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Full Length Research Paper

Fatty acid composition and properties of skin and digestive fat content oils from *Rhynchophorus palmarum* L. larva

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Skin and digestive fat content (DFC) oils from *Rhynchophorus palmarum* L. larva (Curculionidae) were extracted and their physicochemical properties were characterized. Water content (0.41 %) of skin oil was higher than the amount of DFC (0.04 %). While, the lipid fraction of the skin (35.16%) was slightly lower than the DFC (49.05%). The fatty acid compositions of the both oils were determined. Results showed that the most abundant fatty acids in skin and DFC oils were palmitic and oleic acids. In both oils, oleic fatty acid showed the highest percentage of composition of 45.62 and 46.71% for skin and DFC, respectively with palmitic acid followed close by 39.87 and 40.44%, respectively. In this study, saturated fatty acids accounted for 45.06 and 44.97% of total fatty acids, for skin and DFC oils, respectively. Myristic, myristoleic, stearic and linoleic acids were also detected in the both oils. Physicochemical properties of skin and DFC oils respectively include: iodine index, 51.22 and 48.35; acid value, 4.72 and 2.21; saponification value, 189.22 and 198.26; unsaponifiable matter, 0.97 and 0.98; peroxide index, 6.90 and 0; oleic acidity, 7.76 and 0.568; vitamin A, 0 and 12.04 and refractive index, 1.45440 and 1.45424. Results suggested that Skin and DFC oils from *R. palmarum* L. larva could deserve further consideration and investigation as a potential new multi-purpose product for nutritional, industrial, cosmetic and pharmaceutical uses.

Key words: Fatty acids, digestive fat content, skin of larvae, oil, *Rhynchophorus palmarum*.

INTRODUCTION

Insects have played an important role in history of human nutrition in Africa, Asia and Latin America (Bodenheimer, 1951). The larva of *Rhynchophorus palmarum* L., a Coleoptera of Curculionidae family is used as traditional food in several countries (Sanchez et al., 1996; 1997; Cerda et al., 1999). It is a delicacy in many part of Côte d'Ivoire and other countries in Africa where it is found. Generally, the *Rhynchophorus* larvae are strongly looked for the people who believe them to have high nutritive as well as certain pharmaceutical values.

In several countries, these larvae are consumed in their entireties prepared either by stewing, frying in oil with salt and pepper, adding to squash seed paste, or putting on brochettes grilled over coals. In Cameroon for example, these larvae called "FOS" are washed by lot of water and pierced in the abdomen with a sharp pierce of bamboo between each washing to let a white fatty liquid escape before any cooking (brochette, frying etc…) (Grimaldi et Bikia, 1985).

Traditionally, there are many claims that, the *Rhynchophorus* larvae have medicinal properties. In Delta state of Nigeria, the Itskiris believe that *R. phoenisis* larvae could cure a certain ailment in infants which presents such symptoms as the twitching of the hands and feet, restlessness between others (Ekpo, 2003). For certain Amazonian population who live in the forest, *R. palmarum* L. larva's oil is used for treating respiratory sickness (Bourdy et al., 2000). The biochemical basis for this treat-

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ment is not known. The nutritional compositions of this larva used as a food by the Amazonian Indians and its palm substrata have been fairly well investigated by Cerda et al. (1999).

Several authors showed that some of the insects which are pests also have high nutritional qualities. Proximate composition of these insects have been studied from Central Africa (Richards, 1939), South Africa (Quinn, 1959; Dreyer and Weameyer, 1982) and South America (Dufour, 1987). Insects provide high quality of proteins and supplements (minerals and vitamins) (Banjo et al., 2006).

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 larval 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).

However, fatty acid composition and properties of the oil from *R. palmarum* larvae are not yet known.

To evaluate the nutritional value of some parts of this larva eliminated by populations before consumption, we suggested studying in this work the characteristics of skin oil (obtained by extraction in Sohxlet) and digestive fat content (DFC) oil (obtained by heating) extracted from larvae of *R. palmarum* L.

MATERIALS AND METHODS

Materials

Hundred live larvae of *R. palmarum* L. were collected in Epimbé, Adzopé subprefecture (Côte d'Ivoire) palm grove. The species were specifically identified in the entomology department of Abobo-Adjamé University (Côte d'Ivoire).

Methods

Skin and digestive fat content (DFC) obtaining

Larval heads were cut, using a pair of chisel. Press then the abdomen upwards to bring out all the digestive fat content. Skin and digestive fat content are so separated.

Lipid extraction

Skin of larvae was dried at 70°C for seven days, and grinded for obtaining skin powder. Hexan was used as a solvent, and 140 g of skin powder of larva was dissolved in it, in a soxhlet extractor during four hours, to obtain skin oil of larva of the weevil.

Digestive fat content (DFC) oil was extracted from 158 g of DFC of *R. palmarum* L. for 30 min by heating. The extracted lipid was obtained by filtration of the extract.

Physicochemicals analysis of oils extracted from larva

The weights of oils extracted from 110 g of skin powder and 158 g of *R. palmarum* L DFC were determined to calculate the lipid contents. Result was expressed as the percentage of lipids in the dry matter of skin powder or in wet matter of DFC.

Acid index and acidity of *R. palmarum* L oils were determined according to AOCS Ca 5-40 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 values of *R. palmarum* L oils were determined according to NF ISO 3961 (February 1990) method, and ISO 9001, norm. 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 AOCS Cd-8-53/1960 and ISO 9001 norm. 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 values, unsaponifiables and oils moistures were determined according to official method AOCS-Ca-2b-38-T60-2. 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.

Vitamin A was extracted according to the method described by Panfili et al. (1994).

Fatty acid composition was analysed by gas-liquid chromatography after derivatives changed to fatty methyl esters (FAMEs) with 2 M KOH in methanol at room temperature following IUPAC standard method (IUPAC, 1992). Analyses of FAMEs were carried out with a Hewlett-Packard 5890 series gas chromatograph GC system equipped with a hydrogen flame ionisation detector and a capillary column: HP-5 Cross-linked 5% PH ME Siloxane (30 m x 0.32 mm x 0.25 µm film). The column temperature was programmed from 160 to 325°C at 5°C/min and the injector and detector temperatures were set at 275 and 325°C, respectively.

Identification and quantification of FAMEs was accomplished by comparing the retention times of peaks with those of pure standards purchased from Sigma and analysed under the same conditions. The results were expressed as a percentage of indivi-dual fatty acid in the lipid fraction.

Refractive index of *R. palmarum* L oils was determined using a refractometer RFM 81, Multisecale Automatic from Bioblock Scientific.

Densities were determined using the NF T-60-214 method and ISO 9001norm.

All analytical experiments were repeated three times. Values of each parameter were shown as mean ± standard deviation (mean ± S.D.).

Statistical analysis was carried out by Student's *t*-test using SPSS Version 11.0 software and ANOVA (Duncan multiple range test) using SAS system Version 8e. $p < 0.05$., was considered, as level of error.

RESULTS

Table 1 shows Skin and Digestive fat content (DFC) oils moistures and lipid value from *R. palmarum* L. larva. Analysis showed that the water content of skin and digestive fat content (DFC) oils from the larva were respectively 0.41 ± 0.020 and 0.04 ± 0.004%. It also showed that the lipid values obtained from skin (35.16) was lower than the DFC (49.05).

Table 1. Skin and Digestive fat content (DFC) oils water and lipid content (g/100 g of matter of weevil's larva).

	Skin	Digestive fat content (DFC)
Moisture (%H_2O)	0.41 ± 0,020[a]	0.04 ± 0,004[b]
Lipid value (g/100g of matter)	35.16 ± 0,0002[a]	49.05 ± 0,001[b]

All given values are means as three repeats for each experiment. Means for the determined values in the same line followed by the same superscript letter are not significantly different ($p<0.05$).

Table 2. Fatty acid composition (g/100 g of total fatty acid) of skin and digestive fat content oils from *Rhynchophorus palmarum* L larva. Values are all expressed as percentages.

Fatty acid	% composition	
	DFC	Skin
Myristic acid (C14:0)	2.54 ± 0.135[a]	3.02 ± 0.041[a]
Myristoleic acid (C14:1)	2.06 ± 0,002[a]	1.91 ± 0,005[a]
Palmitic acid (C16 :0)	40.44± 0.088[a]	39.87 ± 0.11[a]
Stearic acid (C18 :0)	1.99± 0,01[a]	2.17 ±0,009[a]
Oleic acid (C18 :1)	46.71± 0,008[a]	45.62 ±0,02[a]
Linoleic acid (C18:2)	6.24 ± 0,005[a]	7.37 ± 0,004[a]

All given values are means of three repeats for each experiment. Means for the determined values in the same line followed by the same superscript letter are not significantly different ($p<0.05$).

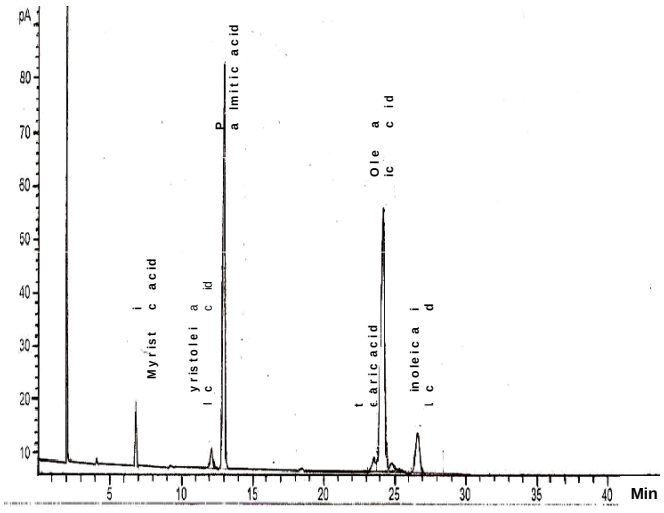

Figure 1. Fatty acid profile of skin oil from *Rhynchophorus palmarum* L larva. Fatty acid composition was analysed by liquid gas chromatography after derivatives changed to fatty methyl esters (FAMEs) with 2 M KOH in methanol at room temperature according to the IUPAC standard method (IUPAC, 1992).

Fatty acid composition determination was another important characteristic carried out on the studied oils from *R. palmarum* L. larva (Table 2). The most abundant fatty acids in the skin and DFC oils were the oleic, palmitic, linoleic followed by the myristic, myristoleic and stearic fatty acids. The major fatty acid were oleic fatty acid with 45.67% (skin oil) and 46.71% (DFC oil), followed by palmitic acid with 39.87 (skin oil) and 40.44% (DFC oil). Figures 1 and 2 show the fatty acid profiles of skin and DFC oils, respectively. There was no significant difference ($p>0.05$) in amounts of the fatty acids in the skin and DFC oil samples. In this study, saturated acids accounted for 45.06 and 44.97% of total fatty acids, for skin and DFC oils respectively (Table 3). However, these oils are enough unsaturated with a total monounsaturated fatty acids as most important in skin and DFC oils.

Table 4 shows the physical characteristics of skin and DFC oils extracted from weevil's larvae.

These oils were clear liquids with light brown and light yellow colours respectively for skin and DFC origins. Refractive index was studied and showed that there was no significant difference ($p>0.05$) in the values of the both oils (1.45440 for skin and 1.45424 for DFC oils).

The chemicals characteristics of skin and DFC oils are given in the Table 5. Peroxide value of DFC oil was nil contrary to that of skin that was relatively high (6.90 ± 0.57). The low values of iodine indexes (51.22 ± 0.25 for skin and 48.35 ± 0.55 for DFC oils) indicated that these oils could not be used mainly as unsaturated oils. The acid value of DFC oil (2.21 ± 0.02) was lower than the skin one (4.72 ± 0.06). This result was similar to oleic acidity of these oils. As a result of determining the saponification indexes, skin oil showed a lower number (189.22 ± 0.92) compared to the DFC one (198.26 ± 0.99) but these indexes were much higher in the both oils. There was significant difference ($p<0.05$) in the saponification numbers and also the density (0.79 ± 0.0 for skin and 0.77 ± 0.00046 for DFC oils) of the both oils.

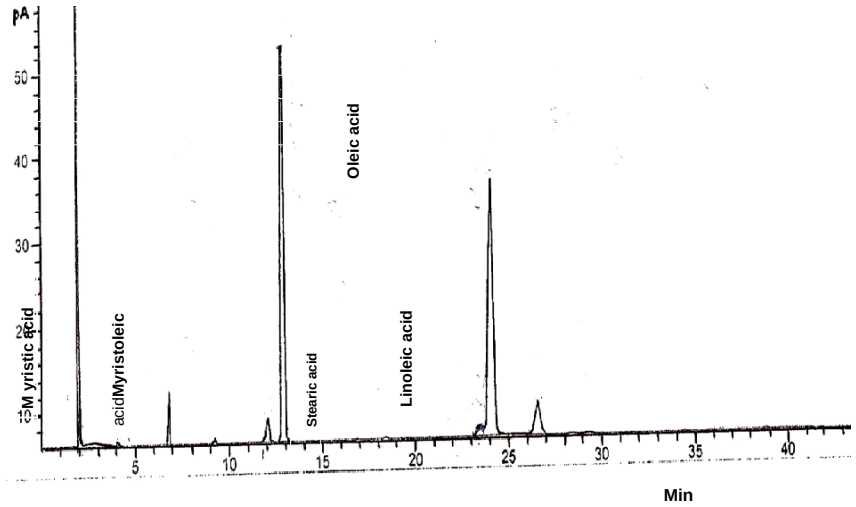

Figure 2. Fatty acid profile of digestive fat content (DFC) oil from *Rhynchophorus palmarum* L larva. Fatty acid composition was analysed by liquid gas chromatography after derivatives changed to fatty methyl esters (FAMEs) with 2 M KOH in methanol at room temperature according to the IUPAC standard method (IUPAC, 1992).

Table 3. Degree of saturation of skin and digestive fat content oils from *Rhynchophorus palmarum* L larva expressed, as percentages.

	DFC	Skin
TUFA	55.00	54.90
TSFA	44.97	45.06
MUFA	48.77	47.53
PUFA	6.24	7.37

TUFA, Total unsaturated fatty acid; TSFA, Total saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, Poly unsaturated fatty acid.

Table 4. Physical characteristics of skin and DFC oils from *Rhynchophorus palmarum* L larva.

Physical characteristics	Values (DFC)	Values (Skin)
Density	0.77 ± 0.00046^{a}	0.79 ± 0.0^{b}
Refractive index	1.45424 ± 0.55^{a}	1.45440 ± 0.25^{a}
Colour	Light yellow	Light brown

All given values are means of three repeats. Means for the determined values in the same line followed by the same superscript letter are not significantly different ($p<0.05$).

Table 5. Chemical characteristics of skin and DFC oils from *Rhynchophorus palmarum* L larva.

Chemical characteristics	Values (DFC)	Values (Skin)
Iodine index (g d'I_2 / 100 g of oil)	48.35 ± 0.55^{a}	51.22 ± 0.25^{b}
Saponification value (mg of KOH / g of oil)	198.26 ± 0.99^{a}	189.22 ± 0.92^{b}
Peroxide index (meq O_2 / kg of oil)	0^{a}	6.90 ± 0.57b
Acid value (mg of KOH / g of oil)	2.21 ± 0.02^{a}	4.72 ± 0.06^{b}
Oleic acidity (%)	0.568 ± 0.02^{a}	7.76 ± 0.14^{b}
Unsaponifiable matter (%)	0.98 ± 0.12^{a}	0.97 ± 0.44^{a}
Vitamin A	12.04 ± 0.021^{a}	0.00^{b}

All values given are means of three determinations. Means for the determined values in the same line followed by the same superscript letter are not significantly different ($p<0.05$).

The unsaponifiable matter of the *R. palmarum* L. larva oils were similar (0.97 ± 0.44 for skin and 0.98 0.12 for DFC oils). However, the vitamin A was present only in DFC oil. As the results of determining the iodine values, *R. palmarum* L. larva oils showed a much lowers numbers. There was also significant difference ($p<0.05$) in these low iodine indexes.

DISCUSSION

This study revealed that the oils from skin and DFC of *R. palmarum* L. larva possess high nutritional qualities.

Water content (0.41%) of skin was higher than the amount of DFC (0.04%) while, the lipid fraction of the skin (35.16 %) was slightly lower than the DFC one (49.05%). The high percentages of *R. palmarum* L larva oils make this insect a distinct potential for the oil industry. Variation in oils yield may be due to the different larval parts; the extraction method used and the larva nutritional metabolism. Skin lipid value was higher than those (dry matter basis) reported from other insects include 3.1 and 4.0%, respectively for the larva and adult beetle of *Lachnosterna* species (Davis, 1918), 7.21% for dried *Melanoplus* (Mc Hargue, 1970), 2.1% for the Japanese beetle, *Popillia japonica* nuwman (Fleming, 1968), 15.5% in the pupae of housefly *Musca domestica* (Calvert et al., 1969). Teotia and Miller (1974) reported the work done by Calvert et al. (1969) and reported the same results except that the lipids content was a little lower.

However, skin lipid value (35.16%) was lower than those reported by Leung (1972) for termites (55.24%); by Ukhun and Osasona (1985) for *Macrotermes bellicosus* (46.10%) and by Ekpo and Onigbinde (2007) for *M. bellicosus* (36.12%). Furthermore, the DFC lipid value (49.05% wet weight basis) was higher than those reportted by Ekpo and Onigbinde (2004, 2005) for *R. pheonicis* (25.30 ± 0.20) and Oryctes rhinoceros larval (14.87 ± 0.33) oils, respectively. The fat content of these larvae could have contributed to its highly acceptable flavour when fried or roasted.

Fatty acid composition study revealed that the most abundant fatty acids in skin and DFC oils were palmitic and oleic acids. In both oils, oleic fatty acid showed the highest percentage of composition of 45.62 ± 0.02 and 46.71 ± 0.008 for skin and DFC, respectively with palmitic acid followed close by with 39.87 and 40.44%, respectively. In this study, saturated acids accounted for 45.06 and 44.97% of total fatty acids, for skin and DFC oils, respectively. Variances analysis showed that there was no significant difference ($p<0.05$) in these different type of fatty acids. Among them, the main saturated normal chain fatty acids were palmitic, myristic and stearic acids.

As shown in Table 2, Skin and DFC oils from *R. palmarum* L larva have unsaturated (TUFA) values similar to that observed for palm oil which is common household oil and the termite *M. bellicosus* oil (Ekpo and Onigbinde, 2007). Insect fatty acids are similar to those poultry and fish in their degree of insaturation, with some groups being higher in oleic and linoleic which are essential fatty acids (De Foliart, 1991). These oils can be used as source of palmitic acid which is an excellent energy-giving food. Industrially, it is used for margarine manufacture, and also hard soaps. These saturated fatty acids are also important for production of oils in paint industry (http://fr.wikipedia.org/wiki/Acide palmitique).

Physical characteristics as refractive index, density and colour of *R. palmarum* L oils were studied. There was no significant difference ($p>0.05$) in the refractive indexes of the both oils (1.45440 ± 0.25 for skin and 1.45424 ± 0.55 for DFC). These refractive indexes were similar to that of olive oils studied by Lalas and Tsakins (2002); that of *Nigella sativa* L. oil (Rouhou- Cheikh et al., 2007) and that those for Arachis and olive oils (Pearson, 1976) . This implies that the oils from this insect are lighter as these oils that have been considered to be of high quality and as such find much use in the pharmaceutical industries. The chemical properties of oil are amongst the most important properties that determines its present condition. Free fatty acid of skin oil (4.72 ± 0.72) was higher than DFC oil one (2.21 ± 0.035) as well as oleic acidity (7.76 ± 0.14 and 0.568 ± 0.02, respectively). These character-ristics in skin oil were significantly higher ($p<0.05$) than those of DFC oil. Furthermore, peroxide index of DFC oil was 0 meqO2/Kg and 6.90 meqO2/Kg ± 0.77 for skin oil. These results showed that DFC oil has high stability to oxidation and its lower acid value is also an indication of its lower susceptibility to rancidity compared to skin oil. The iodine indexes of *R. palmarum* L larva oils studied were lower than those reported for most insect lipids as reported for winged reproductives of the termite *M. Belli-cosus* oil (108 ± 0.15) ; lepidopterous larvae (between 112-159); *Phytophagous chrysomefids* (106.6-118); *Rhynchophorus phoenicis* larvae oil (123.6); *Oryctes rhinoceros* larval oil (140) (EKPO and Onigbindé (2007); Wigglesworth (1976); Ekpo, (2003)).

As a result of determining the saponification values, *R. palmarum* L. larva oils showed 189.22 ± 0.92 and 198.26 ± 0.99 for skin and DFC, respectively. These parameters were higher than those reported for Mounga oleifera seed oil (164.26 ± 1.49 and 163 ± 0.98) by Abdulkarim et al. (2005). However, *R. palmarum* L larva oils saponification values were similar to those showed by Thiégang et al. (2004) for *Ricinodendron heudelotty* (Bail.) oils (between 193 and 195); and by Oomah et al. (2000) for Raspberry (*Rubus idaeus* L.) seed oils (between 191 and 192).

R. palmarum L. larva was shown to be rich in vitamin A (equivalent to 85.0 g of retinol) (Cerda et al., 1999). In the present work, the vitamin A was found in DFC oil (12.04 ± 0.02) but not in skin oil, which suggests that the vitamin A was produced in this part of *R. palmarum* L. larva.

This study showed that the *R. palmarum* L larvae oils have the potential to be developed either for food, pharmaceutical and chemical industries.

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