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CHARACTERIZATION OF FATTY ACID COMPOSITION IN HAPLOZANA NIGROLINEATA, ACANTHACRIS RUFICORNIS, AND PROTOMACRONEMA SPECIES: THREE EDIBLE ARTHROPODS IN THE REPUBLIC OF CONGO

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Abstract:
Lipids, encompassing both plant and animal molecules, are crucial components with diverse roles in biological systems. These molecules are highly efficient energy sources, providing approximately 39.5 kJ per gram, making them vital for sustaining metabolic activities. Apart from their energy reserve function, lipids play essential roles in constructing cell membranes, acting as both inter- and intracellular messengers, and serving as metabolic substrates. In comparison to carbohydrates and proteins, lipids offer a significantly higher energy yield, highlighting their importance in human nutrition. This paper provides an overview of the multifaceted properties and functions of lipids, emphasizing their critical role in supporting various physiological processes and their significance in human dietary intake.

Keywords: Lipids, energy source, cell membranes, metabolic substrates, human nutrition.

INTRODUCTION

Lipids are plant and animal molecules that serve as the g of carbohydrates yields 17.2 kJ. Lipids possess various most efficient energy source, providing 39.5 kJ per gram. properties, such as being an energy reserve, constituting In comparison, 1 g of protein supplies only 23.7 kJ, and 1 cell membranes, acting as inter- and intracellular messengers, and serving as metabolic substrates. They are of significant interest in human nutrition (CSS, 2016).

Lipids are essential sources of fatty acids, including long-chain polyunsaturated fatty acids (LCPUFAs) of the n-3 and n-6 series, as well as fat-soluble vitamins, giving them a crucial nutritional role. They play a role in protecting against cardiovascular disease by reducing cholesterol levels, a benefit not provided by certain saturated fatty acids (Leray, 2020). Lipids can be classified into eight different groups: fatty acids, acylglycerols, phosphoglycerides, sphingolipids, glycolipids, and polyketides, resulting from the condensation of ketoacyl groups, with sterols and phenols (isoprene unit) added. Extraction is typically performed using organic solvents, mechanical action after drying and grinding using sunlight, or artificially using hot air and any other source inducing production (Paul et al., 2016).

Nowadays, research related to the characteristics of arthropod lipids has become topical and is based on the determination of lipid contents, composition and evaluation of fatty acid and triacylglycerol proportions. It is undeniable that lipids play a role in human health, either negatively (certain trans octadecenoic acids) or positively (arachidonic, eicosapentaenoic and docosahexaenoic acids or their precursors). In the Republic of Congo, human consumption of arthropods has long been of great seasonal

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importance, as numerous studies have shown (Mabossy-Mobouna et al., 2021). The nutritional value of insects in the Congo Basin has been confirmed by numerous studies (Ombeni and Munyuli, 2016; Mabossy-Mobouna et al., 2017). In view of the nutritional value of lipids for human health, the present study sets out to assess the fatty acid profile of three Arthropods eaten in Congo.

MATERIALS AND METHODS

Biological

The food material consisted of *Protomacronema* species*, Acanthacris ruficornis* and *Haplozana nigrolineata.* These arthropods were purchased at the Total and Mikalou markets (Talangai) and transported to the laboratory for identification and physicochemical analysis.

Determination of the iodine index

This index is used to determine the degree of overall establishment of lipids in a food. It corresponds to the quantity in grams of iodine fixed per 100 g of fat. The iodine index values have been calculated according to the AOCS (2013) formula:

$$\text{Iodine index} = \sum \frac{127 \times 2 \times n}{MW} \times \%UFA$$

where n = Number of fatty acid double bonds; MW = molecular weight of fatty acid ester, and %UFA = percentage of unsaturated fatty acid.

Determination of lipid content

Lipid content was determined using the Soxhlet extraction method. 20 g of arthropod powder was introduced into the filter paper cartridge, which was placed in the Soxhlet with a cooler on top. A 500 ml flask containing 250 ml Hexane was attached to the Soxhlet and brought to the boil. When the solvent reached a certain level, it initiated the siphon and returned to the flask, carrying the dissolved substance with it. After a dozen siphonings, the solvent is enriched in soluble substances. The lipid fraction is then concentrated using a rotary evaporator. The flask containing the dry residues is weighed before and after extraction to determine the respective content of each of the fractions (Paul et al., 2016); M1 is the mass of the oil vial and Mo is the mass of the empty vial. The oil mass was calculated by the following relation:

Mh = M1- Mo

The lipid content was obtained by the following formula:

$$\% \text{ lipids} = \frac{Mh}{Mp} \times 100$$

Where M1=mass of flask containing oil; M0=mass of empty flask; Mp=mass of powder; and Mh=mass of oil.

Determination of fatty acid profile

After grinding, the lipids were extracted with chloroform/methanol (2:1 v/v) according to the method of Folch et al. (1957) and then concentrated by evaporation at 35°C under reduced pressure using a Büchi-type evaporator and then weighed. The fatty acid methyl esters (EMAGs) were prepared by transesterification catalyzed by boron fluoride sorting (10 mg of sample was placed in Soviet tubes, diluted in 200 µl of n-hexane and then reacted with 0.5 ml of a 20:55:25 mixture of n-hexane, dry methanol and 14% BF3 methanol for 90 min at 70°C. After reaction, 200 µl of 10% m/v sulphuric acid and 500 µl of a saturated sodium chloride solution were added). The resulting solutions containing the EMAGs were finally diluted with 8 ml of n-hexane and analyzed by gas chromatography on an Agilent 6890 chromatograph equipped with a cold-on-column injector and with a FID detector maintained at 260°C according to the following temperature program: 55 to 15°C (30°C/min) then 150°C. Helium was used as a carrier gas at a flow rate of 1.7 ml/min. The EMAGs were separated on a Varian CP9205 VF-Wax ms column (30 m length

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× 0.25 mm internal diameter, df = 0.25 µm) and identified by comparison of the retention times with those of a reference solution containing 37 EMAG (CRM47885 Supelco 37 Component FAME Mix). The results are expressed in area percent with a response factor of 1 for each molecule.

Statistical analysis

The data processing was carried out with the software Epi info, XL stat and Excel 2007. The processing of the data collected, the capture and the production of the raw tables were done with the software Epi info.6.04 and Excel 2007. The quantitative variables are expressed as mean (x) ± standard deviation (s), while indicating the extreme values (minimum and maximum). Qualitative variables are expressed in figures and percentages. The X^2 test is used for comparing qualitative variables. The determination of the degree of significance between the averages of the various quantitative variables studied is carried out by Student's law at (k-1) degree of freedom, with a threshold of significance of 5%.

Table 1. Iodine index for each species.

Fatty acids	**EMAG**	***Protomacronema* spp.**	***Acanthacris ruficornis***	***Haplozana nigrolineata***
C14:1	240	0.74	/	/
C15:1	240	0.85	/	/
C16:1	270	13.13	0.43	0.54
C17:1	282	0.90	0.29	/
C18:1n9t	296	/	0.13	13.16
C18:1n9c	296	10.49	10.42	7.74
C18:2n6t	294	/	0.78	0.28
C18:2n6c	294	9.78	23.86	14.31
C18:3n6	292	0.91	/	/
C18:3n3	292	9.41	100.10	108.93
C20:1	324	0.45	/	0.57
C20:2	322	0.93	/	/
C20:3	320	9.65	/	/
C20:3n6	320	0.35	/	1.20
C20:5n3	316	47.11	/	/
C22:1	352	/	/	0.14
Iodine index		104. 7	136.01	146. 87

Table 2. Total lipid content for the three-arthropod species.

Edible arthropod species *Protomacronema* spp.	***Acanthacris ruficornis***	***Haplozana nigrolineata***
Content (%)	6	9.58	9.83

RESULTS

Iodine index

The results on the index (Table 1) show that *H. nigrolineata* have a higher iodine index (146.87) followed by *A. ruficornis* (136.01) and lastly *Protomacronema* spp.
(104.7).

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Total lipid content

Table 2 reports the total lipid content of the three arthropod species and shows that the lipid content of arthropods varies from one species to another. *H. nigrolineata* had a lipid content of 9.83%, followed by *A. ruficornis* 9.58%, and *Protomacronema* spp. 6%.

Fatty acid profile

Fatty acid profile for three arthropod species

In terms of fatty acid composition (Table 3), all three species have high palmitic acid contents. However, αlinolenic acid (C18:3n3) content is very high for *H. nigrolineata* (41.7428%) and *A. ruficornis* (38.359%), whereas it is low for *Protomacronema* spp. (5.409%). Similarly, oleic acid content (C18:1n9) is high for *A. ruficornis* (12.143%) and *Protomacronema* spp. (12.229%) but low for *Protomacronema* spp. (9.02%). Other fatty acids have very negligible (<1%) or negligible (<2%) contents.

Degree of fatty acid saturation of materials and different ratios

The results on the degree of saturation (Table 4) show that the three Arthropods contain more unsaturated fatty acids (57.12% of *Protomacronema* spp.; 65.71% of *A. ruficornis*; 61.37% of *H. nigrolineata*) than saturated fatty acids (42.88, 34.29, and 38.63%, respectively for *Protomacronema* spp., *A. ruficornis* and *H. nigrolineata*). Others, on the other hand, have more monounsaturated and polyunsaturated fatty acids in almost equal proportions, as in the case of *Protomacronema* spp. The ω6/ω 3 ratio is 0.291 for *Protomacronema* spp, 0.37 for *A. ruficornis* and 0.214 for *H. nigrolineata*. Finally, the PUFA/SFA ratio is 0.651 for *Protomacronema* spp., 1.53 for *A. ruficornis* and 1.312 for *H.a nigrolineata.*

Table 3. Fatty acid profile (g/100 g total fatty acids) for three arthropod species.

Fatty acids	Protomacronema spp.	Acanthacris Ruficornis	Haplozana nigrolineata
C12:0	5.42	1.17	0.35
C13: 0	0.23	/	/
C14:0	5.70	/	1.52
C14:1	0.70	/	/
C15:0	0.76	0.52	0.23
C15:1	0.80	/	/
C16:0	22.10	19.71	22.35
C16:1	13.86	0.46	0.57
C17:0	1.65	2.05	0.59
C17:1	1.00	0.33	/
C18:0	6.28	9.47	9.56
C18:1n9t	/	0.16	0.15
C18:1n9c	12.23	12.14	9.02
C18:2n6t	/	0.45	0.16
C18:2n6c	5.66	13.81	8.28
C18:3n6	0.35	/	/
C18:3n3	5.41	38.36	41.74
C20:0	/	0.78	0.64
C20:1	0.58	/	0.73
C20:2	0.59	/	/
C20:3n6	0.15	/	0.50
C20:3	4.05	/	/

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C20:5n3	11.72	/	/
C21:0	0.21	/	3.22
C22:0	0.52	0.56	0.16
C22:1	/	/	0.19

Table 4. Fatty acid saturation levels and ratios for three species.

Fatty acid saturation (%)	Protomacronema spp.	Acanthacris ruficornis	Haplozana nigrolineata
Saturated fatty acids (SFA)	42.88	34.29	38.63
Monounsaturated fatty acids	29.174	13.087	10.6741
Polyunsaturated fatty acids (PUFA)	27.943	52.626	50.6927
Unsaturated fatty acids	57.12	65.71	61.37
ω6/ω3	0.291	0.37	0.214
PUFA/SFA	0.651	1.53	1.312

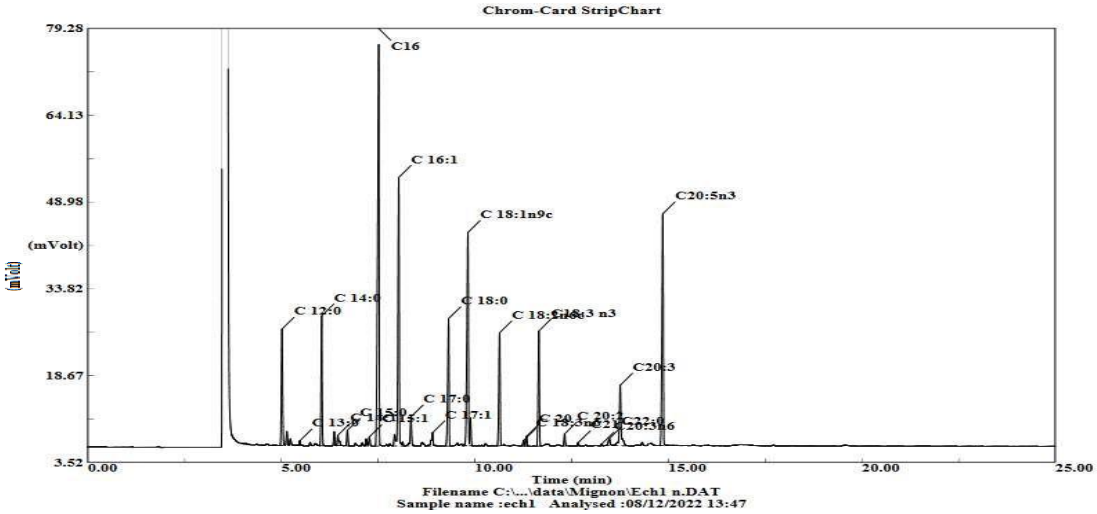

Figure 1. Chromatogram of the fatty acid profile of lipids from *Protomacronema* spp.

Chromatogram of the fatty acid profile of Protomacronema spp. lipids

Figure 1 shows a chromatogram of the fatty acid profile of *Protomacronema* spp. lipids. Fatty acid peaks are revealed according to carbon chain length and degree of establishment. 4 major peaks are revealed according to the fatty acid content of these lipids. In order of revelation, palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), and octadecatetraenoic acid (C20:5) are observed.

Fatty acid profile chromatogram of A. ruficornis lipids

Figure 2 shows a chromatogram of the fatty acid profile of *A. ruficornis* lipids. On this figure, 4 major peaks are revealed. In order of appearance, the peaks are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and α-linolenic acid (C18:3n-3).

Chromatogram of the fatty acid profile of H. nigrolineata lipids

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Figure 3 shows a chromatogram of the fatty acid profile of *H. nigrolineata* lipids. 5 major peaks are revealed. In order of revelation, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6), and α-linolenic acid (C18:3n-3) are observed.

DISCUSSION

Iodine index

The results of the analysis revealed that *Protomacronema* spp., *A. ruficornis,* and *H. nigroneata* had iodine index of 104.7, 136.01, and 146.87, respectively. These results are higher than those obtained by Mabossy-Mobouna and Malaisse (2020) (83.30) on *R. differens*. However, the iodine index of *Protomacronema* spp. Is lower than that of *Imbrasia truncata* caterpillars (134.453) consumed in Congo (Mabossy-Mobouna et al., 2017). The iodine index is proportionally high with the unsaturated fatty acid content. *Protomacronema* spp. has an index below 110, indicating that this fat is non-drying, unlike *H. nigroneata* and *A. ruficornis*, which have index above 110 and are therefore dry due to their high unsaturated fatty acid content. The polyunsaturated nature of the latter two fats may therefore entail the risk of auto-oxidative lipid degradation, which can prove problematic, with the appearance of flavour reversion following prolonged storage.

Total lipid content

The findings revealed that the overall lipid levels of all three species were low (9.83% for *H. nigrolineata, 6% Protomacronemas* spp. and 9.58% *A. ruficornis*). These findings differ from those of Mba et al. (2019), with Imbrasia epimethea caterpillar (22.8%). The low total lipid content of the present study is in line with the findings of authors such as Lautenschläger et al. (2017) with a content of 5.9%. Our results are also lower than those found by Musundire et al. (2016) on Ornithacris turbida (29.4%); by Ayieko et al. (2016) on Acheta domesticus (25.8%) and Loh et al. (2017) with Rhynchophorus phoenicis larvae (56.44 g/100 g). Lipid content varies according to species and development stage. It is generally higher in larvae than in adults (Ghosh et al., 2016).

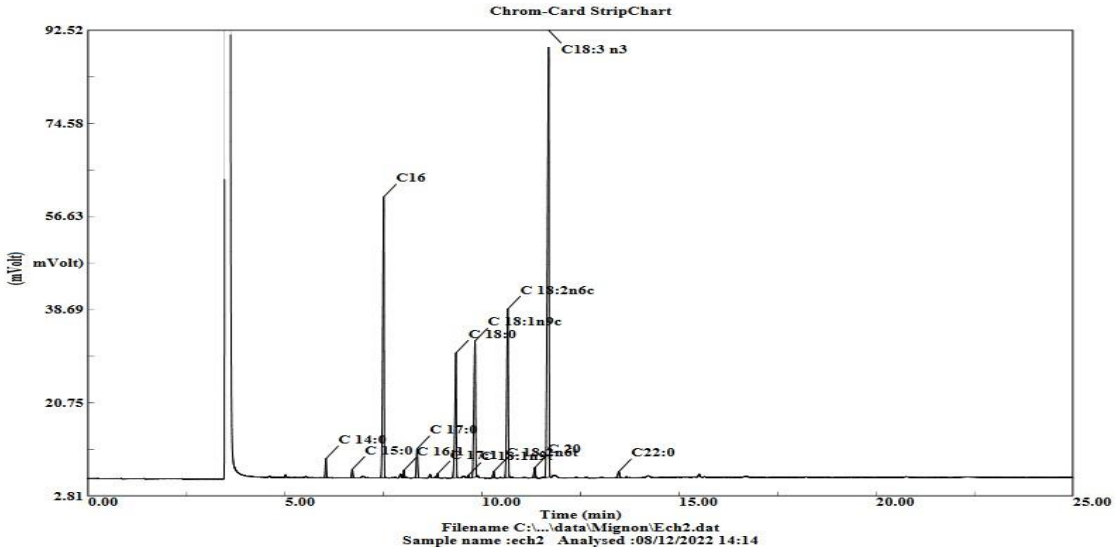

Figure 2. Fatty acid profile chromatogram of *Acanthacris ruficornis* lipids.

Fatty acid profile

The ω6/ω3 ratio is 0.291 for *Protomacronema* spp., 0.37 for *A. ruficornis* and 0.214 for *H. nigrolineata*. The results of the present study are close to those of Mba et al. (2019) with a value of 0.25. These results are lower than those obtained by Finke (2015) (0.96) on *Trimerotropis pallidipennis*.

For all three-arthropod species, this ratio is less than 1, so these three species contribute more ω3 than ω6; which is favorable to the proper functioning of the organism. Indeed, this balance will induce a physiological state characterized by the absence of cardiovascular disease, osteoporosis, obesity, diabetes as well as allergic and inflammatory disorders (Guillocheau et al., 2019; Buaud, 2020; Simopoulos, 2020). Excessive consumption of omega-6 is likely to counteract the positive effects of omega-3 and that the ω6/ω3 ratio is more important than omega-3 content alone (Pisani and Ailhaud, 2019).

The PUFA/SFA ratio of all three species is greater than 0.20, demonstrating low cholesterol levels and a low risk of coronary heart disease following consumption of these three species (Mabossy-Mobouna et al., 2020). Furthermore, the PUFA/SFA ratio of *Protomacronema* spp. is less than 1 (0.65). These results are close to those obtained by Mananga et al. (2015) on blue duiker meat (0.45). This gives *Protomacronema* spp. a low nutritional value in terms of lipids. The PUFA/SFA ratio of two other arthropods is greater than or equal to 1 (1.53 for A. ruficornis and 1.312 for H. nigroneata). These results are similar to those obtained by Finke (2015) on T. pallidipennis. This gives these two arthropods good lipid nutritional value.

Chromatogram analysis revealed 4 major peaks according to the fatty acid content of these lipids. In order of revelation, palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), and octadecatetraenoic acid (C20:5) were observed. The results differ from those of Mananga et al. (2015), who obtained 8 peaks in order of revelation, two of which are unidentified (Mba and Rodrigue, 2018).

Among the fatty acids, a high proportion of oleic (25.52%) and palmitic (24.25%) acids was noticed, followed by linoleic acid (13.80%) and myristic acid (16.22%). This suggests that arthropods are good suppliers of energy. The concentration of α-linoleic acid (18: 2n-6) in the results (9.78, 23.86 and 14.31) is higher than that obtained by Foua et al. (2016) with caterpillars of Imbrasia oyemensis (6.58), I. epimethea (6.3) and I. truncata (11.6).

Conclusion

H. nigrolineata, A. ruficornis, and *Protomacronema* spp. have contents of 9.83, 3.5, and 6% respectively, with iodine indices of 104.7, 136.01, and 146.87. All three species have high palmitic acid contents and contain more unsaturated fatty acids, followed by saturated fatty acids. Others, on the other hand, have more monounsaturated and polyunsaturated fatty acids in almost equal proportions, as in the case of *Protomacronema* spp. The chromatogram of the lipid fatty acid profile shows the presence of 4 major peaks for *Protomacronema* spp., 4 major peaks for *A. ruficornis*, and 5 major peaks for *H. nigrolineata*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFRENCES

American Oil Chemists' Society (AOCS) (2013). Official methods and recommended practices of the American Oil Chemist's Society. Firestone D., ed. 5th Ed. Ce 2-66 (97) procedure.

Ayieko MA, Ogola HJ, Ayieko IA (2016). Introducing rearing crickets (gryllids) at household levels: adoption, processing and nutritional values. Journal of Insects as Food and Feed 2(3):203-211

Buaud B (2020). How fats we eat modulate our immunity? Oilseeds and Fats, Crops and Lipids 27:22.

Conseil Supérieur de la Santé (CSS) (2016). Conseil Supérieur de la santé. Recommandations nutritiolles pour la Belgique-2016. Bruxel. CSS. Avis No 9285 :196p.

https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/css_9285_avis_rec_nutr.pdf

Finke MD (2015). Complete nutrient content of three species of wild caught insects, pallid-winged grasshopper, rhinoceros beetles and white-lined sphinx moth. Journal of Insects as Food and Feed 1(4):281-292.

Folch J, Lees M, Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226(1):497-509.

Foua BF, Meite A, Dally T, Ouattara H, Kati Coulibaly S (2016). Biochemicaland nutritional study of powder from caterpillars Imbrasia oyemensis eaten in west of Côte d' Ivoire. Sky Journal of Biochemistry Research 5(3):24-30.

Ghosh S, Jung C, Meyer-Rochow VB (2016). Nutritional value and chemical composition of larvae, pupae and adults of worker honey bee, Apis mellifera ligustica as a sustainable food source. Journal of Asia-Pacific Entomology 19(2):487-495.

Guillocheau E, Legrand PP, Rioux VV (2019). Benefits of natural dietary trans fatty acids towards inflammation, obesity and type 2 diabetes: defining the n-7 trans fatty acid family. Oilseeds and Fats Crops and Lipids 26:46.

Lautenschläger T, Neinhuis C, Monizi M, Mandombe JL, Förster A, Henle T, Nuss M (2017). Edible insects of Northern Angola. African Invertebrates 58(2):55-82.

Leray C (2020). Lipids and health. Oilseeds and Fats Crops and Lipids 27:25.

Loh AM, Njowe KY, Sop MK (2017). Preliminary proximate composition and mineral contents of five edible insects from Cameroon. International Journal of Current Microbiology and Applied Sciences 6(3):1984-1995.

Mabossy-Mobouna G (2017). Caractérisation et valorisation alimentaire des chenilles d'Imbrasia truncata (Aurivillius, 1908) au CongoBrazzaville. Thèse de doctorat en Nutrition Humaine, Université Marien Ngouabi, Congo Brazzaville 171 p.

Mabossy-Mobouna G, Malaisse F (2020). Caractérisation de la valeur nutritive des orthoptères consommés par l'homme en République du Congo. Geo-Eco-Trop 44(2):255-267.

Mabossy-Mobouna G, Ombeni BJ, Malaisse F, (2021). Consommation et modalités de préparation des chenilles comestible en Republique du Congo. Geo-Eco-Trop 45(3):435-453.

Mananga V, Kinkela T, Elenga M, Loumouamou BW, Kobawila SC, Makosso-Vheiye G, Silou T (2015). Teneur et composition en acides gras des lipides du céphalophe bleu (*Cephalophus monticola*).

Bulletin de la Société Royale des Sciences de Liège 84:4-13.

Mba AR, Kansci G, Viau M, Rougerie R, Genot C (2019). Edible caterpillars of Imbrasia truncata and Imbrasia epimethea contain lipids and protein of high potential for nutrition. Journal of Food Composition and Analysis 79:70-79.

Mba F, Rodrigue A (2018). Etude de quelques propriétés nutritionnelles de trois insectes con-sommés au Cameroun en vue de leur valorisation alimentaire: *Rhynchophorus phoenicis, Imbrasiatruncata et I. epimeathea.* Sciences du Vivant [q-bio]. Université de Yaoundé I. Français. NNT :90-99.

Musundire R, Zvidzai CJ, Chidewe C, Samende BK, Chemura A (2016). Habitats and nutritional composition of selected edible insects in Zimbabwe. Journal of Insects as Food and Feed 2(3):189-198

Ombeni BJ, Munyuli BM (2016). Evaluation de la valeur nutritionnelle des aliments sauvages traditionnels (Règne Animalia) intervenant dans la sécurité alimentaire des communautés rurales du Sud-Kivu (République Démocratique du Congo). Geo-Eco-Trop 40(2):115-132.

Paul A, Frederich M, Uyttenbroeck R, Hatt S, Malik P, Lebecque S, Hamaidia M, Miazek K, Goffin D, Willems L, Deleu M (2016). Grasshoppers as a Food Source. A review. Biotechnologie, Agronomie, Société et Environnement, 20(AgricultureIsLife).

Pisani D, Ailhaud G (2019). Involvement of polyunsaturated fattyacids in the control of energy storage and expenditure. Oilseeds and Fats Crops and Lipids 26:37.

Simopoulos AP (2020). Omega-6 and omega-3 fatty acids: Endocannabinoids, genetics and obesity. Oilseeds and Fats Crops and Lipids 27:7.