



RESEARCH ARTICLE

PHYSICOCHEMICAL CHARACTERIZATION AND TECHNOLOGICAL VALORIZATION
OF PYTHON FAT (*PHYTHONSEBAE*) OF CONGO BRAZZAVILLE

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ABSTRACT

In order to enhance the *Python sebae* fat used as a traditional remedy to treat the boils, hemorrhoids, joint pain, etc., in the Congolese traditional medicine, we realized a physicochemical characterization of this fat. Studied fats come from three locations: Dolisie, Kindamba and Brazzaville. The indices of acid vary between 2,38 and 127,6%, the rate of oxidation of our samples is between 0,28 and 5,75 meq. O₂/Kg; the detergent and foaming power varies between 157.99 and 178.73; the iodine value shows a high level of creation of the order of 127.12. The fatty acids profile reveals 39.19% SFA, 54.15% MUFA and 6,65% PUFA. The main components being (49.47%) oleic acid, Palmitic acid (28,51%), stearic acid (9.38%) and linoleic acid (5.85%). The viscosity of our samples varies between 65.88 and 949.7 mPa.s and a melting point between 38-40 °C. An ointment from the grease and a few ingredients was created, whose virtues have potential: antimicrobial, analgesic and anti-inflammatory.

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INTRODUCTION

In recent years, food fats suffer trends in today's societies (cult of thinness, diets...) and thus their tarnished image. Indeed, the fat consumption is increasingly the object of an alarming situation; many are those who deplore his contribution because it often leads to obesity. However, the use of fat goes back several centuries. They do ensure that our meals are tasty, deliver energy, vitamins and essential fatty acids for the body. They may be of animal or plant origin; the most widely used are those of plant origin for their food and industrial use. Outside this use, some fats are filled with many curative virtues. Natural oils are in general traditional remedies for many ailments.

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Therefore, many authors have studied certain oils of plant and animal origin for their pharmacological application and revealed antimicrobial (Nissen et al., 2010; Dias et al. 2013), antiinflation (Caldefie-Chézet et al., 2006; Ferreira et al., 2010; Cardoso et al., 2011), antioxidant (Sepahvand et al., 2014), and anticancer (Nikolakopoulou et al., 2013) properties. These properties are often due to the fatty acids that make up these oils or fats, especially polyunsaturated fatty acids (Nikolakopoulou et al., 2013; Black and Rhodes, 2006; Lim, et al., 2009; Mandal, et al., 2012; Guesnet et al., 2005). Snake oils are also often used in traditional medicine such as cobra fat oil to treat skin necrosis (Khunsap et al., 2016), the famous oil of snake *Enhydris chinensis* (Chinese water snake) which has anti-inflammatory effects and a therapeutic potential (Kunin, 1989); the oil extracted from boa constrictor fat has anti-inflammatory and antimicrobial properties (Falodun et al., 2008). In Congo, python fat is traditionally used to treat rheumatism, boils, hemorrhoids. It therefore seemed important to us to carry out a study on the physicochemical characterization of this fat to try to understand which are its

constituents which are responsible for these virtues and try to valorize it by a technological application: the production of a ointment made from this fat.

Ointments

Ointments are soft-consistency preparations obtained by mixing drug substances with a suitable excipient, and intended to be applied to the skin for the purpose of administering drugs dermally (Dembélé, 2011). They do not contain water, liquefy on contact with a heat source and consist only of lipids, waxes, fats mixed with finely ground resin powders. Ointments have excipients derived most often from natural plant substances, solid or crushed, pasty or semi liquid and liquid (oils, balms, resin, gums, mixed with finely ground resinous powders ...) or animal (honey, wax, animal fat ...) (Wouessi, 2011). Originally, we mainly used fatty substances of animal or mineral origin (axonge, lanolin, petrolatum), currently we can use many other basic principles (Arditty, 2007).

The python

In the Congo, there are two species of pythons: the Royal Python (*Python regius*) and the Python Seba (*Python sebae*) which is the subject of our study (Fig 1).



Fig. 1. Python sebae

This snake, which belongs to the family Pythonidae is both diurnal and nocturnal, it is not only the most ubiquitous (we meet everywhere) of the Congo, but also by far the heaviest and largest snake in Africa (Figure 2). He is easily differentiated from others by the shape of his head and the color of his body. Two other characters distinguish him at first glance. The very large number of dorsal scale ranks around the middle of the body (ranging from 71 to 95, while the other serpents all have less than 45), and the thermo-sensory dimples on the anterior supra-labial scales (Pauwels and VandeWeghe, 2008).

MATERIAL AND METHODS

Material

Animal material

The animal material used is a fat that is extracted from the *Python sebae* from the following localities: Brazzaville (4 ° 16 '4 "S / 15 ° 16' 31" E), Dolisie (4 ° 11 '54 "S / 12 ° 39' 60" E)

and Kindamba (3 ° 44 '37 "S / 14 ° 31' 34" E). The fat samples that were the subject of our study were purchased in the markets of the aforementioned cities and stored at -20°C.

Ingredient

For the manufacture of our ointment, we used the following ingredients or products:

The python fat that we have characterized.

The camphor of scientific name *Cinnamomum Camphora* which is extracted from the camphor tree. Its chemical formula is $C_{10}H_{16}O$. It is particularly famous for its medicinal properties. Indeed, camphor is a stimulant of the adrenal cortex, releases attacks of asthma and emphysema, and it fights infectious diseases (microbial, viral). It is an antalgic and a powerful antiseptic that heals blows and bruises.

It is also a stimulant of the central nervous system and an anti-inflammatory par excellence;

- Menthol of chemical formula: $C_{10}H_{20}O$ is a covalent organic compound obtained either by synthesis or by extraction from the essential oil of mint. It belongs to the monoterpenol family. Menthol has anti-inflammatory and antiviral properties. It is also used to relieve minor irritation of the throat. It is also a local anesthetic;
- Vaseline is a petroleum jelly, known for decades for its many uses. Its benefits to the skin are well known all over the world. It therefore has a moisturizing and insulating role at the same time.

METHODS

Lipid extraction

The lipids were extracted simply by heating the small pieces of fat from fat bags of fat extracted from the belly of python (Fig 2a and 2b) between 37 and 50 °C until complete dissolution of these fats. Lipids were kept at -20°C until use (Khunsap S. *et al.* 2016)



Fig.2a. Sacs of fat of Python sebae



Fig. 2b. Fat of Python sebae

Methods of analysis

After lipid extraction, we proceeded to the characterization of these lipids, which generally refers to the determination of chemical indices, fatty acid profile, rheological properties and differential thermal analysis.

Physico-chemical analyzes

Determination of chemical indices

The different indices measured in this study were determined according to the following official AOAC standards: Acid Index (AOAC, 969.17, 1997); Iodine Number (AOAC, Standard 993.20, 1997); Peroxide Index (AOAC, Standard 965.33, 1997); Saponification index (AOAC, standard 920.160.1997) (AOCS, 1997; Kyari M.Z., 2008).

Viscosity

The viscosity is measured using the rheometer, Stress Techrometer, REOLOGICA Instruments AB, Sweden calibrated according to the manufacturer's data. 7 μ l of sample are used by varying the stress (from 7 to 20 Pa) as a function of time. The kinematic viscosity η is expressed in mPa.S.

Determination of the fatty acid profile

KOH in solution in 2N methanol is used as a transmethylation reagent to prepare the fatty acid methyl esters of total lipids [Standard ID 182: 1999]. The methyl esters thus formed are analyzed by gas chromatography on a Per 2000 chromatograph (Périchrom, Saulx-le-Chartreux) equipped with a flame ionization detector. This device is equipped with a polyethylene glycol capillary column doped with terephthalic acid (Périchrom), 25 m long, 0.25 mm diameter and 0.5 m thick film. The temperature of the injector and detector is set at 260 °C. The column is raised from 70 to 180 °C (39.9 °C / min), and is maintained at this temperature for 8 minutes. It then undergoes a second heating up to 220 °C (3 °C / min). The carrier gas is nitrogen (1.1 bar). The software Winilab (Périchrom, Saulx-le-Chartreux) allows the integration of chromatograms. The identification of the peaks is done by two standards of fatty acids provided by Supelco (Belle fonte, United States): PUFA1 (marine source) and PUFA2 (animal source).

Differential thermal analysis

Differential Thermal Analysis (DTA) consists in measuring a temperature difference between the sample studied and a reference sample, which does not show any thermal phenomena in the temperature range studied. The thermal profile thus obtained reflects the changes in the state of the analyzed product and the energy variations associated with these changes.

The thermal profile of the fats is determined according to Tan's method, and Che Man, (1999) (Sathivel S., 2005). Weigh 6 to 12 mg of Python sebae fat sample into hermetically sealed aluminum capsules, another empty and hermetically sealed aluminum capsule was used as a reference. The thermal program is as follows: in the chamber at 20 °C., the cell block of the DSC was rapidly heated to 80 °C. at a rate of 40 °C/min and held for 5 minutes at this same temperature to destroy the crystal nuclei. The block is then rapidly cooled from 80 to -30 °C at a rate of 5 °C / min, and held for 5 minutes at this same temperature to allow for polymorphic changes. Finally, the block is new cooled from -30 to -85 °C at a rate of 1 °C / min to set the cooling profile.

Rheological properties

The viscosity is measured using the rheometer, (Stress ® rheometer, REOLOGICA Instruments AB, Sweden). We measured the viscosity by stress (from 1 to 78 Pa) as a function of time and at different temperatures (between 25 °C and 40 °C). Then, the viscosity of the liquid at the plateau of the curve was read.

Making an ointment

For the manufacture of our ointment, we used the Plackett-Burman Experience Plan (Plckett and Burman, 1946; Goupy, 2001). We selected four factors with the following maximum and minimum values: python fat (40.50); Vaseline (42.50); camphor (0.16); menthol (0,2). The results are recorded in the table 1.

Table 1. Experimental design generated according to the Plackett-Burman matrix

OrdEssai	Vaseline	Graisse de python	Campbre	Menthol
1	50	40	16	2
2	42	50	16	2
3	42	40	0	0
4	42	40	16	2
5	42	50	16	0
6	50	40	0	2
7	42	50	0	2
8	50	50	16	0
9	46	45	8	1
10	50	40	16	2
11	50	50	0	0
12	42	40	16	0
13	42	40	0	2
14	50	40	16	0
15	42	50	16	0
16	50	50	0	2
17	42	50	0	2
18	50	50	16	2
19	42	40	0	0
20	50	40	0	0
21	46	45	8	1
22	46	45	8	1
23	50	50	0	0

Table 3. Fatty Acid Profile of Python Sebae Fat

Fatty Acid	Symbol	FPS (%)
<i>SFA</i>		
Myristic acid	C14:0	1.30±0.01
Palmitic acid	C16:0	28.51±0.28
Stearic acid	C18:0	9.38±0.09
<i>MUFA</i>		
Palmitoléic acid	C16:1n7	3.80±0.06
Oléic acid	C18:1n9	49.47±0.31
Vaccénic acid	C18:1n7	0.15±0.04
Gadoléic acid	C20:1n9	0.72±0.05
<i>PUFA</i>		
Linoléic acid	C18:2n6	5.85±0.03
γLinoléic acid	C18:3n6	0.19±0.04
α Linoléic acid	SC18:3n3	0.36±0.06
Arachidonic acid	C20:4n6	0.25±0.02
ΣSFA		39.19
ΣMUFA		54.15
ΣPUFA		6.65
ΣUFA		60.80
ΣPUFA/ΣMUFA		0.12
ΣUFA/ΣSFA		1.55
ΣMUFA/ΣSFA		1.38
ΣPUFA/ΣSFA		0.17
ΣMUFA/ΣPUFA		8.14
Σn-6/Σn-3		16.77
ΣFA		100

FPS : Fat of python *sebae*; FA: Fatty Acid; SFA: Saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids. Les teneurs en ΣAGS, ΣAGMI, ΣAGI et ΣAGPI ont été calculées en considérant tous les acides gras détectés pour chaque classe d'acides gras en fonction du degré d'insaturation. Les données rapportées sont la moyenne ± l'écart-type de trois mesures.

The major unsaturated fatty acid present in *Python sebae* fat is oleic acid. It reduces total cholesterol and low density lipoprotein (LDL) cholesterol without affecting high density lipoprotein (HDL). This leads to a reduction in the risk of cardiovascular disease, since HDL rids LDL-cholesterol blood, known as "bad cholesterol," (Dommels *et al.*, 2002; Mensink RP and Katan MB; 1992; Guesnet P. *et al.*, 2005). We note essentially the presence of saturated fatty acids: myristic acid (C14: 0), palmitic (C16: 0), stearic (C18: 0).

Table 4. Comparison of fatty acid profiles of the fat of a few reptiles

Fattyacids	Symbol	<i>Python sebae</i> (%)	<i>Python regius</i> ²⁵ (%)	<i>Naja kaouthia</i> ¹¹ (%)	<i>Phrynops geoffroanus</i> ²⁴ (%)
Myristic ac.	C14:0	1.30±0.01	-	0.71	-
Pentadecylicac.	C15 :0	-	-	0.18	3.68
Palmitic ac.	C16:0	28.51±0.28	16.05±3.22	17.47	6.82
Stéaric ac.	C18:0	9.38±0.09	10.10±2.87	4.81	-
Arachidicac.	C20:0	-	2.25±0.65	0.25	-
Tétradécénoïcac.	C14:1n-9	-	0.5±0.02	-	-
Palmitoléicac.	C16:1n7	3.80±0.06	4.85±1.45	4.01	58.39
Oléicac.	C18:1n9	49.47±0.31	41.27±5.22	3.84	15.70
Vaccénic ac.	C18:1n7	0.5±0.04	0.15±0.04	22.98	-
Gadoléic ac.	C20:1n9	0.72±0.05	11.87±3.67	-	-
Linoléicac.	C18:2n6	5.85±0.03	7.60±1.95	16.80	4.50
γLinoléicac.	C18:3n6	0.19±0.04	3.00±0.95	-	2.28
α Linoléic ac.	C18:3n3	0.36±0.06	0.36±0.06	-	-
Arachidonic ac.	C20:4n6	0.25±0.02	0.25±0.02	-	-
Erucic ac.	C22 :1n9	-	3.06±1.00	-	3.76
Docosahexaénoïcac. (DHA)	C22 :6n3	-	-	4.45	-

This fat could be a nutritional oil because the ratio of unsaturated fatty acids to saturated fatty acids is greater than 1. These fatty acids are separated on the chromatogram at irregular intervals. In addition, the same number of fatty acids is present in *Python sebae* fats from different localities. These different elements allowed us to extrapolate to deduce some fatty acids present in *Python sebae* fats.

On the other hand, as far as *Python sebae* fat is concerned, no study to our knowledge has been done on its chemical composition, hence the lack of a standard for it. However, we can see that it is a fat that could be very nutritious given its levels of essential fatty acids (oleic acid and linoleic). We have made a table comparing the fatty acid profiles of certain reptilian fats (Table 4). All these fats have fatty acid profiles rich in unsaturated fatty acids (AGI), and they have any ethnopharmacological activity (Khunsap *et al.*, 2016; Dias *et al.*, 2013; Okere *et al.*, 2014), the fatty acid profile of the python fat sebae is closer to that of the fat of python regius. We can therefore say that the fat of the *Python sebae* certainly presents these ethnopharmacological activities, which are recognized in traditional medicine.

Rheological properties

The rheological properties make it possible to study the flow and the deformation of the fats. At 30 ° C, the viscosity of these fats varies between 65.88 and 949.7 mPa.s characterizing very viscous *Python sebae* fats. The high viscosity of *Python sebae* fats indicates that these fats could be used in the manufacture of lubricating greases and fuels (Adebanjo *et al.*, 2005).

Differential thermal analysis

Fig 4 shows the polymorphic changes of the analyzed fats. These changes are observed following a change in temperature. The differential thermal analysis of the samples studied reveals the existence of two peaks. We present in Figure 4 a copy of the thermal profile of our greases. The first peak has a melting point of 0.8 ° C ($\Delta H_f = 17.47 \text{ J / g}$) for the Dolisie sample and 3.08 ° C ($\Delta H_f = 26.86 \text{ J / g}$) for the sample of Brazzaville. The last most important peak is at 40.1 ° C ($\Delta H_f = 28.09 \text{ J / g}$) for the Dolisie sample and 38.8 ° C ($\Delta H_f = 33.74 \text{ J / g}$) for the sample of Brazzaville. This peak represents those of high melting compounds. These peaks correspond to a fusion process because ($\Delta H > 0$).

This explains why this fat is solid at room temperature. The results obtained indicate the interval in which our fats can be heated without affecting their quality. Above 40 °C, there is a structural modification of our greases. Differential thermal analysis is one of the simple ways to study the characteristics of fats. Among other things, the influence of fat composition, aging and heat treatment on fat quality can be explained by the

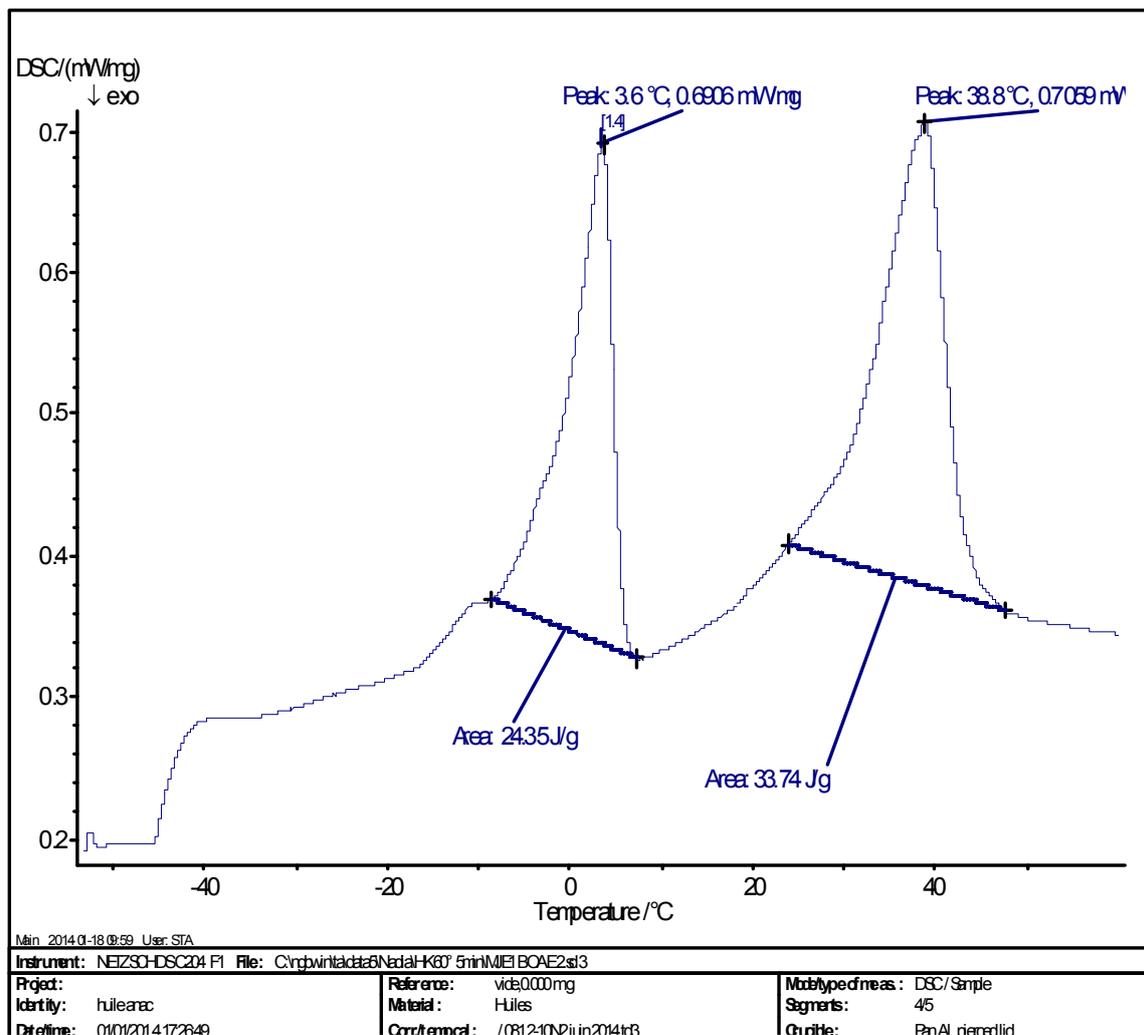


Fig. 4. Thermal Profile of Python Sebae fat

DSC (Tan et Che Man, 2002). DSC provides an approach to physical properties such as melting point, enthalpy, and specific heat capacity of fats (Sathivel, 2005; Sathivel *et al.*, 2008).

Ointment manufacturing

Among the 23 trials, we retained only those whose composition is 100% and therefore the following three compositions or formulations:

Ointment 1: 46 g of petrolatum, 45 g of python fat, 8 g of camphor and 1 g of menthol

Ointment 2: 42 g petrolatum, 40 g python fat, 16 g camphor and 2 g menthol

Ointment 3: 50 g of Vaseline, 50 g of python fat, 0 g of camphor and 0 g of menthol.

The ointments that we manufactured are represented on figure 5

Conclusion

It is clear that the characterization of *Python Sebae* fat in this work has been complete. The results we have just acquired, allow us to know, in the context of the valuation of our products (ointments), the quality of the different *Python Sebae* fats widely encountered in Congo. The first results found show how the environment and geographical conditions could influence the chemical composition of *Python Sebae*'s fats. We note also that *Python Sebae* grease from Kindamba has an acidity and a higher degree of unsaturation than those of Dolisie and Brazzaville, which indicates that this fat is very sensitive to oxygen and therefore very fragile. to rancidity. From the oxidation point of view, all fat samples analyzed are within acceptable limits, in accordance with Codex Alimentarius standards. The saponification index of our fats indicates a high detergency and foaming power. The fatty acid profile shows that our fats contain a lot of unsaturated fatty



Fig.4. Different ointments manufactured

acids. Unsaturated fatty acids, in particular n-3, are precursors of prostaglandins and leukotrienes and have, among other things, a muscular, antimicrobial and anti-inflammatory potential, which confers therapeutic advantages (Guesnet P. *et al.*, 2005; Khunsapet *al.*, 2016). But these unsaturated fatty acids are also known for their hypocholesterolemic and antiatherogenic function.

In addition, the thermal and rheological analyzes indicate the range of melting temperature in which our greases can be heated without affecting the quality of the latter. Seen the ingredients added to our ointment, we can certify the therapeutic virtues (analgesics and analgesics) of the latter. It is important to note the importance of conducting in-depth studies of Python Sebae fats from various localities in our country, to see if the geographical conditions, environment and diet of these latters influence the composition of our fats. It would also be interesting to go deeper into a study on the therapeutic effects of these, in order to know the real and direct effect of the application of our ointments on human health. In view of the nutritional quality of our fat, studies may be conducted for culinary use of our fat.

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