



Characterization of the Unsaponifiable Fraction of Oils from the Pulp of Safous South in West of Democratic Republic of Congo

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Abstract:

The unsaponifiable fraction of oils from the pulp of safous was characterized through the study of essential oils and phytosterols. The essential oils found in the samples of the three zones showed qualitatively, the following composition: α -pinene, β -pinene, α -phellandrine, Benzene-1-methyl, phellandrene, 3-carene, caryophyllene. While higher alcohols and phytosterols found in the unsaponifiable Kinshasa were the 1-eicosanol, 1-Tétracosanol, 5- α -cholestane, 1-hexacosanol, 1-octacosanol, cholesterol, campesterol, Stigmasterol-1, 1-triacontanol, the β -sitosterol, and stigmastanol.

Keywords:

Pulp of safou; unsaponifiable of oils; essential oils; phytosterols; higher alcohols; DRC

I. Introduction

The safou is a fruit known as oilseeds can give up to 50% lipids (Silou, 2000). Its pulp is nutritionally rich. It contains between 50-70% fat, 15-30% protein and 2-5% ash. The pulp oil contains linoleic acid (C18: 2 n-6) between 18-27% and oleic acid (C18: 1 n-9) between 15-30% (Silou, 1996). The unsaponifiable fraction is rich in essential oils, higher alcohols and phytosterols (Obama et al, 2008). Because of his interest in growing food in rural areas and in major cities of Central Africa, the safou becomes a product of an undeniable economic value as a source of income for producers. It thus contributes to the fight against poverty. The post-harvest losses are very high (softened 3 to 4 days after harvest) (Silou, 1996, Tabuna, 1999 and 2004).

Equipment

For the characterization of plant sterols, higher alcohols and essential oils, tests were made on extracts of pulp safou fresh or dried. The material used is powdered dried pulp safou for thin layer chromatography (TLC), while we used the fresh pulp of safou for the determination of phyto-sterols and essential oils on GC-MS. Essential oils and phytosterols have shown similar patterns between areas and comparable to those reported in the literature.

II. Methods

For the study of the unsaponifiable fraction of oils, safou samples were considered for each tree for the oil content for all sites. The sampling for the study of the fatty acid profile has focused on the choice of planting at all sites studied.

2.1 Extraction of Essential Oil

The essential oil is obtained by hydrodistillation of the fresh pulp of safou with a Clevenger apparatus. Oil safou pulp (1.5 g) was refluxed for 30 minutes with 25 ml of alcohol-about 2 N potassium hydroxide. The unsaponifiable fraction was extracted with xylene. Qualitative determination of the components of essential oils from different sources safou (Kinshasa, and Kisantu Tshela) was performed by thin layer chromatography under the following conditions: - Stationary phase: silica gel 60F254 (Merck, United Kingdom) - Mobile phase: toluene / ethyl acetate 95 / 5 - Witnesses: Witness 1: myrcene, Witness 2: transcaryophyllène, Witness 3: alpha-terpineol, Witness 4: alpha-phellandrene (witnesses are the substances found in laboratories) - Detection: ultraviolet light on The thin layer chromatographic profile can characterize safou samples of different origins.

2.2 Analysis of the Essential Oil surGC-MS and GC-FID of Unsaponifiable

Two drops of the distillate collected in the hydrodistillation were diluted in 10.0 ml of trimethylpentane and the resulting solution was injected into a GC-MS instrument Hewlett-Packard 5890A, Electronic Impact 70 eV, 2100, equipped with a column WCOT Fused Silica CP-Wax-58CB (50 m, 0.32 mm ID) (DUEZ et al, 2007). The analysis was performed at a temperature of 250 ° C injector and detector temperature (FID) 250 ° C and a programming column temperature of 60 ° C to 125 ° C, at 4 ° C / min then 125 ° C to 230 ° C at 10 ° C / min. The flow of helium carrier gas was 1.2ml/min.

Identification of constituents was made by comparison to databases available in the literature.

Silylation of the unsaponifiable fraction was carried out by contact for 90 minutes at room temperature with 3 ml of silylating mixture (DUEZ et al, 2007). An amount of 0.5 microL sample of the unsaponifiable fraction and 1 L of sample solution consists of higher alcohols (1-eicosanol, 1-tétracosanol, 1-hexacosanol, 1-octacosanol and 1-triacontanol) and sterols (campesterol, stigmasterol, β -and sisterol stigmastanol) was injected on a gas chromatograph Shimadzu GC brand-17A, equipped with fused silica column WCOT 25 mx 0.32 mm ID coating CP-Sil-5 CB Chrompack. The analysis was performed at a temperature of injector 340 ° C, a temperature detector (FID) 340 ° C and a programming column temperature of 240 ° (1 minute) to 325 ° C, at 8 ° C / min, then 20 minutes at 325 ° C. The flow and pressure of the helium carrier gas were 2.0 mL / min and 49 kPa. Identification of constituents was made by using data from the computer compared to databases available in the literature.

2.3 Statistical Analysis

To better appreciate the results, we used statistical tools with the following software: 2009 Genstat Discovery Edition 3, R, XLSTAT. 2007, Excel 2007 and SPPS.2006. The analysis of descriptive statistics and multivariate analysis is applied in the methodology of Chapter II.

III. Results and Discussion

3.1 Results

a. Identification of the Composition of the Essential Oil of the Pulp Safou

1. Thin layer

Figure 1 shows the spectrum of essential oils of our three samples (middle) thin layer next to the control α -pinene, β -pinene, phellandrene, the first sample (Kinshasa), Terpeneol, Trans caryophyllene, myrcene. Compared to the controls used, it appears that compounds

found in our three samples of *Dacryodes edulis* (4-Kinshasa, 5-and 6-Kisantu Tshela) are not very comparable to the very small number of witnesses. But it is remarkable that the profiles of three samples are similar as shown in Figure 1.

This result shows the three substances visible spectra need further analysis to determine them. Witnesses in our provision does not allow us formally to highlight the differences in the spectra obtained.

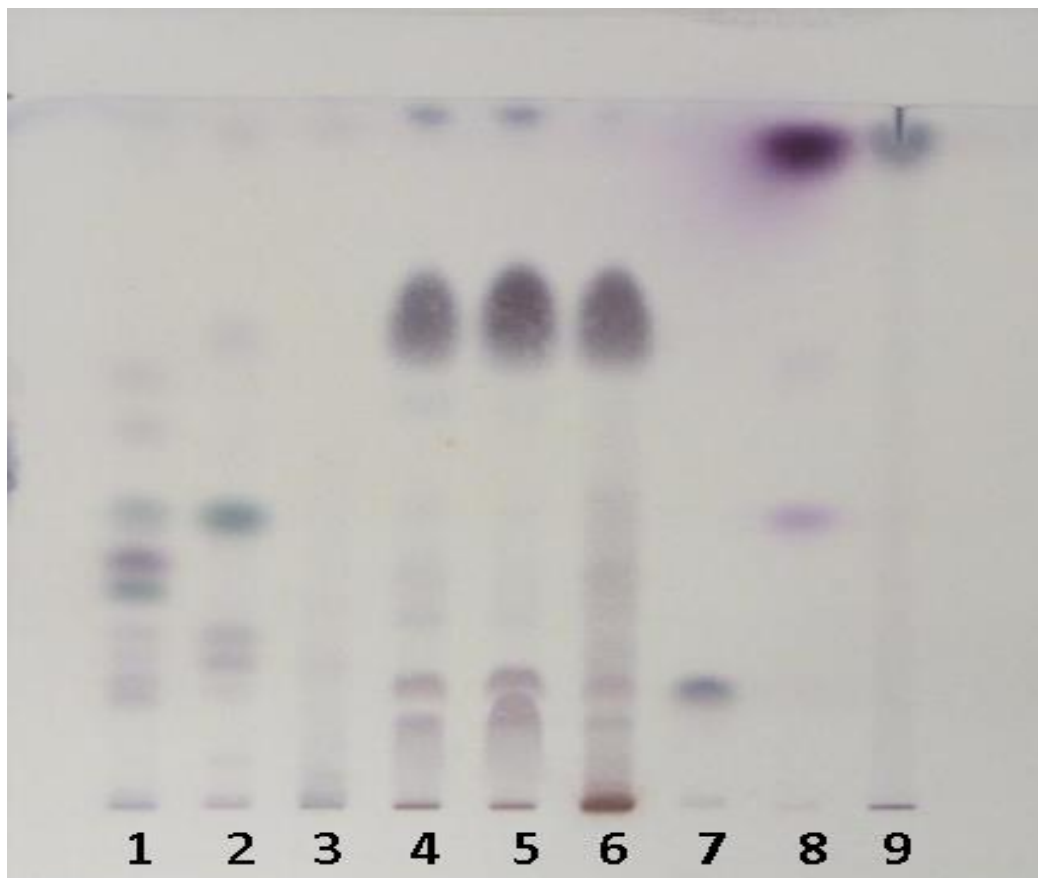


Figure 1. Thin Layer Chromatogram of safou Oil Samples from 3 Sites

The profile of the samples (4, 5 and 6) shows the presence of substances that we can not identify compared to controls. It should be noted that our samples have the same profile in their composition thin layer.

2. On GC-MS

The content of essential oils was determined immediately after extraction by steam distillation of water (the volume read on the graduated part of the device). The hydrodistillation gave us an unsaponifiable content of 0.215%. The chromatogram shown in Figure 39 shows the essential oils found in our sample safou fresh after hydrodistillation. The essential oils found in a qualitative way are: α -pinene, β -pinene, α -phellandrine, Benzene-1-methyl, phellandrene, 3-carene, caryophyllene.

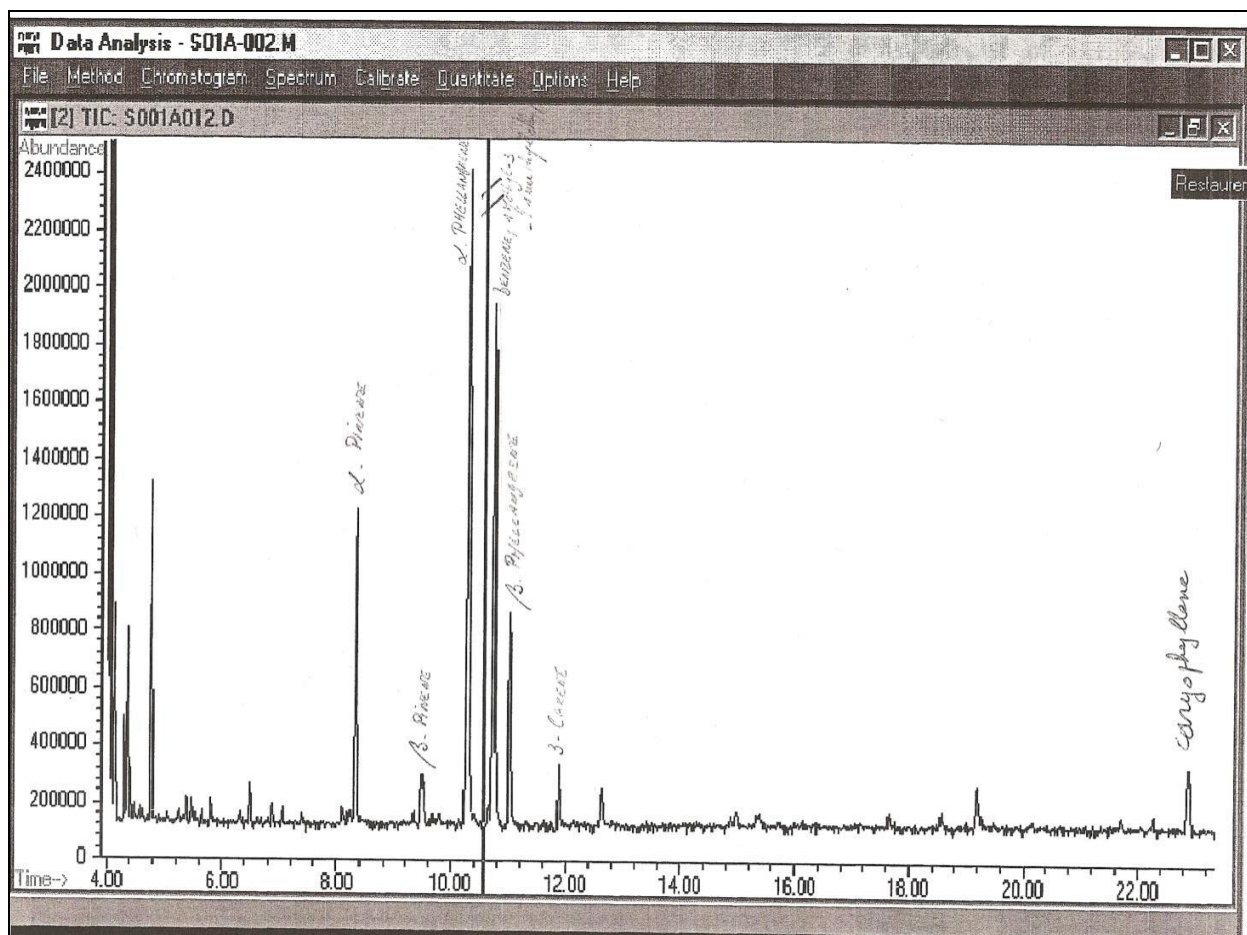


Figure 2. GC-MS Chromatogram of Pulp Essential Oil

b. Composition of Higher Alcohols and Phytosterols

1. Descriptive Statistics of Results of Higher Alcohols and Phytosterols Safou

Table 1 shows the concentrations of the major phytosterols% and higher alcohols found by gas chromatography (GC-FID). We compare the results according to the sources taken in pairs. It turns out that the content of 1-**and** 1-eicosanol tétracosanol differs significantly from between Kinshasa and Kisantu. The rest does not differ significantly in the three sites.

Table 1. Major Phytosterols and Higher Alcohols in the Unsaponifiable Oil from the Pulp of Safous

N°	Phytosterols and higher alcohols	Kinshasa (%)	Kisantu (%)	Tshela (%)
1.	1-eicosanol	1,0 ± 0,0 ^a	0,2 ± 0,2 ^{ba}	0,2 ± 0,2 ^{ca}
2.	1-Tétracosanol	0,9 ± 0,0 ^a	0,4 ± 0,2 ^{ab}	0,2 ± 0,2 ^{ca}
3.	5- α -cholestane	17,6 ± 9,7	10,6 ± 9,3	16,8 ± 11,8
4.	1-hexacosanol	0,45 ± 0,1	0,3 ± 0,1	0,3 ± 0,4
5.	1-octacosanol	1,1 ± 0,3	0,9 ± 0,1	0,7 ± 0,7
6.	Cholestérol	0,3 ± 0,2	0,2 ± 0,2	0,5 ± 0,6
7.	Campestérol	0,3 ± 0,4	0,8 ± 0,5	0,6 ± 0,5
8.	Stigmastérol	0,4 ± 0,0	7,2 ± 0,3	0,7 ± 0,2
9.	1-Triacontanol	1,4 ± 0,4	4,7 ± 3,0	5,5 ± 4,9

10.	β - Sitostérol	$3,8 \pm 0,4$	$5,2 \pm 0,4$	$3,1 \pm 0,9$
11.	Stigmastanol	$1,8 \pm 0,4$	$2,6 \pm 2,1$	$3,6 \pm 1,9$
12.	Total en % (par rapport au % de l'insaponifiable.	$28,6 \pm 10,3$	$32,7 \pm 0,3$	$31,9 \pm 15,1$

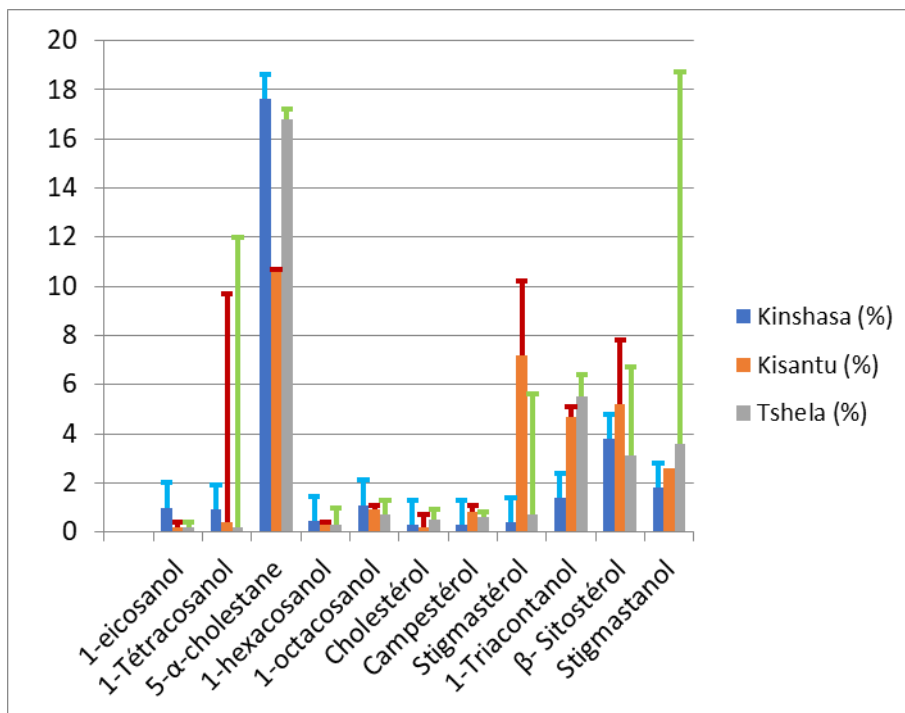


Figure 3. Graphical representation of phytosterols and higher alcohols in the unsaponifiable oils from the pulp of safous south west of the DRC

Statistical analysis by the hierarchical cluster analysis gave the dendrogram in figure 3. It gives us three classes. The three classes include the following: For Class 1, we have samples of Kin 1, one of Kish and Tsh 1. The second class consists of classes 2 and Tsh Kin 2 and the third class the elements of Kish 2. Results from three sites are positively correlated.

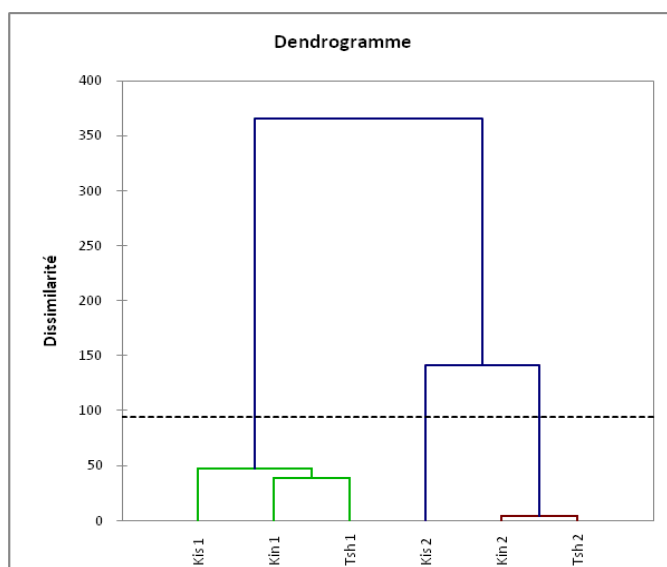


Figure 4. Dendrogram Results Phytosterols and Higher Alcohols

3.2 Discussion

The unsaponifiable matter, we emphasize the presence of essential oils (α -pinene, β -pinene, α -phellandrene, Benzene-1-methyl, β -phellandrene, 3-carene, caryophyllene), phytosterols (cholesterol) and alcohols higher in all samples studied. It turns out that all the oils of safous from Kinshasa, and Kisantu Tshela have the same profile unsaponifiable. We also found the following products as higher alcohols and phytosterols: 5- α -cholestane, 1-hexacosanol, 1-octacosanol, the cholesterol that is representative of the sterols, campesterol, stigmasterol, 1-triacontanol, the β -sitosterol and stigmastanol.

The essential oils found in samples from three sites showed qualitatively, the following composition: α -pinene, β -pinene, α -phellandrine, Benzene-1-methyl, phellandrene, 3-carene, caryophyllene. Phytosterols and higher alcohols found in the unsaponifiable of Kinshasa are: 1-icosanol $1.0 \pm 0.0\%$, 1-Tétracosanol $0.9 \pm 0.0\%$, 5- α -cholestane $17.6 \pm 9.7\%$ 1-hexacosanol $0.45 \pm 0.1\%$, 1-octacosanol $1.1 \pm 0.3\%$, cholesterol $0.3 \pm 0.4\%$, $0.3 \pm 0.4\%$ Campesterol, Stigmasterol 1-0, $4 \pm 0.0\%$, 1-triacontanol $1.4 \pm 0.4\%$ β -Sitosterol $3.8 \pm 0.4\%$, $1.8 \pm 0.4\%$ stigmastanol.

OBAME et al (2008), worked on *Dacryodes edulis* Gabon. The essential oil obtained by hydrodistillation of the resin of *Dacryodes edulis* (G. Don) HJ Lam was simultaneously analyzed on gas chromatography (GC-FID) and gas chromatography coupled to a mass spectrophotometer (GC-MS). Twenty-four compounds were identified in the essential oil. The most notable were sabinene (21.8%), terpinene-4-ol (19.8%), α -pinene (17.5%) and p-cymene (11.3%), respectively....

KUDU et al (2008) also worked on *Dacryodes edulis* Gabon and found the following essential oils: α -pinene: 17.47; sabinene: 21.76, β -pinene: 4.27, p-cementitious, α -terpinene: 1.22; γ -terpinene: 5.8; Limonene: 5.7, terpinene-1-ol: 19.8;-terpineol: 3.0.

JIROVETZ et al (2003) also analyzed the pulp of fruits of *Dacryodes edulis* (G. Don) HJ Lam from Cameroon by HS-SPME analyzer coupled with GC / FID and GC / MS GC / FID. Analyses were performed using a GC-14A with SPME adapted to the injector and FID-CR 6A Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID. The following results were found:

1. Fruit untreated: α -pinene: 20.3%, myrcene: 15.0%, β -pinene: 8.2% limonene and 3.6%.
2. Pulp after cooking: α -pinene: 44.9%, β -pinene: 21.1% myrcene 7.3%, limonene: 6.3% hexanal: 4.6% dimethyl sulfide: 4 1%, sabinene 3.8%.
3. Pulp after roasting α -pinene: 37.1% myrcene 20.9%, β -pinene 16.2%. Hexanal: 8.1% dimethyl sulfide: 7.0% limonene and 3.4%.

The safous contain in their unsaponifiable oil, essential oils and phytosterols interesting food. They also have antioxidant and antibacterial. The characterization of these products led us to see the profile of the lipid fraction is the same in Kinshasa, and Kisantu Tshela. Compared to the work being done in the sub-region, it appears that the profile of the fruit also contains chemical components which are also found in the leaves.

IV. Conclusion

The unsaponifiable oil from the pulp of safous southwest of the DRC is rich in phytosterols and higher alcohols. The safou in that can be incorporated into diets to fight against malnutrition. Just as it is also recommended to reduce the input of exogenous cholesterol. In this, the plant sterols (sitosterol, stigmasterol, and campesterol stigmastanol)

are very important. Indeed, despite their structural similarity with cholesterol, they have small differences of isomerism. These small differences have resulted in their very low absorption by the cells of the intestinal mucosa.

Although they are not absorbed, these sterols effectively inhibit cholesterol absorption by intestinal cells. The marketing of these plant sterols to promote lower cholesterol levels depend as much progress in the study of their structural characteristics as the shape of their administration (Garrett and Grisham, 2000). These plant sterols are present in the lipid fraction of safou and can play an important role in the global diet.

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