrometry. The operational conditions were as follows:

- DB5 MS column: 20 m x 0.18 mm, thickness 0.18 µm;
- a Helium carrier gas: 1 mL/min;
- ionization energy: 70 eV;
- injection temperature: 320°C;
- detector temperature: 280°C;
- temperature of the ion source: 230°C;
- oven programming: from 50°C (2 to 3 min) to 320°C at 8°C/min, 3 min;
- split mode injector 1: 150.

Mass spectra are acquired in the m/z scanning mode RANGE 33 – 450. Compounds are identified by comparing the spectra to those available in databases.

Analysis of the unsaponifiable fraction

The unsaponifiable fraction represents all the substances which do not react with alkaline compounds to produce soap. These compounds remain soluble in organic solvents (hexane, ethyl ether, French standard T 60-205-2).

The extraction of unsaponifiables was carried out according to the NF T 60-205-2 standard and the unsaponifiable fraction was determined by the IUPAC method.

Approximately 5 grams of extracted fat are introduced into a heating tube into which 50mL of 2N ethanolic potassium hydroxide is introduced and then brought to the boil for 1 hour. After cooling, 100mL of distilled water is added. Extraction of the organic fraction is done three times with 50mL of ether-pentane (50:50, V/V). The three organic fractions are combined, then washed three times with distilled water of volume 50mL, then the organic fraction is washed with 40mL of an aqueous solution of potassium hydroxide at 30g/L. A new wash with distilled water containing 5% ethanol is carried out until the pH is no longer basic in the residual water (check made with phenolphthalein). The solution is vaporized using the rotary evaporator in a previously tared flask (A1) and drying in an oven set between 100 and 105°C is carried out for 15 minutes. After cooling, the flask containing the extract of unsaponifiables is weighed (A2). The unsaponifiable content is given by the following formula:

$$\% \text{ Unsaponifiables} = 100 \times A / PE$$

A = A2 - A1: mass of unsaponifiables in grams

Coula edulis seeds

Grinding almonds

Ground material obtained

Determination of hydrocarbon composition

These are various hydrocarbons (saturated and unsaturated aliphatic hydrocarbons, triterpenes or tetraterpenes). The isolation of this unsaponifiable fraction of the *Coula edulis* oil is carried out according to the AFNOR NF method in ISO 15302 and the different classes of constituents of these unsaponifiables are separated by thin layer chromatography (TLC), then the different classes of hydrocarbons are separated by HPLC coupled with Mass Spectrometry.

Determination of total polyphenols

Total polyphenols were obtained by weighing 5g of the *Coula edulis* fat which was added to acetone/water (70:30, V/V). The supernatant composed of polyphenols is recovered by filtration 30 minutes later. For the determination of total polyphenols, we used the Folin-Ciocalteu method, optimized by George (2005): a standard solution of gallic acid (28.9 mg) prepared with distilled water (25 mL) is stored at 4°C. Before the measurement, the solution was diluted ten times with distilled water before preparing the calibration solutions 20, 40, 60, 80µL (water is added to obtain a volume of 500µL). 2.5mL of Folin-Ciocalteu reagent (diluted ten times) are added to 500µL of sample diluted ten times, 500µL of water (blank) or 500µL of calibration solution. The mixture is exposed for 2 minutes at room temperature, then 2 mL of a sodium carbonate solution (75 g/L) is added. The optical density is immediately measured with a spectrophotometer.

Determination of the composition and contents of tocopherols by HPLC-UV

Tocopherols were determined according to the ISO 9936 standard, using the Thermo Fischer HPLC chain with a silica column (25 cm x 0.4 cm). The mobile phase with a flow rate of 1mL/min and the eluent consisted of a mixture of solvent: hexane (97%) and dioxane (3%). The detector used was a Thermo Fischer brand spectrofluorimeter (FL 3000). The recording of the chromatograms is carried out on a microcomputer with Chromquest 4.2 Thermo Fischer data processing software.

The analysis of tocopherols in the oil was carried out by HPLC in normal phase. A 20 mg/mL solution of hexane and isopropanol (99:1) was filtered using a 0.45 µm diameter millipore filter. The device and its accessories (pump, injector, detector) are products from Agilent Series 1100 (France), comprising a quaternary pump, a manual injector equipped with a 20 µL injection loop and a DAD detector (with strips diode). The column was Luna SI 60, 5 µm, 4.6 x 250 mm (Phenomenex, France). The solvent mixture under isocratic conditions was composed of hexane and isopropanol (99:1, %V: V). The column flow rate was 1 mL/min and the pressure was 33 bars with a DAD detector at a wavelength of 295 nm. The peaks were identified by injection of tocopherol standard (Sigma Aldrich products). Calibration curves were obtained using a dilution range of 0.3 to 8 µL/mL.

Determination of the sterol composition of *Coula edulis* oil

To determine the total sterols, we weighed 0.5 g of fat and 1 mL of alcoholic KOH which we introduced into a flask with 2 grains of pumice stone and refluxed for 15 minutes. 5mL of ethanol are then introduced into the refrigerant. 10 mL of this solution are introduced into a chromatography column filled with aluminum oxide (0.063 < I < 0.2 mm). The elutions were carried out successively with 5 mL of ethanol and 30 mL of diethyl ether. The fraction obtained was, after evaporation of the solvent, dissolved in 1 mL of chloroform.

Preparation of the sterol fraction: Thin Layer Chromatography: 20 µL of a cholesterol standard solution and 400 µL of a solution of the unsaponifiable fraction of the *Coula edulis* oil are successively deposited using a Linomat IV-Y CAMAG depositor (Merck, Ref 022-786) on a silica 60 plate (Alltech, 20 x 10 cm, 250 µm thick). The elution is carried out with a chloroform/diethyl ether mixture (90/10; %v/v). The part containing the cholesterol deposit is revealed by nebulization of a Cu^{++}/H_3PO_4 mixture (1/1; % v/v) and baking at 180°C for 10 min.

The sterol band corresponding to the cholesterol spot was scratched and the sterols were extracted using chloroform (10 mL/g of silica) at room temperature with magnetic stirring for 5 min. The solution obtained is passed through a Millipore membrane filter (0.45 µm, Ref. SLFH 013 NL) in order to recover the total sterols.

Composition and sterol content: 1µL of the sterol fraction obtained was injected into the gas chromatograph in order to determine the contents of the different classes of sterols in the solution containing the total sterols of the unsaponifiable fraction extracted from the oil of the *Coula edulis* almonds. This analysis was carried out under isothermal conditions (285°C) in a CG 8000 GC apparatus (Fisons Instruments) equipped with a SAC-5 type column (Sigma-Aldrich, USA), (length 30 m, 0. 25mm internal diameter and 0.25µm film thickness). The temperature of the flame ionization detector was maintained at 300°C and that of the injector in split mode, ratio 1/100 at 300°C. The peaks were integrated using a Merck D2000 integrator. Helium served as carrier gas (1.5 mL/min). To identify the peaks, we injected standards of cholesterol, β-sitosterol and stigmasterol (Sigma quality products, concentration of 1 mg/mL). To check the reproducibility of the results, each injection was repeated three times under the same operating conditions. Total sterols were calculated as follows:

(Ax X ms X K X 100)

Total sterols (mg/g) = Σx

(As x m)

Ax = Peak area of sterol

As = cholesterol peak area

ms = mass of added cholesterol

K = sterol response factor calculated based on the area of the internal standard for an identical concentration.

Table 1. *Composition of Tocopherols, Tocotrienols of Coula edulis oil*

Tocopherols or Tocotrienols	Contents (mg/kg of MG)
Acetate α-tocopherol <5	<5
α-tocopherol	<2
β-tocopherol	<2
γ-tocopherol	<2
Δ-tocopherol	<2
α-tocotrienol	<2
β-tocotrienol	<2
γ-tocotrienol	<2
Δ-tocotrienrol	<2

Table 2. *Sterol composition of Coula edulis oil*

Sterols	Contents (%)
Cholesterol	0.8
Brassicasterol	<0.1
24 methyl-cholesterol	<0.1
Campesterol	1.5
Campestanol	<0.1
Stigmasterol	41.3
Δ7-Campesterol	<0.1
Δ5,23 Stigmastadienol	<0.1
Clerosterol	1.0
β-Sitosterol	49.3
Sitostanol	0.9
Δ5-Avenasterol	3.0
Δ5,24 Stigmastadienol	0.8
Δ-7-Stigmastenol	1.2
Δ7-Avenasterol	<0.1

Table 3. *Composition of aliphatic alcohols and terpenes of Coula edulis oil*

Settings	Contents (%)
Aliphatic Alcohols	**9.8 mg/kg**
Terpene alcohols	**620.9 mg/kg**
α-Amyrin	2.3 %
β-amyrin	1.7 %
Butyrospermol	1.1 %
Lupeol	17.4 %
Cycloartenol	7.6 %
Tirucallol	0.1 %
Unidentified triterpene alcohols	**70 %**

Results and discussion

We obtained the following results:

Extracted fat content: 25.91±0.16%;

Total sterols: 959 mg/Kg; Total polyphenols: 34 mg/Kg; Alkanes: 98 mg/Kg;

Tocopherols and Tocotrienols (α-tocopherol acetate <5mg/Kg of fat, α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, Δ-tocotrienol < 2 mg/Kg of fat for each molecule) ; Sterols: β-sitosterol (49.3%), Stigmasterol (41.3%), Δ5-avenasterol (3%), Campesterol (1.5%), Δ7-stigmastenol (1.2%), Clerosterol (1%), Sitostanol (0.9%), Cholesterol (0.8%), Δ5,24-Stigmastadienol (0.8%), Brassicasterol, 24-methyl-cholesterol, Campestanol, Δ5,23-stigmastadienol and Δ7-avenasterol 0 .1% for each of the molecules.

The most represented molecules are sterols with a content of 959 mg/Kg (including: β-sistosterol (49.3%), Stigmasterol (41.3%), Δ5-avenasterol (3%) and Campesterol (1.5 %), are the most representative) followed by alkanes (98 mg/Kg) and total polyphenols (34 mg/Kg). Despite its low content in vegetable oils, the unsaponifiable fraction contains noble compounds such as tocopherols which have provitamin E activity and play an important role in the stability of the oil during storage or culinary processing [5].

The unsaponifiable fraction of the Coula edulis oil studied is less rich in β-sitosterol (49.3%) compared to that of the argan tree (58.89% for Morocco and 64.22% for Algeria), very weakly rich in Campesterol (1.5%) compared to argan (19.2% for Morocco and 17.7% Algeria) and richer in Stigmasterol (41.3%) compared to argan tree (8.5% for Morocco and 17.4% for Algeria) [6].

The total sterol content obtained in this study (959mg/Kg) is higher than that obtained by certain authors working on the same product in Ivory Coast [7].

This total sterol content obtained in this study (959 mg/Kg) is much higher than those obtained by other authors, working on oils extracted from the seeds of *Neocarya macrophylla* (24.42 mg/Kg), of *Anona muricata* (32.40mg/Kg) and *Terminalia catappa* (30.48mg/Kg) [8]. *Coula edulis* seed oil is richer in sterols than the seed oils of *Neocarya macrophylla, Anona muricata* and *Terminalia catappa*.

The rate of β-sistosterol (49.3%) obtained on the oil of *Coula edulis* is higher than the value obtained by the previous authors, working on the oils extracted from the seeds of *Neocarya macrophylla* (39.44%) and lower than the values obtained by the same authors on the seeds of *Anona muricata* (55.23%) and *Terminalia catappa* (80.60%) [8].

The campesterol content (1.5%) obtained in the seed oil of Coula edulis is very low compared to the values obtained by the same authors on the seed oils of *Neocarya macrophylla* (7.38%), of *Anona muricata*. (15.33%) and *Terminalia catappa* (4.50%). However, the oil extracted from the seeds of *Coula edulis* is richer in stigmasterol (41.3%) than the oils from the seeds of *Anona muricata* (26.27%) and *Terminalia catappa* (9.00%) but slightly less rich. compared to *Neocarya macrophylla* oil (45.48%) [8].

The level of Δ5-avenasterol (3%) in the fat of *Coula edulis* is lower than those of the oils extracted from the seeds of *Neocarya macrophylla* (7.69%) and *Terminalia catappa* (5.90%) but substantially identical to that of *Anona muricata* seed oil (3.17%).

The *Coula edulis* oil studied is less rich in α-tocopherol, Δ-tocopherol, γ-tocopherol (values <2 mg/Kg for each) than *Moringa oleifera* oil (α-tocopherol: 101.11 mg/Kg; Δ-tocopherol: 10.36 mg/Kg and γ-tocopherol: 86.87 mg/Kg) [9]. It is also less rich in sterols (959mg/Kg) than the *Moringa oleifera* oil studied by previous authors and who obtained a value of 2896.2mg/Kg.

Coula edulis oil has a higher content of tocopherols and all classes of tocopherols are represented with contents lower than 2 mg/Kg of fat for α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol for each molecule, unlike *Neocarya macrophylla* oil including α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol which do not exist [8]. On the other hand, *Neocarya macrophylla* oil essentially only contains γ-tocopherol as tocopherol. The tocopherol contents are also lower in the fat of *Anona muricata* studied by the authors cited above compared to the oil of *Coula edulis* studied with values lower than <1 mg/Kg of fat for α-tocopherol. , γ-tocopherol and Δ-tocopherol, the others not existing. The same authors, on the other hand, obtained significant values by working on *Terminalia catappa* oil. The latter obtained: α-tocopherol (4.09mg/Kg), γ-tocopherol

(8mg/Kg), the others exist in trace form.

Argania spinosa L. oil is richer in sterols (1837.25 mg/Kg) than the *Coula edulis* oil studied here (959 mg/Kg) [10]. The *Coula edulis* oil studied is less rich in α-tocopherol, Δ-tocopherol, γ-tocopherol (values <2 mg/Kg for each) than the oil of *Argania spinosa* L. (α-tocopherol: 31.6 mg/ Kg; Δ-tocopherol: 49.52 mg/ Kg and γ-tocopherol: 615.65 mg/Kg) [10].

Selma Ş. Uras Güngör and Gamze Kökdil, working on the oil extracted from the seeds of *Trigonella cariensis*, obtained the following values: α-tocopherol (2335.4 mg/Kg), β-tocopherol (362.9 mg/Kg), γ- tocopherol (7.8mg/Kg); all these values are much higher than the values obtained on the *Coula edulis* oil studied, the values found of which are: α-tocopherol, β-tocopherol, γ-tocopherol all <2 mg/Kg of fat for each molecule [11].

The *Coula edulis* oil studied is less rich in campesterol (1.5%) than the oils of *Passiflora edulis* and *Passiflora foetida* with the following values: *P. edulis* (12.3%) and *P. foetida* (10.3%) [12]. The Passiflora oils studied by these previously cited authors are less rich in stigmasterol (P. edulis: 28.6% and *P. foetida*: 18.3%) than the *Coula edulis* oil studied here (41.3%). The β-sitosterol contents are substantially identical in *Passiflora* oils (*P edulis*: 48.8%; *P. foetida*: 48.6%) as in the *Coula edulis* oil studied (49.3%) [12] . The Δ5-avenasterol contents of the three oils are very close (3% for *Coula edulis*, 4.5% for *P. edulis* and 4.6% for *P. foetida*) [12].

Some authors have obtained the following tocopherol values on the seed oils of some varieties of argan tree from Morocco: the total tocopherol contents vary from 323.86 to 553.12 mg/ Kg distributed as follows: α- tocopherols, the values vary from 25.72 to 57.69 mg/Kg; Δ-tocopherols, the values vary from 18.62 to 45.09, and γ-tocopherols, the values vary from 247.25 to 465.04mg/Kg [13]. These values are much higher than the values obtained for the same molecules on the Coula edulis oil studied (α-tocopherol, γ-tocopherol, Δ-tocopherol <2 mg/Kg of fat for each of the molecules). *Coula edulis* oil is very low in vitamin E compared to argan oils.

Coula edulis oil is very rich in β-sitosterol (472.79mg/ Kg) than the oil extracted from green plum seeds whose β-sitosterol content is 76.95mg/Kg corresponding to 80.70% total tocopherols [14]. For total tocopherols from green plum seed oil, the following values were obtained by the same authors: α-tocopherol (34.42 mg/Kg), β-tocopherol (1.14 mg/ Kg), γ-tocopherol (52.30mg/Kg), and Δ-tocopherol (1.14mg/ Kg) [14]. These values are much higher than those of the *Coula edulis* oil studied and which are: α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol <2 mg/Kg of fat for each of the molecules.

The *Coula edulis* almond oil studied is as rich in total sterols (959 mg/Kg) as soybean (800-1700mg/Kg) and sunflower (700-1200mg/Kg) oils [15]. On the other hand, it is less rich in total sterols than wheat germ oil (3000-3500 mg/Kg) also studied by these previous authors.

The *Coula edulis* fat studied gave a total polyphenol content of 34 mg/Kg. This value is higher compared to the values found by certain authors, working on the oils of *Anona muricata* and *Terminalia catappa* and who obtained respectively: 21.20 mg/ Kg of polyphenols for the first and 17.70 mg/Kg for the last [8]. However, it is less rich in total polyphenols than the *Neocarya macrophylla* oil studied by the same authors and who obtained a value of 533% mg/Kg [8].

In this study, we obtained a total polyphenol content of 34 mg/Kg. This value is very low compared to the average values obtained by certain authors, working on sunflower seeds (6863 mg/Kg), rapeseed (6141 mg/Kg) and soya (4154 mg/Kg) [16]. It is also low compared to the values obtained by other authors, working on oils extracted from grape seeds and who obtained values ranging from 240 to 1130 mg/Kg of material [17] and also very low compared to oils from four varieties of olives from the Saïs region of Morocco studied by other authors and who obtained values ranging from 320 to 360 mg/Kg [18]. This value is also low compared to sesame oil whose value is 138 mg/Kg of fat [19].

Conclusion and perspectives

This study allowed us to affirm that sterols are the most representative molecules (959 mg/Kg), followed by hydrocarbons (98 mg/Kg) and finally polyphenols (34 mg/ Kg) in the unsaponifiable fraction of the oil. extracted from the kernels of ripe *Coula edulis* fruits. All classes of unsaponifiables of a vegetable oil are represented.

The oil extracted from the kernels of *Coula edulis* seeds is very rich in sterols and less rich in polyphenols.

The high level of sterols in *Coula edulis* oil studied shows that this oil has significant vitamin E power. On the other hand, its low polyphenol content allows us to conclude that this oil is less stable and has low antioxidant activity.

The contents of tocopherols and tocotrienols (α-tocopherol acetate <5 mg/Kg of fat, α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol , Δ-tocotriénrol <2 mg/Kg of fat for each molecule) are low. From the identified sterols, we obtained: β-sistosterol (49.3%), Stigmasterol (41.3%), Δ5-avenasterol (3%), Campesterol (1.5%), Δ7-stigmastenol (1.2%), Clerosterol (1%), Sitostanol (0.9%), Cholesterol (0.8%), Δ5,24-Stigmastadienol (0.8%), Brassicasterol, 24-methyl-cholesterol, Campestanol, Δ5,23- stigmastadienol and Δ7-avenasterol 0.1% for each of the molecules.

Much work still remains to be done; it is:

- determine the biochemical composition of the glyceric and phospholipid fractions of the oil, the protein and carbohydrate fractions of the almonds of this agro-resource in order to compare it with others;
- determine the different allergens of *Coula edulis* oil and compare to other vegetable oils;
- bring back to the laboratory the information that will allow us to consider a large-scale commercial application of these hazelnuts, or even their protection as seeds.

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