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Effect of dehulling method on the chemical composition of the lipid constituents of the kernels and oils of *Ricinodendron heudelotii* seeds

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ABSTRACT

The aim of this study was to characterize the lipids present in the oil and kernels of *Ricinodendron heudelotii* seeds. Two dehulling methods were used to extract kernels from their husks: a heat treatment and a manual mechanical dehulling technique. Oil from kernels obtained by traditional heat treatment of seeds had a kinematic viscosity of 169 ± 0.5 mPa.s, and an iodine value of 175.6 ± 1.1 g/100 g oil. The oil from kernels isolated by mechanical dehulling had a kinematic viscosity of 128 ± 0.11 mPa.s and an iodine value of 195.2 ± 2.0 g/100 g oil. Determination of the minor compound profile of the oil revealed a total tocopherol content of 135.0 ± 0.4 mg oil from kernels isolated by traditional heat treatment. The oil obtained from kernels isolated by manual mechanical dehulling had a total tocopherol content of 178.3 ± 0.4 mg/100 g. An analysis of the fatty-acid profile of the oil from kernels isolated by traditional heat treatment revealed the presence of α -eleostearic acid ($50.5\% \pm 0.1$), linoleic acid ($24.0\% \pm 0.0$), β -eleostearic ($8.4\% \pm 0.0$) and catalpic acid ($0.40\% \pm 0.1$). By contrast, the fatty acid composition of oil from kernels isolated by manual mechanical dehulling was of α -eleostearic acid ($60.1\% \pm 0.2$), linoleic acid ($22.8\% \pm 0.1$).

Ricinodendron heudelotii oil has a very high level ($84.4 \pm 0.4\%$) of polyunsaturated fatty acids (C18A). Some analysis of the triglycerides present in the oil revealed the potential isomerization of α -eleostearic acid to form β -eleostearic acid and catalpic acid.

1. Introduction

Ricinodendron or *Jatropha heudelotii* is a tree belonging to the Euphorbiaceae family. It is known under various common names in Africa: djansan in Cameroon, akpi in Côte d'Ivoire and wama in Ghana. It can grow to a height of almost 30 m, and it produces drupes, each of which contains no more than four seeds. It grows in humid tropical zones and its distribution range in Africa extends from Senegal to Kenya. The fruits of *Ricinodendron heudelotii* reach maturity between June and September. They are harvested periodically, under the trees, by women.

Since the United Nations Conference on Environment and Development (UNCED) in Rio in 1992, the ecological and socio economic importance of non ligneous forest products (NLFPs) has been recognized in the management of forest ecosystems. In Cameroon, an economic and social model based on NLFPs was recently implemented, in order to structure the NLFP sectors, including the *Ricinodendron*

heudelotii sector. This sector was included in the model based on the properties of the lipids this tree produces, its cultural importance, its dietary value and its potential profitability (Roques et al., 2019). The seeds are extracted from the fruits and boiled to extract the kernels, which are used as aroma providing condiments in traditional African cooking. The oil has an agreeable flavor and is very popular for cooking. In the last few years, *Ricinodendron heudelotii* has been used by the National Center for Agronomic Research (CNRA) in Côte d'Ivoire as part of an agroforestry system based on cocoa plantations, and in experimental fields, with a view to its domestication. There are two main reasons for wanting to domesticate *Ricinodendron heudelotii*: (i) the fruits, the kernels of which are rich in oil and sold on most of the markets of West Africa for use as a seasoning and thickening agent in various African dishes (Coulibaly et al., 2018) and (ii) *Ricinodendron heudelotii* is accepted by foresters in Côte d'Ivoire as a canopy tree in cocoa plantations.

This tree has been the subject of several scientific studies in recent

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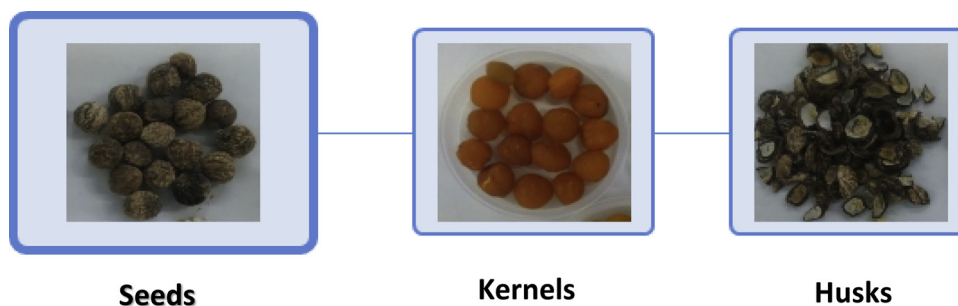


Fig. 1. *Ricinodendron heudelotii* (seeds, kernels, husks).

years. Studies of its kernels have reported an oil content of 42 to 60%, depending on the extraction technique used (Dandjouma et al., 2008). This is a potentially important oleaginous natural resource, due to the high oil content of its seeds. According to studies performed by César Kapseu in 1995 and confirmed by Tchiégang et al. in 1996, the oil from kernels isolated by traditional heat treatment has α eleostearic acid (51%) and linoleic acid (28%) as its major fatty acids (Kapseu and Tchiégang, 1995; Tchiégang et al., 1997). By contrast, Samuel and coworkers found, in 2011, that the major fatty acid of the oil obtained from kernels isolated in the same conditions was linoleic acid (58.7%) (Yeboah et al., 2011). Leudeu et al., in their 2009 study on oil from kernels isolated by traditional heat treatment, found that the major fatty acid was linoleic acid, which accounted for 36% of the fatty acids present (Leudeu et al., 2009). By contrast, according to Sompila et al. (2014), the major fatty acid in *Ricinodendron heudelotii* oil is α eleostearic acid, which accounts for 35% of the fatty acids present (Sompila et al., 2014).

The α eleostearic acid (C18:3 n 5) is a fatty acid containing 18 carbon atoms and three conjugated double bonds (9c, 11 t, 13 t) with the following physico chemical data : molecular weight 278.42 g/mol, melting point 49 °C, boiling point 235/12 (°C/mm Hg), density 0.902 g/ml (at 50 °C), iodine value 274. This polyunsaturated fatty acid and its isomers (punicic acid, catalpic acid, β eleostearic acid and jacaric acid) have demonstrated many health benefits. Studies have shown that these bioactive molecules may be used as dietary supplements for treatment of diseases such as obesity, cancer and diabetes (Bialek et al., 2017; Yuan et al., 2014).

Ricinodendron heudelotii oil is a siccative oil, it can be used as a renewable raw material for the formulation of surface coating systems, paints and adhesives, as demonstrated by the work of Assanvo et al. (Assanvo et al., 2015). α eleostearic acid, β eleostearic acid and cat alpic acid from this oil are polymerizable fatty acids. They can be used in the polymer industry for the synthesis of biodegradable polymers (biopolymers) like tung oil. The importance of CLnA in Phytomedicine may promote the use of *Ricinodendron heudelotii* oil in the pharmaceutical industry.

This overview of the state of the art reveals a variability of fatty acid profiles for oils extracted from kernels isolated by traditional heat treatment. Certain factors, such as heat, humidity, ultraviolet radiation, and storage time and conditions, can change oil fatty acid composition.

In addition to contributing to the structuring of NLFP sectors, the study performed in Cameroon by Roques et al. in 2019 highlighted the need to improve knowledge of these products and the procedures for their transformation, to target and optimize the parameters likely to influence product quality, including, in particular, oxidation factors. We performed a survey to obtain reliable data concerning the sites at which *Ricinodendron heudelotii* is grown and fruits are harvested in 23 regions of Côte d'Ivoire. We present here a comparative study of the chemical (protein content, oil content, fatty acid profiles, minor compounds) and physicochemical (kinematic viscosity, iodine value, acid value) properties of oils and kernels obtained by traditional heat treatment or by manual mechanical dehulling.

2. Materials and methods

2.1. Materials

The seeds of *Ricinodendron heudelotii* used in this study came from Sinfra (6°36'29.4"N 6°01'18.2"W), a community in the Marahoué region of Côte d'Ivoire, in West Africa. The fruits were collected from under the trees, during the month of August 2014. The seeds were extracted from the pulp of the fruit by hand and dried in a ventilated shelter for five days. The seeds were then frozen and stored loose in freezer bags at +4 °C until their transport to the agro industrial chemistry laboratory in Toulouse, France. They were then stored in a cold room at +4 °C.

2.2. Methods of kernel isolation

Two methods were used to isolate the kernels from the seeds during this study. The first was the traditional heat treatment, performed in a ventilated shelter, and the second was manual mechanical dehulling, performed in the agro industrial chemistry laboratory in Toulouse, France.

2.2.1. Traditional heat treatment

The seeds of *Ricinodendron heudelotii* consist of a kernel surrounded by a husk (Fig. 1).

The most widely used technique for separating the kernel from the husk is the traditional three step heat treatment. In the first step, soaking, 5 kg of seeds are immersed in tepid water in a round stainless steel 20 liter cuvette for 24 h. In the second stage, the seeds are boiled in water over a wood fire for 12 h. This process generates sufficient heat to fissure the husk. The final step is the release of the kernels with the aid of a nail or small knife. For the traditional heat treatment in this study, we dehulled the seeds in this way, then dried the kernels in the sun for about 10 days, after which they were frozen and stored in freezer bags at +4 °C.

2.2.2. Manual mechanical dehulling

In this technique, the kernels are released from the seeds without prior treatment. The seeds (Fig. 1) of *Ricinodendron heudelotii* fruits were directly dehulled by hand, one by one. A standard pincer type nutcracker was used, with the seed placed where the walnut would usually be inserted. The two arms of the pincer were closed, exerting sufficient pressure on the seed to break the husk without shattering the kernel inside it. The kernel was then removed from the husk with a small stainless steel spatula.

2.3. Extraction of oil from the *Ricinodendron heudelotii* kernels by two methods

We used two methods of oil extraction, depending on the amount and quality of oil desired for the various analyses. We extracted lipids in solvent with a Soxhlet extractor for measurements of oil content, and

we used the cold extraction of lipids in cyclohexane to obtain oil for chemical and physicochemical characterization. The different solvent mediated lipid extractions were performed at least three times.

2.3.1. Extraction of lipids with solvent in a soxhlet extractor

The kernels were ground with a bladed grinder. A mass of 15 g of ground kernels was placed in the extraction cartridge and inserted into the Soxhlet extractor. With the aid of 150 ml of cyclohexane in a 200 ml balloon flask, the lipid matter within the ground kernels was extracted by solvent reflux (at about 80 °C) for six hours. The oil was obtained by evaporating off the solvent in a rotary evaporator at 50 °C, under vacuum, at 200 mbars. The amount of oil extracted was then determined relative to the mass of ground material.

2.3.2. Cold extraction in cyclohexane

A mass of 2.5 g of ground kernels (ground in a bladed grinder, as described above) was placed in a 30 ml centrifuge tube with 25 ml of cyclohexane. The tube was vortexed for 30 s and then centrifuged for 15 min at 10,000 x g. The supernatant was decanted off and the organic phase (lipid matter) was recovered. This process was repeated twice. All of the organic phases collected were combined and filtered through a funnel containing a glass fiber filter paper on which a few grams of anhydrous sodium sulfate were placed. The oil was recovered by evaporating off the cyclohexane in a rotary evaporator at 50 °C, under vacuum, at 200 mbars. The amount of oil extracted was then determined relative to the mass of ground material.

2.4. Physicochemical characterization of the kernels by the ADF NDF method

We used the revised version (March 23, 2011) of the Van Soest three part sequential analysis procedure to determine the lignocellulosic composition of the *Ricinodendron heudelotii* kernels. The first step was the determination of the insoluble fiber content of 1 g of ground *Ricinodendron heudelotii* kernels from which the lipids had been removed by Soxhlet extraction (cyclohexane), by solubilizing and eliminating the proteins and water soluble material with a neutral detergent solution (sodium lauryl sulfate) at 95 °C. The residue obtained is known as NDF (neutral detergent fiber). It consists of hemicelluloses, celluloses, lignins and ash. It was filtered and washed with demineralized water heated to 90 °C. Calcination at 450 °C for 6 h was performed to determine the ash content.

The second stage of the procedure was the solubilization of hemicellulose in an acid detergent solution consisting of acetyl trimethyl ammonium bromide in 0.5 M sulfuric acid. The residue obtained by this procedure is called the ADF (acid detergent fiber) and consists of cellulose and lignin. This residue was filtered, washed in hot water and dried.

Finally, the ADF residue was treated with potassium permanganate to oxidize and eliminate lignins, for the determination of cellulose content.

2.5. Determination of protein content

We used an indirect method based on the determination of the percent total nitrogen (% N) by the Kjeldahl method, in accordance with the NF V 18 199 standard. Kjeldahl's method involves mineralization of the organic nitrogen present in the sample to generate ammoniacal nitrogen, which is then determined by titration against acid. For mineralization, a mass of 0.8 g of dried kernels delipidated by the Soxhlet method was placed in a glass tube, to which 12.5 ml of 95% sulfuric acid was added. The mixture was placed in a fume hood for 16 h. It was then mineralized by heating at 400 °C for 1 h. Finally, the analysis was performed with a Kjeltec™ 8400 analyzer.

2.6. Determination of standard values for the oils

Acid value, iodine value, and acidity were determined by volumetric methods, according to the protocols of standards, NF EN ISO 3961 for iodine value and NF EN ISO 660 for acid value and acidity/eleostearic acid equivalent. Acidity was assessed by a theoretical method based on the fatty acid profile of *Ricinodendron heudelotii* oil.

2.7. Fatty acid composition determination by gas chromatography (GC)

The fatty acid profile of the oil was determined by the trimethylsulfonium hydroxide (TMSH) method (standard NF EN ISO 12966 3, 2016). The oil (15 mg) was solubilized in 1 ml of TBME (tert butyl methyl ether), methylated with trimethyl sulfonium hydroxide (TMSH, 0.2 ml/l in methanol) and the resulting fatty acid methyl esters (FAMES) were analyzed with a gas chromatograph (Varian 3900) equipped with a CP Select CB column (50 m, 0.25 mm i.d., 0.25 µm film thickness). We injected 1 µl, using the following parameters: injector in split mode, ratio of 1/100, 250 °C (55 min). The carrier gas was helium, at a flow rate of 1.2 ml/min. The oven temperature was initially set at 185 °C for 40 min, then increased to 250 °C at a rate of 15 °C/minute and maintained at 250 °C for 11 min. The temperature of the flame ionisation detector was set at 360 °C. FAMES were identified by comparing the retention times obtained with those for a mixture of commercial standards, Mix 37 (Sigma Aldrich), tung oil and oil from *Catalpa bignonioides*.

2.8. Determination of tocopherol content by HPLC

The tocopherol content of the oil was determined by HPLC fluorimetry, according to the analytical method described in the AFNOR NF ISO 9936 standard. We used a liquid phase chromatograph coupled to a Dionex fluorescence detector (λ_{ex} 290 nm; λ_{em} 317 nm) and an oven heated to a fixed temperature of 22 °C. We injected 20 µl of a 10 mg/ml solution of oil in cyclohexane into the HPLC apparatus. The mobile phase was a mixture of isooctane and isopropanol (99.5/0.5 v/v), at a flow rate of 1.1 ml/minute. Quantification was based on external calibration with standards (α tocopherol, γ tocopherol, and δ tocopherol) from Sigma.

2.9. Determination of sterol content by GC

The determination of sterol content began with the preparation of a 2 mg/ml solution of cholestanol (internal standard). We added precisely 50 µl of this solution (corresponding to 100 µg of cholestanol) to a 15 ml screw top tube. After chloroform evaporation, 100 mg of oil was added. Saponification was carried out through addition of 2 ml of 1 M KOH in ethanol. The mixture was vortexed and heated for 20 min in a water bath at 75 °C. The reaction mixture was allowed to cool, 1 ml of demineralized water plus 6 ml of cyclohexane were added and the contents of the tube were mixed by vortexing. We allowed the mixture to separate and recovered the organic phase containing the unsaponifiable matter. We mixed 160 µl of this solution with 40 µl of silylation reagent (99% N,O bis[trimethylsilyl]trifluoroacetamide] plus 1% trimethyl chlorosilane: BSTFA/TMCS, 99/1). Sterol determinations were performed by GC with a Perkin Elmer Auto System XL equipped with a CPSil 8CB (Varian) 30 m x 0.25 mm column, with a film thickness of 0.25 µm. The carrier gas was helium, at a column head pressure of 100 kPa. We injected 1 µl of solution in the on column mode. The oven temperature was maintained at 160 °C for 0.5 min and was then increased gradually to 260 °C at a rate of 20 °C/minute. Oven temperature was then increased to 300 °C at a rate of 2 °C/minute, and finally to 350 °C at a rate of 45 °C/minute. The total analysis time was 50 min. The temperature of the flame ionisation detector (FID) was set at 360 °C (Roche et al., 2006).

3. Results and discussion

3.1. Mapping *Ricinodendron heudelotii* in côte d'ivoire

The localization of *Ricinodendron heudelotii* trees and fruit harvesting was studied at 23 sites: Abidjan, Yamoussoukro, Sinfra, Gagnoa, Tiassalé, Toumodi, Oumé, Divo, Danané, Man, Lakota, San Pedro, Soubré, Adiaké, Adzopé, Kotobi, Bouaké, Arrah, Akoupé, Daloa, Sikensi, Agboville, and Rubino. With the aim of collecting as much information as possible, three villages were selected per site for the collection of information on the presence of *Ricinodendron heudelotii* and kernel production. According to this survey, this plant was present at all the sites evaluated. *Ricinodendron heudelotii* seed harvest and kernel production were strongly linked to the dietary habits of the local population. We identified two large production areas in the south east and south west of the country. Overall, 82.5% of the sites studied produced *Ricinodendron heudelotii* kernels, but there were no harvesting or transformation activities at the remaining 17.5% of the sites at which this plant was found.

In Côte d'Ivoire, the annual harvest of *Ricinodendron heudelotii* seeds generates about 100 tonnes of kernels. The fruits are harvested by the women in certain parts of the country. The kernels are almost exclusively destined for human consumption, by local populations. Much of this production is destined for the supply of large towns, such as Abidjan, Yamoussoukro and Bouaké, for reasons of profitability. The buyers in these towns are solvent and the kernels are sold at 8000 Francs CFA (equivalent to 12 Euros) per kilogram.

3.2. Composition of *Ricinodendron heudelotii* kernels

Table 1 shows a breakdown of kernel contents for kernels obtained by the two dehulling methods. Similar kernel content results were obtained for the two dehulling methods.

Water and dry matter contents were 7% and 93%, respectively, cellulose content was 6%, hemicellulose content was 7.5% and lignin content was 3%. These values indicate that *Ricinodendron heudelotii* kernels have low contents of cellulose, hemicellulose and lignin. By contrast, they have a high protein content, at $21.3 \pm 1.9\%$. This protein content is consistent with the protein contents of 21% reported by Saki et al. (2005), and 24% reported by Coulibaly et al. (2018). According to these results, regardless of the method of kernel isolation used, the same amount of lipid is extracted, corresponding to $47.4 \pm 0.2\%$ of the kernel contents. Similar results were obtained by Dandjouma et al. in 2008 and Saki et al. in 2005, with these authors reporting rates of oil extraction by the Soxhlet method of 50% and 48.9% for kernels isolated by the traditional heating method. The results obtained for protein content and lipid extraction rate indicate that *Ricinodendron heudelotii* seeds can be considered as oleoproteaginous. Such seeds are a good source of both lipids and proteins.

Table 1
Characterization of kernel components.

| Kernel samples isolated: | | |
|--------------------------|-------------------------------|--------------------------------|
| Constituent contents (%) | By traditional heat treatment | By manual mechanical dehulling |
| Water | 7.3 ± 0.2 | 7.5 ± 0.1 |
| Dry matter | 93.1 ± 0.2 | 92.5 ± 0.1 |
| Ash | 7.3 ± 0.2 | 7.5 ± 0.1 |
| Cellulose | 6.5 ± 0.2 | 6.1 ± 0.3 |
| Hemicellulose | 7.8 ± 0.3 | 7.5 ± 0.2 |
| Lignin | 3.0 ± 0.2 | 3.2 ± 0.2 |
| Protein | 21.3 ± 1.9 | 21.1 ± 0.2 |
| Oil (Soxhlet extraction) | 47.4 ± 0.2 | 47.3 ± 0.6 |

Table 2

Physicochemical characteristics of the oils.

| Samples of oil from kernels isolated: | | |
|---------------------------------------|-------------------------------|--------------------------------|
| Physical characteristics of the oil | By traditional heat treatment | By manual mechanical dehulling |
| Density (g /ml at 25 °C) | 0.92 | 0.94 |
| Viscosity (mPa.s) | 169 ± 0.5 | 128.0 ± 0.1 |
| Acid value | 5.7 ± 0.3 | 3.7 ± 0.3 |
| Acidity (% by mass) | 2.8 ± 0.2 | 1.8 ± 0.1 |
| Iodine value | 179.6 ± 1.1 | 195.2 ± 1.7 |

3.3. Physicochemical characterization of *Ricinodendron heudelotii* oil

Table 2 summarizes the results for kinematic viscosity, iodine value, acid value and acidity for the oils extracted from kernels by the traditional heating method and by manual mechanical dehulling.

The densities of the oil samples obtained following the two dehulling methods were almost identical, at 0.92 g/ml and 0.94 g/ml. This demonstrates the reproducibility of the mass of oil obtained from kernels isolated by the two methods of dehulling. The oil obtained from kernels isolated by traditional heat treatment had a higher viscosity (169 mPa.s), a higher acid value (5.7 ± 0.3) a higher acidity (2.8 ± 0.2) and a lower iodine value (179.6 ± 1.1) than the oil obtained from kernels isolated by manual hulling. This reflects a slight deterioration of the oil during the traditional heat treatment. Indeed, the traditional method for isolating kernels from *Ricinodendron heudelotii* seeds involves uncontrolled techniques, such as a long period of heating in boiling water and the drying of the kernels in the sun, both of which are factors favoring oxidation of the oil. These results demonstrate that the mode of seed treatment for kernel isolation has a significant influence on the iodine value of the extracted oil. Iodine value is lower following the traditional heat treatment of the seeds to isolate the kernels. The iodine values obtained here are different from those reported in previous studies. According to the studies performed by the groups of Tchiégang and Assanvo, the iodine value of this oil is 150 (Tchiégang et al., 2003; Assanvo et al., 2015). In this study, we obtained iodine values of 179.6 ± 1.1 and 195.2 ± 1.7 for the oils extracted from kernels isolated by the two dehulling methods. These values were thus high, for both methods. They indicate that this oil from *Ricinodendron heudelotii* is highly siccative, even more so than tung oil, which has an iodine value of 170 according to Samadzadeh et al. (2011).

3.4. Fatty acid composition of the oils

Ricinodendron heudelotii is one of the rare highly polyunsaturated plant oils, with a CLnA content of at least 84%, depending on the method used to dehull the kernels. An analysis of the CLnA content of the oils obtained revealed that α eleostearic acid and linoleic acid were the two principal fatty acids present. Nevertheless, their contents varied with the method used to obtain the kernels. Oil from kernels isolated by manual mechanical hulling without prior treatment contained $60.1 \pm 0.2\%$ α eleostearic acid and $22.8 \pm 0.1\%$ linoleic acid, whereas oil extracted from kernels obtained by traditional heat treatment contained $50.5 \pm 0.1\%$ α eleostearic acid and $24 \pm 0.0\%$ linoleic acid. These two oils also contained CLnA known to be isomers of α eleostearic acid: β eleostearic acid ($1.1 \pm 0.1\%$ in oil from kernels isolated by manual mechanical hulling and $8.4 \pm 0.0\%$ in oil from kernels isolated by traditional heat treatment) and catalpic acid, which was present only in oil from kernels isolated by traditional heat treatment ($0.40 \pm 0.1\%$). Published results for the CLnA content of *Ricinodendron* oils are shown in Table 3.

Oils extracted from kernels obtained by traditional heat treatment have α eleostearic acid contents of 32.5% to 51.1% and linoleic acid contents of 28.1% to 36.0%. This composition is similar to the CLnA

Table 3Published results concerning the chemical composition of *Ricinodendron heudelotii* oil.

| Fatty acid | Kapseu 1995 [*] | Dandjouma 2007 [*] | Sompila 2014 [*] | Leudeu 2009 [*] | This study | |
|-------------------------------------|--------------------------|-----------------------------|---------------------------|--------------------------|---------------------|---------------------|
| | | | | | Kernel ^a | Kernel ^b |
| Palmitic acid C16:0 | 5.5 | 6.4 | 5.4 | 9.4 | 3.8 ± 0.0 | 4.1 ± 0.0 |
| Stearic acid C18:0 | 6.4 | 7.5 | 8.0 | 10.6 | 5.1 ± 0.0 | 5.3 ± 0.0 |
| Oleic acid C18 :1n-9 | 7.4 | 7.18 | 11.2 | 10.3 | 6.7 ± 0.0 | 7.1 ± 0.0 |
| Linoleic acid C18:2n-6 | 28.3 | 30.7 | 28.1 | 36.0 | 22.8 ± 0.1 | 24.0 ± 0.0 |
| α-eleostearic acid C18:3n-5(c,t,t) | 51.1 | 48.4 | 35 | 32.5 | 60.1 ± 0.2 | 50.5 ± 0.1 |
| β-eleostearic acid C18:3n-5(t,t,t) | — | — | 9.7 | — | 1.1 ± 0.1 | 8.4 ± 0.0 |
| Catalpic acid C18 :3n-5 (t,t,c) | — | — | 1.3 | — | 0.0 | 0.40 ± 0.1 |
| Saturated fatty acids (SFAs) | 11.9 | 13.9 | 13.4 | 20.0 | 8.9 ± 0.1 | 9.4 ± 0.0 |
| Monounsaturated fatty acids (MUFAs) | 7.4 | 7.18 | 11.2 | 10.3 | 6.7 ± 0.0 | 7.0 ± 0.0 |
| Polyunsaturated fatty acids (CLnA) | 79.4 | 79.07 | 74.1 | 68.5 | 84.4 ± 0.4 | 83.3 ± 0.1 |

^{*} kernels bought on the market.^a kernels isolated by manual mechanical dehulling.^b kernels isolated by traditional heat treatment.

profile of our oil extracted from kernels obtained by traditional heat treatment: 50.5 ± 0.1% α eleostearic acid and 24.0 ± 0.0% linoleic acid.

Two parameters must be factored into this comparative study: the location and type of kernel production. Even with the same method of oil extraction in solvent (Soxhlet extraction), CLnA content varied considerably, ranging from 35% to 60% for α eleostearic acid and 22% to 36% for linoleic acid. Furthermore, another study have reported the presence of β eleostearic acid and catalpic acid (Sompila et al., 2014). An examination of our data and those of Sompila and al revealed a clear tendency towards a decrease in α eleostearic acid content in favor of linoleic acid and β eleostearic acid. For an oil obtained by the traditional dehulling method, an 8.4% β eleostearic acid content was determined in our study, and 9.7% were found by Sompila and al.

The traditional dehulling method involves some isomerization of α eleostearic acid. This acid is isomerized into β eleostearic acid and catalpic acid, in the form of triglycerides in native oil.

The oil enriched in β eleostearic acid have beneficial effects on human health. According to some studies, this fatty acid may has cytotoxic effects on cancer cells (Shinohara et al., 2012; Sun et al., 2012).

Alpha eleostearic acid, β eleostearic acid and catalpic acid in *Ricinodendron heudelotii* oil are isomers of conjugated linolenic acid (CLnA). There are other isomeric fatty acids of CLnA, namely punicic acid (C18:3 9c, 11 t, 13c) in *Punica granatum* oil (79.3%), jacaric acid (C18:3 8 t, 10 t, 12 t) in *Jacaranda mimosifolia* seed oil (62%) (Hernandez et al., 2000; Takagi and Itabashi, 1981).

Recent research shows that these fatty acids from CLnA family are used in phytomedicine. They have a beneficial effect on health. They have favourable physiological effects, such as anti arteriosclerosis, anti obesity, anti tumour, anti hypertension and anti cancer (Gasmi and Thomas Sanderson, 2013; Yuan et al., 2014).

α eleostearic acid in *Momordica charantia* seed oil is a potential therapeutic agent in the treatment of breast cancer and leads to self destruction of colon cancer cells (Caco 2) (Shinohara et al., 2012).

Punicic acid in *Punica granatum* improve insulin secretion without altering fasting blood sugar (Nekooeian et al., 2014). Catalpic acid in *Catalpa bignonioides* seed oil causes a decrease in fasting blood sugar and insulin (Hontecillas et al., 2008).

3.5. Characterization of the minor constituents of *Ricinodendron heudelotii* oils

Table 4 summarizes the tocopherol content of the oils.

Total tocopherol content was 178.3 mg/100 g for oil extracted from kernels isolated by manual mechanical dehulling and 135 mg/ 100 g for oil extracted from kernels isolated by traditional heat treatment. This value was influenced by the treatment of the seeds before oil extraction

Table 4

Chemical characteristics of the minor components of the oil.

| Minor components | Samples of oil from kernels isolated: | |
|--------------------------|---------------------------------------|--------------------------------|
| Tocopherols (%) | By traditional heat treatment | By manual mechanical dehulling |
| α-tocopherol | 4.0 ± 0.01 | 7.9 ± 0.02 |
| γ-tocopherol | 92.9 ± 0.02 | 89.9 ± 0.18 |
| δ-Tocopherol | 3.1 ± 0.02 | 2.5 ± 0.46 |
| Total (mg/100 g) | 135.0 ± 0.4 | 178.3 ± 0.4 |
| Sterols % | | |
| Campesterol (%) | 7.2 ± 0.11 | 7.2 ± 0.19 |
| Stigmasterol (%) | 3.1 ± 0.1 | 2.9 ± 0.2 |
| β-sitosterol(%) | 83.6 ± 0.5 | 83.7 ± 0.3 |
| Stigmastanol (%) | 1.7 ± 0.01 | 1.5 ± 0.9 |
| 5-avenasterol (%) | 4.3 ± 0.11 | 4.6 ± 0.4 |
| Total sterols (mg/100 g) | 412.0 ± 8.0 | 402.5 ± 8.0 |

from the kernels. This tocopherol content is similar to that of poly unsaturated oils such as linseed oil, which contain 110–280 mg/100 g. Regardless of the pretreatment method during dehulling, the major constituent was γ tocopherol, at 92 mg/100 g. However, these results show that the traditional heat treatment degrades the tocopherols present in the oil within the seeds.

An analysis of the sterol composition of *Ricinodendron heudelotii* oil showed that β sitosterol was the major component, accounting for 83% of the sterols present, and that total sterol content stays the same between seed treatments, ranging from 402.5 ± 8.0 to 412.0 ± 8.0 mg/100 g. *Ricinodendron heudelotii* oil is, thus, moderately rich in sterols, including β sitosterol in particular.

4. Conclusion

This study focused on the characterization of two sorts of oil from *Ricinodendron heudelotii* kernels: oil from kernels isolated by the traditional heat treatment and oil from kernels obtained by manual mechanical dehulling. The two dehulling methods tested resulted in a different chemical composition readily identifiable in terms of CLnA contents. Oil from kernels isolated by manual mechanical dehulling contained 60.1% ± 0.2 α eleostearic acid, 22.8% ± 0.1 linoleic acid, and 1.1% ± 0.1 β eleostearic acid, whereas oil from kernels isolated by the traditional method contained 50.5% ± 0.1 α eleostearic acid, 24% ± 0.0 linoleic acid, 8.4% ± 0.0 β eleostearic acid and 0.40% ± 0.1 catalpic acid. Thus, oils from mechanically dehulled kernels were very rich in α eleostearic acid, whereas oils from kernels dehulled by the traditional method displayed enrichment in β eleostearic acid and catalpic acid, due to the isomerization of α eleostearic acid. Oils are rich in CLnA (84%), with high iodine values (180 and 195, respectively). The minor constituents, including γ tocopherol and

β sitosterol, were conserved. The kernels contained $21.3\% \pm 1.9$ protein and $47.4\% \pm 0.2$ oil. It is a source of dietary fiber and of polyunsaturated oils, rich in ALA and CLnA. These characteristics make this oil a good candidate for alimentary and nutritional uses.

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