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# FATTY ACIDS, MINERAL COMPOSITION AND PHYSICO-CHEMICAL PARAMETERS OF *IMBRASIA OYEMENSIS* LARVAE OIL WITH UNUSUAL ARACHIDONIC ACID CONTENT

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**Abstract:** *Imbrasia oyemensis larva is a widely prized and consumed caterpillar in Côte d'Ivoire. The oil extracted from this insect was analyzed for physicochemical properties, mineral composition and fatty acid constituents using standard methods. The analysis revealed a refractive index of 1.4675 ± 0.0002, specific gravity of 0.942 ± 0.02, moisture and volatile matter of 7.51 ± 0.1, acid value of 2.81 ± 0.02, iodine value of 105.19 ± 1.62, saponification value of 184.20 ± 1.62, unsaponifiable matter of 45.0 ± 1 and free fatty acid of 1.49 ± 0.37 (% oleic acid). This oil was found to be a very rich source of mineral elements with a 100 g dry sample containing phosphorus (26.82 g), potassium (14.97 g), magnesium (5.63 g), calcium (5.39 g), zinc (0.63 g) and iron (0.31 g). As regards fatty acids profile, the major unsaturated fatty acids of Imbrasia oyemensis oil were linolenic (26.22 ± 0.03 %), linoleic (11.33 ± 0.02 %) and arachidonic (10.26 ± 0.04 %), while the major saturated fatty acids were stearic (20.92 ± 0.03 %) and palmitic (18.65 ± 0.05 %). The unsaturated fatty acids accounted for 55.7 % of the total fatty acids, whereas the saturated fatty acids constituted 44.3 % of the fatty acids. These values when compared with those observed in oils which have been considered to be of high quality, suggest that Imbrasia oyemensis oil has potentials that could be exploited by the nutritional and pharmaceutical companies.*

**Keywords:** *fatty acids profile, Imbrasia oyemensis, mineral composition, oil, physico-chemical parameters.*

## 1. Introduction

Insects are often considered a nuisance to human beings and mere pests for crops and animals. Yet this is far from the truth. They provide food at low environmental cost, contribute positively to livelihoods, and play a fundamental role in nature [1, 2, 3]. However, these benefits are largely unknown to the public. Contrary to popular belief, insects are not merely "famine foods", eaten in times of food scarcity or when purchasing and harvesting "conventional foods" becomes difficult; many people around the world eat insects out of choice, largely because of the palatability of the insects and their established place in local food cultures [4]. People in the world eat insects as regular parts of their diets [2]. They may do so not only because conventional meats such as beef, fish and chicken are unavailable and insects therefore are vital sources of protein, but also because insects are considered important food items, often delicacies [5, 6, 7]. In countries where edible insects constitute regular elements in traditional diets, the shift towards Western foods constitutes a real threat to entomophagy. To counter this, efforts are being made to merge the traditional practice of insect eating with more popular foods [1].

Caterpillars are among the world's most diverse groups of edible insects. They are not only valuable sources of protein and other micronutrients; they also make valuable contributions to livelihoods in many parts of the world. The consumption of caterpillars is especially pervasive in sub-Saharan Africa, where 30 percent of all edible insect species are caterpillars [4]. In the Democratic Republic of the Congo, caterpillars make up 40 percent of the total animal protein consumed [8]. The most popular and profitable caterpillar on the African continent is undoubtedly the mopane caterpillar, *Imbrasia sp*. Although the caterpillars are important sources of nutrition in lean times, they also form a regular part of the diet [9]. The protein content of the mopane caterpillar is 48–61 percent and fat content is 16–20 percent, of which 40 percent is essential fatty acids. Mopane caterpillars are also a good source of calcium, zinc and iron [10, 11].

In Cote d'Ivoire, *Imbrasia oyemensis* is one of the most widely eaten insects. This caterpillar, sold at high prices on Abidjan markets, is consumed by an important population fringe of the country in replacement of meat and fish [12]. The present paper investigates the content and composition of oil extracted from *Imbrasia oyemensis* larvae. Indeed, edible insects are a considerable source of fat [13]. Womeni *et al.* [14] investigated the content and composition of oils extracted from several insects. These authors showed that insect oils are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids. The nutritional importance of these two essential fatty acids is well recognized, mainly for the healthy development of children and infants [15]. Furthermore, the fatty acid composition of insects appears to be influenced by the plants on which they feed [16, 17].

## 2. Materials and methods

### Larvae collection and sample preparation

Dried caterpillars *I. oyemensis* were obtained from the "Gouro" market of Adjamé (Abidjan, Côte d'Ivoire). After collection, they were sorted and free from any kind of waste. Then, dried caterpillars (2 kg) were ground using a porcelain motar to obtain the full-fat flour.

### Oil extraction

Oil was extracted from 3 g of caterpillar flour (full-fat flour) with 70 mL of n-hexane in a Soxhlet extractor [18]. Then, the solvent was gently evaporated with a rotary evaporatorty (Heidolph, Hei-Vap, Germany). The extracted lipid was weighted to determine the oil content of caterpillar. Crude oil was stored at 4 °C in airtight brown sterile glass bottle until further use for physicochemical analysis.

### Physicochemical analysis

### Moisture content

10 g of oil was weighed into a Petri-dish of known weight. The weighed sample was put into an oven pre-set at 110 °C for 3 h. The sample was removed and cooled in a dessicator at room temperature and the weight was determined after which it was returned into the oven at 110 °C for 30 min until constant weight was obtained [18].

**Ash content**

Ash content was determined according AOAC [18] method. 5 g of oil was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3–5 h at 600 °C. It was cooled in a desiccator and weighed to ensure completion of ashing. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content.

**Specific gravity and refractive index**

Specific gravity and refractive index of *I. oyemensis* oil was determined at 25 °C following the IUPAC [19] method by using a pycnometer and a refractometer (Abbe, Optic Ivymen, Spain), respectively.

**UV-Vis spectra**

UV-Vis spectra of oil sample was determined by measuring absorbance of hexanic oil solution (1%) by using a UV-Vis spectrophotometer (T80+, PG Instruments, England) in the range of 200 to 600 nm [20].

**Acid, peroxide, iodine and saponification values**

Acid, peroxide, iodine and saponification values were determined following the AOAC [18] methods.
Acid index of *I. oyemensis* oil was determined according to AOAC official method with the morn ISO-9001. A volume of 100 mL ethanol was neutralised with a solution of NaOH (N/10). The titration was performed using a solution of KOH (1N) in the presence of phenolphthalein.
Iodine value of *I. oyemensis* oil was determined according to AOAC [18] official method. A volume of 30 mL carbon tetrachloride was used to dilute 0.4 g oil in the presence of 25 mL of Wijs reagent and 10 mL of acetate mercury. The titration was carried out using a solution of sodium thiosulfate (0.1 N).
The peroxide value was determined according to AOAC [18] method. A solution made out of potassium iodine added to a mixture of acetic acid – chloroform: 3/2 (v/v) has been used. The titration was carried out using a solution of sodium thiosulfate (N/100).
Saponification value was determined according to AOAC [18] official method. An amount of 2 g oil has been treated using alcoholic potash (0.5 N) and titrated hot with hydrochloric acid (0.5 N) in the presence of phenolphthalein.

**Unsaponifiable matter**

Unsaponifiable matter content of oil sample was determined following the IUPAC [19] method. For this, 100 mg of unsaponifiable fraction was dissolved in 2 mL of chloroform. To 1 mL of aliquot, 4 mL of a trifluoroacetic-chloroform (1:3, v/v) solution was added. The absorbance was measured at 620 nm using a spectrophotometer (T80+, PG instruments, England).

**Mineral composition**

Mineral composition was determined by Scanning Electron Microscopy / Energy Dispersion Spectrometric (SEM/EDS) methods, from 2 g of ash.

**Fatty acid composition**

The fatty acids were converted to their methyl esters (FAMEs) as described by the European Communities [21]. About 0.1 g of oil sample was mixed with 2 mL of n-heptane and 0.2 mL of a methanolic solution of potassium hydroxide (2N). The whole mixture was shaken up for 30 s and allowed to settle for 5 min. The top layer containing the FAMEs was used for gas chromatography (GC) analysis.

FAMEs solution (1 µL) containing the internal standard (erucic acid) was injected into a gas chromatograph (Shimadzu, GC 14 A, Japan) equipped with a flame ionization detector (FID) and a capillary column TRD1 (60 m X 0.25 mm i.d. X 0.25 µm film thickness). The carrier gas was nitrogen and the flow rate was adjusted to 23 mL/min. Temperatures of detector and injector were 250°C. The initial column temperature was fixed to 100°C and programmed to increase by 5°C per min intervals until 220°C and, kept for 10 min at this temperature. The fatty acid methyl esters peaks were identified by comparing their retention times with those of standards. After adjusting areas with the internal standard (erucic acid), the yield of each fatty acid was calculated as follow: area of the fatty acid/areas of total fatty acids in the oil sample × 100 (%).

**Statistical analysis**

Each sample was analyzed in triplicates and data are reported as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using SPSS version 11.0 software. Statistical significance was set at $p \leq 0.05$.

## 3. RESULTS AND DISCUSSION

### Extraction yield and physicochemical composition

Edible insects are well known as fat and oil sources [14, 22]. As far as concerned *I. oyemensis*, its oil content was amounted to 23.96 ± 0.82 % (dry matter) (Table 1). With regard to this value, *I. oyemensis* larvae are lipid-rich than most of edible insects such as *Cirina forda* [23], *Zonocerus variegates* [14], *Oryctes rhinoceros* and *Rhynchophorus pheonicis* [23].

Physicochemical characteristics of *I. oyemensis* oil are shown in Table 1. Values of specific gravity and refractive index were found to be 0.942 ± 0.02 and 1.4675 ± 0.0002 respectively (Table 1). The refractive index value obtained for *I. oyemensis* (1.4675) was in close agreement with values reported for conventional edible oils [24]. The high refractive index of this oil seems to confirm the high number of carbon atoms in its fatty acids [25]. Refractive index also increases as the double bond increases [26]. Furthermore, *I. oyemensis* oil could be classified as non-drying fats in view to its refractive index value [27]. This property would underline its quality of edible fat and also disqualify him for varnish manufacturing in chemical industry. As regards specific gravity (0.942), it is reported for denser than specific gravity (0.89) reported for *Rhynchophorus phoenicis* [22]. The specific gravity and refractive index for *I oyemensis* oil is similar to those for Arachis oil, linseed oil and Olive oil [28]. This implies that the oil from this insect is lighter as these oils that have been considered to be of high quality and as such find much use in the pharmaceutical industries. In addition the oil is also unsaturated as these oils, which suggests that it might be fluid at room temperature [22].

The acid value is low, acid value of 0.00 to 3.00 mg KOH/g oil is recommended for oil to find application in cooking [29]. Thus the oil from *I. oyemensis* larvae could be suitable for cooking. The free fatty acid value of 1.49 ± 0.37 falls within the maximum limit of 5% for free fatty acids in high grade palm oil in Nigeria [30]. The data relevant to acid value and free fatty acid further indicate that *I. oyemensis* oil is not much susceptible for fat degradation process during oil extraction too.

Saponification value is an index of average molecular mass of fatty acids in oil sample. The higher saponification value (184.20 ± 1.62) in the *I. oyemensis* oil suggest that the mean molecular weight of fatty acids is higher as that of *Rhynchophorus palmarum* larvae (189.22 ± 0.92) oil or that the number of ester bonds is similar to that of *Rhynchophorus palmarum* larvae [31]. This might imply that the fat molecules were intact. High saponification value indicated the presence of greater number of ester bonds, suggesting that the fat molecules were intact. These properties make it useful in soap making industry [32].

**Table 1**

**Extraction yield and physicochemical properties of *I. oyemensis* oil**

| **Parameters** | **Values** |
|---|---|
| Extraction yield (%) | 23.96 ± 0.82 |
| Moisture and volatile matter at 105 °C (%) | 7.51 ± 0.1 |
| Acid value (mg KOH/g mg) | 2.81 ± 0.02 |
| Free fatty acid (% oleic acid) | 1.49 ± 0.37 |
| Saponification value (mg KOH/g mg) | 184.20 ± 1.62 |
| Iodine value (g $I_2$/100 g) | 105.19 ± 1.62 |
| Refraction index 26°C | 1.4675 ± 0.0002 |
| Specific gravity | 0.942 ± 0.02 |
| Unsaponifiables (g/kg) | 45.0 ± 1 |
| Total sterols content (mg / kg) | 1589.0 ± 2 |

Values are mean ± standard deviation of three measurements (n = 3).

## Mineral composition

It is noteworthy that minerals play an important part in biological processes. The recommended dietary allowance (RDA) and adequate intake are generally used to quantify suggested daily intake of minerals. The mineral composition of oil from *I. oyemensis* larva is shown in Table 2. This oil was found to be a very rich source of mineral elements with a 100 g dry sample containing phosphorus (26.82 g), potassium (14.97 g), magnesium (5.63 g), calcium (5.39 g), zinc (0.63 g) and iron (0.31 g). The consumption of 100 g dry oil would provide 1407 %, 3831 %, 318 %, 539 %, 3875 %, and 5727 % of the Recommended Dietary Allowances of magnesium, phosphorus, potassium, calcium, iron and zinc respectively [33]. Therefore, this oil can be used in diet to prevent against some mineral deficiencies. For example, Zinc deficiency is a core public health problem, especially for child and maternal health. Zinc deficiencies can lead to growth retardation, delayed sexual and bone maturation, skin lesions, diarrhea, alopecia, impaired appetite and increased susceptibility to infections mediated via defects in the immune system [34].

Otherwise, the sodium and potassium levels in the larva oil are 0.67 g/100 g and 14.97 g/100 g, respectively, resulting in potassium to sodium ratio of approximately 22:1. This favourable potassium/sodium ratio renders the oil from *I. oyemensis* larva a potential component of diets for the management of hypertension. Potassium intake has been found to lower blood pressure by antagonizing the biological effect of sodium [35].

**Table 2**

**Mineral composition of *I. oyemensis* oil**

| Minerals | Values (g/100 g) | Intake recommendation for 25-year-old males (g per day)* |
|---|---|---|
| **Na** | 0.67 ± 0,08 | 1.50 |
| **Mg** | 5.63 ± 0,05 | 0.40 |
| **Al** | 0.00 | |
| **Si** | 0.00 | |
| **P** | 26.82 ± 0.24 | 0.70 |
| **S** | 0.00 | |
| **Cl** | 0.00 | |
| **K** | 14.97 ± 0.15 | 4.70 |
| **Ca** | 5.39 ± 0.14 | 1.00 |
| **Mn** | 0.85 ± 0.04 | 0.0023 |
| **Fe** | 0.31 ± 0.03 | 0.008 |
| **Cu** | 0.36 ± 0.16 | 0.0009 |
| **Zn** | 0.63 ± 0.15 | 0.011 |

Values are mean ± standard deviation of three measurements (n = 3).
*Dietary reference intakes (DRIs): recommended dietary allowances and adequate intakes, minerals, Food and Nutrition Board, Institute of Medicine, National Academies.

## UV-Vis spectrum

The UV-Vis spectrum of *I. oyemensis* oil is depicted in Figure 1.

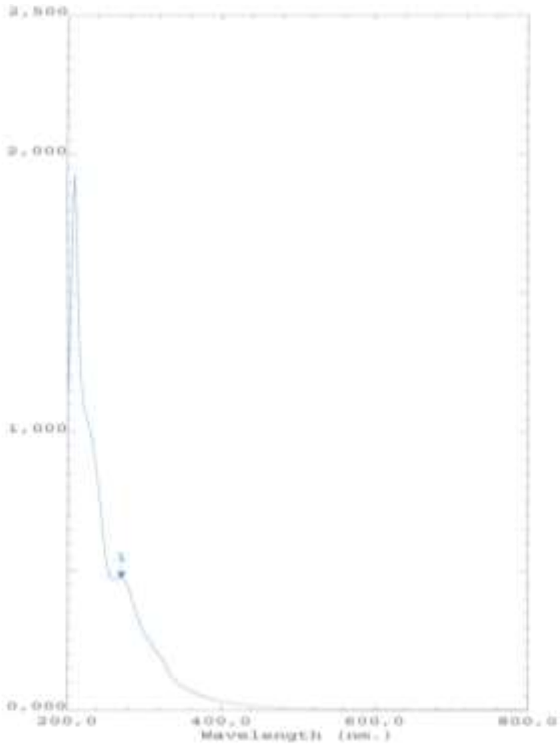


**Fig. 1. UV-Vis spectrum of *I. oyemensis* oil**

Maximum absorbencies were obtained at 220 and 300 nm corresponding respectively to absorbance values of 1.9 and 0.5. Absorbance of the studied oil decreases quickly from 0.5 to 0 in the range of 250 – 400 nm. This wavelength interval includes that of the UV-B and UV-A range varying from 290 to 400 nm. Given that in the UV-B and UV-A wavelengths range, absorption of UV light is responsible for most of cellular damages, *I. oyemensis* oil may be used in cosmetic formulations of UV protectors that provide protection against both UV-B and UV-A [20].

## Fatty acids composition

Figure 2 depict the fatty acids profile of *I. oyemensis*. The data given in the Table 3 showed that unsaturated fatty acids (55.7 %) are higher than saturated (44.3 %). The high percentage of polyunsaturated fatty acids in the oil from *I. oyemensis* is supported by the high iodine value obtained for the oil in this study. The abundances of unsaturated fatty acids in the oils were desirable from the nutritional and health view points as unsaturated fatty acids consumption will not lead to heart related diseases while the consumption of foods rich in saturated fatty acids is implicated with certain cardiovascular disorders like atherosclerosis, cancer and aging [36, 37]. The major unsaturated fatty acids in *I. oyemensis* oil were linolenic (26.22 ± 0.03 %), linoleic (11.33 ± 0.02) and arachidonic (10.26 ± 0.04), while the major saturated fatty acids were stearic (20.92 ± 0.03) and palmitic (18.65 ± 0.05). The unsaturated fatty acids accounted for 55.7 % while the saturated acids accounted for 44.3% of total fatty acids (Table 3). The presence of both saturated and unsaturated fatty acids in this insect could be an advantage since they may complement the functions of one another. Linoleic, linolenic and arachidonic acids are important essential fatty acids required for growth, physiological functions and body maintenance [38]. As far as concerned palmitic and stearic acids, which are the main saturated fatty acids of the studied oil, previous studies have shown that they are free from deleterious effect on plasma cholesterol [39]. In addition, they are often used in food industries to provide texture and softness to products [40].

Ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) has been used widely to indicate the cholesterol lowering potential of a food. A PUFA/SFA ratio of 0.2 has been associated with high cholesterol level with high risk of coronary heart disorders while a ratio as high as 0.8 is associated with desirable levels of cholesterol and reduced coronary heart diseases [41]. The PUFA/SFA ratio of 1.07 in *I. oyemensis* larva (Table 3) tends to suggest that the larva oil has the potential of being used in the dietetic management of certain coronary heart diseases.

Ratios of linoleic and arachidonic acids to the linolenic one (n-6/n-3), calculated for *I. oyemensis* (0.82), are extremely lower than the critical ratio (5.00) recommended by nutritionists to reflect the need for a nutritional equilibrium [42]. Therefore, *I. oyemensis* oil would be nutritionally balanced than most of the conventional oils such as soybean, walnut, olive, corn and sunflower oils with respective n-6/n-3 ratios of 8, 8.1, 34, 72.5 and 670 [24].

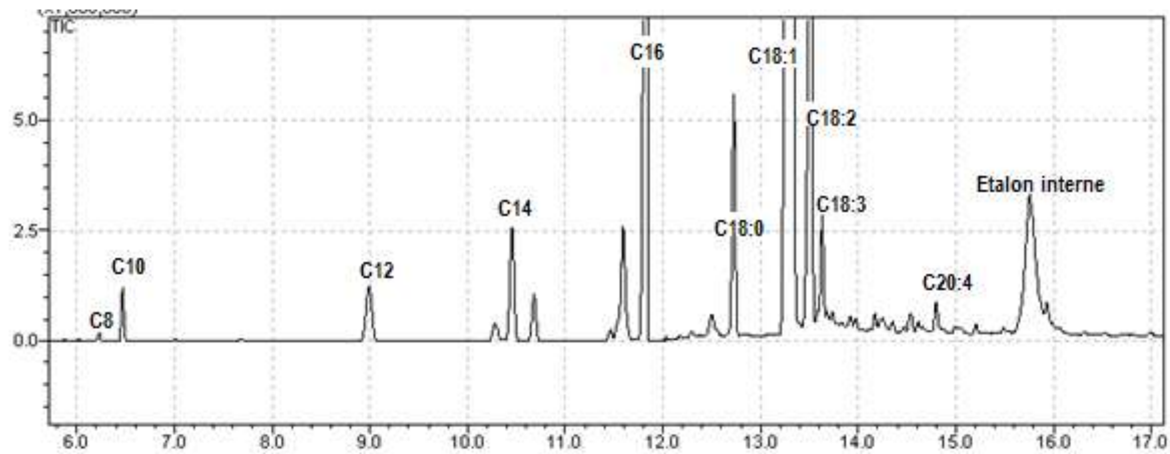


**Fig. 2. Gas chromatograms of fatty acids methyl esters of fatty acids methyl esters of *I. oyemensis* oil analysed by flame ionization detector**

**Table 3**

**Fatty acids composition of *I. oyemensis* oil**

| Fatty acid | Saturation | Values (%) |
|---|---|---|
| **Essential** | | |
| Linoleic Acid | Omega 6 polyunsaturated | 11.33 ± 0.02 |
| Linolenic Acid | Omega 3 polyunsaturated | 26.22 ± 0.03 |
| Arachidonic Acid | Omega 6 polyunsaturated | 10.26 ± 0.04 |
| **Non-essential** | | |
| Caprylic Acid | Saturated | 0.56 ± 0.045 |
| Capric Acid | Saturated | 1.47 ± 0.02 |
| Lauric Acid | Saturated | 1.38 ± 0.01 |
| Myristic Acid | Saturated | 1.30 ± 0.03 |
| Palmitic Acid | Saturated | 18.65 ± 0.05 |
| Stearic Acid | Saturated | 20.92 ± 0.03 |
| Oleic Acid | Omega 9 monounsaturated | 7.91 ± 0.01 |
| **Ratios** | | |
| SFA | | 44.3 |
| MUFA | | 7.91 |
| PUFA | | 47.79 |
| PUFA / SFA | | 1.08 |
| n6 / n3 | | 0.82 |

Values are mean ± standard deviation of three measurements (n = 3).

## 4. Conclusion

Based on the present study, it appears that *I. oyemensis* larva is a good source of edible oil, contains high percentage of polyunsaturated fatty acids (55 %) mainly consisting of arachidonic, linolenic and linoleic acids which were desirable from the nutritional and health view points. Furthermore, the PUFA/SFA and n-6/n-3 ratios suggest that *I. oyemensis* oil would be nutritionally more balanced than most of the conventional oils such as soybean, walnut, olive, corn and sunflower. This oil was also found to be a very rich source of minerals especially phosphorus, potassium, magnesium, calcium, zinc and iron. Therefore, it can be used in diet to prevent against some mineral deficiencies. The oil extract exhibited good physicochemical properties and could be useful for nutritional, pharmaceutical and industrial applications.

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