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EVALUATION OF THE THERMAL STABILITY OF THE SEED OIL OF *ALCHORNEA CORDIFOLIA* FROM CONGO BRAZZAVILLE

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ABSTRACT
This article describes the chemical changes resulting from heat treatment at 100 °C at different times of oil from *Alchornea cordifolia* seeds extracted by Folch, Water and Soxhlet methods. A comparative approach of quantitative analyzes of acid and peroxide values and oxirane content of oil before and after heat treatment formed the basis of our assessment of its thermal stability. The oils obtained by the Folch and water methods give better thermal stability when heated at 100 °C in an oven for 300 minutes compared to oils obtained by the Soxhlet method. All the oils studied showed a reduction of more than 80 % in their oxirane content. The use of the ascending hierarchical classification (AHC) clearly confirms the influence of the extraction method on the oxirane content in *Alchornea cordifolia* oils. This oil should not be heated to high temperatures and for long periods in order to preserve the oxirane content.

KEYWORDS:
Thermal stability, *Alchornea cordifolia*, oil extraction methods, chemical changes, oxirane content

INTRODUCTION

Thanks to its fatty acid composition and its oxirane content [1], the oil from the seeds of *Alchornea cordifolia* from Congo-Brazzaville is without doubt classified as one of the naturally epoxidized oils. If the influence of extraction methods on the quality and fatty acid composition of *Alchornea cordifolia* seed oils from Congo-Brazzaville is now well known, the temperature and time limit for use of *Alchornea cordifolia* seed oils remain the major concerns of producers and industrialists. Indeed, no scientific study has been carried out on the thermal stability of the oils in the seeds of *Alchornea cordifolia*.

Like all oils, *Alchornea cordifolia* seed oil may go rancid depending on time and temperature. This rancidity is due to the oxidation of fatty acids.

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Oxidation comes from four main factors: light, air, heat and contact with certain metals. Therefore, whenever the oil from the seeds of *Alchornea cordifolia* is in the presence of one of these factors, it spends part of its antioxidants for its own resistance in order to fight against oxidation.

In order to assess the thermal stability of *Alchorena cordifolia* seed oil, we simulated the phenomenon by heating the studied oils at different times in an oven. This stability is assessed by measurements of the peroxide and acid and oxirane content.

OBJECTIVES

The main objective of this present work is to make a significant contribution to the study of naturally epoxidized oils as an alternative raw material to petroleum for their potential direct use. More specifically, to study the impact of the temperature and the heating time of oils on their chemical properties, including the acid, peroxide and oxirane values.

MATERIAL AND METHODS

Plant material

Alchornea cordifolia is known in southern Congo under the vernacular names of "Mbunzila" in the Pool department, "Mbunzi" in the Niari department and "Abunzi" in northern Congo. It is a shrub up to four to five meters tall. The fruits of *Alchornea cordifolia* are two-lobed capsular, about 1.5 cm × 1.5 cm, lobes slightly compressed, smooth, briefly hairy, green to red, with two seeds (photo 1). The seeds are ovoid-ellipsoid, about 6 mm long, smooth, bright red. In Congo-Brazzaville, the fruits of *Alchornea cordifolia* mature in the period from December to February and its red seeds are used as bait to trap birds.

Photo 1: Plant and fruit of *Alchornea cordifolia*

Sampling

The sample consists of six oils, including three oils extracted from seeds of a tree of *Alchornea cordifolia* from Dolisie by three different methods, water, Folch and soxhlet noted: WD, FD and SD on the one hand and three other oils extracted from seeds of a tree of *Alchornea cordifolia* from Goma tsetse by three different methods, water, Folch and Soxhlet noted: WG, FG and SG on the other hand.

Methods

Oil extraction from Alchornea cordifolia seeds

Three different methods were used to extract lipid materials: the Folch method (F), the Soxhlet method (S),) and the water method (W).

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- In the method of Folch [2], the solvent mixture is composed of chloroform-methanol (2: 1, v / v). The extract is purified by washing the organic phase with a saline solution (NaCl, 0.73%). The extraction is carried out cold.
- The Soxhlet method is based on the hot solubilization of the fat in a solvent (hexane); then the solvent is evaporated in order to separate and recover the pure products (oil and solvent).
- In the water method, the powder of 100 g of the seeds dried in an oven for 24 hours at 100 °C is mixed with 500 ml of water in an aluminum pan. The mixture is heated for 1 hour 30 minutes at a temperature of 150 °C. After boiling, two phases are obtained. The oil floats in the upper phase and the rest in the lower phase. The upper phase is taken, which is placed in a separating funnel for 24 hours and the oily phase is separated with the aqueous phase. The oil is dried using anhydrous sodium sulfate.

Evaluation of the stability of the oil on heating.

The alteration was evaluated by measuring the variation in the overall chemical characteristics of the oils heated to 100 °C. The oils were homogenized by heating at 60 °C for 15 minutes; they were then distributed in petri dishes, and heated at a temperature of 100 °C for 5 hours (300 minutes). These samples thus formed are placed in an oven which is adjusted to the study temperature (100 °C.). At 30 minutes time intervals, two petri dishes are removed for analysis [3].

Determination of chemical properties

Peroxide value (PV)

The peroxide value was determined according to the IUPAC method 2.501 [4].
1 gram of oil is introduced into a conical flask fitted with a stopper, which is rapidly dissolved in 10 ml of chloroform. 15 ml of acetic acid are added, then 1 ml of the potassium iodide solution and immediately the flask is capped and stirred for one minute and then left for exactly 5 minutes, protected from light. 75 ml of distilled water are added with vigorous stirring and in the presence of starch paste as an indicator. The released iodine is titrated with the 0.0105 N sodium thiosulfate solution. The blank is then assayed under the same conditions.
The peroxide index expressed in milliequivalents of active oxygen per kilogram of fat is given by the formula: PV = 1000 x (VT - VE) x T / m with: VE: Volume (ml) of the thiosulfate solution used to dose the test portion; VT: Volume (ml) of the thiosulfate solution used for the blank test; T: Exact normality of the sodium thiosulfate solution used;

Acid value (IA)

The acid value was determined according to the IUPAC method 2.201 [4].
0.5 to 1 g of oil is dissolved in 150 ml of the solvent mixture (1/1, V / V) of 95% ethanol and diethyl ether. The solution obtained is titrated in the presence of a few drops (3) of phenolphthalein, with stirring, with the 0.1N potassium hydroxide solution. The acid value is given by the following formula: AV = (MKOH x VE x 0.1) / m with: MKOH: Molar mass of KOH; VE: Volume of alcoholic potash (in ml); m: Test sample (in grams)

Oxyrane content (OC)

The oxyrane value was determined according to AOCS Cd 9-57 standard [5].
0.3-0.5g of oil is dissolved in 10mL of glacial acetic acid. The solution obtained is titrated in the presence of a few drops of gentian violet, while stirring, with the 30-32% solution of hydrogen bromide at 0.1mol / L (100ml) until the blue-green turn. The oxyran index is calculated by the following formula: OC = V × C × 16 / m × 10; With: V: volume of HBr at equivalence (mL); c: exact concentration of the HBr solution (mol / L); m: mass of the sample (g); 16: molar mass of oxygen (g / mol).

RESULTS AND DISCUSSION

The chemical changes of *Alchornea cordifolia* oil (which naturally contains epoxidized triglycerides) upon heating was evaluated and is reported in this manuscript.

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Peroxide value (PV)

Free fatty acids or acylglycerols participate in the oxidation reaction that is the most important alteration reaction of oils and fats. Free fatty acids are more sensitive to oxidation than acylglycerols.
The oxidation of an oil or fat takes place according to the radical mechanism, which includes an initiation stage, a propagation stage and a stopping stage. There is formation of intermediate peroxides (ROOH, ROOR) which evolve into stable products of oxidation [6]; the peroxide number thus makes it possible to measure the degree of oxidation of an oil or a fat.

The results of the peroxide value of six samples heated to 100 °C at different times are given in table 1.

Table 1: Variation of the peroxide indices of the different samples of *Alchornea cordifolia* oils at T = 100 ° C

Heating time (min)	FG		WG		SG		FD		WD		SD	
	I_P (Méq (O_2)/kg)	R	I_P (Méq (o_2)/kg	R	I_P (Méq (o_2)/kg)	R	I_P (Méq (O_2)/kg)	R	I_P (Méq (o_2)/kg	R	I_P (Méq (o_2)/kg	R
0	1,80	1	1,90	1	1,88	1	1,53	1	1,88	1	1,88	1
30	1,88	1,04	1,92	1,01	1,87	0,99	1,84	1,20	1,80	0,96	1,89	1,00
60	1,88	1,04	2	1,05	1,89	1,00	1,94	1,27	1,98	1,05	1,90	1,01
90	1,88	1,04	2	1,05	1,92	1,02	1,96	1,28	1,98	1,05	1,92	1,02
120	1,98	1,1	1,94	1,02	1,94	1,03	1,93	1,26	1,98	1,05	1,92	1,02
150	1,96	1,08	15,88	8,36	1,94	1,03	1,96	1,28	2	1,06	1,96	1,04
180	2	1,1	25,47	13,41	1,94	1,03	1,96	1,28	1,87	0,99	1,98	1,05
240	2	1,1	26	13,68	3	1,60	2	1,31	6	3,19	2,94	1,56
300	2,06	1,1	26,50	13,95	3,96	2,10	2,10	1,37	20,79	11,06	3,74	1,99

FG: *Alchornea cordifolia* oil from Gomatsétsé extracted by the Folch method
WG: *Alchornea cordifolia* oil from Gomatsétsé extracted by the water method
SG: *Alchornea cordifolia* oil from Gomatsétsé extracted by the Soxhlet method
FD: *Alchornea cordifolia* oil from Dolisie extracted by Folch method
WD: *Alchornea cordifolia* oil from Dolisie extracted by the water method
SG: *Alchornea cordifolia* oil from Dolisie extracted by the Soxhlet method
$R=[PV(t_{xmin})]/[PV(t_{0min})]$

After 300 minutes of heating at 100 ° C, the highest variation in the peroxide number (PI) is observed with the oils extracted by the water method for R values varying from 1 to 13.95 (WG oil) , and from 1 to 11.06 (WD oil) (Table 1).

By visualizing the shapes of FIGS. 1a and 1b deduced from tables IA and IB, it is constant that after a period of "latency" which lasts about 2 hours, for WG oil, 3 hours for SG oil, and 3 hours for oils WD and SD, the peroxide number begins to increase. This increase is probably due to the formation of hydro peroxides during heating. These hydro peroxides are formed by the oxidation of unsaturated fatty acids constituting the oils studied. On the other hand, the peroxide index of FG and FD oils remains almost constant throughout the heating.

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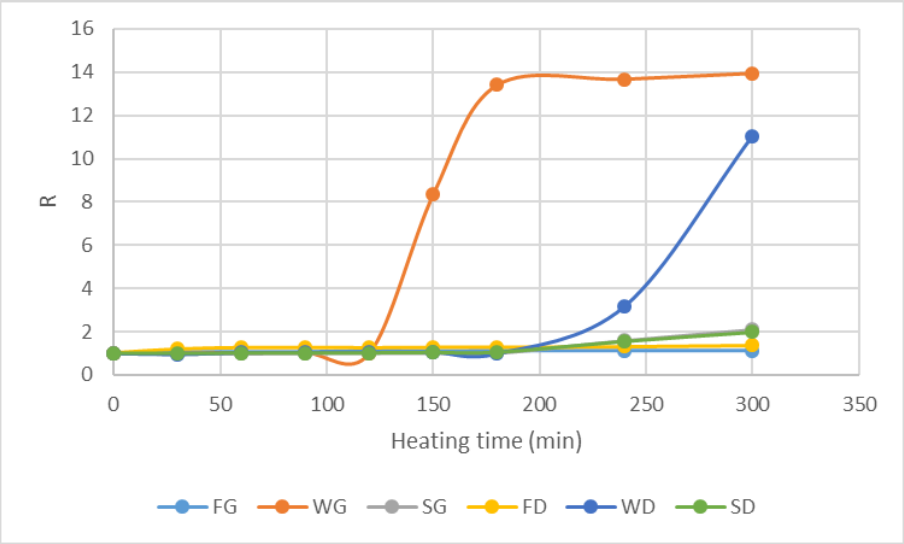

Figure 1: Evolution of the peroxide value of the different samples of *Alchornea cordifolia* oils

The oils obtained by the Folch method (FG and FD) give better thermal stability during heating at 100 ° C in an oven for 300 minutes compared to the oils obtained by the water and Soxhlet methods (WG, WD, SG, SD). The low thermal stability of oils extracted by water and Soxhlet methods (WG, WD, SG, SD) could then be explained by the use of water or heat during their extractions. Our results are also in agreement with those of the study of the effect of the mode of extraction on the thermal stability of oils of safou, olive and kernels of *Ricinodendron heudelotii* [7, 8].

Kama-Niamayoua found that hot extracted safou oil (Soxhlet) exhibits, after 6 days of heating at 105 ° C, an overall peroxide value twice as high as that of cold extracted oil (Folch).

Tchiegang et al (2003) [8] found that the peroxide value of the oil of *Ricinodendron heudelotii* kernels which had previously undergone a wet embrittlement treatment for 60 minutes is higher than the peroxide value of the oil obtained from almonds dry treated at 90 ° C for 60 minutes. The method of extraction influences the thermal stability of *Alchornea cordifolia* seed oils.

Acid value (AV)

The acidity of an oil or fat has at least two origins.
Fatty acids are naturally present in the pulp or seeds of oleaginous fruits;They participate in the synthesis of lipids. There are also endogenous enzymes in lipids which, if they are not deactivated when the oil is extracted, passes into the oil; they are responsible for the hydrolysis reactions of acylglycerols (mono-, di, and tri-), resulting in the release of fatty acids in the oil or fat.

The free fatty acids thus formed catalyze the hydrolysis reaction for the formation of new free fatty acids and so on ... [7]. In addition, the presence of water, heat and pressure is a reinforcing factor in the hydrolysis of triglycerides [9, 10]. The acid value therefore makes it possible to assess the quality at the time of extraction and to monitor its evolution over time or following specific technological treatments [7].

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Table 2: Variation of the acid indices of the different samples of *Alchornea cordifolia* oils at T = 100 ° C

Heating time (min)	FG		WG		SG		FD		WD		SD	
	I_A (mg de KOH/g d'huile)	R	I_A (mg de KOH/g d'huile)	R	I_A (mg de KOH/g d'huile)	R	I_A (mg de KOH/g d'huile)	R	I_A (mg de KOH/g d'huile)	R	I_A (mg de KOH/g d'huile)	R
0	48,25	1	63,53	1	64,06	1	54,11	1	63,35	1	63,98	1
30	51,95	1,08	64,21	1,01	66,69	1,04	60,43	1,12	74,11	1,17	64,98	1,02
60	55,03	1,14	69,14	1,09	75,85	1,18	69,58	1,29	74,11	1,17	87,53	1,37
90	53,95	1,11	71,51	1,13	75,92	1,19	55,07	1,02	66,31	1,05	87,82	1,37
120	49,50	1,03	72,94	1,15	120,85	1,89	47,48	0,88	66,31	1,05	103,42	1,62
150	48,25	1	72,96	1,15	126,52	1,98	46,01	0,85	60,82	0,96	102,02	1,59
180	46,76	0,97	59,41	0,94	115,39	1,80	45,11	0,83	57,92	0,91	95,28	1,49
240	38,15	0,79	58,18	0,92	75,53	1,18	37,05	0,68	50,61	0,80	82,57	1,29
300	34,53	0,72	49,50	0,78	71,69	1,12	35,33	0,65	47,40	0,75	62,46	0,98

$$R=[AV(t_{xmin})]/[AV(t_{0min})]$$

The acid value (AV) varies unexpectedly (Table II, Figure 2). While one would expect a steady increase in AV with the duration of heating, there are two phases:

• First phase: we observe the expected behavior, ie AV increases. From 0 to 60 minutes IV increases for WG, FD and WD oils; from 0 to 120 minutes for SD oil; from 0 to 150 minutes for WG and SG oils. One of the possible explanations for this behavior would be the intervention of reactions other than the hydrolysis of the TAGs constituting the oils studied.

• Second phase: we observe the unexpected behavior, ie AV decreases.

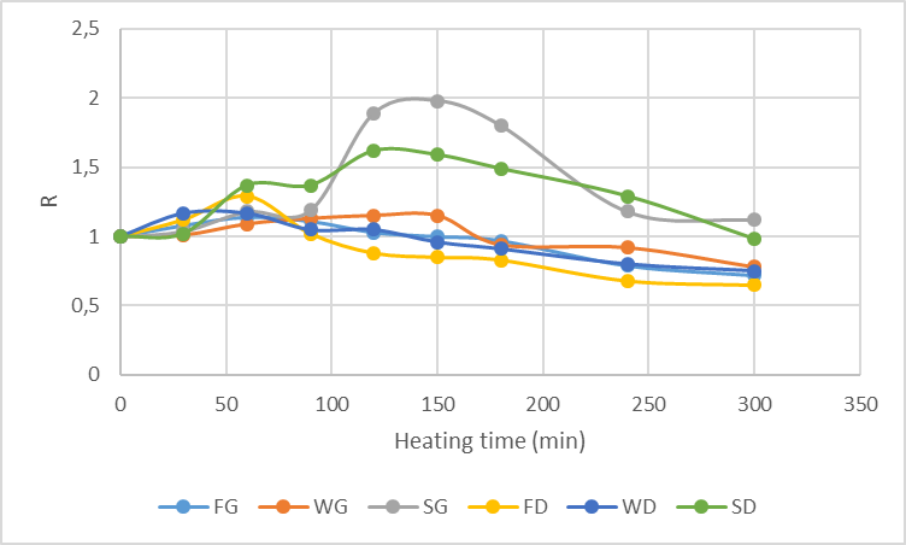

Figure 2: Evolution of the acid value of the different samples of *Alchornea cordifolia* oils

From 60 minutes to 300 minutes IA decreases for FG, FD and WD oils; from 120 to 300 minutes IA decreases for SD oil; from 150 to 300 minutes IA decreases for WG and SG oils.

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The decrease in the acid numbers of oils heated to 100 ° C can be explained on the one hand, because the heating promotes the evaporation of volatile free fatty acids; on the other hand, the polymerization of free fatty acids, which takes place during the heating of the oil, blocks certain acid functions, hence their reduction [11].

The oils obtained by Folch methods and with water (FG, FD, WG, WD), give better thermal stability during heating at 100 °C in an oven for 300 minutes compared to oils obtained by the method to Soxhlet (SG, SG). Hot extraction (Soxhlet) tends to promote the degradation of oils by hydrolysis due to lipase which leads to the release of free fatty acids by the effect of heat extraction.

These results show that the method of extraction has an influence on the thermal stability of the seed oils of *Alchornea cordifolia*.

Oxirane content (OC)

The variations of the oxirane content of *Alchornea cordifolia* oils (which naturally contains epoxidized triglycerides) upon heating at the temperature of 100 °C for different times were evaluated and are reported in Tables 3. All these oils showed a reduction in their oxirane content from 2.22 meq O2 / kg (before heating) to 0.43 meq O2 / kg (after 300 min of heating), from 1.97 meq O2 / kg (before heating) to 0.19 meq O2 / kg (after 300 min of heating) and from 2.22 meq O2 / kg (before heating) to 0.097 meq O2 / kg (after 300 min of heating) respectively for the oils extracted by the Folch method, by the water method and by the Soxhlet method. This means a drop of over 80% of the oxirane content for a heating time of 300 minutes.

Table 3: Variation of oxirane content of different samples of *Alchornea cordifolia* oils at T = 100 ° C

Heating time (min)	OC (meq O_2 /kg d'huile)					
	FG	WG	SG	FD	WD	SD
0	2.22	2.20	1.97	2.27	2.21	1.96
30	1.90	1.80	1.20	1.98	1.80	1.14
60	1.08	0.97	0.63	1.01	0.96	0.61
90	0.97	0.79	0.60	0.91	0.81	0.58
120	0.93	0.68	0.57	0.88	0.62	0.49
150	0.88	0.50	0.52	0.78	0.57	0.38
180	0.86	0.39	0.40	0.64	0.40	0.29
240	0.59	0.25	0.19	0.49	0.21	0.17
300	0.43	0.19	0.10	0.35	0.13	0.074

Using the titrimetric method to follow the changes in oxygen concentration of oxirane, it is evident that there is a correlation between the oxirane content and the heating time of *Alchornea cordifolia* oil (Table 3). The results indicate that the oxygen concentration of the oxirane in the oil decreases with increasing time for heating the oil to 100 ° C. This finding is in agreement with that made by Wamalwa et al (2000) [12] on the study of the thermal stability of vernonia oil heated to different temperatures. *Alchornea cordifolia* oil obtained by solvent extraction at room temperature (Foch method) has a higher oxirane content than that obtained by the Soxhlet extraction procedure at much higher temperature. Gringberg et al (1994) [13] made the same finding on vernonia oil obtained by solvent extraction at room temperature.

The rationalization of this correlation between the oxygen content of oxirane and the heating time is based on the fact that the epoxy ring is a highly stressed heterocyclic group of oloyl glycerides with a 60 ° angle between the cyclic ether bonds. Although the epoxy moiety is sterically hindered due to the conformation of the triglyceride molecule, the epoxy moiety remains sensitive to thermal disturbances due to its high energy resulting from angular distortions. The implications are that, large attacking groups cannot affect the cleavage of

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the epoxy ring. However, the small angular deformation of the cycle in combination with increased binding vibrations due to thermal energy is sufficient to break the cycle, forming a transient and intramolecular carbocation-anion pair that have high energy potential and charge for engage in an intermolecular reaction with an identical neighbor molecules. This results in simple dimers and / or polymer formations [12].

In order to understand whether the method of extracting the oils or the origin of the seeds indeed influences the oxyran content of the extracted oils, we used the hierarchical ascending classification. There are many statistical techniques aimed at dividing a population into different classes or subgroups. The ascending hierarchical classification (CAH) is one of them. We want the individuals grouped together within the same class (intra-class homogeneity) to be as similar as possible while the classes to be the most dissimilar (inter-class heterogeneity).

Figure 3 below is the dendrogram. It clearly represents how the algorithm proceeds to group individuals and then subgroups. In the end, the algorithm gradually regrouped all the observations. The dotted line represents the truncation and makes it possible to visualize that three homogeneous groups have been identified. The oils are grouped according to the extraction method and not the origin of the seeds. The first group includes the oils extracted with Soxhlet (SG and SD), the second group more homogeneous than the other two groups, includes the oils extracted by the water method (WG and WD) and the third group includes the oils extracted by the method of Folch (FG and FD).

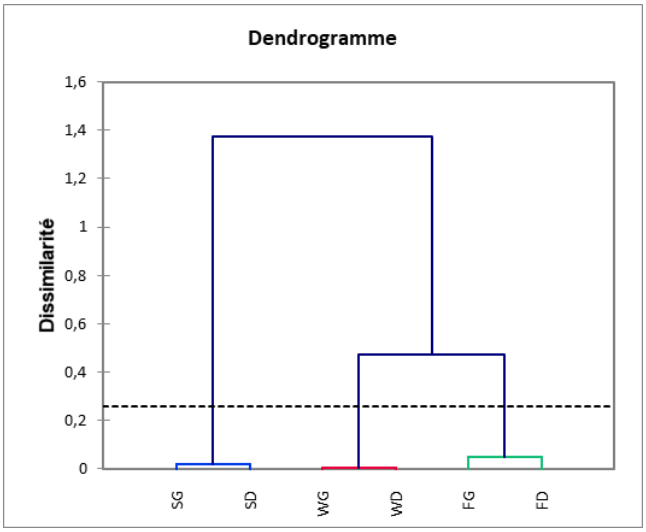

Figure 3: CAH dendrogram of *Alchornéa cordifolia* oils from Dolisie and Gomatsétsé extracted by different methods.

In conclusion, the CAH confirms the influence of the extraction method on the oxygen content of oxyrane in *Alchornea cordifolia* oils.

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CONCLUSION

The seeds of *Alchornea cordifolia* provide an oil with multiple chemical functionalities that could act as an alternative to petroleum as a raw material. However, this oil poses exceptional challenges for workers looking

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for their potential applications. This work reports the thermal instability character of this oil. This oil should not be heated to high temperatures and for long heating times in order to preserve the oxirane content. *Alchornea cordifolia* oil is therefore not suitable as a raw material in industrial operations for upgrading conventional oil at high temperature.

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