IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 15, Issue 9 Ser. II (September 2021), PP 35-46
www.iosrjournals.org

Effect of culinary treatments on the nutritional and microbiological properties of *Macrotermes subhyalinus* and *Imbrasia obscura*: two insects consumed in the Adamawa and Est regions of Cameroon

DJOUFFA KOUABITHEU Marivaux Lesage[2]
SAÏDOU Clément [1*]
MOHAMMADOU Bouba Adji [1]
ALI Ahmed [1]

1 University of Ngaoundere, University Institute of Technology ; P.O. BOX, 454 Ngaoundere-Cameroon
[2] University of Ngaoundere, National School of Agro-Industrial Sciences ; P.O. BOX, 455 Ngaoundere-Cameroon
**Corresponding author: saidouclement2015@gmail.com*

Abstract:
The effects of various culinary methods notably scalding, frying, grilling, roasting, and drying of Macrotermes subhyalinus and Imbrasia obscura on the physicochemical and microbiological characteristics and the digestibility of their proteins were studied for the two insects consumed in Adamawa and East regions of Cameroon. The fat content of fried samples significantly ($p < 0.05$) increased due to absorption of oil from the processing medium. It was not the case with the samples cooked using other methods. The comparison of results of raw and cooked samples showed that with respect to the cooking method, there is significant increase or reduction ($p < 0.05$) of the contents of nutrients contents analysed. The variation of dry mater, proteins and ash contents were revealed to be important based on all the cooking methods. The thermal treatments enabled total or partial reduction of the microbial load to acceptable values. The digestibility of crude samples proteins of (M. subhyalinus (86.65%) and I. obscura (85.20%)) reduced with the type of thermal treatment. Frying and toasting on one hand for M. subhyalinus then boiling and the combination of boiling and drying on the other hand for I. obscura are the best cooking methods for healthy eating. Nevertheless, out of the different processing unit operations involved in insects preparation, cooking seems to be the one which highly influences proteins digestion as well as microbiological parameters.

Keyword*: culinary treatments, physicochemical characteristics, digestibility, entomophagy, Imbrasia obscura, Macrotermes Subhyalinus, Cameroon*

Date of Submission: 01-09-2021 Date of Acceptance: 15-09-2021

I. Introduction

In Africa, several insect species have been used as traditional food within autochthonous people. They contribute to subsistence means and for food security in households **(1).** Surveys conducted in the Adamawa and East regions of Cameroon contributed to show that insects are part of the food habits of most populations among which *Macrotermes subhyalinus* and *Imbrasia obscura* (**2**). However, contamination of insects by undesired microorganisms is a consequence of combination of substrates, insect species, collection, environmental sites, and steps of treatments applied **(2,3).** The culinary treatment represents a defence line against potential dangers. It stops deterioration processes, as such, improve the quality of the product and minimises losses. Thus, recent works **(3,4,5)** concluded that it is necessary to revise and normalise the processing methods in order to guarantee the security and nutritional values of insects**.** The methods were identified in the course of the survey conducted in the Adamawa and Est regions of Cameroon (**2)**. A significant reduction of microbiological dangers can be obtained by approaches like thermal treatment, but some procedures may not attain adequate or required results and consequently have an impact on the chemical composition and the digestibility of proteins **(6).** Evaluation and validation of these methods based on traditional knowledge may be a starting point to develop and implement food security mechanisms especially in the above mention regions where insect collection can have more impact on securing subsistence means. Therefore, the objective of this work is to study the impact of few culinary transformation techniques on the microbial load and nutritional properties of insects obtained.

II. Materials And Methods

II.1. Sources and collection of insects

M. subhyalinus and *I. obscura* sample whose images are visible on figure 1 were collected early in the morning in the localities of Ngaoundere 3 (Latitude/Longitude : 7°19'39" N/7° 19' 39 E, Adamawa region of Cameroon) in June 2020 for the first sample, and in Garoua- Boulaï (Latitude/Longitude : 5°53′00″N/14°33′ 00″E, Adamawa region of Cameroon) in July of the same Year.

A **B**

Figure 1: Photographs of *M. subhyalinus* (A) and *I. obscura* (B)

II.2. Preparation of insects

Preparatory methods retained for insects were identified from the population consuming these two insects in Adamawa and East Regions (figure 2 and 3). The raw sample was used as control. As far as the sample of *M. subhyalinus* is concerned, after the selection operation, to remove impurities and the wings, the insects were then washed using distilled water and the following processing techniques were applied.

Toasting: A washed and dried frying pan made of stainless steel was used. The frying pan was put on the flame (gas) at about 100° C and about 250g of raw *M. subhyalinus* were introduced in the hot frying pan and the content toasted at 160 °C for 12 minutes while stirring.

Scalding: A quantity of 250 g of each of the two raw insect samples (*M. subhyalinus* and *I. obscura*) were introduced in a metallic kitchen sieve and immerge in a water bath (w/v de 1/3 at 28.3 °C) and heated for 20 minutes for *I. obscura*, meanwhile *M. subhyalinus* was heated for 15 minutes at 93.8±0,3 °C.

Frying: A cleaned and driednon-oxidable frying pan was used this purpose. Five mL of refined oil was introduced in the frying pan, heated up to 94 °C, and 250g of *M. subhyalinus* (w/v of 1/0.02) was added in the hot fried oil for 12 minutes at 165 °C with constant stirring.

Sun drying: An amount 250g of two insect samples were dried under sun light till constant weight. The process took 3 days for *M. subhyalinus* and 4 days for pour *I. obscura*. The temperature varied between 25 and 35 °C. The samples were considered dried when the weight variation was less than 1% for triplicate determinations in the interval of an hour.

Quantities of 250g of boiled samples of *I. obscura* in the conditions described above was used for additional processing techniques. These samples were spined, and dried till constant weight then fried at 160°C for 10min in 12.5 mL of supplementary refined (vegetable oil), roasted for 12 min in boiling water (90°C) while adding 5 mL of oil.

For each of the products obtained, they were then subdivided into two packaged separately for microbial and physicochemical analyses.

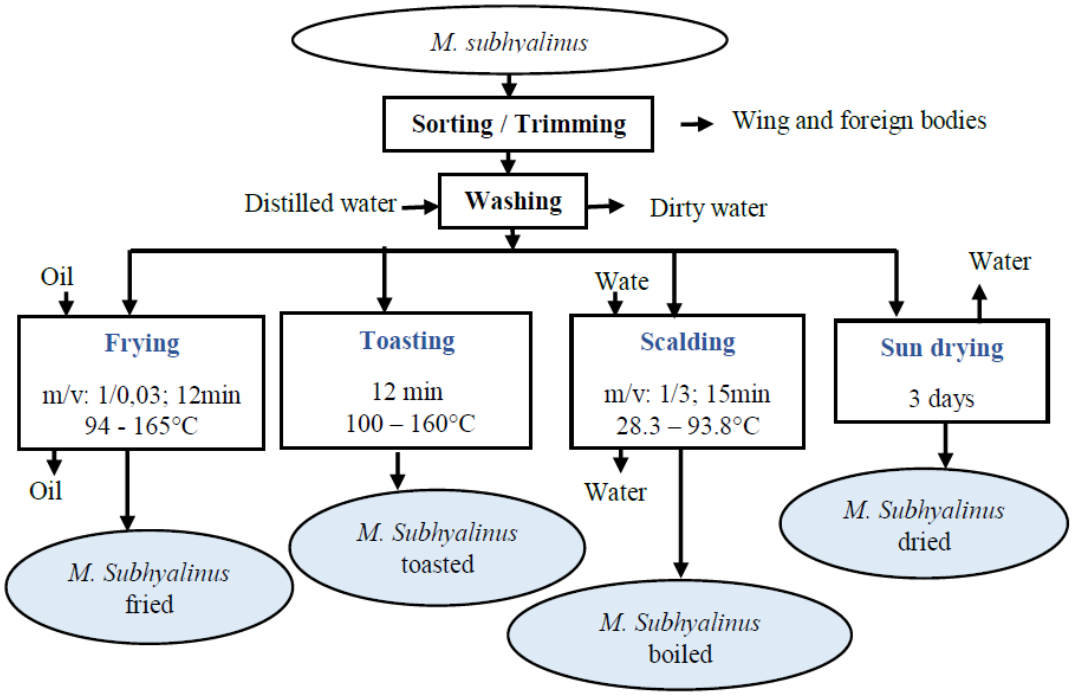

Figure 2 : Culinary treatments of *M. subhyalinus* to cooking methods

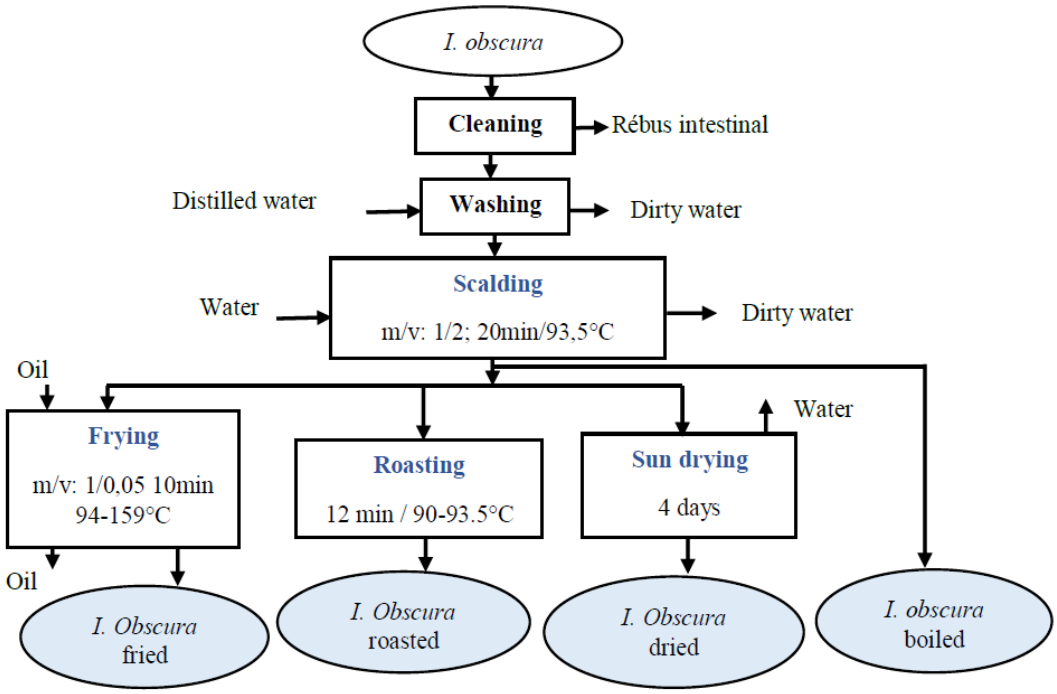

Figure 3 : Culinary treatments of *I. obscura* to cooking methods

II.3. Physicochemical analyses of raw and transformed insects

The determination of dry matter was done with the method described by **AFNOR** (**7**). The total ash content was evaluated by **AOAC** (**8**) method, the total lipid content was determined by the Soxhlet extraction method described by **Bourely** (**9**), while the total nitrogen content was determined after mineralization of the samples according to Kjeldahl method (**10**) was assessed by the colorimetric technique of **Devani *et al.*** (**11**). The protein content was calculated by multiplying the nitrogen content by the conversion factor of nitrogen to proteins (6.25). The total sugar content was computed through differential method by subtracting the percentages of other constituents as reported by **Stadlmayr *et al.*** (**12**): Sugars= 100- (water+proteins+lipids+ ashes). The energetic value was calculated based on the total protein content, fat content and sugars content,

applying conversion factors into energy the formula proposed by Stadlmayr *et al.* (**12**). The *in vitro* digestibility of proteins was determined using the method reported by Hsu *et al.* (13) which is modified from that of Maga *et al.* **(14)**.

II.4. Microbiological analysis of raw and transformed samples

Microbiological analyses carried out were focused on certain microbiological quality indicators for various samples. The search of TAMF on insect samples was done using the method described in the French norm NF V 08-051. Total coliforms (CT) count was done according to the method of the norm NF V 08-051 and the norm NF V 08-060 was exploited for total faecal Coliform count (CF). Moulds and yeasts were assed with the specifications of the norm NF V 08-059. The strict anaerobic sporulated flora was determined based on the norm NF V 08-061 relative to "enumeration in anaerobic condition of sulphide-reducing bacteria through colonies count. *Staphylococcus aureus* was enumerated by the routine normalised method NF V 08-57 partial which technique is confirmed by colonies. Enumeration of positive coagulase *Staphylococcus* was done by colonies count at 37 °C, while *Salmonella* assessment was done by the method NF V 08-52.

II.5. Statistical analyses

Various results obtained for nutritional analyses are means of three replicates and are expressed as mean ± standard deviation. Analysis of variance was used to compare the means. The Duncant multiple range test was used to classify the means that are statistically significant different with the help of Statgraphics Centurion XV.II. software. XLSTAT 2007 and SigmaPlot 11.0. software were used to do the Principal Component Analysis (PCA) and to plot histograms respectively.

III. Results and discussion

III.1. Effect of transformations on the nutritional properties of *M. subhyalinus* and *I. obscura*

Results presented in table 1 show how most comestible insects than *M. subhyalinus* and *I. obscura* are mainly made up of fats (54,25% and 18,67% respectively) and proteins (36,84% and 59,04% respectively) for raw samples. The different culinary treatments significantly ($P<0.05$) influenced on these parameters.

The moisture content (MC) of *M. subhyalinus* and *I. obscura* which undergo diverse transformations vary between 1.40±0.14to 61.74±0.11 g/100g of DM and 7.28±0.02 to 68.41±0.62 g/100g of DM respectively (table1). There is no similarity at 5% probability between the different samples. Scalded sample have higher moisture content than sample which were processed with other treatments. Boiling enhanced humidity absoption of 17.64% and 19.43% for *M. subhyalinus* and *I. obscura* respectively, compared to raw samples. This can be explained by hydration or increase of linkages of water and hydrophilic tissues of the samples. Similar humidity increase has respectively been reported in *Ruspolia differens* and *Spodoptera littoralis*, 17.0% and 17.8% **(15).** The humidity increase was however less important for scalded *Gryllodes sigillatus* **(16)**, and for *A. domesticus* (8.9%) and *Hermetia illucens* (9.9%). Previous research works **(17)** showed differences at the level of hydration properties, and the situation was attributed to the variability of the composition of samples, which influences the number and the linkage strength with water and hydrophilic sites of the material. Toasted sample of *M. subhyalinus* is the one which had the least moisture content, characterised by water loss of 97.33% of crude raw sample. This high loss may be attributed to the cooking temperature, which led to evaporation of water contained in the insect. Drying reduces humidity form hole insects from 90.62 and 87.29% respectively for *M. subhyalinus* and *I. obscura.M. subhyalinus* which absorbed more humidity during scalding had a contrary effect during toasting, frying and drying compared to *I. obscura*. This indicates differences related to hydration properties compared to *I. obscura*.

Proteins contents differed significatively. The proteins content of *M. subhyalinus* presented the following order: Scalded *M. subhyalinus* < raw *M. subhyalinus* < dried *M. subhyalinus* < dried *M. subhyalinus* < toasted *M. subhyalinus* with respective values of 34.20; 36.84; 37.03; 38.57; 38.72 g/100 g of DM. That of *I. obscura* had the following order: scalded/fried *I. obscura* < scalded and toasted *I. obscura* < raw *I. obscura* < scalded *I. obscura* < scalded and dried *I. obscura* with the following 55.53; 57.67; 59.04; 60.10 and 62.16 g/100 g of dry matter. These protein contents of *M. subhyalinus* are in line with those obtained by **Malaisse (18**) which shows that the protein content of flying and matured termites is found between 35% et 42% with respect to the dry matter. However, these values are significantly higher than those of other species of termites, among which dried *M. bellicosus* (20.4%) and dried *Macrotermes notallensis* (22.1%) as reported by **Banjo *et al.* (19)**. Moreover, there is significant difference at 5% probability of the protein content of raw and fried *M. subhyalinus* on one hand, and that between the toasted and dried samples on the other hand. The increase of protein content during drying and toasting is significantly different meanwhile during scalding was a significant decrease ($P<0.05$). As for *I. obscura*, there was a significant difference ($P<0.05$) between the protein content of samples obtained from various treatments. Other authors **(20)** mentioned a decrease of protein content when *Eulepida mashona* and *Henicus whellani* were scalded between 30 to 60 min (*E. Mashona*: 1.2-14.7%; *H.*

whellani 9.5-10.1%) but later noticed no change when these insects were toasted. The decrease was probably due to dissolution of proteins or disintegration and lost of conjunctive tissues in the form of colloidal constituents in boiling water. A reduction of crude protein was also mentioned in studied involving *Imbrasia belina***(20)** and *Hemijana variegata***(21)**. Some authors **(22)** did not find any significant difference in the protein content when comestible caterpillar, *I. epimethea* was thermally treated (scalding, scalding and sun drying). A reduction of the nitrogen content can also occur during thermal treatments due to loss of amides and amines **(23)** or formation of complexes with primary and secondary lipids oxidation products **(24)**. These results suggest that these effects were veiled by other dynamics such as loss of dry mater constituents, mainly lipids for fried and dried *M. subhyalinus*. Higher crude lipid levels after frying probably induce a proportional dilution of protein content. Very minute differences observed on the protein content between treatments may be due to the loss of small soluble fractions of proteins in the insect exudate. In fact, the increase of protein content was correlated to the crude fat loss ($r = -0.69$; $p < 0.001$). Though the protein content of insect samples was different between treatments, the digestibility of insect proteins may be modified by these treatments.

The main effects of independent or individual treatments (drying, toasting, scalding) contributed to the drop of the lipid content from 4.77%, 4.55% and 0.53% respectively compared to raw samples of *M. subhyalinus* and scalding treatments and the operation combining scalding/drying respectively reduced fats between 8.81 and 1.07% of *I. obscura* compared to the raw sample. However, during frying of *M. subhyalinus*, there was a gain of 1.62% of lipids and the combined treatments of scalded/fried *I. obscura* and scalded/toasted enabled gains of 16.87 and 40.49% respectively. In fact, compared to raw samples these results corroborate those of **Malaisse (18)** which estimated that the level of fat of flying and matured termites is between 42% and 53% with respect to dry matter. This lipid content is higher than most conventional protein food products like beef, chicken meat, mackerel, egg, and milk **(25)**. The loss of lipid during scalding may be due to melting of fat globules in boiling water. The drop of lipids content was positively correlated with the fat content of raw insects *M. subhyalinus* ($r = 0.57$; $p < 0.001$). All the same frying might have led to melting of fats that exuded following the concentration of tissues, meanwhile part of this may have been loss by thermal decomposition as described by **Knothe et Dunn (26)**. The reduction of the lipid content during drying suggests that certain fats may have been pierced near water vapour or may have been oxidated into other compounds **(26,28)**. According to **Guil-Guerrero *et al.* (29),** lipids of different insect species are made up of fatty acid profiles of different physicochemical varieties. This may partly explain the variability of lipids amplitude losses during thermal treatments. Comparing the lipid content of *M. subhyalinus* to values found in the literature, those obtained in this study were higher to the ones reported by **Kinyuru *et al*. (30)** who found values of 44.82 g / 100g of dry matter. The central role of termites (*M. subhyalinus*) as comestible insects owes its prestigious status from its fat (44.82 to 47.31 g/100 g) and protein content (33.51 to 39.74 g / 100 g) **(30,31)** compared to *I. obscura* which has higher protein content (55.53 – 62.16 g / 100 g). The decrease of ashes in boiled samples results from the solubilisation of these nutrients in water during cooking. This result corroborates those of **Hosseini *et al*. (32); Manditsera *et al.* (20)** who demonstrated losses of minerals by percolation during cooking of certain food products. Meanwhile the increase in toasted insects may be due to effects of concentration. It is contrary to conclusions drown by other authors **(33)**. They however justified this effect by contamination of samples by ashes during frying. There was no significant change in the ashes content when samples were subjected to drying, except *I obscura* which was particular.

Table 1: Physicochemical composition of raw and transformed insects (*M. subhyalinus* and *I. obscura*) (in (g/100 g of DM)

Treatments	Moisture content (%)	Crude proteins (Nx6.25)	Crude fats	Ashes	Sugars	Energetic values (Kcal/100 g of DM)
			M. subhyalinus			
Raw	**52.48±0.33^{d}**	**36.84±0.44^{b}**	**54.25±0.01^{c}**	**5.83±0.12^{b}**	**3.04±0.36^{c}**	**647.77±1.26^{c}**
Scalded	61.74±0.11^{e} *(+17.64)**	34.20±0.44^{a} *(-7.16)*	53.96±0.04^{b} *(-0.53)*	4.17±0.10^{a} *(-28.47)*	3.66±0.50^{d} *(+20.39)*	653.08±1.19^{d} *(+0.81)*
Fried	6.98±0.19^{c} *(-86.69)*	37.03±0.10^{b} *(+0.51)*	55.13±0.05^{d} *(+1.62)*	6.74±0.32^{c} *(+15.60)*	1.09±0.16^{a} *(-64.14)*	648.65±0.82^{c} *(+0.13)*
Toasted	1.40±0.14^{a} *(-97.33)*	38.72±0.00^{c} *(+5.10)*	51.78±0.03^{a} *(-4.55)*	7.17±0.03^{d} *(+22.98)*	2.28±0.03^{b} *(-25.00)*	630.02±0.23^{a} *(-2.74)*
Sun drying	4.92±0.03^{b} *(-90.62)*	38.57±0.39^{c} *(+4.69)*	51.68±0.24^{a} *(-4.73)*	6.49±0.02^{c} *(+11.32)*	3.30±0.34^{c} *(+8.55)*	632.6±1.02^{b} *(-2.34)*

			I. obscura			
Raw	**57.28±0.43^{c}**	**59.04±0.46^{c}**	**18.67±0.26^{b}**	**7.82±0.13^{b}**	**14.45±0.74^{b}**	**461.99±2.02^{b}**
Scalded	68.41±0.62^{e} *(+19.43)*	60.10±0.44^{d} *(+1.79)*	17.30±0.19^{a} *(-7.33)*	7.24±0.09^{a} *(-7.41)*	15.34±0.24^{c} *(+6.52)*	457.46±1.58^{a} *(-0.98)*
Scalded + fried	20.52±0.98^{b} *(-64.17)*	55.53±0.88^{a} *(-5.94)*	26.23±0.36^{d} *(+40.49)*	8.27±0.13^{c} *(+5.75)*	9.95±0.45^{a} *(-30.90)*	497.99±2.8^{d} *(+7.79)*
Scalded + roasted	65.19±1.03^{d} *(+13.8)*	57.67±0.26^{b} *(-2.32)*	21.82±0.54^{c} *(+16.87)*	7.13±0.05^{a} *(-8.82)*	13.36±0.48^{b} *(-7.22)*	480.50±2.36^{c} *(+4.01)*
Scalded + sun drying	7.28±0.02^{a} *(-87.29)*	62.16±0.41^{e} *(+5.28)*	18.47±0.02^{b} *(-1.07)*	8.80±0.09^{d} *(+12.53)*	10.56±0.38^{a} *(-26.66)*	457.11±0.92^{a} *(-1.05)*

III.2. Effects of treatments on the *in vitro* digestibility of proteins

Figures 4 and 5 show the digestibility of insect proteins before and after thermal treatments. Compared to the digestibility of raw and fresh insects which is higher, a significant ($p < 0.05$) reduction is noted for these parameters for treated insects. The digestion of casein protein was taken as a positive control to provide an indication of absolute efficiency of digestion.

Digestibility of isoptera (*M. subhyalinus*) (figure 4) after thermal treatment of fried samples (81.98%), toasted samples (80. 44%), scalded sample (81.10%) was significatively different ($p < 0.05$) from a sample to the other. Scalding treatments, scalding and fried, scalding and roasting on Lepidopteran *I. obscura* (figure 5) which had digestibility values of 79.65%, 80.26% and 79.77% respectively were not significatively different at 5% between the samples. Nevertheless, dried samples in the case of *M. subhyalinus* and scalded/dried in the case of *I. obscura* presented values which were significatively different ($p < 0.05$) compared to other treatments.

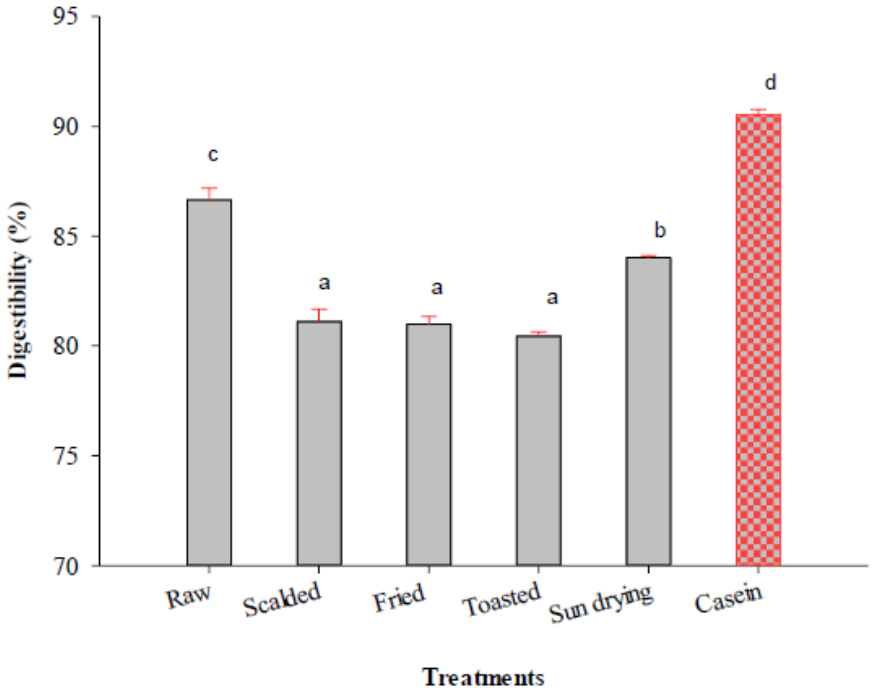

Figure 4: *In vitro* digestibility (%) of *M. subhyalinus* proteins

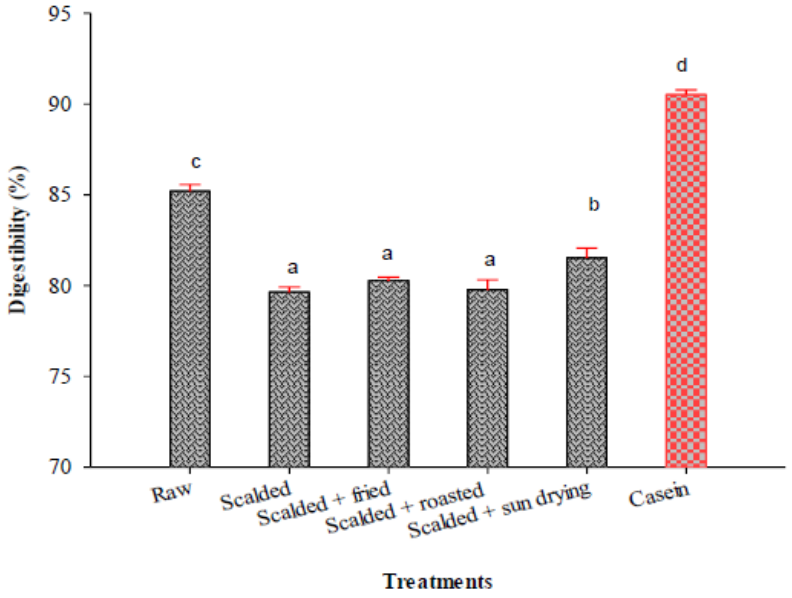

Figure 5: *In vitro* digestibility (%) of *I. obscura* proteins

Other studies have reported decrease of protein digestibility after treatment of comestible insects. It is the case witk boiled or fried sudanese tree crickets **(34),** for fried or roasted grasshopper and termites **(35)**, for oven roasted flour worms and domestic crickets **(36)**, for caterpillar and boiled cricket, boiled and toasted crickets (*Eulepida mashona*) **(20**). Depending on treatment conditions, heat can reduce or increase the digestibility of proteins. Exposition of samples to denaturing temperatures can increase the digestibility of native proteins by unfolding the peptide chains and by rending the protein more accessible to digestive enzymes **(37**). On the other hand, when proteins are exposed to a certain thermal treatment, the digestibility can be reduced due to the formation of disulphide bonds within the protein **(38,39)**. The present study shows that thermal treatments considerably reduce the digestibility of proteins, **Poelaert *et al.* (36)** reported a similar result. Nevertheless, these authors used higher temperatures (200°C) and the heating time were longer than those used in the present study. These factors increase the formation of disulphide bonds, protein oxidation and formation of Schiff base, by so doing favours aggregation of proteins and reducing the bio-accessibility of enzymes cleavage sites **(40)**.

Also, lipid oxidation products make complexes with proteins, leading to modification of chemical composition, the structure and the functionality of proteins.

Particularly, the digestibility of proteins is altered because the protein-lipid complexes are less sensible to enzymatic proteolysis **(41).** The thermal treatments can alter the protein structure by rupture of hydrogen and electrostatic bonds. Consequently, thermal denaturation, induces hydrophobic amino acids exposure at the surface of proteins, which most often are aromatic amino acids and long chain aliphatic amino acids can be produced **(42)**. This favours the tendency of protein to form aggregates.

Variable results on the effects of transformation on the digestibility of proteins may be due to the multiples differences linked to the variability of species. For examples, it has been reported that the presence of anti-nutrients that can link to proteins affect the digestion of proteins **(43)**. Moreover, **Nafisa *et al.* (44)**, reported that toasting and scalding of arboreal crickets increase the tannins and phytate content of entire insects, which then leads to a reduction of the *in vitro* proteins digestibility of scalded and fried samples. Apart from the digestibility, other properties of proteins can be affected by similar methods of treatment. **Babiker *et al.* (45)** studied functional properties of sudanese arboreal cricket proteins and they discovered that the protein solubility was considerable reduced after frying. This treatment reduced the digestibility of proteins. Another reason of differences of protein digestibility observed among the insect species may be the differences in the types of proteins **(46)**. **Jonas-Levi et Martinez (47**) also showed that certain insect proteins are not digestible by Man. It is the case of chitin which may interfere with the digestibility of proteins. The level of digestibility observed with fresh *M. subhyalinus* (86.65%) and fresh *I. obscura* is well compared to the values reported in the conventional sources of animal and plant proteins. **Bodwell *et al.*(48)** reported proteins digestibility values of 89% for entire beef, 90% for pork, 78% for turkey and 85% salmon. These results suggest that insect proteins are highly digestible.

III.3 Principal components analysis of nutritional properties with respect to the processing method

A Principal Component Analysis (PCA) which aim is to group cooking (processing) time that could have an influence on the nutritional properties was done. The PCA enabled the establishment of a grouping plan between constituents (dry matter, lipids, proteins, protein digestibility, and ashes). Figure 6 and 7 present the bidimensional distribution of cooked *I. obscura* and *M. subhyalinus* by diverse methods and their constituents which influences nutritional properties on F1 and F2 principal axes. It is noted that variable contributions for F1 principal axis formation like protein content, protein digestibility, and lipids content are highly associated with F1 axes. The distribution of constituents following F1 shows a distinction between this axis with respect to cooking time. It can be deduced that:

- Fried *M. subhyalinus* presents a maximum of proteins and ashes contents.
- Scalded and dried *I. obscura* presents a maximum of proteins content, protein digestibility and ashes.

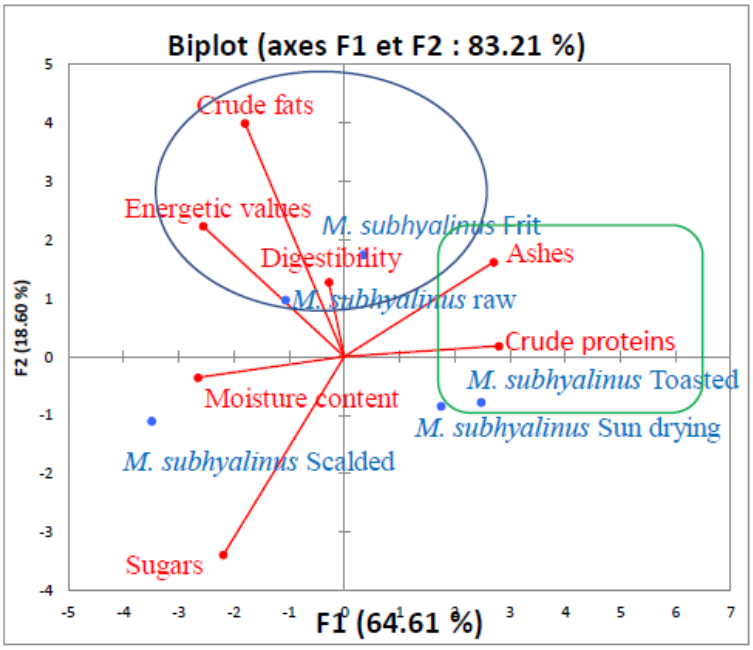

Figure 6: Bidimensional distribution of technological treatments of *M. subhyalinus* and their components on F1 F2 principal components coordinates

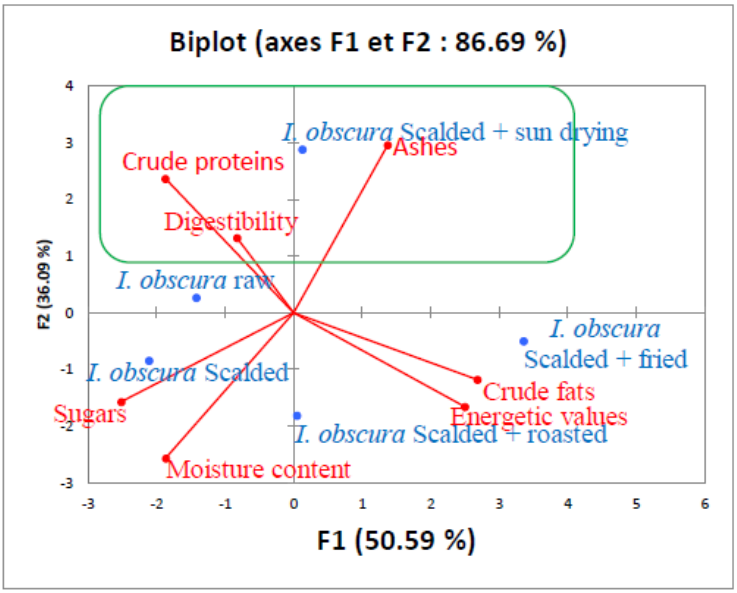

Figure 7: Bidimensional distribution of technological treatments of *I. obscura* and their components on F1 F2 principal components coordinates

It clearly appears that constituents like lipids, proteins, protein digestibility, energetic value and ashes which are the constituents of interests for nutritional analyses are well represented for fried and dried *M. subhyalinus* and for scalded and dried *I. obscura.* These treatments are retained as the best methods of insects transformation.

III.4. Effect of treatments on the microbiological quality of *M. subhyalinus* and *I. obscura*

Table 2 groups microbiological analysis results done on *M. subhyalinus* and *I. obscura* that were subjected to different treatments.

Table 2: Results of microbiological analyses on raw and transformed *M. subhyalinus* and *I. obscura* in $\log_{10}$ UFC/g

Samples en $\log_{10}$ UFC/g	**TAMF**	**Total coliforms**	**Feacal coliforms**	**Yeast and Moisture**	***Staphylococcus aureus***	**Anaerobic sulphide-reductors**	***Salmonella***
			M. subhyalinus				
Raw	5.76 ± 0.05^{c}	5.12 ± 0.02^{a}	6.08 ± 0.01^{b}	5.06 ± 0.04^{a}	4.52 ± 0.06^{a}	/	P
Scalded	3.59 ± 0.01^{b} (-37.67%)	/	/	/	/	/	A
Fried	3.09 ± 0.07^{a} (-46.35%)	/	/	/	/	/	A
Toasted	3.10 ± 0.02^{a} (-46.18%)	/	/	/	/	/	A
Dried	6.16 ± 0.02^{d} (+6.94%)	5.85 ± 0.02^{b} (+14.26%)	4.79 ± 0.01^{a} (-21.22%)	5.58 ± 0.03^{b} (+10.28%)	5.25 ± 0.02^{b} (+16.15%)	/	P
			I. obscura				
Raw	**8.32 ± 0.03^{e}**	**7.49 ± 0.02^{c}**	**6.56 ± 0.08^{d}**	**7.64 ± 0.13^{b}**	**6.51 ± 0.03^{e}**	**1.38 ± 0.12^{b}**	**P**
Scalded	3.66 ± 0.18^{c} *(-5.,01%)*	1.66 ± 0.06^{a} *(-7.83%)*	1.49 ± 0.02^{b} *(-77.28%)*	/	1.85 ± 0.05^{c} *(-71.71%)*	/	A
Scalded + fried	1.33 ± 0.08^{a} *(-8.,01%)*	/	/	/	1.51 ± 0.03^{a} *(-76.80%)*	/	A
Scalded + roasting	1.97 ± 0.03^{b} *(-7.32%)*	/	1.09 ± 0.02^{a} *(-83.38%)*	/	1.73 ± 0.005^{b} (-73.42%)	/	A
Scalding + Drying	6.88 ± 0.03^{d} *(-17.30%)*	$2.66\pm0,11^{b}$ *(-64.48%)*	2.50 ± 0.03^{c} *(-61.89%)*	2.75 ± 0.02^{a} *(-64.00%)*	2.04 ± 0.02^{d} *(-68.66%)*	0.38 ± 0.12^{a} *(-72.46%)*	P
Standard*	**< 5.48**	**< 3**	**< 3**	**< 3**	**< 2**	**< 1.48**	**Absent /25g**

Means in the same colon for each specie followed by the same capital letters are not significantly different *($p < 0.05$).* Values in brackets represent the increasing or decreasing percentages of parameters analysed with respect to raw samples; **A** :*Absence; **P** : Present*

*Microbiological security parameters recommended in European countries (according to (49))

The Total Aerobic Mesophilic Flora (TAMF) of raw and transformed products are presented in table 2. The TAMF determined on raw insects (5.76 and 8.32 Log UFC / g respectively for *M. subhyalinus* and *I. obscura*) is comparable to those of reported elsewhere by Klunder***et al.* (6), Vandeweyer *et al.* (16), Wynants *et al.* (50)** on comestible insects. The limit authorised by TAMF for comestible foods is 5.48 log UFC / g **(49)**. Consequently, raw *M. subhyalinus* and *I. obscura* are above the limits. This high level of contamination may be explained by the presence of microorganism in the intestines of insects.

Scalding, frying, toasting reduced the TAMF respectively by 2.17; 2.67 and 2.66 cycles logarithmic for *M. subhyalinus* and completely eliminated yeasts, moulds, coliforms, staphylococci, anaerobic sulphide reductors and *salmonella*. Similar results were reported for for boiled 5-10 min) *G. sigillatus***(16),** boiled (93-93 °C; 30 min) *I. belina* **(51)** and fried (10 min) *R. differens***(52).** Frying and toasting were more efficient than boiling to reduce the TAMF. These results are contrary to those of **Nyangena *et al.* (15)** who indicated that scalding was more efficient due to its adequate transfer of heat within tissues. However, they equally show differences in the reduction of TAMF, coliforms, staphylococci, yeasts and moulds within insect species (see table 2). This may be due to a combination of factors such as initial levels of contamination, and the size of the insect.

Yeasts and moulds for raw *M. subhyalinus* and *I. obscura* are respectively 5.6 log UFC / g and 6.51 log UFC / g (table 2). These values were higher by 1 to 3 logarithmic cycles than yeasts and mould reported in worms (*Alphitobius diaperinus*) from industrial breeding flours **(50),** But within the limits given for domestic

breeding crikects *G. sigillatus***(16)**. A significant difference was noted (P<0.05) between raw and dried samples. The later is more contaminated than the first. The raw and dried samples had quantities of yeast and moulds greater than the limit recommended by 3 Log CFU / g (**53**). Though yeasts known not to enhance food intoxication, some are mould strains are capable of producing mycotoxins having undesired consequences on human and animal health **(54,55).** Thermal treatments applied completely eliminated yeasts and moulds in *M. subhyalinus* sample and considerably reduced their values in *I. obscura.* There exists a positive correlation (r = 0.96) between treatments and elimination of yeasts and moulds.

Coliforms: The presence of germs in raw and dried insects might be from environmental non-hygienic conditions, the sol or poor handling as stipulated by **Ali *et al.*(56).** In dried insect products, destined to the preparation of animal feed, the total number of according to **KEBS (57)** must not be above 500 UFC/g. Elsewhere, a contamination by coliforms including *E. coli* was mentioned in butterfly caterpillar (not transformed) **(51,54)** and grasshoppers (**58**). Scalding, frying and toasting proceduces applied in the present study potentially eliminated coliforms. Other authors made similar observations on boiled of *I. belina* lava **(51)**, toasted *R. differens***(52),** boiled and toasted *A. domesticus, H. illucens, S. littoralis* and *R. differens***(15).**

Staphylococcus aureuswas present in all the raw samples (table2) with loads comprised between 4.52±0.06 log10 UFC / g for *M. subhyalinus* and 7.64±0.06 log10 UFC for *I. obscura.* These values are higher than the recommended upper limits 4 Log CFU / g **(53).** Housing manual collection, package and transport may be the causes of such high and a significant contamination by germs in *I. obscura* compared to *M. subhyalinus.* The treatment reduced the contamination levels from 0 to 0.9 Log UFC / g according to the insect specie and the treatment method. Scalding, frying, toasting considerably reduced *S. aureus* from 3.8 ; 4.5 to 4.4 logarithmic cycle in *I. obscura* and totally eliminated it in *M. subhyalinus.*

Salmonella :Microbiological recommendations for foodstuffs stipulate total absence of these germs in 25g of sample **(53,57,59)**. Data of table 2 show that compared to raw insects which are all contaminated with *Salmonella*, scalding, frying, toasting treatments contributed to destroy these germs. Nevertheless, dried products remain contaminated unlike raw ones. Similar results were brought out by **Nyangena *et al.* (15)** on *A. domesticus, H. illucens, S. littoralis et R. differens* samples. According to **Grabowski et Klein, (60),** the presence of Salmonella on dried insects was probably due to the resistant nature of this pathogen to dry heat or to a recontamination. Likewise, other authors mentioned the presence of Salmonella on dried insects treated thermally. It is the case of roasted *B. alcinoe* 5min) and dried under the sun light **(54),** Dried **g**rasshoppers under sun light and fried (15-20 min) **(58).** Recontamination resulting from a treatment and from poor hygienic handling was associated to these observations.

Clostridium sulphide-reducers are gastro-intestinal germs, soil and organic matter undergoing putrefaction. They are involved in food intoxication **(61).** *Clostridium sulphide-reducers* were present in raw *I. obscura* samples at a load of 1.38±0.12 log10 UFC. This value respects microbiological criteria within which processed meat products should obey (< 1.48 according to **Grabowski *et al.* (49)**). Scalding for 15min followed by sun drying of this sample for 48 hours contributed to significantly reduce its load by 72.46 %. Frying and roasting also contributed to completely eliminate these germs. These results suggest that contaminated and traded samples by *Clostridium sulphide-reducers* results from exogenous origin due to poor hygienic handling conditions and good preparation practices. This suggestion is in corroborates the finding of **Mbawala *et al.* (62)** for *kilishi* sold in the town of Ngaoundere. *Clostridium sulphide-reducers* were not found in *M. subhyalinus* samples

IV. Conclusion

Raw and dried *M. subhyalinus* and *I. obscura* are highly contaminated by microorganisms (TAMF, TC, FC, CSR, *S. aureus*, Salmonelle) and their microbial loads are greater than recommended values. Thermal treatments applied in this study enabled the reduction of microbial load to recommended values. Insect processing by frying and toasting one hand for *M. subhyalinus* and processing involving scalding and the process combining scalding and drying on the other hand for *I. obscura* are culinary methods which better preserve nutritional parameters. Protein digestibility of *M. subhyalinus* and *I. obscura* reduces when subjected to thermal treatments, compared to raw products which are more digestible.

References

[1]. **Baiyegunhi, L. J. S., Oppong, B. B., and Senyolo, G. M,. (2016). Mopane** worm **(Imbrasia belina)** and rural household food security in Limpopo province, South Africa. Food Security,8, 153-165. doi:10.1007/s12571-015-0536-8.

[2]. **Djouffa, K.M.L., Saïdou, C., ALI, A., Mohammadou, B. A., and Tchiegang, C., (2021).** Edible insects in the Adamawa and Eastern regions of Cameroon : collection, processing and consumption. Journal of Insects as Food and Feed, 7(4), 483-494. DOI 10.3920/JIFF2020.0015

[3]. **Van der Fels-Klerx, H.J., Camenzuli, L., Belluco, S., Meijer, N., Ricci, A,. (2018).** Food Safety Issues Related to Uses of Insects for Feeds and Foods. Comprehensive Reviews in Food Science and Food Safety, 17, 1172-1183. doi:10.1111/1541- 4337.12385.

[4]. **Mutungi, C., Irungu, F.G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., Ekesi, S., Fiaboe, K. K. M,. (2019).** Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. Critical Reviews in Food Science and Nutrition,59, 276-298. doi:10.1080/10408398.2017.1365330.

[5]. **Rumpold, B.A.; and Schlüter, O.K., (2013).** Nutritional composition and safety aspects of edible insects. Molecular Nutrition & Food Research,57, 802-823. doi:10.1002/mnfr.201200735.

[6]. **Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., and Nout, M. J. R,. (2012).** Microbiological aspects of processing and storage of edible insects. Food Control, 26, 628-631. doi:10.1016/j.foodcont.2012.02.013.

[7]. **AFNOR (Association Française de Normalisation). (1982).** Recueil des normes françaises des produits dérivés des fruits et légumes. Jus de fruits. 1ère éd., Paris la défense (France).

[8]. **AOAC. (1990).** Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edition. (The Association of Official Analytical Chemists, Arlington, 1990)

[9]. **Bourely, J. (1982).** Observation sur le dosage de l'huile des graines de cotonnier. Coton et Fibres Tropicales, 27(2), 183-196.

[10]. **AFNOR. (1984).** Recueil de normes françaises. Produits agricoles alimentaires : directives générales pour le dosage de l'azote avec minéralisation selon la méthode de Kjedahl. AFNOR, Paris (France).

[11]. **Devani, M.B., Shishoo, J.C.,. Shal, S. A., and Suhagia, B.N., (1989).** Spectrophotometrical method for determination of nitrogen in Kjeldahl digest. Journal of AOAC International, 72, 953-956.

[12]. **Stadlmayr B., Charrondiere R.U., Enujiugha N.V., Bayili G.R., Fagbohoun G.E., Samb B., Addy P., Barikmo I., Ouattara F., Oshaug A., Akinyele I., Amponsah A.G., Bomfeh K., EneObong H., Ifeyironwa F.S., Thiam I., and Burlingame B., (2012).** West African Food Composition Table. Publisher: Vialle delle Terme di Caracalla, 00153 Rome, Italy: p 57- 75.

[13]. **Hsu, H.W., D.L. Vavak, L.D. Satterlee, and G.A. Miller. (1977)**. A multienzyme technique for estimating protein digestibility. Journal of Food Science, 42(5), 1269 - 1273

[14]. **Maga, J. A., Lorenz, K., and Onayemi, O., (1973).** Digestive acceptability of proteins as measured by the initial rate *in vitro* proteolysis. Journal of Food Sciences, 38, 173.

[15]. **Nyangena., Dorothy, N., Mutungi, C., Imathiu, S., Kinyuru, John., Affognon, H., Ekesi, S., Nakimbugwe D., and Fiaboe. K K.M., (2020).** Effects of Traditional Processing Techniques on the Nutritional and Microbiological Quality of Four Edible Insect Species Used for Food and Feed in East Africa. Foods, 9, 574. doi:10.3390/foods9050574

[16]. **Vandeweyer, D., Wynants, E., Crauwels, S., Verreth, C., Viaene, N., Claes, J., Lievens, B., Van Campenhout, L,. (2018).** Microbial dynamics during industrial rearing, processing, and storage of tropical house crickets (Gryllodes sigillatus) for human consumption. Applied and Environmental Microbiology, 84, 00255-00218. doi:10.1128/aem.00255-18.

[17]. **Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., Ekesi, S., Nakimbugwe, D., and Fiaboe, K.K.M., (2018).** Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket Acheta domesticus (L.) and black soldier fly larvae Hermetia illucens (L.). Food Research International, 106, 420–427. doi:10.1016/j.foodres.2018.01.012.

[18]. **Malaisse F., (2004).** Human consumption of Lepidoptera, Termites, Orthoptera and Ants in Africa. In M. Malaisse F., Ressources alimentaires non conventionnelles.Tropicultura, SPE, pp. 30-36

[19]. **Banjo, A. D., Lawal O. A., and Songonuga, E. A., (2006).** The nutritional value of fourteen species of edible insects in southwestern Nigeria. African Journal of Biotechnology, 5 (3), 298-301.

[20]. **Manditsera, F.A.; Luning, P.A.; Fogliano, V.; and Lakemond, C.M.M., (2019).** Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. Food Research International, 121, 404–411. doi:10.1016/j.foodres.2019.03.052.

[21]. **Egan, B.A., Toms, R., Minter, L.R., Addo-Bediako, A., Masoko, P., Mphosi, M., and Olivier, P.A.S., (2014).** Nutritional Significance of the Edible Insect, Hemijana variegata Rothschild (Lepidoptera: Eupterotidae), of the Blouberg Region, Limpopo, South Africa. African Entomology, 22, 15–23.

[22]. **Lautenschläger, T., Neinhuis, C., Kikongo, E., Henle, T., and Förster, A,. (2017).** Impact of different preparations on the nutritional value of the edible caterpillar Imbrasia epimethea from northern Angola. European Food Research and Technology,243, 769–778, doi:10.1007/s00217-016-2791-0.

[23]. **Hosseini Bai, S., Darby, I., Nevenimo, T., Hannet, G., Hannet, D., Poienou, M., Grant, E., Brooks, P., Walton, D., and Randall, B., (2017).** Effects of roasting on kernel peroxide value, free fatty acid, fatty acid composition and crude protein content. PLoS ONE 12, e0184279, doi:10.1371/journal.pone.0184279.

[24]. **Lira, G.M., Barros Silva, K.W., Figueirêdo, B.C., and Bragagnolo, N., (2014).** Impact of smoking on the lipid fraction and nutritional value of seabob shrimp (Xiphopenaeus kroyeri, Heller, 1862). LWT Food Science and Technology, 58, 183–187. doi:10.1016/j.lwt.2014.02.057.

[25]. **Pyke M., (1979).** The science of nutrition. In: Success in nutrition. John Murray Ltd (Publishers) London. p.175.

[26]. **Knothe, G., and Dunn, R.O. A.,(2009).** Comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. Journal of the American Oil Chemists' Society, 86, 843–856. doi:10.1007/s11746-009-1423-2.

[27]. **Wu, T., and Mao, L., (2008).** Influences of hot air drying and microwave drying on nutritional and odorous properties of grass carp (Ctenopharyngodon idellus) fillets. Food Chemistry, 110, 647–653. doi:10.1016/j.foodchem.2008.02.058.

[28]. **Akonor, P.T., Ofori, H., Dziedzoave, N.T., Kortei, N.K., (2016).** Drying characteristics and physical and nutritional properties of shrimp meat as affected by different traditional drying techniques. International Journal of Food Science and Technology, 2016, 5p. doi:10.1155/2016/7879097.

[29]. **Guil-Guerrero, J.L., Ramos-Bueno, R.P., González-Fernández, M.J., Fabrikov, D., Sánchez-Muros, M.J., and Barroso, F.G., (2018).** Insects as Food: Fatty Acid Profiles, Lipid Classes, and sn-2 Fatty Acid Distribution of Lepidoptera Larvae. European Journal of Lipid Science and Technology, 120, 370-391. doi:10.1002/ejlt.201700391.

[30]. **Kinyuru, J. N., Konyole, S. O., Roos, N., Onyango, C. A., Owino, V. O., Owuor, B. O., Estambale, B. B., Friis, H., Aagaard-Hansen, J., and Kenji G. M., (2013).** Nutrient composition of four species of winged termites consumed in Kenya. Journal of Food Composition and Analysis, 30(2),120–124.

[31]. **Bukkens S. G. F., (1997).** The nutritional value of edible insects. Ecology of Food and Nutrition, 36(2-4), 287-319.

[32]. **Hosseini, H., Mahmoudzadeh, M., Rezaei, M., Mahmoudzadeh, L., Khaksar, R., Khosroshahi, N.K., and Babakhani, A., (2014).** Effect of different cooking methods on minerals, vitamins and nutritional quality indices of kutum roach (Rutilus frisii kutum). Food Chemistry, 148, 86–91. doi:10.1016/j.foodchem.2013.10.012.

[33]. **Madibela, O.R., Seitiso, T.K., Thema, T. F., and Letso, M., (2007).** Effect of traditional processing methods on chemical composition and in vitro true dry matter digestibility of the Mophane worm (Imbrasia belina). Journal of Arid Environments, 68, 492–500. doi:10.1016/j.jaridenv.2006.06.002.

[34]. **El Hassan, N. M., Hamed, S. Y., Hassan, A. B., Eltayeb, M. M., and Babiker, E. E,. (2008).** Nutritional evaluation and Physicochemiacla properties of boiled and fried tree locust. Pakistan Journal of Nutrition, 7(2), 325–329.

[35]. **Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., and Ayieko, M., (2010).** Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (Macrotermes subhyalinus) and grasshopper (Ruspolia differens). Food Bioprocess Technology, 3(5), 778–782.

[36]. **Poelaert, C., Beckers, Y., Despret, X., Portetelle, D., Francis, F., and Bindelle, J., (2016).**In vitro evaluation of fermentation characteristics of two types of insects as potential novel protein feeds for pigs. Journal of Animal Science, 94, 198–201. https://doi.org/10.2527/jas2015-9533

[37]. **Opstvedt, J., Nygard, E., Samuelsen, T. A., Venturini, G., Luzzana, U., and Mundheim, H,. (2003).** Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. Journal of the Science Food and Agriculture, 83, 775–782.

[38]. **Stanley, D. W., (1998).** Protein reactions during extrusion processing in extrusion cooking. In C. Mercier, P. Linko, and J. M. Harper (Eds.), St. Paul, MN, USA: American Association of Cereal Chemists, Inc pp. 321–341.

[39]. **Oria, M. F., Hamaker, B. R., and Shull, J. M,. (1995).** Resistant of sorghum and kafirins to pepsin digestion. Journal of Agricultural and Food Chemistry, 43, 2148–2153.

[40]. **Bax, M.-L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., and Santé-Lhoutellier, V., (2012)**. Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. Journal of Agricultural and Food Chemistry, 60(10), 2569–2576. https://doi.org/10.1021/jf205280y

[41]. **Hes, M., (2017).** Protein-Lipid Interactions in Different Meat Systems in the Presence of Natural Antioxidants – A Review. Polish Journal of Food and Nutrition Sciences, 67(1), 5-17. https://doi.org/10.1515/pjfns-2016-0024

[42]. **Davies, K. J. A., (1987).** Protein damage and degradation by oxygenradicals. I: General aspects. Journal ofBiological Chemistry, 262(20), 9895–9901.

[43]. **Caparros Megido, R., Poelaert, C., Ernens, M., Liotta, M., Blecker, C., Danthine, S., Francis, F,. (2018).** Effect of household cooking techniques on the microbiological load and the nutritional quality of mealworms (Tenebrio molitor L. 1758). Food Research International, 106, 503–508.

[44]. **Nafisa, M. E., Hassan, S. Y., Hamed, A. B., Hassan, M. M., & Elfadil, E. B., (2008).** Nutritional evaluation and physiochemical properties of boiled and fried tree locust. Pakistan Journal of Nutrition, 7(2), 325–329.

[45]. **Babiker, E. E., Hassan, A. B., and Eltayeb, M. M., (2007).** Solubility and functional properties of boiled and fried Sudanese tree locust flour as a function of NaCL concentration. Journal of Food Technology, 5(3), 210-214

[46]. **Bosch, G., Zhang, S., Oonincx, D. G., & Hendriks, W. H., (2014).** Protein quality of insects as potential ingredients for dog and cat foods. Journal of Nutritional Science, 3(29), 1–4.

[47]. **Jonas-Levi, A., and Martinez, J.-J. I,. (2017).** The high level of protein content reported in insects for food and feed is overestimated. Journal of Food Composition and Analysis, 62, 184-188.

[48]. **Bodwell, C. E., Satterlee, L. D., and Ree, L., (1980).** Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. American Journal of Clinical Nutrition, 33, 677–686.

[49]. **Grabowski, N.T., Ahlfeld, B., Lis, K.A., Jansen, W., and Kehrenberg, C., (2019).** The current legal status of edible insects in Europe. Berliner und Münchener Tierärztliche Wochenschrift, 132, 295–311

[50]. **Wynants, E., Crauwels, S., Verreth, C., Gianotten, N., Lievens, B., Claes, J., Van Campenhout, L., (2018).** Microbial dynamics during production of lesser mealworms (Alphitobius diaperinus) for human consumption at industrial scale. Food Microbiology, 70, 181-191. doi:10.1016/j.fm.2017.09.012.

[51]. **Ng'ang'a, J., Imathiu, S., Fombong, F., Ayieko, M., Vanden Broeck, J., and Kinyuru, J., 2019.** Microbial quality of edible grasshoppers Ruspolia differens (Orthoptera: Tettigoniidae): From wild harvesting to fork in the Kagera Region, Tanzania. Journal of Food Safety, 39, e12549. doi:10.1111/jfs.12549.

[52]. **Todd, E.C.D., (2004).** Microbiological safety standards and public health goals to reduce foodborne disease. Meat Sciences, 66, 33-43. doi:10.1016/S0309-1740(03)00023-8.

[53]. **Gashe, B.A.; Mpuchane, S.F.; Siame, B.A.; Allotey, J.; Teferra, G. 1997.** The microbiology of Phane, an edible caterpillar of the emperor moth, Imbrasia belina. Journal of Food Protection, 60, 1376-1380. doi:10.4315/0362-028x- 60.11.1376.

[54]. **Braide, W., Oranusi, S., IUdegbunam, I., Oguoma, O., Akobondu, C., and Nwaoguikpe, R,. (2011).** Microbiological quality of an edible caterpillar of an emperor moth, Bunaea alcinoe**.** Journal of Ecology and the Natural Environment, 3(5), 176-180

[55]. **Shigehisa, T., Nakagami, T., Taji, S., and Sakaguchi, G., (1985).** Destruction of salmonellae, Escherichia coli, and Staphylococcus aureus inoculated into and onto beef during dry-oven roasting. Japanese Journal of Veterinary Research, 47, 251257. doi:10.1292/jvms1939.47.251.

[56]. **Ali, A., Mohammadou, B. A., Saidou. C., Aoudou, Y., Tchiegang, C., (2010).** Physico-chemical proprieties and safety of Grasshoppers, important contribution to food security in the far North region of Cameroon. Research Journal of Animal Sciences, 4(5), 108-111. doi:10.3923/rjnasci.2010.108.111.

[57]. **KEBS., (2017).**Dried Insect Products for Compounding Animal Feeds - Specification KS 2117: ICS 65.120; Kenya Bureau of Standards: Nairobi, Kenya, p. 9.

[58]. **Kukier, E., Goldsztejn, M., Grenda, T., Kwiatek, K., and Bocian, L., (2013).** Microbiological quality of feed materials used between 2009 and 2012 in Poland. Bulletin of the Veterinary Institute in Pulawy, 57, 535-543.

[59]. **Mpuchane, S., Gashe, B.A., Allotey, J., Siame, B., Teferra, G., Ditlhogo, M,. (2000).** Quality deterioration of phane, the edible caterpillar of an emperor moth Imbrasia belina. Food Control, 11, 453-458. doi:10.1016/S0956- 7135(00)00010-4.

[60]. **Grabowski, N.T.; and Klein, G,. (2017).** Microbiology of processed edible insect products - Results of a preliminary survey. International Journal of Food Microbiology,243, 103-107. doi:10.1016/j.ijfoodmicro.2016.11.005.

[61]. **Joffin, C., and Joffin, J.N., (1985)**. Microbiologie Alimentaire. Ed. CRDP (Centre Régional de Documentation Pédagogique), Bordeaux, p. 45.

[62]. **Mbawala, A., Daoudou, B., et Ngassoum, M.B., (2010).** Qualité microbiologique du kilishi (produit carné séché) produit dans la ville de Ngaoundéré (Cameroun) Tropicultura, 28(3), 153-160.

DJOUFFA KOUABITHEU Marivaux Lesage, et. al. "Effect of culinary treatments on the nutritional and microbiological properties of *Macrotermes subhyalinus* and *Imbrasia obscura*: two insects consumed in the Adamawa and Est regions of Cameroon."*IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 15(9), (2021): pp 35-46.