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Characterisation and Nutritional Potentials of *Rhynchophorus phoenicis* Larva Consumed in Congo-Brazzaville

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Abstract: In this study, the proximate composition and physicochemical analyses were carried out on the larva and extracted oil of *Rhynchophorus phoenicis*. The results showed that the larva contained 0% moisture, 24% crude protein, 6.3% ash, 68.5% fat and 1.2% carbohydrate. The larvas were found to be good sources of minerals. Sodium (832.59±0.5 mg/100g) was the highest, followed in descending order by magnesium (132.7±0.20 mg/100g), calcium (72.4±0.72mg/100g) and potassium (22.89±1.7 mg/100g). The physical properties of the oil extracts showed the state to be semi-solid at room temperature. The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 41.20%) and linoleic (up to 12.50%). The dominant saturated acid was palmitic (up to 31.10%). The oil extracts exhibited good physicochemical properties and could be useful as edible oils and for industrial applications.

Key words: DSC, Essential fatty acid, oil yield, physicochemical properties, proximate composition, *Rhynchophorus phoenicis*

INTRODUCTION

The larva of the beetle *Rhynchophorus phoenicis* popularly know as "Edible worm" is delicacy in many parts of Congo and other countries in Africa where it is found.The larva is known by The name of "Tsombé" by the many ethnic groups in Congo, who strongly believe it to have high nutritive as well as certain pharmaceutical potentials. The mode of preparing for eating differs from one geographical locality to another. In some places, it is boiled (ethnics groups in north of Congo) while others smoke, fry or simply eat it raw (ethnics groups in south of Congo).

The use of the larva of *Rhynchophorus phoenicis* is believed to extend beyond the nutritional value. Traditionally, many claim that the larva has medicinal properties. For example, The Nigerian believe that the live larva could cure a certain aliment in infants which presents such symptoms as the twitching of the hands and feet, restlessness and other such movements. To effect a cure for these conditions, the larvae are left in water which is then used to wash the child for several days at the end of which the larvae are crushed together with alligator pepper and administered orally to the child. The biochemical basis for this treatment is not known (Ekpo and Onigbinde, 2005).

Evaluation of the nutritive value of this larva becomes important as the insect larva could form a base for new food/feed product of considerable nutritive value.

This study was, therefore, conducted to determine selected nutritional and physicochemical properties of the larva and oil extracted from the larvae of *Rhynchophorus phoenicis*.

MATERIALS AND METHODS

This study was led to the Laboratory of Engineering And Biomolecule of the ENSAIA-INPL, Vandoeuvre - lès-Nancy (France) for the period of Jan. 5, to Feb. 27, 2010. The larva of *Rhynchophorus phoenicis* were bought from Total-Bacongo market, Brazzaville. Only larvae that were not damaged were chosen and stored under cool dry storage conditions until needed. Proximate analysis of *Rhynchophorus phoenicis* larva moisture, crude protein (micro-Kjeldahl) and oil (Soxhlet) contents were determined using the methods described by Person (1976), whereas the ash content was determined using the method of Pomeranz and Meloan (1994), and total

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carbohydrate was determined by difference. All determinations were done in triplicate.

Oil extraction: Dried *Rhynchophorus phoenicis* larvae were ground in a Moulinex Model SeB PREP'LINE 850 (Moulin cafe). For solvent extraction (soxlhet method), 50 g of ground larva were placed into a cellulose paper cone and extracted using light petroleum ether (b.p 40-60ºC) in a 5-l Soxhlet extractor for 8 h (Pena *et al*., 1992). The oil was then recovered by evaporating of the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60ºC for 1 h and flushing with 99.9% nitrogen. For methanol/chloroform extraction of Bligh and Dyer (1959), 100 g of the ground larvae were homogenised with a chloroform mixture methanol (1:1) and water. Two phases was obtained, aqueous layer (methanol-water) and organic layer (chloroform). Oil was recovered by evaporating of the solvent (chloroform) using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60ºC for 1 h and flushing with 99.9% nitrogen All experiments were done in triplicates and the mean and standard deviations were calculated.

Physical and chemical analysis of crude oil:

Thermal behaviour: The thermal property of the oil samples was investigated by differential scanning calorimetry using a Perkin-Elmer Diamond DSC (Norwalk, USA). The instrument was calibrated using indium and zinc. The purge gas used was 99.99% nitrogen with a flow rate of 100 ml.min^{-1} and a pressure of 20 psi. Sample weights ranged from 5-7 mg and were subjected to the following temperature program: Frozen oil sample was heated at 50ºC in an oven until completely melted. Oil sample was placed in an aluminium volatile pan and was cooled to -50ºC and held for 2 min, it was then heated from - 50 to 50ºC at the rate of 5ºC.min^{-1} (normal rate) (Che Man and Swe, 1995), and held -50ºC isothermally for 2 min and cooled from -50 to 50ºC at the rate of 5ºC min^{-1}. The heating and cooling thermograms for the normal and the fast (hyper DSC) scan rates were recorded and the onset, peak, and offset temperatures were tabulated. These values provide information on the temperature at which the melting process starts, the temperature at which most of the TAG have melted, and the complete melting temperature of the oil, respectively.

Viscosity measurements: A rheometer as described by Nzikou *et al*. (2007) was used to measure the different oil viscosities. By this procedure, a concentric cylinder system is submerged in the oil and the force necessary to overcome the resistance of the viscosity to the rotation is measured. The viscosity value, in mPa.s, is automatically calculated on the basis of the speed and the geometry of the probe. Temperature (20ºC) was controlled with a water bath connected to the rheometer. The experiment was carried out by putting 3 ml of sample in a concentric cylinder system using 100 s^{-1} as shear rate.

Chemical analysis: Determinations for peroxide, iodine, and saponification values, unsaponifiable matter and free fatty acid contents were carried out using Pena *et al*. (1992) standard analytical methods. The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 µl of n-hexane 50 mg of oil followed by 50 µl of sodium methoxide using the method of Cocks *et al*. (1966). The mixtures were vortex for 5 sec and allowed to settle for 5 min. The top layer (1 µl) was injected into a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionisation detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 µm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240ºC and column temperature was 110ºC held for one minute and increased at the rate of 8ºC.min^{-1} to 220°C and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample. The minerals were determined by atomicabsorption spectrophotometry. One gram samples, in triplicate, were dry ashed in a muffle furnace at 550ºC for 8 h until a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20.0 ml of 2.5% HCl, heated in a steam bath to reduce the volume to about 7.0 ml, and this was transferred quantitatively to a 50 ml volumetric flask. It was diluted to volume (50 ml) with deionised water, stored in clean polyethylene bottles and mineral contents determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). These bottles and flasks were rinsed in dilute hydrochloric acid (0.10 M HCl) to arrest microbial action, which may affect the concentrations of the anions and cations in the samples. The instrument was calibrated with standard solutions.

Statistical analysis: Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Excel Version 8.0 software. Significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Proximate analysis of *Rhynchophorus phoenicis* larva oil: Results obtained showed that the seeds contained 0%

moisture, 68.5% crude oil, 24% crude proteins, 1.2% carbohydrate (by difference) and 6.3% ash (Table 1). The high percentage of oil makes this seed a distinct potential for the oil industry. The oil output is higher than that obtained by Ekpo and Onigbinde (2005). Variation in oil yield may be due to the differences in geographical locality, climate, ripening stage, the harvesting time of the larvae and the extraction method used.

Minerals: The *Rhynchophorus phoenicis* larvae contained significant amount of important minerals (Table 2). The Sodium concentration (832.59±0.5 mg/100g dry mater) was the highest, followed in descending order by Magnesium (132.7±0.20 mg/100g dry mater), calcium (72.4±0.72 mg/100g dry mater) and potassium (22.89±1.7 mg/100g dry mater). Potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins (Malik and Srivastava, 1982). Calcium and magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Russel, 1973). Calcium assists in teeth development (Brody, 1994). Magnesium is essential mineral for enzyme activity, like calcium and chloride; magnesium also plays a role in regulating the acid-alkaline balance in the body (Fallon and Enig, 1999).

Oil extraction: Characteristics of the oil were compared with *Rhynchophorus phoenicis* species described by Ekpo and Onigbinde (2005). The extracted oils were semi-solid at room temperature. The oil content of *Rhynchophorus phoenicis* "Congo-Brazzaville" larvae for the two methods utilised and the level at which the differences are significant are shown in Table 3. The oil extraction with the Soxlhet method had the highest yield, due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley and Colwell, 1991). The Bligh and Dyer (1959) method, showed the low yield due to losses during the separation of the two phases, aqueous layer (methanol-water) and organic layer (chloroform). The results of the above authors agree with those of the present work.

Physical and chemical properties of oil:

Physical properties:

Differential Scanning Calorimetry (DSC): DSC is suitable to determine these physical properties. The results of thermal analysis of oils are presented in Table 4. The obtained peaks were asymmetries and may indicate the presence of three components in oil extracted from the two methods. The first peaks at low melting points appear at -20.43ºC (H_f = +2.09 J.g^{-1}) for Bligh and Dyer (1959) method and -21.37°C (H_f = +1.55 J.g^{-1}) for Soxlhet method. These peaks correspond to triglycerides formed by poly unsaturated acids (PUFA). The second peaks appear to +1.10ºC (H_f = +0.36 J.g^{-1}) for Bligh and Dyer method and +1.06ºC (H_f = +1.24 J.g^{-1}) for Soxlhet method. This is a characteristic of mono unsaturated acids 'MUFA). The last peaks appear to +13.53ºC (H_f = +0.34 J.g^{-1}) for Bligh and Dyer method and +14.02ºC (H_f = +1.12 J.g^{-1}) for Soxlhet method, suggest the presence of mixed triglycerides groups with different melting points.

Table 1: Proximate analysis (g/100 g dry weight) of *Rhynchophorus phoenicis* larva

Characteristic	Obtained values[a] (M±SD)	Reported values[b] 1
Moisture content (%)	-	-
Crude protein[c](%)	24.0±0.18	22.06
Ether extract (%)	68.5±0.42	66.61
Ash content (%)	6.3±0.12	5.79
Total carbohydrate[d] (%)	1.2	5.53

[a]: M ± S.D. mean ± standard deviation.
[b]: (1) Ekpo and Onigbinde (2005).
[c]: Crude protein = N (%) x 6.25
[d]: Non-fiber carbohydrate was estimated by difference of mean values i.e 100-(sum of percentages of moisture, ash, protein and lipid)

Table 2: Mineral elemental composition of *Rhynchophorus phoenicis* larva

Mineral elements	Composition (mg/100g) of Seed
Calcium, Ca	072.4±0.72
Magnesium, Mg	132.7±0.20
Potassium, K	22.89±1.7 0
Sodium, Na	832.59±0.50

Values are mean±S.D of triplicate determinations

Table 3: Physical and chemical properties of *Rhynchophorus phoenicis* larva

Properties	Obtained values: Blye and Dyer	Obtained values: Soxlhet
Oil[a] (%)	66.80±0.27^{B}	70.2±1.18^{A}
PV	0.04±0.12^{A}	0.05±0.35^{A}
FFA (as % oleic acid)	3.8±0.12^{A}	4.2±0.24^{A}
IV (wijs)	126.4±0.15^{A}	127.2±1.22^{A}
Saponification value	201.2±1.42^{A}	206.5±0.31^{A}
Unsaponifiable matter Content (%)	8.21±0.21^{A}	9.07±0.72^{B}
Viscosity (mPa.s) at 20ºC	26.90	17.00
Ea (KJ. mol^{-1})	28.36	44.59

Means for the determined values in the same row followed by the same superscript letter are not significantly different (p<0.05),
[a]: Oil = weight of extracted oil x 100/weight of seed,
PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value

Table 4: Melting behaviour of *Rhynchophorus phoenicis* larva oil using different scan rates. Experimental conditions: temperature program set at -50°C for 10 min, rising to 50ºC at rate of 5°C.min^{-1}

Thermogram	5ºC.min^{-1}: Blye and Dyer	5ºC.min^{-1}: Soxlhet
Peak 1 [ºC]	-20.43	-21.37
ΔH_f [J.g^{-1}]	+2.09	+1.55
Peak 2 [ºC]	+1.10	+1.06
ΔH_f [J.g^{-1}]	+0.36	+1.24
Peak 3 [ºC]	+13.53	+14.02
ΔH_f [J.g^{-1}]	+0.34	+1.12

Viscosity: Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring. Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. In optics to know the rheological proprieties of these oils, we studied the influence of temperature on viscosity. Activation energies

Table 5: Oil viscosity at various temperature in degree celsuis

	η (mPa.s)	
T (ºC)	Bligh and Dyer	Soxlhet
5	47.70	39.40
10	38.90	30.40
15	32.90	22.95
20	26.90	17.00
25	21.30	12.15
30	17.40	08.30
35	14.20	05.80
40	11.70	04.50
45	10.10	03.80
50	09.60	03.10

Table 6: Energie plot derived from the Arrhenius equation

	Ln η (mPa.s)	
1/T (K^{-1})	Blye and Dyer	Soxlhet
0.00359712	3.86493140	3.67376582
0.00353357	3.67630067	3.41444261
0.00347222	3.49347266	3.13331794
0.00341297	3.29212629	2.83321334
0.00335570	3.05870707	2.49732917
0.00330033	2.85647021	2.11625551
0.00324675	2.65324196	1.75785792
0.00319489	2.45958884	1.50407740
0.00314465	2.31253542	1.33500107
0.00309598	2.26176310	1.13140211

of the various classes of fatty acids contained in these oils were given Table 3. When the temperature increases, viscosity decreases exponentially (Fig. 1) some is the extraction method (Arslan *et al*., 2005; Nzikou *et al*., 2007). Viscosity varies between 39.40 and 3.10 mPa.s when temperature decreases of 50 to 5ºC by Soxlhet method, by Bligh and Dyer method, the viscosity of oil decreases of 47.70 to 9.60 mPa.s (Fig. 1, Table 5). The viscosity of the oil obtained by Bligh and Dyer (1959) method was highest, possibly because of the water that was absorbed by the gums (phospholipids) during extraction. This calculator calculates the effect of temperature on reaction rates using the Arrhenius equation.

$$\eta = A*\exp^{(-Ea/R*T)}$$

Where η is the viscosity, A is constant, Ea is the activation energy (in KJ mol^{-1}), R is the universal gas constant and T is the temperature (in degrees Kelvin). R has the value of 8.314x10-3 KJ mol^{-1} K^{-1}. We should use this calculator to investigate the influence of temperature on viscosity. Linear regression analysis was applied to the logarithmic form of Arrhenius equation in order to determine the parameters of the relation (Fig. 2, Table 6). lnη against 1/T, -Ea/RT is the slope from which Ea was evaluated. Activation energies of oils are given in Table 3. The highest value of activation energy is obtained by Soxlhet method (44.59 and 28.36 KJ.mol^{-1}) by Bligh and Dyer (1959). The higher the activation energy, the more stable the fatty acid is.

Chemical properties: The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide

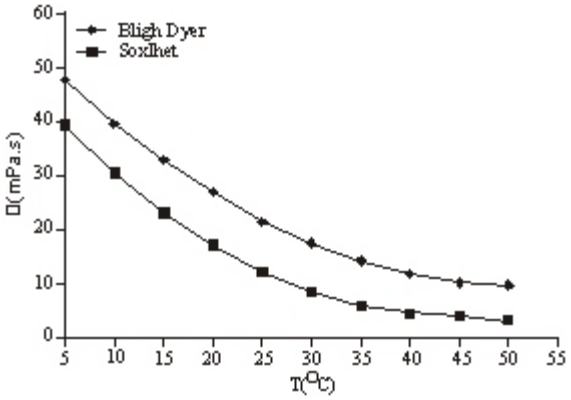

Fig. 1: Effet of *Rhynchophorus phoenicis* temperature on larva oil

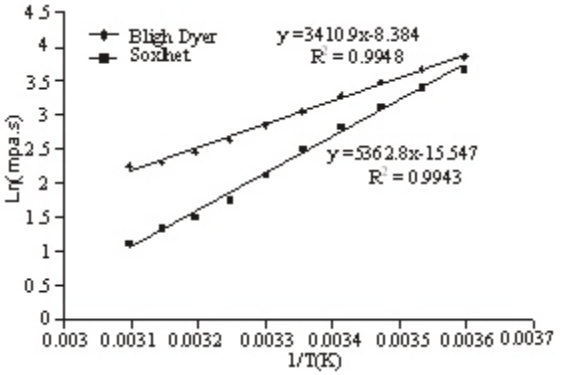

Fig. 2: Relationship between viscosity and temperature *Rhynchophorus phoenicis* larva oil extracted by Bligh and Dyer and Soxlhet. solid line arrhenius model

values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The free fatty acid and the unsaponifiable matter content of the Soxlhet method were significantly higher ($p<0.05$) than those of the Bligh and Dyer (1959) method (Table 3). There was no significant difference in the iodine and saponification values, in the two extraction methods ($p>0.05$). The slightly higher value of unsaponifiable matter in the Soxlhet method may be due to the ability of the solvent to extract other lipid associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic *et al*., 1978; Salunke *et al*., 1992).

Fatty acid composition: The major saturated fatty acids in *Rhynchophorus phoenicis* larva oil were palmitic (30.87%) and stearic (3.75%) acids. The main unsaturated fatty acids are oleic (41.41%) and linoleic (30.60%) acids (Table 7). There was no significant difference ($p>0.05$) in the amounts of the major fatty acids in the two oil samples. The two oil samples of *Rhynchophorus phoenicis* contained saturated and unsaturated acids

Table 7: Relative percent composition of fatty acid in *Rhynchophorus phoenicis* larva oil

	Determined values	
Fatty acid	Bligh and Dyer	Soxlhet
Lauric (C12:0)	0.18±0.74^{A}	0.22±1.24^{A}
Myristic C14 :0	3.82±0.32^{B}	3.69±0.94^{A}
Palmitic (C16 :0)	30.64±1.54^{A}	31.10±0.58^{A}
Palmitoleic (C16 :1)	3.12±0.12^{A}	2.96±0.25^{B}
Stearic (C18 :0)	3.63±0. 81^{A}	3.59±0.58^{B}
Oleic (C18 : 1)	41.20±0.20^{B}	41.09±0.53^{A}
Linoleic (C18 :2)	12.77±0.29^{A}	12.50±0.44^{A}
Linolenic (C18 :3)	3.34±1.74^{A}	3.70±0.12^{A}
Arachidonic (C20:4)	1.3±0.84^{A}	1.15±0.22^{A}
Saturated38.27	38.60	
Unsaturated	61.73	61.40

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p<0.05$)

(38.44 and 61.56%) respectively. The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil.

The level of unsaturation observed in *Rhynchophorus phoenicis* larva oil (Table 7) is higher than what is obtainable in most animal lipids, as well as for palm oil and coconut oil, which are common household oils (Ekpo and Onigbinde, 2005). Insect fatty acids are similar to these of poultry and fish in their degree of unsaturation (DeFoliart, 1991).

Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders (Rahman *et al*., 1995). The presence of the essential fatty acids such as linoleic, linolenic and arachidonic acids in substantial amounts further points to the nutritional value of the larva oil. One implication of the high fat content in the insect larva is that it may increase susceptibility of the undefatted larva to storage deterioration via lipid oxidation (Greene and Cumuze, 1982). This may then be accompanied by increased browning reactions concurrent with reduced lysine availability (Pokorny, 1981). Another implication of the relative proportions of the other nutrients encompassed in the proximate composition.

CONCLUSION

This study showed that the *Rhynchophorus phoenicis* larva is a good source rich in minerals and oil. *Rhynchophorus phoenicis* larva contains mainly the fatty acids oleic C18:1 (41.15%), palmitic C16:0 (30.82) and linoleic C18:2 (12.63%). High unsaponifiable matters content (8.64%) guarantees the use the oils in cosmetics industry. The oil extracts exhibited good physicochemical properties and could be useful for industrial applications.

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