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Physicochemical Properties, Fatty Acids and Epoxidation Reaction *of Cucumeropsis Edulis* Seeds Oil (Cucurbitaceae) from Gabon

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Abstract

The *Cucumeropsis edulis* seeds oil was extracted by the Soxhlet method with a yield of 51.17%. This oil has been tested for its physicochemical properties, fatty acids and for epoxidation reaction. The results showed that the refractive index was 1.4708. The density and Kinematic viscosity values were 0.9211 g / Cm^3 and 33 mm^2.s respectively. The acid and peroxide values were 5.7 mg KOH / g and 2.6 meq O_2 / Kg. The saponification and esterification number values were 193.4 and 192.2 mg KOH / g respectively. The water content (7.56%) and the calorific value (39695.2 KJ / Kg). H-NMR revealed that the main fatty acids were linoleic acid (67.29%), oleic acid (10%) and saturated fatty acid (22.54%).

The epoxidation reaction gave the following results: conversion (96%), reaction yield (50%) and selectivity (52%).

Keywords: oil characteristics, *Cucumeropsis edulis*, fatty acids, epoxidation reaction.

Introduction

The *Cucumeropsis edulis* is an important native resource of the central Africa. indeed, it is a multifunctional plant and valued for its medicinal, nutritional, economic, and social uses. The *Cucumeropsis edulis* is a plant cultivated by the natives, its seeds are edible and produce vegetable oil.

vegetable Oil is important nutritional components with diversity of functions in our body as an energy source, membrane structures, regulating body temperature and insulate organs (Ichu et al, 2019; Yonnas et al, 2019). Vegetable oils apart from meeting the dietary needs of man, are also used in cosmetic industry, bioresources and biodiesel (Zang et al, 2017; Aremu et al, 2015; Sharma et al, 2006; Jun Liu Zheng, 2016). Indeed, many studies have shown its potential as a raw material for food, pharmaceutical, and cosmetic products. the vegetable oil can also undergo chemical modifications by different methods. Among these methods, the epoxidation of vegetable oils has received much attention. because, in epoxidized form, vegetable oils can be used as PVC (polyvinyl chloride) additives, lubricants, and polymer precursors. the *Cucumeropsis edulis* seed oil could be is a good source of raw material for food, cosmetic, biofuel, paint, metallurgy, and pharmaceutical industries.

The objective of the present study is to quantitatively determine the physicochemical properties, fatty acids and to perform the epoxidation reaction of *Cucumeropsis edulis* seeds oil.

Materials and Methods

Vegetable Material

The fruits of *Cucumeropsis edulis* (Cucurbitaceae) were collected in the north of Gabon (Momo village). Unhulled seeds were washed, dried at room temperature and under the sunlight After extraction of shells, the seeds were dried on stove at 90°C for 24 h and pummeled to powder utilizing the mechanical processor. The powder obtained was kept in the refrigerator at 4°C.

Oil Extraction

The extraction of oil was made by the Soxhlet method. Thirty grams of dried and crushed *Cucumeropsis edulis* seed was placed in a cartridge of the Soxhlet apparatus. After a 6 hours extraction with cyclohexane as solvent, the solvent was evaporated under reduced pressure and solvent traces eliminated by oven drying after extraction.

Oil Characteristics

- ***Relative density (or specific gravity)***

Relative density is described as mass per unit volume of a fluid. Oil density was determined a precision DMA 4100 M density meter.

- ***Refractive index***

The refractive value of oil was measured using a precision Abbemat 300 refractometer,

- ***Viscosity***

The viscosity of oil is characterized by its resistance to internal flow and an indication of its oiliness in the lubrication of surfaces. The kinematic viscosity of cucumeropsis edulis seed oil was measured using a precision Lovis 2000 ME microviscometer.

- ***Acid value***

Ethanol was heated on a water bath for a few minutes to remove dissolved gases. The boiled ethanol was counteracted by adding a few drops of phenolphthalein and about 10 ml 0.1N potassium hydroxide until a pale pink color was obtained. 2 g of oil was weighed into a 250 mL conical flask and 50 mL of hot previously neutralized ethanol was added. The mixture was then carried to a boil on a water bath and the hot mixture was titrated

with 0.1N potassium hydroxide solution until the pink color (stable for few minutes) returned. The acid value was determined from the relation shown in the equation:

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

Where, V is titer value (mL), N is normality of KOH = 0.1N and 56.1 = molar mass of KOH and W is the weight of sample (Ondo-Azi et al, 2020).

- ***Saponification value***

About 2 g of oil was weigh up into a conical flask and mixed with 25 ml of 0.5 N ethanolic KOH. A blank was also concocted by taking 25 mL of alcoholic KOH in a similar flask. Reflux condensers were fixed to both flasks and the contents were heated in a water bath for one hour, swirling the flask from time to time. The flasks were then permitted to cool a little and the condensers washed down with a little distilled water. The abundance KOH was titrated with 0.5 N HCl acid utilizing phenolphthalein as a pointer. The saponification value was calculated using the equation:

$$\text{Saponification value (mg KOH/g)} = \frac{(a-b)*F*56.1}{W}$$

Where, b = titer value of blank (mL), a = titer value of 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 sample (Ondo-Azi et al, 2020).

- ***Peroxide value***

Two grams of oil test was weighed into a 500 mL conical flask and 10 mL of chloroform was added to disband the sample. This was ensued by the addition of 15 ml of acetic acid and 1ml of freshly prepared saturated potassium iodide solution. The flask was immediately clogged, stirred for about 1 minute, and kept at room temperature away from light for exactly 5minutes. Around 75 mL of refined water and 3ml of starch poison were added to the substance of the flask and afterward shaken vigorously. Scarcely any drops of starch poison were added as a pointer. The freed iodine was titrated against 0.01N sodium thiosulphate solution. A similar technique was done for blank, and the

peroxide value communicated in milliequivalent of dynamic oxygen per kilogram of test was determined utilizing.

Peroxide value (mg O_2/Kg) = $\frac{(V1-V0)*T*1000}{W}$

Where, V0 is the volume of the sodium thiosulphate solution used for blank, V1 is the volume of the sodium thiosulphate solution used for determination of sample, T is the normality of the sodium thiosulphate used, and W is the mass of the test sample in grams (Ondo-Azi et al, 2020).

- ***Iodine value***

0.2 g of oil sample was weighed into a dry 250 mL glass stopper bottle and 10ml of chloroform was added to the oil. 10 mL of Hanus solution was then added and allowed to stand in the dark for 1 hour. 10 mL of 10% Potassium Iodide with 100 mL of water were added and the resulting mixture was then titrated with 0.1N Sodium thiosulphate solution using starch (0.5%) as indicator just. A blank determination was brought out alongside the oil samples.

Iodine value was calculated thus:

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

Where, V1 = titer value for blank, V0 = titer value for sample and 1269 = Concentration conversion coefficient and W is weight of sample (g).

- ***ester index***

The ester number of a fatty substance is the number of milligrams of hydroxide potassium (KOH) required for neutralization of acids released by hydrolysis of esters contained in 1 g of fat body. In particular, the ester index is equal to the saponification index for pure glycerides. In practice, this index is not measured experimentally, but it is rather deduced by making the difference between the saponification index and the index of acid (Novidzro et al, 2019).

- ***Calorific value***

The calorific value (PC) was calculated using the formula of literature (Novidzro et al, 2019).

PC = 47645 - 4.187* Iodine value - 38.31*Saponification value

Fatty acids

The *Cucumeropsis edulis* seed oil was characterized by ^{1}H NMR with a Bruker device 300 MHz. The attribution of chemical shifts to the different protons belonging to the glycerol and fatty acids is easy because it is described in the literature (Zovi, 2009) and the calculation of integrations allows us to determine the mass composition of fatty acids in oils, the average number of unsaturations per triglyceride molecule, Total fatty acid mass per triglyceride molecule, Molecular weight oil (g/mol) and iodine number.

Epoxidation reaction

The epoxidation was carried out in a 500 mL lab-scale reactor equipped with mechanical agitation, heating glass jacket and reflux condenser in semi-batch mode. The *Cucumeropsis edulis* seed oil and formic acid were charged into the reactor and heated to 55 °C and then, the hydrogen peroxide solution was added dropwise with a pump for 1 h. The mixing share of reactants was 1:0.5:4 M of double bond/formic acid/hydrogen peroxide. The reaction while was 16 h, and the stirring speed was fixed to 350 RPM. Then, the system could settle for 15 min, and the lower aqueous phase was removed. The upper organic phase was washed six times with distilled water, and the remaining water was removed by vacuum rotating evaporation. The iodine value and oxirane oxygen content were measured to the final product to estimate the conversion and yield of the process (Guzmán et al, 2020). By following this protocol, the double bond conversion was around 96 %, and the epoxide selectivity was 52%.

$$\% \text{ conversion} = \frac{Initial\ Iodine\ Value - Final\ Iodine\ Value}{Initial\ Iodine\ Value} * 100$$

$$\% \text{ Yied} = \frac{Oxirane\ value}{Theoretical\ oxirane\ value} * 100$$

$$\% \text{ Selectivity} = \frac{\% Yied}{\% Conversion} * 100$$

Results and Discussion

- ***Physicochemical characteristics***

The physicochemical characteristics of *Cucumeropsis edulis* seeds oil are presented in Table 1. The extracted oil gave a yellow color. The oil and water contents were 51.17% and 7.56% respectively.

The physical properties that were studied are following: refractive index, relative density, kinematic viscosity, and calorific value. The value of refractive index was 1.4708 while the relative density was about 0.9211 ± 0.0002. The relative density and refractive index of *Cucumeropsis edulis* seeds oil are within the range of those reported by literature (Ondo-azi et al, 2020; Codex Alimentarius, 2019) for most conventional edible oils. Viscosity value obtained was 33 ± 0.8 mm^2/ s. This value is within the range of the viscosity of corn oil (31-35) (Blin et al, 2013). The calorific value was 39695.2 ± 81.4 KJ / Kg.

Table 1: physicochemical characteristics of *Cucumeropsis edulis* seeds oil

parameters	**Values**
Oil content (%)	51.17
Color	Yellow
water content (%)	7.56
Réfractive index (nD, 20°C)	1.4708
Relative density (g/cm^3)	0.9211 ± 0.0002
Kinematic viscosity (mm^2.s, 40°C)	33 ± 0.8
Calorific value (KJ/Kg)	39695.2 ± 81.4
Iodine value (g I_2/100g oil)	125.4 ± 3.3
Acide value (mg KOH/g oil)	5.7 ± 0.6
Peroxide value (méq O_2/Kg oil)	2.6 ± 0.04
Saponifiable value (mg KOH/g oil)	193.4 ± 2.5
Esterifiable value (mg KOH/g oil)	192.2 ± 2.1

The chemical parameters that were studied are following: peroxide value, saponifiable value, iodine value, acid value and esterifiable value. The saponification value was 193.4 ± 2.5 mg KOH/g, which suggests that *Cucumeropsis edulis* seeds oil is suitable for soap making and the manufacture of lather shaving creams. The high saponification value recorded for the seed oil suggest that the oil contain high molecular weight fatty acids and low levels of impurities. This is evidence that the oil could be used in the soap making industry (Ondo-azi et al, 2020; Enengedi et al, 2019). Iodine and peroxide values were 125.4 ± 3.3 mg I_2/100g oil and 2.6 ± 0.04 méq O_2/Kg huile. Iodine value of *Cucumeropsis edulis* seeds oil is within the range of corn oil (103-128) and sunflower oil (118-141) (Blin et al, 2013; Codex Alimentarius,1999). Likewise, saponifiable value conforms to the standard of the Codex Alimentarius (up to 10 milliequivalents of active oxygen/kg oil) (Codex Alimentarius, 2019). Acid value was 5.7 mg KOH/g oil. However, this value is lower than the maximum value for virgin palm oils (10 mg KOH/g oil) (Codex Alimentarius,1999).

Cucumeropsis edulis seeds oil has shown quality parameters in accordance with the standards of Codex Alimentarius (Codex Alimentarius, 1999; Codex Alimentarius, 2019). This justifies that the *Cucumeropsis edulis* seeds oil is to be used as an edible oil.

- ***Fatty Acid Composition***

Fatty acids are the lipid. They can be saturated or unsaturated (Nagy et al, 2017). The saturated fatty acid content in *Cucumeropsis edulis* seeds oil was 22.54%. However, the main unsaturated fatty acids were oleic acid (10%) and linoleic acid (67.29%). The molecular weight of the oil and total mass of fatty acids were 870.2 g/mol and 832.2 g respectively. The unsaturations number per triglyceride molecule was 4.3 in Table 2. the essential fatty acids have therapeutic effects (Ondo-azi et al, 2020). Indeed, the role of linoleic acids is not fully elucidated yet, however, they have anti-inflammatory properties.

Table 2: Fatty acid composition of Cucumeropsis edulis seeds oil

Fatty acids	Values
Linolenic acid (ω3, %) $C_{18:3}$	0
Linoleic acid (ω6, %) $C_{18:2}$	67.29
Oleic acid (ω3, %) $C_{18:1}$	10
Acide Gras saturé (%)	22.54
Unsaturations number per triglyceride molecule	4.3
Molecular weight of the oil (g/mol)	870.2
Total mass of fatty acid mass (g)	832.2

Cucumerospsis edulis seed oil is thus an important source of unsaturated fatty acids. Thus, the high presence of polyunsaturated fatty acids in *Cucumeropsis edulis* seed oil demonstrates its potential as a raw material for food, pharmaceutical, and cosmetic products since polyunsaturated fatty acids may have bioactive properties of industrial interest (Montserrat et al, 2013; Medeiros et al, 2020; De Carvalho et al, 2018; Koubaa et al, 2017).

- ***Epoxidation reaction***

The simplified mechanism of the different reactions occurring during the epoxidation of *Cucumeropsis edulis* seed oil by peroxycarboxylic acids (Leveneur et al, 2014; Santacesaria et al, 2011; Santacesaria et al, 2012; Leveneur et al, 2012; Xiaoshuang et al, 2018; Zheng et al, 2016) is shown in fig 1. In a first step, peroxyformic acid is formed in situ by formic acid perhydrolysis. Then, peroxyformic acid transfers to the organic phase to epoxidize the unsaturated fatty acids double bonds of cucumeropsis edulis seed oil. Ring opening

occurs between epoxidized unsaturated fatty acids and water from the aqueous phase at the interface (Santacesaria et al, 2011). According to literature (Leveneur et al, 2014; Santacesaria et al, 2011; Santacesaria et al, 2012; Leveneur et al, 2012; Xiaoshuang et al, 2018; Zheng et al, 2016), the solubility of hydrogen peroxide in the organic phase is negligible.

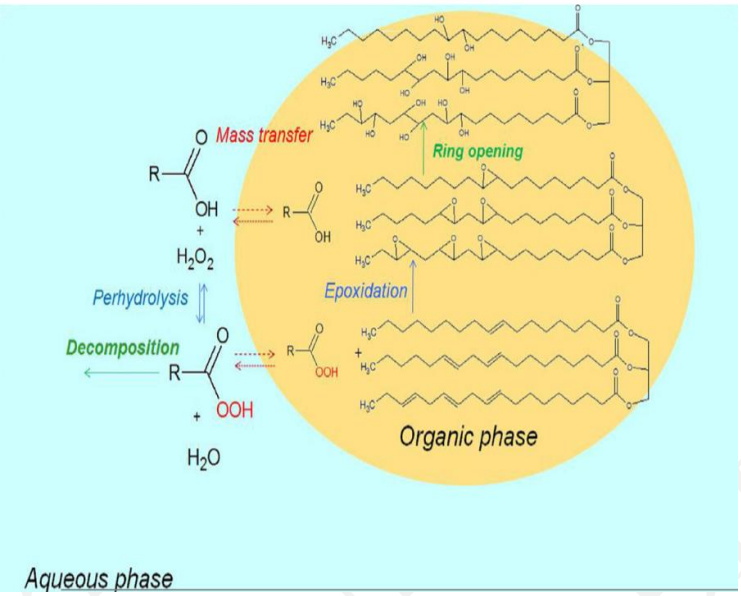

Fig. 1. Simplified mechanism for fatty acid epoxidation.

Numerous studies have shown different mechanisms of ring opening (Leveneur et al, 2014; Santacesaria et al, 2011; Santacesaria et al, 2012; Leveneur et al, 2012; Xiaoshuang et al, 2018; Zheng et al, 2016). Indeed, this reaction is complex, because different nucleophilic agents can be involved, i.e., hydrogen peroxide, formic acid, water or peroxyformic acids. For the sake of simplicity, only the ring opening reaction due to the hydroxonium ions was considered (Santacesaria et al, 2011). As shows in Fig. 2, the activation of oxirane by protons is the first step of the ring opening reaction.

The peroxyformic acid synthesis was described by literature (Leveneur S et al 2012). Two main reactions occur in the aqueous phase, formic acid perhydrolysis and peroxyformic acid decomposition, respectively. The rate equations are shown below:

$$R_{perh} = K_{perh}*([HCO_2H]_{aq}*[H_2O_2]_{aq} - (1/K^C)*[HCO_3H]_{aq}*[H_2O]_{aq}) \quad (1)$$

$$R_{decomp} = K_{decomp}*[HCO_3H]_{aq} \quad (2)$$

where K^C is the equilibrium constant of the perhydrolysis reaction, which has been determined by Leveneur Sébastien (Leveneur S et al 2012).

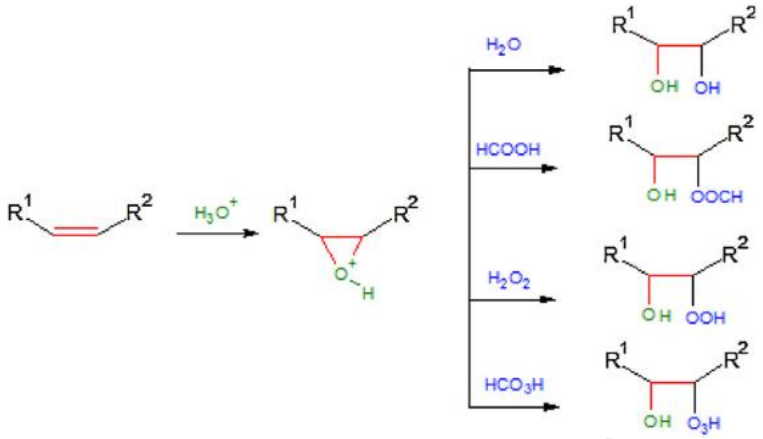

Fig. 2. Ring opening reactions.

In the organic phase, epoxidation and ring opening proceed simultaneously. The rate equations for the organic phase are summarized below:

- **Epoxidation**:

$$R_{Ep} = K_{Ep}*[HCO_3H]_{org}*[\text{ unsaturated fatty acids}]_{org} \quad (3)$$

- **Ring opening**:

$$R_{ro} = K_{ro}*[\text{Epoxide}]_{org}*[H_3O^+]_{org} \quad (4)$$

By considering rapid phase equilibria, the rate expressions for the epoxidation and ring opening can be rearranged to:

$$R_{Ep} = K_{Ep}*[HCO_3H]_{org}*[\text{ unsaturated fatty acids}]_{org} = K_{Ep}*[\text{ unsaturated fatty acids}]_{org}*([HCO_3H]_{aq}/K_{HCO_3H})$$

$$= K'_{Ep}*[\text{ unsaturated fatty acids}]_{org}*[HCO_3H]_{aq} \quad (5)$$

$$R_{ro} = K'_{ro}*[\text{Epoxide}]_{org}*[H_3O^+]_{aq} \text{ ; with } K'_{ro} = K_{Ep}/K_{H_3O+} \quad (6)$$

where $K_{HCO_3H} = [HCO_3H]_{aq}/[HCO_3H]_{org}$ is the distribution coefficient of peroxyformic acid between the aqueous and organic phase, and $K_{H_3O^+} = [H_3O^+]_{aq}/[H_3O^+]_{aq}$ is the distribution coefficient of hydroxonium ions.

Table 3 shows conversion was 96%, selectivity and yield were 52% and 50% respectively. The conversion rate only provides information on the proportion of reagent which has disappeared, but not on the amount of product formed which itself depends on the selectivity. So, we can say that in view of the results obtained, the chemical reaction mainly gave the desired product (oxirane) although the side reaction (Ring opening) is important.

Table 3 : epoxidation reaction balance

Parameters	**Values**
theoretical oxirane Content (mol/L)	7.3
Oxirane obtained Content (mol/L)	3.7 ± 0.1
Conversion (%)	96%
Yield (%)	50%
Selectivity (%)	52%

Conclusion

The present study on the chemical composition, physicochemical properties, and epoxidation reaction of *Cucumeropsis edulis* seed oil suggest that this oil could be a good source of raw material for the food, pharmaceutical and cosmetic industries. Indeed, the high saponification value allows many oil based products (soap, shampoo, lubricants, etc); the high presence of unsaturated fatty acids favors bioactive properties and physicochemical parameters are conform to the standards of Codex Alimentarius.

the epoxidation reaction gave several products: oxirane (majority) and the products resulting from ring opening.

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Nomenclature

K_{decomp}: decomposition rate constant [s]

$K_{perh:}$ perhydrolysis rate constant [l/mol/s]

org: organic phase

aq: aqueous phase

decomp: decomposition

perh: perhydrolysis

R_{perh} : perhydrolysis reaction rate [mol/l/s]

R_{decomp}: decomposition reaction rate [mol/l/s]

ro: ring opening

Ep: Epoxidation

R_{Ep} : Epoxidation reaction rate [mol/l/s]

R_{ro}: ring opening reaction rate [mol/l/s]

K_{Ep} : Epoxidation rate constant [l/mol/s]

K_{ro} : ring opening rate constant [l/mol/s]