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Optimization of the Soxhlet Extraction of Oil from Safou Pulp (*Dacryodes Deulis*)

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Abstract: The aim of this study was to investigate Soxhlet extraction of oil from safou using various organic solvents. The safou, fruit of the safou tree (*Dacryodes edulis*) is very fragile. Post-hervest spoilage, essentially due to the softening of the pulp, can affect 50% of production. Extracting oil from the pulp could offer a way to reduce losses. The safou contains 30-70% of oil in its pulp and about 10% in its seeds. It is a major oilseed plant in the countries of the Congo Bassin, where unfortunately it is still underexploited. One possibility is to extract fresh oil by cold pressing. This oil would be characteristic of a geographical area, the Congo Bassin, much like olive oil is of the Meditarranean. Soxhlet extraction of oil from safou using various organic solvents was carried out to obtain optimization data for the assessment of cold pressing extraction rates. Using a 2^3 factorial design and a centred composite design for the sample studied, we obtained an optimal yield of 52% after 2 h of extraction from a finely ground safou powder containing 8% of residual moisture and with a ratio of pulp mass to solvent volume of 45 g/250 mL.

Keywords: *Dacryodes edulis*, FA, oil, organic solvents, safou, soxhlet extraction, TAG

INTRODUCTION

The reference method for the extraction of plant oils is by organic solvents in the soxhlet apparatus (AFNOR, 1981). We used this method to extract oil from safou pulp (*Dacryodes edulis*) using a range of organic solvents.

The safou tree, a major oil bearing plant that is still underexploited in the Congo Basin is a very fragile fruit. Post-harvest spoilage, due essentially to the softening of the pulp, can affect 50% of production in less than one week of storage. The extraction of oil from the pulp could offer a way to reduce these losses. The optimization of soxhlet extraction to determine the maximum oil content of samples is a preliminary step in assessing cold pressing extraction rates. Knowledge of these rates is needed to study the cost effectiveness of cold pressing.

First we defined the main parameters expected to influence the extraction yield. These were identified as the extraction time, the temperature, the particle size of the plant material and the nature of the solvent and its recovery rate. We then assessed the influence of the nature of the solvent and the extraction time on the yield, in relation to the quality of the oil obtained. Preliminary results enabled us to limit the factors included in the optimization process with a 2^3 factorial and a centred composite design.

MATERIALS AND METHODS

Plant material: This study was conducted 2010, the plant material used was safou from Pointe Noire in Congo-Brazzaville. Chemical and nutritional characteristics of oils from this origin have already been studied (Kinkela, 2003). The safou pulp was dried and powdered.

Solvent: Trichloroethane (density 1.29-1.35, boiling point 74-76°C), chloroform (boiling point 61.2°C, density 1.47-1.49), hexane (boiling point 78°C) and petroleum ether (boiling point 35-60°C, density 0.63-0.64), supplied by Prolabo (France) were used as extraction solvents.

Extraction: The oil was extracted from dried ground safou pulp by standard methods using 500 mL Soxhlet extractors (AFNOR, 1981).

Physical characteristics: Specific gravity and viscosity were determined using AFNOR standard methods (AFNOR, 1981).

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Analysis of Fatty Acids (FAs): An oil sample (100 mg), weighed to within 5 mg in a test tube was dissolved in 5 mL of hexane. To this was added 0.2 mL of transesterification agent (11.2 g of potassium hydroxide dissolved in 100 mL of methanol). The tube was stoppered and the contents vigorously mixed with a vortex-type blender for 1 min. The mixture was left for 5 min and 0.5 g of solid $NaHSO_4$ was added.

The sample was homogenized and centrifuged for 3 min. at ambient temperature and an aliquot of supernatant was sampled for analysis.

The methyl esters obtained were analysed by Gas Phase Chromatography (GPC) using a Perichrom (France) type PERI 2000 apparatus, equipped with a glass capillary column 30 m long and 0.4 m in internal diameter, impregnated with Carbowax 20 M (Applied Science Labs, State College, PA, USA).

The analysis was carried out at a constant temperature of 195°C with a nitrogen flow rate of 3 mL/min and a pressure of 0.5 bar. The injector temperature was 220°C and the flame ionization detector temperature was 215°C (Kinkela, 2003).

Data acquisition (peak areas and mass fraction of FAs), was carried out with Azur v. 2.0 software (Datalys, Saint-Martin-d'Hères, France).

The response of the detector to the different methyl ester was studied with quantitative mixtures (Nu Chek-Prep. Elysian, MN, USA).

Determination of the Triacylglycerol (TAG) composition: The TAGs purified by column chromatography were analysed by reversed-phase High-Performance Liquid Chromatography (HPLC) using a stainless steel column 250 mm long and 4.6 mm in internal diameter packed with silica (4 µm diameter) grafted with octadecyl radicals (HibarLichrospher 100 CH-18, Merck).

The column was set up on a Waters 717 Plus Autosampler equipped with a differential refractometer (Waters 996 Photodiode Array Detector, Waters, Milford, MA, USA). The analysis conditions were as follows: isocratic analysis at ambient temperature (21°C), using an acetone-acetonitrile (47:33, v/v) mobile phase with a flow rate of 1 mL/min).

Data acquisition (peak area, mass fraction of TAG) was carried out with Azurv. 2.0 software

Optimization by the Design of Experiment (DOE) method:

2^3 factorial design: The DOE method uses a factorial design, i.e., simultaneous variation of all the factors. The influence of each factor is then determined by calculating its principal and interactive effects.

The listed variables that influence the oil extraction yield were: time, temperature, particle size of plant material, residual moisture content of pulp, mass of the sample and nature and volume of solvent.

A model with seven variables, even for a first degree model, would need 2^7 = 128 experiments (Goupy, 2004). To reduce the number of experiments, four variables, namely the solvent (hexane), the volume of the solvent (250 mL), the sample mass (50 g) and the temperature (boiling point of hexane) were kept constant, reducing the experiment to the following three variables:

X_1 = Particle size of safou pulp
X_2 = Residual water content of safou pulp
X_3 = Extraction time

The safou pulp oil extraction yield Y depends on the variables X_1, X_2 and X_3, i.e., mathematically:

$$Y = f(X_1, X_2, X_3)$$

where,
Y : The yield or response
f : The response function
X_1, X_2 & X_3: The variables, or factors

The purpose of the experiments was thus to determine the effects of certain variables or factors on the response. In effect, the following two-part question was addressed: does a particular factor have a specific effect on the response and if so what is the relation between that factor and the response?

The two-level factorial design as developed by Davies (1954) appeared well suited to this type of problem and it offered the advantage of requiring only very basic mathematical knowledge (Ortigosa, 1993) and no cumbersome machine calculation.

The general formula for the number (N) of experiments for a complete factorial design is:

$N = 2^k$, where k is the number of variables in the factorial design.

If $k = 3$, then $N = 2^K = 2^3 = 8$ experiments

To construct the DOE matrix, we defined:

- Reduced variable x_i such that:
  - $x_i = (X_i - X_{i0}) / \Delta X$
  - X_{i0} = The base value, at the centre of the experimental domain (level 0)
  - ΔX = The variation increment, i.e., the unit of variation of the variables
- Two levels of the variables: High (+1) and low (-1)

This operation enables us to replace the experimental domain by the domain (-1, +1), with eight responses described by the matrix (Table 1) after randomization.

For a first-degree model with interactions, the points representative of a three-variable experimental design are located in three-dimensional space (cube).

Table 1: Design of experiment matrix (2^3 factorial design)

x_1	x_2	x_3
-1	-1	-1
+1	-1	-1
-1	+1	-1
+1	+1	-1
-1	-1	+1
+1	-1	+1
-1	+1	+1
+1	+1	+1

Table 2: Experimental domain (centred composite design)

Variable levels	Variable X_1	Variable X_2
Centre (0)	45 g/250 mL	80 min
Low level (-1)	20 g/250 mL	50 min
High level (+1)	70 g/250 mL	110 min
Very low level (-α)	9.65 g/250 mL	37.58 min
Very high level (+α)	80.35 g/250 mL	122.42 min

Table 3: Experimental design (centred composite design)

Experiments	x_1	x_2
1	-1	-1
2	+1	-1
3	-1	+1
4	+1	+1
5	-α	0
6	+α	0
7	0	-α
8	0	+α
9	0	0
10	0	0
11	0	0

The corresponding response function is a first degree polynomial for each factor taken independently. It is noted:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

As the mathematical model associated with the factorial design is built with centred, reduced variables, the coefficients of the polynomial have very straightforward meanings: mean: b_0, principal effects: b_i, interactions: b_{ij} and b_{ijk} (Goupy, 2004). The experimental data were processed using NemrodW software (lprai@nemrodw.com).

Centred composite design: The model used here was a second degree one with two variables:

- X_1 = Ratio of mass of plant material to solvent volume
- X_2 = Reaction time or duration of extraction

The response is *Y*, the percentage of oil extracted.

The mathematical expression of the response using the reduced variables takes the form:

$$y = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2$$

The number of experiments is given by the expression:

$$N = N_F + N_A + N_0 = 2^2 + (2 \times 2) + 3 = 11$$

Table 4: Variation in extraction yield (%) according to the extraction time

Time (min)	Yield (%)	$(1/t)\ 10^3$
43	11.36	23.3
114	27.60	8.8
147	54.40	6.8
221	55.40	4.5
343	56.96	2.9
427	57.72	2.3

Table 5: Effect of the solvent on the extraction yield

Solvent	Trichloro ethane	chloroform	Petroleum ether	Hexane
Yeild (%)	54.1	50.0	56.5	51.6

where,

N_F : The number of experiments in relation to the number of variables

N_A : The number of experiments in relation to the variation increment

N_0 : The number of experiments at the centre of the matrix

With base points of 45 g/250 mL for X_1 and 80 min for X_2 and increments of 25 g/250 mL and 30 min, we arrive at the levels of variation and the experimental design presented respectively in Tables 2 and 3.

RESULTS AND DISCUSSION

Influence of extraction time on yield: Finely ground powdered safou pulp (50 g) was extracted in a 500 mL Soxhlet apparatus with 250 mL of solvent. Extraction times extended to 2 h 30 min. The results obtained are given in Table 4.

Figure 1, which gives the plot of the variation in yield according to the extraction time, presents the usual profile of a substance being gradually extracted to exhaustion from a plant matrix.

The function $Y = f(1/t)$ takes the form Y (%) = 62.8-2.3 ($1/t$), with coefficient $R^2 = 0.85$. This equation, by extrapolation to $1/t = 0$, gives the maximum yield of extractable oil contained in the sample, i.e., 62.8%.

This relation is also important for technological applications, because it provides the extraction rate, which is the ratio of the real yield to the maximum theoretical yield obtained by extrapolation of the $Y = f(1/t)$ plot. Also, this result shows that almost 90% of the oil contained in the pulp is extracted after an extraction time of 2 h 30 min longer extraction times are therefore not worthwhile.

Effect of the solvent on the extraction yield: Four organic solvents were used in this work. Trichloro ethane and petroleum ether gave yields of respectively 54.1 and 56.5% (Table 5). These were the highest extraction yields. Chloroform had the weakest extractive power, with a yield of 50.0%. Hexane gave yields between these two extremes (51.6%). All the yields were greater than 50%.

Given that chlorinated solvents are toxic and petroleum ether is a mixture that is difficult to eliminate from extracts and is also extremely volatile, hexane was selected as a solvent to model the soxhlet extraction of safou pulp oil.

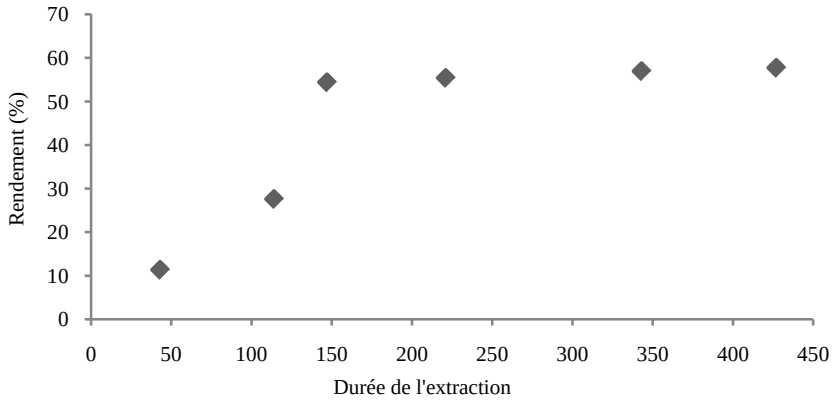

Fig. 1: Variation in extraction yield according to the extraction time (Yeild = f (extraction time))

Table 6: Influence of the extraction solvent on: physical and chemical characteristics, FA and TAG composition

	Trichloro ethane	Petroleum ether	Chloroform	Hexane
Specific gravity (T °C)	0.923 (23.4)	0.924 (25.8)	0.924 (25.8)	0.914 (24.4)
Viscosity (T °C)	72.90 (40)	61.03 (40)	70.97 (40)	34.87 (40)
Fatty Acids (FA)				
C16: 0	50.11	48.91	49.90	50.25
C18: 0	4.31	4.27	4.21	2.61
C18: 1 n-9	30.24	30.15	29.95	32.13
C18: 2 n-6	13.14	14.59	13.58	13.74
Triacylglycerols (TAG)				
PLL	3.29	4.24	4.08	4.50
POL	9.01	10.59	10.43	-
PPL	19.37	21.22	20.36	15.00
POO	20.60	19.92	20.86	14.80
PPO	47.73	44.28	44.28	58.30

Influence of the extraction solvent on: physical and chemical characteristics, FA and TAG composition of the oils obtained: The oils obtained were semi-solid at ambient temperature and presented very similar physical and chemical characteristics. Their density varied only slightly according to the extraction solvent used. Hexane gave oil that was half as viscous as that obtained with the other solvents. It probably extracted less wax and other heavy fractions than the other solvents (Table 6).

All the oils were shades of green in colour. The chlorophyll pigments, which predominated in the green oils and reddish-yellow coloured carotenoids absorb in the visible range between 400 and 700 nm. The absorption in this range is a measure of the intensity of the colour of the oil or fat studied. This intensity is in principle proportional to the quantity of pigments present in the oil when the Beer-Lambert law is obeyed.

In studies of oils extracted from citrus and gourd seeds, Helmy (1990) identified absorption maxima at 400, 425, 455 and 480 nm for carotenoids, 610 and 670 nm for chlorophylls and 525, 570 and 595 nm for an unknown pigment, respectively.

Whichever the extraction solvent used, the visible spectrum of the safou pulp oil obtained presented a small peak at about 500 nm corresponding to the absorption of carotenoids, which thus occur in small quantities and a double peak between 630 and 670 nm corresponding to the absorption of chlorophyll pigments, responsible for its greenish colour (Fig. 2).

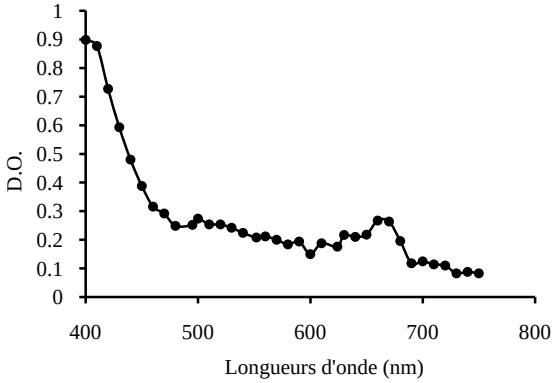

Fig. 2: Absorption spectrum in the visible range of safou pulp oil (Absorbance = f (wave number (nm)))

The oils extracted with the different solvents also presented similar FA and TAG compositions (Table 6).

These oils contained three major fatty acids representing more than 90% of the total FAs. These were: palmitic (P or C16:0), oleic (O or C18:1 *n*-9) and linoleic acids (L or C18:2 *n*-6). Then came stearic acid (S or C18:0) at about 2-3% and then minor

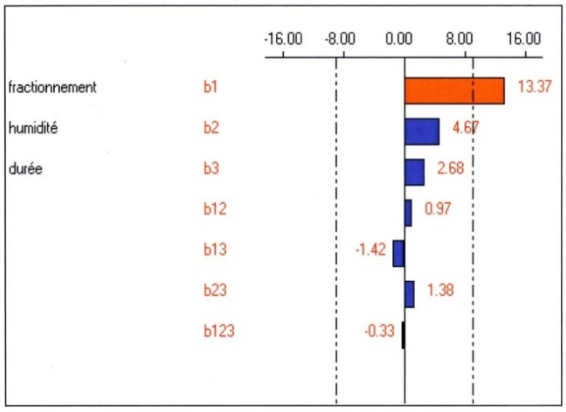

Fig. 3: Graphical illustration of the effects and interactions of variables during soxhlet extraction of safou pulp oil b_1: Particle size effect; b_2: Moisture effect; b_3: Extraction time effect

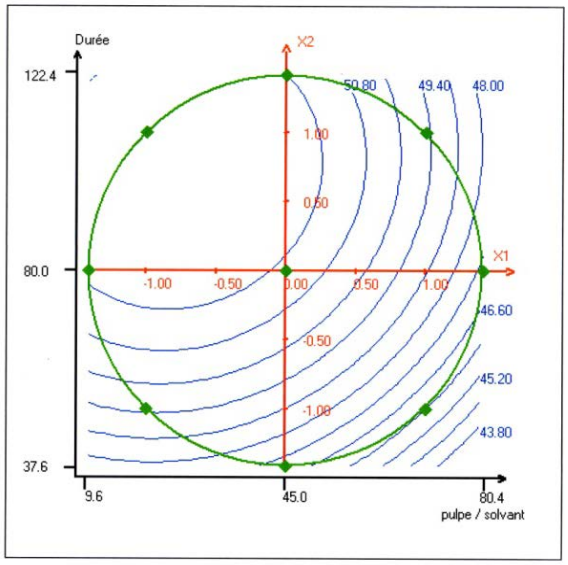

Fig. 4: Response diagram of safou pulp oil extraction Extraction time = f (ration of pulp mass to solvent volume)

components, linolenic acid (Ln or C18:3 *n*-3), arachidic acid (or C20:0) andeicosaenoic acid (or C20:1 *n*-9) at levels less than 1%.

For the major FAs we find here the characteristic profile of safou pulp oil as reported in the literature (Silou, 1996; Mbofung *et al*., 2002; Ondo, 2004; Ondo-Azi *et al*., 2009), i.e.:

%P>%O>%L

We note the presence of four major TAGs in the oils extracted from pulps with a cumulated content of more than 95% of the total TAGs and the following profile:

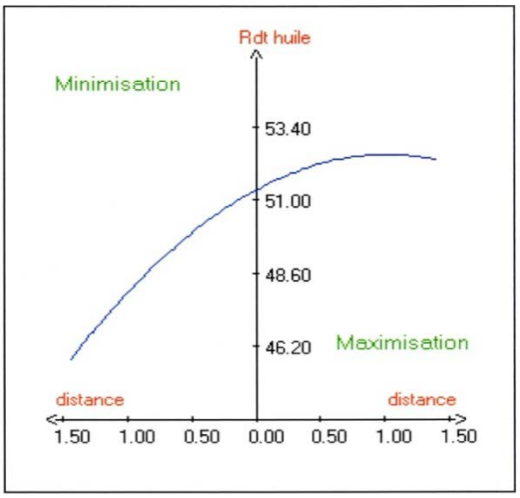

Fig. 5: Optimum yield (%) of safou pulp oil extraction

%PPO>%POO>%PPL>%POL

This work thus enabled us to collect important data for subsequent modelling of the process of safou oil extraction with the Soxhlet apparatus, justifying the use of hexane as solvent and the extraction time, generally no longer than 3 h in the laboratory. We also established that the physical and chemical quality of the oil did not vary significantly with the nature of the extraction solvent. Overall, this quality was compliant with standards applicable to edible oils and was consistent with the results available in the literature.

Optimization of safou pulp oil extraction by the DOE method:

2^3 factorial design: The mathematical model representing the yield of oil extraction (y) from safou pulp as a function of reduced centred variables (x_1, x_2, x_3) in a 2^3 factorial design is:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

The effects (x_i) and interactions (x_{ij}, x_{ijk}) determined using the NemrodW software, shown in Fig. 3, gave the following coefficients:

$$y = 28.9 + 13.7x_1 + 4.67x_2 + 2.68x_3 + 0.97x_1x_2 - 1.42\,x_1x_3 + 1.38x_2x_3 - 0.33x_1x_2x_3$$

The fractioning of the pulp was by far the factor most strongly influencing the extraction. It was followed, with a division factor of about 3, by the moisture content of the pulp feedstock. The extraction time came third.

Centred composite design: We repeated the optimization, keeping constant the most influential variables (fine grinding and 8% pulp moisture) using a

centred composite design, with the extraction time (X_1) and ratio of pulp mass to solvent mass (X_2) as variables.

The results of automatic data processing with the NemrodW software are given in Fig. 4 and 5.

These two figures show that the maximum yield, about 52% for this sample, corresponds to a pulp mass /solvent volume ratio of 45 g/ 250 mL and an extraction time of 122.4 min.

CONCLUSION

These two treatments show that the optimal conditions for oil extraction from this sample by hexane in a soxhlet apparatus (Y_{max} = 52%) were: fractionation, fine grinding, 8% residual moisture in the pulp, a ratio of pulp mass to solvent volume of 45 g/ 250 mL and an extraction time of 122.4 min.

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