



## Profile in amino-acids and fatty-acids of *Imbrasia epimethea* caterpillar eaten in the Northern area of the Republic of the Congo

### Profil en acides aminés et en acides gras de la chenille d'*Imbrasia epimethea* consommée dans la zone Nord de la République du Congo

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**Résumé :** Les chenilles d'*Imbrasia epimethea* représentent un met apprécié par de nombreuses populations de la zone septentrionale de la République du Congo. La présente étude visait à évaluer le profil en acides aminés (AA) et en acides gras (AG) de la chenille *Imbrasia epimethea*. Cette chenille est univoltine et polyphage. Ses plantes-hôtes les plus connues sont *Ricinodendron heudelotii*, *Petertianthus macrocarpus* et *Pycnanthus angolensis*. Cent cinquante larves de dernier stade ont été récoltées dans la nature, ramenées au laboratoire et déshydratées à 65°C pendant 72 heures puis incinérées à l'étuve à 550°C. Les matières sèches de ces larves obtenues ont été analysées par les méthodes de Kjeldal pour les protéines et Folch pour les lipides. Les résultats obtenus montrent des taux importants de protéines et de lipides totaux évalués respectivement à 70.63 et 15.22%. Ils révèlent en outre, la présence en quantité appréciable de tous les AA essentiels dont la thréonine, le tryptophane et les AA aromatiques. Les lipides sont riches en AG polyinsaturés : acide linoléique C18 : 2 ω6 (8.67%) et en acide α-linolénique C18 : 3 ω3 (42.63%). L'ensemble des résultats obtenus montre que les chenilles d'*Imbrasia epimethea* sont une source importante en nutriments, riche en protéines animales et très appréciables susceptibles de contribuer au développement harmonieux de l'organisme humain. Les résultats de cette étude démontrent donc à suffisance que la chenille *I. epimethea*, est un produit forestier non ligneux de grande valeur dans la sécurité alimentaire et que sa gestion durable doit être assurée par les différents services environnementaux de la forêt.

**Mots clés :** *Imbrasia epimethea*. Chenilles comestibles. Analyse des aliments. Composition des aliments. Acide aminé. Acide gras. Valeur nutritionnelle. République du Congo.

**Abstract:** *Imbrasia epimethea* caterpillars represent a delicacy appreciated by many populations in the northern part of the Republic of the Congo. The present study aimed to evaluate the amino acid (AA) and fatty acid (FA) profile of *Imbrasia epimethea* caterpillar. This caterpillar is univoltine and polyphagous. Its host plants most well-known are *Ricinodendron heudelotii*, *Petertianthus macrocarpus* and *Pycnanthus angolensis*. One hundred and fifty last instar larvae were collected in the wild, brought to the laboratory and dehydrated at 65°C for 72 hours, then incinerated in an oven at 550°C. The dry matter of these larvae obtained was analysed by Kjeldal methods for proteins and Folch for lipids. The results obtained show significant levels of total protein and lipids, evaluated at 70.63 and 15.22% respectively. They also reveal the presence in appreciable quantities of all the essential AA, including threonine, tryptophan and aromatic. The lipids are rich in polyunsaturated fatty acids PUFAs: linoleic acid C18: 2 ω6 (8.67%) and α-linolenic acid C18: 3 ω3 (42.63%). The overall results obtained show that *Imbrasia epimethea* caterpillars are an important source of nutrients, rich in animal proteins and very appreciable that can contribute to harmonious development of human body. The results of this study therefore provide sufficient evidence that the *I. epimethea* caterpillar is a valuable non-timber forest product NTFP for food security and its sustainable management should be ensured by the various forest environmental services.

**Keywords:** *Imbrasia epimethea*, Edible caterpillars, Food analysis, Food composition, Amino acid, Fatty acid, Nutritional value, Republic of the Congo.

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## INTRODUCTION

The world's population is expected to reach 10 billion by 2050 (UN, 2019). This increase leads to a global food demand of up to 70% compared to the current needs (ZIELIŃSKA et al., 2015; FAO, 2017), yet food security based on three pillars: food availability, food accessibility and food utilisation (DUBE et al., 2013). Today, approximately 795 million people are undernourished in the world, with nearly 220 million people in sub-Saharan African countries (FAO-IFAD-WFP, 2015).

In the Republic of the Congo, the problem of malnutrition inherent in food insecurity is acute (FAO, 2013). Indeed, Congo is heavily dependent on external sources of animal products: 95.2% for meat; 99.6% for poultry; 86.7% for eggs and 100% for milk and dairy products (FAO, 2013). The Congolese diet has a protein deficit (9% of the total energy intake instead of 12 to 15% recommended); this deficit is more important for animal proteins, which provide only 37.3% against 62.7% for vegetable proteins. It turns out that animal proteins have a high biological value. Fishing and livestock farming are unable to make up this protein deficit. There is therefore a need to resort to other alternative solutions. The alternative resource must allow for a sustainable, culturally and economically accessible diet with good nutritional value. It should provide sufficient amounts of macronutrients and micronutrients, sufficient amounts of all essential AAs according to the reference protein (WHO/FAO/UNU, 2007), vitamins, minerals, essential FAs and dietary fibre.

Insects are a good alternative resource with protein levels fit above those of conventional meat (15-81% vs. 13-28%) (LAVALETTE, 2013 a, b). They supplement the diets of about 2 billion people and have always been part of the human diet. However, it is only recently that entomophagy has captured the attention of the media, research institutes, chefs and other members of the food industry, legislators and other institutions concerned with food and feed (HALLOTAN & VANTOMME, 2013). Insects are an obvious alternative source recommended by the FAO (VAN HUIS, 2013). Eloquent proof is provided by the recent creation, in 2014, of a new Journal, namely "Journal of Insects as Food and Feed" (MABOSSY-MOBOUNA & MALAISSE, 2020). Insect proteins are of high biological value: all essential AAs in appreciable quantities including threonine, tryptophan and aromatic AAs. They contain abundant reserves of lysine, a cereals-limiting amino acid (DEFOLIART, 1992) and are richer in isoleucine, leucine, valine, tyrosine and alanine than beef proteins (FINKE, 2002). Their leucine/isoleucine and leucine/lysine ratios are favourable and would not pose any problem of imbalance in essential AA. Indeed, their leucine/lysine ratio of less than 4.6 is considered nutritionally correct (DEOSTHALE et al., 1970). The ratio of the sum of essential AA to the sum of total AA in the protein is greater than 33% (BLANKERSHIP & ALFORD, 1983). Insect proteins have a good chemical balance between its different AAs. The AAs derived from the proteins of most insects have higher contents than the AAs in poultry feed supplement formulations (BUKKENS, 2005). The digestibility of insect proteins is higher than that of legume grains such as *Phaseolus vulgaris* (78.5%), *Lentil esculenta* (80.3%), *Cajanus cajan* (59.9) (HSU et al., 1977; MBA, 1986; OSHODI et al., 1999). As well as fishmeal (74.7%) and casein (78.4%) (ALETOR, 2012). Insects contain low levels of anti-nutritional substances (AJAYI, 2012). The digestibility and bioavailability of AAs are factors that determine the nutritional quality of a protein. Worldwide, intakes of PUFAs are well below those recommended (MICHA et al., 2015).

Their fats are rich in PUFAs (linoleic acid C18:2 n-6 and  $\alpha$ -linolenic acid C18:3 n-3) (MABOSSY-MOBOUNA et al., 2017; OMBENI et al., 2018). The high level of omega-3 acids is beneficial; it is a positive factor in a diet where the omega-6/omega-3 ratio is often high. The consumption of insects certainly helps to restore a better balance between the various PUFAs. They are particularly important as food supplements for undernourished children, as most insect species are rich in these fats. In addition, these FAs have favourable effects on improving blood glucose, insulin resistance and insulin secretion compared to carbohydrates, SFAs or MUFAs (IMAMURA et al., 2016).

In the Republic of the Congo, edible insects constitute an important food and economic component (LENGA et al., 2012; MABOSSY-MOBOUNA et al., 2017; MABOSSY-MOBOUNA & MALAISSE, 2020). Among these insects, the *Imbrasia epimethea* caterpillars represent a delicacy appreciated by many populations in the northern part of the Republic of the Congo. However, their AA and FA profile is not known. Indeed, it is already known that the nutritional value of insects depends on their life stage (metamorphic stage), their habitat and their diet (VAN HUIS, 2013). This observation poses the problem of knowing the AA and FA profile of *Imbrasia epimethea* caterpillars collected in the localities of Northern Congo.

## MATERIALS AND METHODS

**Field material:** Five litre jars, covered with 2 mm mesh, were used to store the caterpillars collected from the wild. The Samsung camera A20KC9AC90119A was used for taking images.

**Biological material:** One hundred and fifty *Imbrasia epimethea* caterpillars of stage 5 (Photo 1), weighing 1,200g were collected in the dense equatorial forest field, Likouala department in Northern Congo on 18 August 2017 and airlifted to Brazzaville.



**Photo 1.-** Fresh *Imbrasia epimethea* caterpillars on last stage called “kukula” or “nkukuka” in Sangha and Likouala Departments. © Germain MABOSSY-MOBOUNA, Photo taken on 18<sup>th</sup> August 2017 at Ipendja Thanry (Republic of the Congo)..



**Photo 2.-** *Imbrasia epimethea* caterpillars on *Petertianthus macrocarpus*, caterpillar of penultimate stage, called “beta”, “mbèmbo” or “matèmbeo” in Sangha and Likouala Departments, © Paul LATHAM, Photo taken on 27<sup>th</sup> March 2002 at Ndembo Mission (Democratic Republic of Congo).

**Analysis methods:** The collected caterpillars were analyzed at the Laboratory of General and Organic Chemistry at Gembloux Agro-Bio Tech of the Liège University.

### **Protein analysis**

Crude protein was determined by Kjeldahl method. The total nitrogen content (TNC) or total crude protein (TCP) was determined by multiplying the nitrogen content obtained with a Kjeltac automatic analyser by 6.25 (method 981.10; AOAC, 1990). The AA obtained after hydrolysis was determined by the method of MOORE et al. (1958) on a Biochrom 20 Alpha Plus type instrument. The non-sulphur AAs were quantified after 24 hours of acid hydrolysis at 110°C, under nitrogen, in 6N HCl containing 0.1% phenol. However, due to their instability in hydrochloric acid, the sulphur-containing AAs (cysteine and methionine) were determined after oxidation with performic acid (LEWIS, 1966) (transformation into cysteic acid and methionine sulphone respectively) and 24-hour acid hydrolysis at 110°C in 6N HCl containing 0.1% phenol. Tryptophan was determined separately after a 15-hour hydrolysis at 110°C, under nitrogen, in a solution of Ba(OH)2.8H2O at 8.4 g/16 ml according to LETERME & MONMART (1990).

The chemical indices were calculated on FAO/UNU/WHO (2007) basis data. According to NGUDI et al. (2003), this calculation gives a correct prediction of the amount of protein needed to cover the requirements of essential AAs during the growing period.

The chemical index of an AA was calculated using the formula below:

$$\text{Chemical Index} = \frac{\text{mg of AA in 1g of protein analyzed}}{\text{mg of AA in 1g of reference protein}} \times 100$$

The AA with the lowest index is the limiting AA of the studied protein.

### **Lipid analysis**

After grinding, lipids were extracted with chloroform/methanol (2:1 v/v) according to FOLCH et al. (1957) method and then concentrated by evaporation at 35°C under reduced pressure using a Büchi type evaporator and weighed. The fatty acids methyl esters (FAME) were prepared by catalysed transesterification by boron trifluoride (10 mg of sample was placed in soviel tubes, diluted in 200µl of n-hexane and then reacted with 0.5 ml of a 20:55:25 mixture of n-hexane, dry methanol and 14% BF<sub>3</sub> methanol for 90 min at 70°C. After reaction, 200 µl of 10% w/v sulphuric acid and 500 µl of a saturated sodium chloride solution were added. The resulting solutions containing FAMES were finally diluted with 8ml of n-hexane and analysed by gas chromatography on an Agilent 6890 chromatograph equipped with a cold on column injector and an FID detector maintained at 260°C according to the following temperature program: from 55°C to 150°C (30°C/min) then from 150°C to 250°C (5°C/min). Helium was used as a carrier gas at a flow rate of 1.7 ml/min. FAMES were separated on a Varian CP9205 VF-Wax ms column (30 m length x 0.25 mm inner diameter. df = 0.25 µm) and identified by comparing retention times with those of a reference solution containing 37 FAMES (CRM47885 Supelco 37 Component FAME Mix). The results are expressed as area % with a response factor of 1 for each molecule.

The iodine index values were calculated according to AOCS (1998) formula:

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

with n= number of double bonds in the FA; MM = molecular mass of FA ester and %UFA= percentage of UFA.

This index makes possible to determine the overall unsaturation degree of lipids in a food. It corresponds to the quantity in grams of iodine fixed per 100g of fat.

### **Statistical data processing and analysis**

Data processing was carried out with SPSS version 20 and Excel 2013 software. The raw tables were entered and produced with SPSS and Excel software. The quantitative variables are expressed as an average (x) ± standard deviation (s) while indicating the extreme values (minimum and maximum).

## **RESULTS**

### **Overall protein and lipid composition**

It was found that *Imbrasia epimethea* caterpillars are very rich in protein (68.69%) and lipids (8.8%) (Table 1).

**Table 1:** Chemical composition of dried *Imbrasia epimethea* caterpillars

Overall chemical composition	<i>Imbrasia epimethea</i>
Total nitrogen	10.67
Proteins	68.69
Total fats	8.80

**Profile in amino acids of three samples of *I. epimethea* caterpillars and evaluation of the ratio between the sum of essential AA and the sum of total AA.**

The proteins of *Imbrasia epimethea* caterpillars contain all essential AAs in appreciable amounts. The aromatic AAs (tyrosine + phenylalanine), lysine, leucine and threonine are major AAs. The sulphur AAs (methionine + cysteine) and tryptophan are the least represented. The ratio of the sum of essential AAs to the sum of total AAs in the proteins of these caterpillars shows a value of over 33%.

**Table 2:** Composition in amino acids of *Imbrasia epimethea* caterpillars

Amino-acids	<i>Imbrasia epimethea</i>	
	g/100g DM	g/16g N
Aspartic acid	3.843 ± 0.05	4.39 ± 0.06
Threonine	2.341 ± 0.02	2.68 ± 0.02
Serine	2.496 ± 0.006	2.86 ± 0.007
Glutamic acid	5.010 ± 0.04	5.73 ± 0.05
Proline	2.712 ± 0.12	3.10 ± 0.14
Glycine	2.227 ± 0.001	2.55 ± 0.007
Alanine	2.424 ± 0.02	2.77 ± 0.03
Valine	2.347 ± 0.008	2.68 ± 0.014
Methionine	0.791 ± 0.03	0.91 ± 0.04
Isoleucine	2.038 ± 0.02	2.33 ± 0.03
Leucine	2.745 ± 0.03	3.14 ± 0.04
Tyrosine	3.033 ± 0.001	3.46 ± 0.014
Phenylalanine	2.324 ± 0.03	2.66 ± 0.03
Histidine	1.317 ± 0.02	1.51 ± 0.02
Lysine	2.943 ± 0.005	3.37 ± 0.007
Arginine	2.448 ± 0.05	2.85 ± 0.05
Tryptophane	0.866 ± 0.02	0.99
Total	41.94 ± 0.4	47.94 ± 0.53
Sum of essentials AA		23.73
Ratio (in %)		49.50

However, compared to the reference protein composition (Table 3), these proteins have a lot of limiting AA because their chemical indices are below 100%. This is the case for valine, Sulphur AA: leucine, isoleucine and lysine. Leucine is the primary limiting factor with a chemical index of 53.22. Sulphur AAs constitute the second limiting factor. Overall, these AAs have chemical indices above 50%. In addition, these proteins contain AAs with chemical indices above 100%. These are threonine, aromatic amino acids and tryptophan.

**Table 3:** *Imbrasia epimethea* chemical index based on FAO/UNU/WHO (2007) values.

Essential amino acids	Reference proteins FAO/UNU/OMS (g/100g proteins)	<i>Imbrasia epimethea</i> chemical index
Threonine	2.3	116.52
Valine	3.9	68.71
Methionine + Cysteine	2.2	56.56
Isoleucine	3	77.66
Leucine	5.9	53.22
Tyrosine + Phenylalanine	3.8	161.05
Histidine	1.5	100.66
Lysine	4.5	74.88
Tryptophane	0.6	165

Table 4 shows the fatty acids composition and iodine index of *Imbrasia epimethea* caterpillars analysed.

Relating to the fatty acids' composition (Table 4), *Imbrasia epimethea* caterpillars have high contents of  $\alpha$ -linolenic, palmitic and stearic acids. These are followed by oleic and linoleic acids. Myristic acid, arachidic acid and arachidonic acid have very low contents. Concerning the degree of saturation, these caterpillars contain more PUFA (45.07%) followed by SFA (32.22%). The fat content of these caterpillars is above 50% with a higher content of essential fatty acids (C18:3n 3 and C18:2n 6). The fats extracted from *Imbrasia epimethea* caterpillars are therefore characterised by high levels of both  $\alpha$ -linolenic acid (36.14%) and linoleic acid (8.63%). Arachidonic acid is present in small quantities (about 0.30%).

**Table 4:** Fatty acid composition of *Imbrasia epimethea* caterpillars (in % mass of fat) and iodine indices

Fatty acids	Mass of molecular ions (FAME)	<i>Imbrasia epimethea</i>
Myristic acid (C14:0)	242	0.18
Pentadecanoic acid (C15:0)	256	0.13
Pentadecaenoic acid (C15:1)	254	0.18 $\pm$ 0.007
Palmitic acid (C16:0)	270	12.38 $\pm$ 0.16
Hexadecaenoic acid (C16 :1)	268	0.12 $\pm$ 0.007
Heptadecanoic acid (C17:0)	284	0.68 $\pm$ 0.021
Heptadecaenoic acid (C17:1)	282	0.14
Stearic acid (C18:0)	298	18.45 $\pm$ 0.14

Oleic acid (C18:1 n-9)	296	4.64 ± 0.035
Linoleic acid (C18:2 n-6)	294	8.63 ± 0.14
Alpha linolenic acid (C18:3 n-3)	292	36.14 ± 0.13
Arachidic acid (C20:0)	326	0.39
Gadoleic acid (C20:1)	324	0.11 ± 0.02
Arachidonic acid (C20:4 n-6)	318	0.30 ± 0.007
Saturated fatty acids (SFA)		32.22 ± 0.32
Monounsaturated fatty acids (MSFA)		5.21 ± 0.014
Polyunsaturated fatty acids (PUFA)		45.07 ± 0.3
Total		82.50 ± 0.063
Others (not identified)		17.49 ± 0.06
Iodine index (calculated)		<b>114.668</b>
Omega-6 (ω6)		8.93
Omega-3 (ω3)		36.14
Ratio ω6/ω3		0.25
Ratio PUFA/SFA		1.40

The iodine value is high (114.668), which justifies the high level of UFAs (50.27%) compared to the proportion of SFA (32.22%).

The ratio ω6/ω3 of their fat is equal to 1/5. It is therefore rich in linolenic acid (C18-3 ω3), which is good from a nutritional point of view for improving the ω6/ω3 ratio. The PUFA/SFA ratio is higher than 1.

## DISCUSSION AND CONCLUSION

Caterpillars are an important source of proteins. The most appreciated species vary according to countries. Just as example, *Bunaeopsis aurantica* are welcome in South Kivu Province of the Democratic Republic of Congo (MUVUNDJA et al., 2013 ; OMBENI et al., 2018), whilst *Imbrasia epimethea* caterpillars are an important source of animal protein (68.69%) in Republic of the Congo. The protein content comparison of these caterpillars to other consumed caterpillars and popular foods like fish and meat shows that they have a similar protein content to *Imbrasia obscura* (MABOSSY-MOBOUNA et al., 2018), *Imbrasia truncata* (MABOSSY-MOBOUNA et al., 2017) and *Hadraphe ethiopica* caterpillars (MALAISSE et al., 2003). They are richer in protein than *Bunaeopsis aurantiaca* (49%) (MUVUNDJA et al., 2013; OMBENI et al., 2018) and *Antheua insignata* caterpillars (61%) (MALAISSE et al., 2003), beef (18.2%) and fresh (18.3%) or dried and salted fish (47.3%) (WU LEUNG et al., 1970).

The protein amount in *Imbrasia epimethea* caterpillars in this study (68.69%) is similar to that obtained (68.6± 1.6% DM) by FOGANG MBA et al. (2019) on the same caterpillar species in Cameroon and JANSEN et al. (2017) on *Alphitobius diaperinus* larvae (63.8± 0.3%). But it is higher than that of dried *Imbrasia oyemensis* caterpillar flour (57.77%) sold at Adjamé market in Abidjan (AKPOSSAN et al., 2009) and that of winged *Macrotermes subhyalinus* termite flour (38.20%) captured during its nuptial flight at the swarming in Abobo-Doumé in Côte d'Ivoire (NIABA KOFFI et al., 2013). This quantity is also higher than that of *Macrotermes bellicosus* (38.36%) as well as those of *Oryctes rhinoceros* larvae (30.15%) and *Rhynchophorus phoenicis* (22.06%) obtained by EKPO et al. (2009). Similarly, the protein content of these larvae obtained by LENGA et al. (2012) is lower than that of *Imbrasia epimethea* caterpillars, 21.21% for *Rhynchophorus phoenicis* and 42.66% for *Oryctes rhinoceros*. *Imbrasia epimethea* caterpillars have a higher protein content than *Cirina forda* caterpillars (51.43-52.39%) obtained by BADANARO et al. (2014) and those obtained by JANSEN et al. (2017) from *Tenebrio molitor* (58.8 ± 0.2%) and *Hermetia illucens* (48.1 ± 0.4%) larvae. This protein content is in agreement with that of FAO (2004) who noted a high protein content in caterpillars favouring their

incorporation in low protein flours in order to fight against child malnutrition. The protein content of *Imbrasia epimehea* caterpillars studied is higher than that obtained by KODONDI et al. (1987), ADRIAENS (1953) and MALAISSE & PARENT (1980) which are respectively 58.1%, 64.5%, 65.9%. But lower than those obtained by LAUTENSCHLÄGER et al. (2017) which is 73.1%. This difference could be due either to the botanical host species of these caterpillars, which is not the same, as this caterpillar is polyphagous, its best-known hosts being *Ricinodendron heudelotii*, *Petertianthus macrocarpus* and *Pycnanthus angolensis*, or to the developmental stage of the caterpillars analysed (RUMPOLD & SCHLÜTER, 2013), or to the difference in the methods used by laboratories to analyse these compounds. Thus, our study shows that *Imbrasia epimethea* caterpillars rank among the most protein-rich foods.

The result on amino-acids profile indicates that all essential AAs are present in the studied caterpillar. The AAs with the highest concentrations in these caterpillars are tyrosine, lysine, leucine and threonine; the essential AAs with the lowest levels are: sulphur AAs and tryptophan. These results are in agreement with those of DEFOLIART (1992) who considers that insect proteins tend to be low in particular AA, such as methionine and cysteine, and contain many other types, particularly lysine and threonine. As lysine is deficient in cereal proteins (SOLTENER, 2021), these caterpillars can be incorporated into maize porridges, given to infants in some Congolese households, to enrich them with lysine.

These caterpillars have a high arginine content which, according to VOET et al. (2006), is essential for children growth as it promotes collagen formation and osteoblasts growth from which real bone mass can develop (WILLIAMS et al., 2002). To make up for the sulphur AAs deficiency (methionine and cysteine) in *Imbrasia epimethea* caterpillars, it is recommended that they be combined with cereals such as maize, as the MOSSÉ (1990) work shown that cereals are rich in sulphur AAs. The essential AAs profile we obtained is different from the one obtained by AKPOSSAN et al., (2014) on *Imbrasia oyemensis* caterpillars and OMBENI et al., (2018) on *Bunaeopsis aurantiaca* caterpillars where they noted a high lysine content followed by leucine and valine.

According to DA SILVA et al. (2006), it is essential to take into account the essential AAs in a balanced proportion, as an excess of leucine in food interferes with the use of isoleucine (HARPER et al., 1955). The work of DEOSTHALE et al. (1970) showed that a leucine/lysine ratio of less than 4.6 is considered nutritionally correct. In our study, this ratio is 1.31 and the leucine/isoleucine ratio is 1.96. The protein from *Imbrasia epimethea* caterpillars therefore has favourable leucine/isoleucine and leucine/lysine ratios and would not pose any problem of essential AA imbalance. These results are comparable to those obtained by NIABA et al. (2011) in their study on *Macrotermes subhyalinus* meal and by FOUA BI et al. (2015) on *Imbrasia oyemensis* caterpillar powder.

Essential AAs represent 49.50g out of 100g of total AAs. This ratio is slightly lower than that obtained by FOGANG MBA et al. (2019) on the same caterpillar species (51.3g/100g of total AA). This ratio is higher than 33%, reflecting a chemical balance between these different AA (BLANKERSHIP & ALFORD, 1983). However, compared to the reference protein composition (FAO/UNU/WHO, 2007), the protein of *Imbrasia epimethea* caterpillars in this study contains many limiting AAs, as their chemical indices are below 100. This is the case for valine (68.71), sulphur AAs (56.56), leucine (53.22) and lysine (74.88). The percentage deficit is 31.29% for valine, 43.44% for sulphur AA, 46.78% for leucine and 25.02% for lysine. Leucine is the primary limiting factor with a chemical index of 53.22 and a percentage deficit of 31.29%; sulphur-containing AA is the second limiting factor. This result differs from that of KODONDI et al. (1987) who showed only one limiting amino acid, isoleucine, for the protein of *Imbrasia epimethea* caterpillars. Overall, these AAs have chemical indices above 50. The chemical index of the *Imbrasia epimethea* caterpillar protein is therefore 53.22. This is lower than that of *Hadraphe ethiopica* caterpillars, which varies between 69.9 and 87.2 (MALAISSE et al., 2003). Furthermore, these proteins contain AAs with chemical indices higher than 100, namely threonine (116.52), aromatic AAs (161.05) and tryptophan (165). According to NGUDI et al. (2003), the calculation of the chemical index of each essential amino acid allows a correct precision of the quantity of protein necessary to cover the needs in essential AA during the growth period. Thus, *Imbrasia epimethea* caterpillars can be used to supplement tryptophan-poor feeds such as chicken, dried fish and shellfish (ADRIAN et al., 1995) with threonine and aromatic AAs. Therefore, these caterpillars must be supplemented in the dish with foods that will provide 1.13g of lysine, 2.76g of leucine, 1.29g of methionine and 1.22g of valine per 100g of protein to have a chemical index of 100. However, 178g of *Imbrasia epimethea* caterpillar protein is sufficient to cover the daily requirement of essential AAs as recommended by the joint FAO/UNU/WHO (2007) commission.

The fat content of *Imbrasia epimethea* caterpillars analysed in this study was 8.8%. This result is similar to that obtained by ADRIAENS (1953) on the same caterpillar species which is 9.1%. It is lower than those obtained by KODONDI (1987), MALAISSE & PARENT (1980), and FOGANG MBA et al. (2019) which are respectively 14.2%, 12.4% and 22.8±0.8%; higher than that obtained by LAUTENSCHLÄGER et al. (2017) which is 5.9%. Furthermore, this amount is lower than that of most foods such as lean mutton, veal tongue, camembert-type cheese, avocado, chicken meat, mackerel, fresh eggs, most dairy products (women's milk, liquid

whole cow's milk, yoghurt and others) and lean beef (ADRIAN et al, 1995) and is very high compared to fresh or dried and salted fish (WU LEUNG et al., 1970).

However, these caterpillars are less rich in fats than *Oryctes rhinoceros* (28.85%) and *Rhynchophorus phoenicis* (28.85%) larvae studied by LENGA et al. (2012), and *Tenebrio molitor*, *Alphitobius diaperinus* and *Hermetia illucens* larvae studied by JANSEN et al. (2017) which ranged from 21 to 24%. They are also less rich in fats than *Macrotermes subhyalinus* meal, whose fat content was estimated at 46.3% by NIABA et al. (2011). Nevertheless, this fat amount is equivalent to an energy value of 136.68kcal and represents 31.73% of the total energy intake of this food, which is within the normal range of the recommended value for fats of 30-35% (EFSA, 2004).

The iodine index of this fat, evaluated at 114.668, is similar to that of soybean (*Glycine max*) and sunflower (*Helianthus annuus*), but higher than those of rapeseed (*Brassica campestris*), cotton (*Gossypium* sp.) and maize (*Zea mays*) reported by ADRIAN et al. (1995). This index reflects a high fat content of *Imbrasia truncata* caterpillars in UFA. Indeed, the higher the iodine value of a figure, the higher its UFA content (ALAIS & LINDEN, 1997). Unsaturated fats are present in significant quantities in this fat (50.28%). This level of unsaturated fat is lower than that of winged *Macrotermes subhyalinus* meal which is 55.84% (NIABA et al., 2011).

The fat of *Imbrasia epimethea* caterpillars is rich in  $\alpha$ -linolenic acid (36.14%), which is good from a nutritional point of view for improving the  $\omega 6/\omega 3$  ratio. In addition, a high level of n-3 PUFA has suppressive effects on the pathogenesis of many diseases such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases (SIMOPOLUS, 2002). The presence of  $\alpha$ -linolenic acid in sn-2 position in TFAs is of great importance in nutrition on their bioavailability (GUIL-GUERRERO et al., 2018). This material also contains quite significant amounts of linoleic acid (8.63%) and oleic acid (4.64%). These results are almost similar to those obtained by KODONDI et al. (1987) and recently by OMBENI et al. (2018) on *Bunaeopsis aurantiaca* caterpillar commonly consumed and vended on local markets of Bukavu town, in South Kivu province, DR Congo.

Linoleic acid is responsible for cardiovascular and immune balance, and acts on cholesterol regulation (DEMAISON & MOREAU, 2002). Oleic acid has favourable effects on health by increasing "good" cholesterol and is relatively insensitive to oxidation (FAO/OMS, 1993), which guarantees good stability during frying and cooking (NZIKOU et al., 2010).

The SFA content of studied caterpillars is lower (32.22%) than that obtained by PAGEZY (1988) (59.8%). Stearic acid is more abundant (18.45%) followed by palmitic acid (12.38%). Palmitic acid is a fatty acid known to be atherogenic. Nevertheless, the action of this acid could be mitigated by the simultaneous presence of stearic acid and oleic acid. Indeed, these two acids each contribute to HDL-cholesterol increase (FAO/WHO, 1993; VANIER & JOSIANE, 2006).

The major fatty acids profile of *Imbrasia epimethea* caterpillars studied (C18:3>C18:0>C16:0>C18:2>C18:1) is similar to the one obtained by FOGANG MBA et al. (2019) on caterpillars of the same species, but, it is different from that obtained by LENGA et al. (2012) for *Rhynchophorus phoenicis* larvae (C18:1>C16:0>C18:0>C18:2>C18:3) and *Oryctes rhinoceros* larvae (C18:1>C16:0>C18:2>C18:0>C18:3>C14:0) and by NIABA et al. (2011) for winged *Macrotermes subhyalinus* flour (C18:1>C16:0>C18:0>C18:2).

The PUFA/SFA ratio equal to 1.40 indicates a very high nutritional value of *Imbrasia epimethea* caterpillars in terms of fats (LEGRAND, 2010). This ratio >0.20 is associated with a low cholesterol level and a low risk of coronary heart disease (MABOSSY-MOBOUNA et al., 2020; KINYURU et al., 2013). It is almost similar to that obtained by FOGANG MBA et al. (2019) on caterpillars of the same species. These caterpillars have a good nutritional value in terms of fats than *Rhynchophorus phoenicis* and *Oryctes rhinoceros* larvae (LENGA et al., 2012) and *Cephalophus monticola* meat (MANANGA et al., 2015). Their fat has almost the same nutritional value as pork fat (WOOD & ENSER, 1997; MOUROT, 2001) and fish fat (KINKELA & BEZARD, 1993) whose PUFA/SFA ratio is equal to 1.

The ratio  $\omega 6/\omega 3$  of caterpillars in this study is less than 1 (0.25). This result is similar to the one obtained by FOGANG MBA et al. (2019) on caterpillars of the same species which was 0.3. It corroborates the results of GUIL-GUERRERO et al. (2018) who had lower ratios for 12 Lepidoptera larvae analysed. It is different from the result obtained by FOUA BI et al. (2015) on *Imbrasia oyemensis* caterpillar powder which is 8.02. This ratio is lower than the optimal value proposed by ANSES (2015). Thus, *Imbrasia epimethea* caterpillars should be consumed with  $\omega 6$ -rich foods such as *Imbrasia oyemensis* caterpillars to optimise this ratio

in the range of 1 to 4. They are therefore balancing foods because they correct a  $\omega 6/\omega 3$  ratio that is too high in the usual diet (BOURRE, 2004).

The results of this work show that *Imbrasia epimethea* caterpillars are rich in protein and fats. They contain all the essential amino acids and fatty acids in suitable proportions ( $\alpha$ -linolenic acid and linoleic acid) with a  $\omega 6/\omega 3$  ratio of less than 1 and an iodine index of over 100. Knowledge of the amino acids and fatty acids profile of these caterpillars will make it possible to improve the nutritional value of population food ration suffering from various nutritional deficiencies and of vulnerable people by supplementation. The incorporation of these caterpillars in powder form into local foods that are poor in nutritional nutrients in order to overcome cultural constraints should make it possible to offer them to children and adults for their appreciable nutritional value.

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