# Effect of epoxidation on the physicochemical properties of fruit oil of *RaphiahookeriG. Mann & H. Wendl*from Mebole (Gabon)

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**Abstract:** Vegetable oils obtained from fruits and seeds have a rather important part of the diet. They are some underutilized oilseeds species as Raphiahookeri. Physicochemical properties of oil extracted from Raphiahookerimesocarp was analyzed before and afterepoxidation reaction. The results showed that the extraction yield was 53.46%. The epoxidation reaction gave a conversion of 99.8% and a selectivity of 52.4%. Before epoxidation, refractive index, relative density kinematic viscosity, iodine index, acid index, peroxide index, saponification index, unsaponifiable matter were 1.4677nD, 0.9052  $\pm$  0.003 g/cm<sup>3</sup>,56.6  $\pm$  0.6 mm<sup>2</sup>.s<sup>-</sup>1, 87.2  $\pm$  2.3 g  $I_2/100g$  oil, 3.7  $\pm$  0.1 mg KOH/g oil,12.7  $\pm$  1.2 meq  $O_2/Kg$ , 194.7  $\pm$  1.5 mg KOH/g oil oil, 2.23% respectively. After epoxidation index were 1.4675 nD, 0.9636  $\pm$  0.001 g/cm<sup>3</sup>, 257.9  $\pm$  0.3 mm<sup>2</sup>.s<sup>-</sup>1, 0.2  $\pm$  0.01 g  $I_2/100g$  oil, 3.0  $\pm$  0.1 mg KOH/g oil, 62.7  $\pm$  2.2 meq  $O_2/Kg$ , 226.6  $\pm$  1.3 mg KOH/g oil oilrespectively **Keywords:** Raphiahookeri G. Mann & H. Wendl;mesocarp of fruit, oil, physicochemichal properties; epoxidation reaction.

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# I. Introduction

Gabon, a Central African country that is part of the Congo Basin, is more than 80% covered by forest [1]. It therefore has a rich and diverse flora with fruit trees whose different parts are sometimes consumed by certain populations. This is the case of certain populations of northern Gabon who consume the mesocarp of *Raphiahookeri G. Mann & H. Wendl* fruit after steaming without apparent danger [2]. However, its fruits are still under-exploited. The fruits of *R.hookeri* are therefore non-conventional oilseeds, whose mesocarp is rich in fats. The oil extracted from these fruits could be an alternative to common oils or other non-conventional oils. Indeed, the vegetable oil of the mesocarp of the fruits of *R. hookeri* could present interesting specific properties, related in part to the presence of fatty acids and to the quality of their physicochemical properties. Moreover, this oil could undergo a chemical modification such as epoxidation reaction. This study is thus a contribution to the valorization of the oil of the mesocarp of the fruits of *Raphiahookeri* of Gabon, a non-conventional oleaginous.

# II. Materials and methods

**Plant material:** The fruits of *Raphiahookeri* were collected from Mebole Village in northern Gabon (Figure 1). Once the fruits were in the laboratory, we removed the mesocarps and dried them in an oven at 103°C for 24 hours. The dried mesocarps were ground using a mechanical grinder and the powder obtained waskept in the refrigeratorat 4 °C until analyses. Plant wasidentified in The National Herbarium of Gabon Pharmacopoeia Institute of TraditionalMedicine (Iphametra) of Libreville in Gabon.

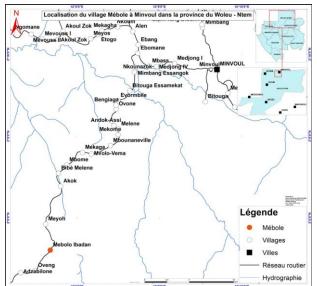


Figure 1: Location of Mebole in Minvoul in the province of Woleu-Ntem (Gabon)

**Oil extraction:** Oil extraction from the mesocarp of *Raphiahookeri* fruit was performed using a Soxhlet with a capacity of 100mL. 30g of *Raphiahookeri* fruit mesocarp powder was collected in a cartridge and placed in the Soxhlet apparatus. Extraction was carried out for 6 hours with 150 mL of cyclohexane (solvent). After extraction, the solvent was removed using a rotary evaporator, and then the traces of the solvent were removed in an oven at 85°C for 24 hours.

**Epoxidation reaction:** The epoxidation reaction was performed according to the protocol described by Guzmán[3]. Forty grams of *Raphiahookeri* oil and 3.1 g of formic acid are introduced into the reactor and heated to  $55^{\circ}$ C with stirring. The hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>) is added to the reactor using a metering pump with a flow rate of 1 mL/min for 50 minutes. The reaction lasted 16 h, and the stirring speed is set to 350 rpm. Then, the system was left to stand for 15 minutes, and two phases were formed.

The lower aqueous phase was removed while the upper organic phase was washed six times with distilled water. The remaining water in the organic phase after washing is removed by a rotary evaporator under a vacuum. The iodine value and oxirane oxygen content were measured for the final product to estimate the conversion, yield, and selectivity of the process (Guzmán et al., 2020).

% conversion =  $\frac{InitialIodineValue}{InitialIodineValue} + 100$ 

% Vield =  $\frac{Oxirane \ value}{Theoretical \ oxirane \ value} * 100$ % Selectivity =  $\frac{\% \ Yied}{\% \ Conversion} * 100$ 

**Relative density:** A volume of 3mL of *Raohiahookeri* fruit mesocarp oil is injected into the DMA 4100M densimeter using a 5mL syringe. Measurements are made in triplicate.

**Refractive index:** A volume of 0.5mL of *Raphiahookeri* oil is placed in the sample cuvette of the refractometer (Abbemat 300) using the 5mL syringe. The temperature of the refractometer is set to 20°C and measurements are performed in triplicate.

**Viscosity:** Approximately  $100\mu$ L of *Raphiahookeri* fruit mesocarp oil is introduced into a  $\Theta$ 1.59 (23427488) glass capillary tube using the 5mL syringe. An aspherical steel ball is inserted into the capillary tube containing the oil. Then the capillary tube is placed in the micro-viscometer (Lovis 2000 ME) and measurements are performed in triplicate at a fixed temperature of  $20^{\circ}$ C.

Acid index: In an Erlenmeyer flask, 2g of oil are dissolved in 50mL of previously neutralized ethanol. The solution is titrated under magnetic stirring with 0.1N ethanolic KOH in the presence of 1% phenolphthalein (2 or 3 drops). The acid value was determined from the relation shown in the equation

Acid value (mgKOH/g) = 
$$\frac{V*N*56.1}{W}$$

V is the titer value (mL), N is the normality of KOH = 0.1N and 56.1 = molar mass of KOH and W is the weight of the sample.

**Saponificationindex:** In an Erlenmeyer flask with a round neck, 2g of oil is introduced with 25 mL of ethanolic solution of KOH (0.5N). The mixture is boiled (using a pumice stone) under a reflux condenser for 1h with occasional stirring. The mixture is titrated while hotly with a 0.5N hydrochloric acid solution in the presence of the colored indicator (1% phenolphthalein). The saponification value was calculated using the equation :

Saponification value (mgKOH/g) =  $\frac{(a-b)*F*56.1}{u}$ 

With b = titer value of a blank (mL), a = titer value of the sample (mL), F = factor of 0.5 N HCl =1 (in this case) and 28.05 = mg of KOH equivalent to 1 ml of 0.5 N HCl and W is weight of the sample.

**Peroxideindex:** In an Erlenmeyer flask, 2g of oil were dissolved in 10mL of chloroform, and then successively 15mL of acetic acid and 1mL of saturated potassium iodide were added. The mixture was stirred for one minute and allowed to stand in the dark for 5 minutes after sealing the Erlenmeyer flask. After incubation, 75mL of distilled water and 3mL of starch was added. The solution was titrated with 0.01N sodium thiosulfate until the blue color disappeared. A blank test was performed under the same operating conditions. The analyses were performed in triplicate. The peroxide value was determined by the following formula(): Peroxide value (mgO<sub>2</sub>/Kg) =  $\frac{(V1-V0)*T*1000}{V}$ 

With

V0 is the volume of the sodium thiosulphate solution used for the blank,

V1 is the volume of the sodium thiosulphate solution used for the determination of the sample, T is the normality of the sodium thiosulphate used,

and W is the mass of the test sample in grams.

**Iodine index:** In an Erlenmeyer flask, 0.2 g of oil is dissolved in 15 mL of chloroform, and 25 mL of Hanus solution is added. The Erlenmeyer flask is stoppered, shaken lightly, and placed in the dark for one hour. Next, a 10% potassium iodide (KI) solution is added with 150 mL of distilled water and 2 mL of starch. The mixture is stirred and then titrated with a 0.1N sodium thiosulfate solution. A blank determination was brought out alongside the oil samples.

Iodine value was calculated thus (Mefouet et al., 2021):

Iodine value = 
$$\frac{(V1-V0)*T*1269}{W}$$

V1 = the titer value for the blank,

V0 = the titer value for the sample,

1269 = Concentration conversion coefficient,

and W is the weight of the sample (g).

**Unsaponifiable value:** Five grams of *Raphiahookeri* fruit mesocarp oil is saponified with 30ml of alcoholic potash (2N). To the yellowish solution obtained are added 50ml of hexane, then 30ml of distilled water. The organic phase is recovered, and the aqueous phase is treated with 15 ml of hexane, three times. The organic fractions containing the unsaponifiable matter are collected and dried with magnesium sulfate. The recycling of the hexane in the rotavapor allows for to recovery of the unsaponifiable part, which is then weighed [4].

## III. Results and Discussion

The oil extracted from the mesocarp of fruit of *Raphiahookeri*wasobtened with 53.46% yield. This result shows that the mesocarp of these fruits is rich in fat.

The epoxidation reaction of the *R. hookeri* fruit mesocarp oil gave a conversion of 99.8%. This conversion value shows that almost all double bonds of unsaturated fatty acids in *Raphiahookeri* oil were reacted with peroxyformic acid. The selectivity represents the amount of oxirane formed, in other words, it is the yield of an epoxide. The value obtained was 52.4%. It thus appears that 52.4% of oxiranes were produced in the majority against 47.6% of the products of secondary reactions such as the opening of the ring (Figure 2). The high number of secondary reactions is due to the non-use of a phase catalyst [5].

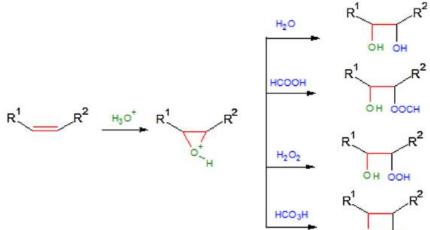


Figure2: Cycle opening reactions

The simplified mechanism of the different reactions occurring during the epoxidation of the oil of the fruit mesocarp of *R. hookeri* by peroxycarboxylic acids [6] is shown in Figure 3. In the first step, peroxyformic acid is produced in situ by formic acid perhydrolysis. Then, peroxyformic acid moves to the organic phase to epoxidize the unsaturated fatty acids double bonds of the oil of the fruit mesocarp of *Raphiahookeri*. Ringopening strikes between epoxidized unsaturated fatty acids and water from the aqueous phase at the interface. According to literature [6,7], the solubility of hydrogen peroxide in the organic phase is negligible.

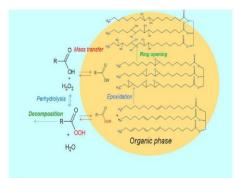


Figure3: Simplified mechanism for fatty acid epoxidation.

The physicochemical properties of the mesocarp oil of fruit of *Raphia. hookeri* fruitbefore epoxidation are recorded in Table 1.

Parameters	Values
Refractive index (nD, 20°C)	1.4675
Relative density(g/cm <sup>3</sup> )	0.9052±0.003
Kinematic viscosity (mm <sup>2</sup> .s <sup>-1</sup> , 20°C)	56.6± 0.6
Iodine value (g I <sub>2</sub> /100goil)	87.2 ± 2.3
Acid value (mg KOH/g oil)	3.7 ± 0.1
Peroxide value (meq O <sub>2</sub> /Kg oil)	12.7 ± 1.2
Saponifiable value (mg KOH/g oil)	$194.7\pm1.5$
Unsaponifiable value (%)	2.23

Table 1: Physicochemical properties of the mesocarp oil of fruit of Raphiahookeri

The relative density of the oil of *Raphiahookeri* fruit mesocarp was  $0.9052 \pm 0.003$  g/cm<sup>3</sup>. This value of density is by the standard of the food codex [8]. The density of our oil is comparable to that of hazelnut oil (0.898 - 0.915 g/cm<sup>3</sup>). Generally, the relative density of most oils, whether mineral or vegetable, is between

0.840 and 0.960 at 20°C. The density of oil is also a criterion of purity. Therefore, the density value of the oil from the mesocarp of this fruit shows that this oil contains few foreign bodies.

The refractive index of the oil of *R. hookeri* fruit mesocarp was 1.4675 nD at 20 °C. This value agrees with the food codex standard[8]. Thus, for example, the refractive index of the oil in our study is comparable to olive oil (1.4677 - 1.4707) or almond oil (1.467 - 1.475). The refractive index of the oil in our study shows that this oil is nondrying (1.467 < Refractive Index < 1.472) and pure[9]. The refractive index confirms that this oil has few foreign bodies, and its fatty acids are of high molecular weight [10].

The kinematic viscosity of *R.hookeri* fruit mesocarp oil was  $56.6 \pm 0.6 \text{ mm}^2 \text{.s}^{-1}$  at 20 °C. This value is very close to soybean oil  $57.1 \pm 1.1$  at  $22 \pm 1$  °C [11].

The acid value of the oil of *R. hookeri* fruit mesocarp was  $3.7 \pm 0.1$  mg KOH/g oil. This value is by the food codex standard [8]. For, the acid value of our oil is comparable to that of virgin oils (4.0 mg KOH/g oil). The acid value of the oil in this study shows that it (oil) is of good quality and confirms a low presence of free fatty acids. Indeed, a good quality oil should have zero or low acidity. The maximum accepted value is 2% oleic acid, corresponding to an acid number of about 4.0 mg KOH/g oil [9].

The iodine value of *R. hookeri* fruit mesocarp oil was  $87.2 \pm 2.3$  g I<sub>2</sub>/100g oil. This value is lower than 110 g I<sub>2</sub>/100g oil so the oil in our study is indeed non-drying [12]. The iodine value highlights the degree of unsaturation of the oil. Thus, the more unsaturated oil is, the higher its iodine value [10]. However, the iodine values of vegetable oils should be between 75 and 141 g of I<sub>2</sub>/100 g oil[13]. The iodine value of the oil in this study is high, so it is an unsaturated oil. The iodine value of the mesocarp oil of this fruit is comparable to that of olive oil (77 - 94 g I<sub>2</sub>/100 g) and peanut oil (77 - 107 g I<sub>2</sub>/100 g) [8]. The value of the iodine index obtained is therefore by the food standard.

The peroxide value of *Raphiahookeri* fruit mesocarp oil was  $12.7 \pm 1.2 \text{ meq } O_2/Kg$  oil. This value is by the food codex standard[8]. Indeed, the peroxide value of the oil in our study is less than 15 milliequivalents of active oxygen/kg oil (food standard). The value of the peroxide value obtained confirms that the oil in our study is of good quality. Indeed, a fatty substance presents an alteration when the peroxide index reaches a value of 100 to 320 meq  $O_2/Kg$  oil, which corresponds to a value of 10 to 20 millimolecules of peroxides per kilogram [9]. However, the peroxide value of the mesocarp oil of this fruit is less than 15 meq $O_2/Kg$  oil.

The saponification value of the oil from the fruit mesocarp of *Raphiahookeri* was 194.7  $\pm$  1.5 mg KOH/g oil. This value is by food standards, as saponification numbers of vegetable oils should be in the range of 189 to 202 mg KOH/g oil [8,13]. The saponification value of the oil in our study which is less than 202 mg KOH/g oil shows that this oil contains long carbon chain fatty acids (high molecular weight fatty acids) and few impurities [11]. *Raphiahookeri* fruit mesocarp oil would therefore be suitable for the manufacture of soaps and shaving creams [14,15].

The unsaponifiable content of the oil from the fruit mesocarp of *Raphiahookeri* was 2.23%. This value represents the amount of the residual fraction that is insoluble in water after saponification [16]. It is a complex mixture including polyphenols, phytosterols, triterpenes, saturated hydrocarbons, fatty alcohols (waxes), fatsoluble pigments, vitamins (A, D, E, and K), and extremely diverse constituents found in small quantities [16].

The physicochemical properties of the mesocarp oil of fruit of *Raphia. hookeri* fruitafter epoxidation are recorded in Table 2.

Parameters	Values
Refractive index (nD, 20°C)	1.4675
Relative density(g/cm <sup>3</sup> )	0.9636± 0.001
Kinematic viscosity (mm <sup>2</sup> .s <sup>-1</sup> , 20°C)	$257.9{\pm}0.3$
Iodine value (g I <sub>2</sub> /100goil)	0.2 ± 0.01
Acid value (mg KOH/g oil)	$3.0\pm0.01$
Peroxide value (meq O <sub>2</sub> /Kg oil)	$62.7\pm2.2$
Saponifiable value (mg KOH/g oil)	226.6 + 1.3

**Table 2:** Physicochemical properties of the mesocarp oil of fruit of Raphiahookeriafter epoxidation

The refractive index changes from 1.4677 nD for the initial oil to 1.4675 nD for the epoxidized oil. This change is considered negligible. Therefore, the epoxidation reaction does not influence the refractive index of this oil. The values obtained show that the epoxidized oil is of the same degree of purity as the initial oil.

The relative density increases from  $0.9052 \pm 0.003$  g/cm<sup>3</sup> for the initial oil to  $0.9636 \pm 0.001$  g/cm<sup>3</sup> for the epoxidized oil. This increase is justified by the formation of epoxides and other products from ring-opening

(Figure 4).Indeed, the epoxidation reaction allowed the insertion of oxygen atoms on the double bonds of unsaturated fatty acids. This insertion led to an oil-rich in saturated fatty acids. This type of oil is either semisolid or solid. The density varies from semi-solid to solid or from liquid to semi-solid. In other words, the density has increased because the initial (non-drying) oil has become semi-drying due to the epoxidation reaction. The epoxidation reaction significantly influences the density of this oil.

The kinematic viscosity increases from  $56.6 \pm 0.6$  mm2.s for the initial oil to  $257.9 \pm 0.3$  mm2.s for the epoxidized oil. Epoxidation allows the transformation of unsaturated fatty acids into saturated fatty acids, hence the increase in viscosity. Indeed, oils rich in saturated fatty acids tend to be solid [17]. The epoxidation reaction has a very strong influence on the kinematic viscosity of this oil.

The iodine value decreases from  $87.2 \pm 2.3$  g I<sub>2</sub>/100g oil for the initial oil to  $0.2 \pm 0.01$  g I<sub>2</sub>/100g oil for the epoxidized oil. These results reflect the almost complete disappearance of the unsaturated fatty acid double bonds in the initial oil because of the epoxidation reaction. The epoxidation reaction influences very considerably the iodine value of this oil.

The iodine value increases from  $3.7 \pm 0.1$  mg KOH/g oil for the initial oil to  $3.0 \pm 0.01$  mg KOH/g oil for the epoxidized oil. These results show a slight decrease in free fatty acids following oxidation. This decrease in free fatty acids can be explained by the formation of hydroperoxide[18]. The epoxidation reaction, therefore, influences the acid number of this oil.

The saponification value increases from  $194.7 \pm 1.5$  mg KOH/g oil for the initial oil to  $226.6 \pm 1.3$  mg KOH/g oil for the epoxidized oil. These results confirm the oxidation of the initial oil by the epoxidation reaction and reflect the formation of short carbon chain fatty acids. Indeed, the saponification index and the size of fatty acids are inversely proportional. That is, when the saponification index increases, the size of the fatty acids decreases [10]. The epoxidation reaction significantly influences the saponification number of this oil.

The peroxide value increases from  $12.7 \pm 1.2 \text{ meq } O_2/\text{ Kg}$  oil for the initial oil to  $62.7 \pm 2.2 \text{ meq } O_2/\text{ Kg}$  oil for the epoxidized oil. These results confirm the oxidation of the vegetable oil by the epoxidation reaction but do not necessarily reflect the alteration of the epoxidized oil. Indeed, the high value of the peroxide index is justified by the formation of peroxides and hydroperoxides due to the opening of the epoxy rings (Figure 3) [19]. It is important to combine this index with the measurement of the secondary products of oxidation (volatile substances) to know the real state of alteration of the oil. Aldehydes (volatiles) are a reliable indicator of oil rancidity and the para-anisidine index correlates well with the total volatile content [19]. However, since the peroxide value of epoxidized*R.hookeri* oil is always less than 100 meq O<sub>2</sub>/ Kg oil, it can be concluded that epoxidized*R.hookeri* oil is of good quality[9].

### **IV.** Conclusion

The physicochemical properties of the oil of mesocarp of the fruit of *Raphiahookeri*were conform with the food standards. The oil of mesocarp of the fruit of *Raphiahookeri*can thus be regarded as pure, good quality and not siccative. It can be used in the food industry as a substitute for vegetable fat or in cosmetics. The epoxidation of *Raphiahookeri* fruit mesocarp oil showed that the conversion rate is 99.8% and the yield of epoxide is 52.4%. This reaction converted the initial unsaturated oil to a saturated oil. The epoxidation does not influence the refractive index, unlike the other physicochemical parameters. This influence allowed to improve these parameters and give the epoxidized*Raphiahookeri* oil better oxidative stability while keeping its purity, its good quality and finally by widening its fields of applications.

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