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Nutritional, functional properties and estimated shelf-life of defatted *Rhynchophorus phoenicis* (Fabricius, 1801) larvae flours

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ABSTRACT

Rhynchophorus phoenicis (Fabricius, 1801) is an insect of the coleopteran family widely consumed in Africa and particularly in Cameroon. Their dehydrated larvae are rich in lipids and proteins that can help in combating malnutrition. The present work aimed at producing low-lipid content larvae flours of *R. phoenicis* and to determine their nutritional and functional properties as well as their estimated shelf-life. The flour was obtained by the cooking-pressing process. The shelf-life of the flour was estimated using adsorption isotherms. The results showed that the flours obtained had low lipids (8 g/100 g of flour) and high protein content (69 g/100 g of flour). Their water and oil absorption capacities, swelling power, foaming properties and foam stability make them good ingredients for food formulations. As regards of storage capacity, the adsorption isotherms obtained can be modelled by the Guggenheim-Anderson-and-de-Boer (GAB) and Brunauer-Emmett-and-Teller (BET) equations. The shelf-life was estimated at 300 days when flour is packed in polyethylene bag and stored at 20°C in enclosure with a relative humidity of 90%. In short, the cooking pressing process makes it possible to obtain flours of high nutritional and technological values. The resulting flours can be used as ingredients to fortify food with poor proteins content.

Keywords: Cooking-pressing, food security, adsorption isotherm, entomophagy.

RESUME

Rhynchophorus phoenicis (Fabricius, 1801) est un insecte de la famille des coléoptères largement consommé en Afrique et particulièrement au Cameroun. Ses larves déshydratées sont riches en lipides et en protéines qui peuvent aider à la lutte contre la malnutrition. Le présent travail a pour but la production de farine délipidée de larves de *Rhynchophorus phoenicis* et la détermination de leurs propriétés nutritionnelles, fonctionnelles ainsi que de leur durée de vie estimative. La farine a été obtenue par le procédé de cuisson-pressage. L'estimation de la durée de vie s'est faite en utilisant les isothermes d'adsorption. Les résultats montrent que les farines obtenues ont des faibles teneurs en lipides (8g/100g de farine) et des teneurs élevées en protéines (69 g/100g de farine). Leurs capacités d'absorption d'eau, d'huile, les pouvoirs gélifiant, moussant et la stabilité des mousses en font de bons ingrédients pour les formulations alimentaires. En ce qui concerne la capacité de stockage, les isothermes d'adsorption obtenus peuvent être modélisés par l'équation de Guggenheim-Anderson-and-de-Boer (GAB) et celle de Brunauer-Emmett-and-Teller (BET). La durée de vie estimée est de

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300 jours quand les farines sont emballées dans des sacs en polyéthylène et stockées dans des enceintes d'humidité relative de 90%. En sommes, le procédé de cuisson-pressage permet d'obtenir des farines de larves de *R. phoenicis* à hautes valeurs nutritionnelles et technologiques. Les farines qui en résultent peuvent être utilisées pour fortifier les aliments pauvres en protéines.

Mots clés : Cuisson-pressage, sécurité alimentaire, isothermes d'adsorption, entomophagie.

INTRODUCTION

The world's ever-growing population will reach 10 billion by 2050 with this, food demand will increase by 70% (Zielińska et al., 2015). The current dietary challenges of quantitative and qualitative intake of animal proteins and micronutrients will be increasingly acute. In order to meet this demand for food, men, in particular industrialists, have introduced techniques for intensifying and diversifying livestock production. This industrialization of livestock production leads to the large-scale supply of animal protein. However, this type of farming poses many environmental and health problems, including the occupation of increasingly growing areas, deforestation and the release of greenhouse gases, all of which lead to the rapid destruction of the ozone layer. We should also note the very high water consumption, the conflicts between herders and farmers and finally the competition between animals and people for increasingly scarce food resources. In addition, there are also risks of zoonotic diseases and wildlife infections (Van Huis, 2014). All these constraints have led to the searches for alternative sources of animal proteins for human consumption. It has emerged that between 1900 and 2500 species of insects are edible in the world, and occupy an important place in the diets of the people of Asia, Africa and Latin America (Vantomme and Halloran, 2013). The latter are rich in lipids, proteins and minerals (Womeni et al., 2012; Gbangboche et al., 2016; Fogang, 2018; Badanaro et al., 2021). Because of their accessibility, rapid growing and richness in macronutrients, insects are consumed as a substitute for high-cost meat and fish proteins in emerging countries. They can improve the diet of vulnerable populations (Van Huis, 2014).

In Cameroon, insects are consumed throughout the country (Balinga, 2003). The best known are grasshoppers, locusts, termites, ants and beetles (Fogoh et al., 2015). Of all these insects, only the *Rhynchophorus phoenicis* (Fabricius, 1801) larvae are consumed by almost all tribes in the seven regions of southern Cameroon (Balinga, 2003). Fresh larvae are rich in water, lipids and proteins (Ekpo and Onigbinde, 2005; Womeni et al., 2012; Fogang et al., 2018). Once larvae are dehydrated, their macronutrients levels are doubled or tripled (Womeni et al., 2012; Fogang et al., 2018). This makes these larvae interesting for human consumption. They can thus be consumed as such or incorporated into foods in the form of flours to increase their nutritional value. The latter, which are easily transportable, also have less risk of degradation, which lengthens their shelf-life. Works have been carried out on the production of flour from *R. phoenicis* larvae. However, the flours obtained were pasty and high in lipids (71.62 ± 7.61 g/100g) (Fogang, 2018), which is likely to cause high oxidation and may increase browning reaction thus reduce lysine activity. Thus, the aim of this work was to produce flour from *Rhynchophorus phoenicis* larvae with a reduced lipid content by the cooking-pressing method and to determine some nutritional and functional properties as well as their estimated shelf-life.

MATERIALS AND METHODS

Material

White larvae of *Rhynchophorus phoenicis* (Fabricius, 1801) were purchased in Ayos, in the Centre Region of Cameroon. They were transported in a wicker basket with their

food to the IRAD Central Laboratory of Entomology and Agro-Food Technology laboratory in Nkolbisson for identification and treatment respectively. Work was done with the larvae 24 hours after collection.

Methods

Determination of morphometric parameters of larvae of Rhynchophorus phoenicis

Morphometric parameters of larvae sacrificed by freezing at -26°C including mass, length, abdominal width, and cephalic capsule length were measured on larvae sacrificed by freezing at -26°C. Measurements of the length, width and circumference of the abdomen were carried out using an ITimo electronic slide foot, and the mass was measured using a RADWAG balance. The measures were made on frozen larvae because they were more precise than the measures on the living one.

Production of larvae flours of Rhynchophorus phoenicis by cooking-pressing

The larvae flours of *Rhynchophorus phoenicis* (Fabricius, 1801) with reduced lipid content were obtained according to the modified fish flour protocol described by Linder et al. (2004). The *R. phoenicis* larvae were washed with running water and then sacrificed by freezing at -26°C for 24 hours in a SANYO freezer. The sacrificed larvae were deiced, washed and then boiled. They were then cooled and ground using a Sylver Crest blender. The crushes were pressed until the cooking juice stopped flowing. The press cakes were then dried on stainless steel trays at 50°C. In a Panasonic oven up to constant weight and then ground in a HOME FLOWER blender. The resulting flour was packed in 80 micrometres thick high density polyethylene and stored away from light and heat in a cupboard at 25°C for further use.

Determination of proximal composition of larval flours of Rhyncophorus phoenicis

The water content was determined by the A.O.A.C. method (1996). The lipid and total nitrogen contents were determined by the AOAC method (1980) and the protein mass obtained by multiplying the nitrogen content by the coefficient 6.19 (Fogang et al., 2018). Total carbohydrate (G) was obtained by difference.

Determination of functional properties of low-fat flours

Determination of density

The densities of the samples were evaluated by the Kinsella method (1976). Fifty grams of flour sample were introduced into a 100 mL measuring cylinder. The measuring cylinder was continuously compacted until a constant volume of flour was obtained. The density (D) in g/mL was calculated as the mass (m) of the flour (g) divided by the volume (v) of the flour after compaction (mL) according to equation (1):

$$D = \frac{m}{V} \qquad (1)$$

With, m= mass of powder; v= settlement volume (mL); D = density (g/mL).

Determination of water absorption capacity and oil absorption capacity

The water and oil absorption capacities of the samples were determined by the method described by Womeni et al. (2012). One gram of flour was mixed with 10 ml of distilled water or sunflower oil for 30 seconds. The mixture was incubated at 20°C for 30 min and then centrifuged in a HIMAC CT15E centrifuge at 13 600 g for 10 min at 25°C. The supernatant was decanted and drained at 45°C for 20 min. The volume of water or oil absorbed was divided by the test sample to obtain the water or oil absorption capacity. Water absorption capacity (WAC) and oil absorption capacity (WAC) are given by the equation (2):

$$CAE\ ou\ CAH(mL/g) = \frac{Vi-Vf}{M} \qquad (2)$$

Where:

Vi: initial volume of water or oil introduced into the tube (mL)

Vf: volume of oil or water collected after drainage (mL)

M: test sample (g)

Determination of the swelling capacity of flours

It was determined using the method described by Leach et al. (1959) with modification for small samples. One gram of flour was mixed with 10 mL of distilled water

in a centrifuge tube and heated to 80°C for 30 min. This was continuously agitated during the heating period. After heating, the suspension was centrifuged at 280 g for 15 min. The supernatant was decanted and the weight of the pellet weighed. The swelling ca pacity was calculated by Equation 3.

$$G = \frac{P_c}{P_f} \quad (3)$$

With: Pc = pellet weight (g)
Pf = flour weight (g)
G = swelling capacity (g/g)

Determination of foaming power and foam sta bility

The foaming power and the stability of the foam were determined by the method described by Lin et *al*. (1976). In a 100 mL sa mple, 2 g of flour sample were added and then 50 mL of distilled water at 30±2°C. The susp ension is mixed and stirred to form the foam a nd the volume of foam after 30 seconds is not ed. Foaming power (Pm) was expressed as a percentage of increase in initial foam volume. The foam was left at rest for 1 h to determine its stability (Sm) as a percentage of the initial volume of the foam. The foaming power and t he stability of the foams are given by equations 4a and 4b.

$$P_m = \frac{V_1 - V_0}{V_0} \times 100 \quad (4a)$$

$$S_m = \frac{V_1 - V_2}{V_1} \times 100 \quad (4b)$$

With: Pm=foaming power, Sm= Foam stabilit y, V0=volume before stirring (mL),
V1 = volume after stirring (mL) and V2 = vol ume after rest for one hour (mL).

Determination of adsorption isotherms of lo w lipid content Rhynchophorus phoenicis flours

Adsorption isotherms were determined by the modified static gravimetric method of Chuzel and Zakhia (1991). The samples were dried beforehand at 60°C on phosphorus pentoxyde for 10 days to reduce their water co ntent to a minimum value and the mass denoted M_1. The second step consisted in prep aring sulfuric acid solutions at different concentrations. The acid solutions used, their water activities and working temperatures are presented in Table 1. The solutions were introduced into desiccators which were filled at a quarter. A sample tripod was deposited in each desiccator and then glass crucibles containing 100 mg of flour were deposited on the sample holder. The desiccators were place d in ovens adjusted to the desired temperature (20, 30, 40°C) which are the average temperat ures in some cities of Cameroon. To determine the achievement of equilibrium, the samples were weighed every two days until the changes in mass between two successive measurements became less than 1%. The time interval to remove the sample from desiccator, weigh it and re-introduce it was less than one minute to minimize the effect of desiccator opening on the results. The steam mass was measured after dehydration at 102°C for 48 hours in a Panasonic forced convection oven, denoted M_2.

Each determination was made 3 times. The equilibrium moisture content of insect powders, expressed as g/g dry matter (Me), was calculated using the equation 5 as follows:

$$Me = \left(\frac{M2 - M1}{M1}\right) \quad (5)$$

Mathematical Modelling

To model the adsorption curves, five models from the literature were used and are presented in appendix B.

Statistical validity of the model

The statistical validity of the model w as evaluated using 3 statistical parameters:

- The coefficient of determination R^2 is one of the first criteria for predicting the best equation describing the adsorption isotherm.

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(Mcal - M)^2}{\sum_{i=1}^{N}(\bar{M} - M)^2} \quad (6)$$

- The relative average error (EMR. /MRD)

$$MRD = \frac{1}{n}\sum_{i=1}^{1}\left|\frac{(M - Mcal)}{M}\right| \quad (7)$$

- The standard error of the water conte nt of the product (EST or SE)

$$SE = \sqrt{\frac{\sum_{i=1}^{n}(M-Mcal)}{df}} = \sqrt{\frac{RSS}{df}} \qquad (8)$$

Where

M: Equilibrium water content

Mcal: Predicted water content from the model studied

df: Degree of freedom of the model

$\bar{M}$: Average water content

Determination of the isosteric heat of adsorption

Isosteric heat was estimated using adsorption curves obtained at different temperatures and the Clausius Clapeyron equation (Labuza and Altunakar, 2008).

$$\ln(aw) = \frac{-qst}{RT} + \frac{Sd}{R} \qquad (9)$$

Where

Sd is differential entropy ($J.mol^{-1}. K^{-1}$);

qst is the net isosteric heat of sorption ($J.mol^{-1}$)

G is Gibbs free energy ($J.mol^{-1}$)

R is the constant of the perfect gases (8.314 $J. mol^{-1}. K^{-1}$)

T is the temperature (K^{-1}) and aw is water activity.

The net isosteric heat (qst) and the differential entropy of adsorption were calculated by plotting the graph ln(aw) as a function of 1/T, for a specific water content and determining the slope of the curve (qst/R) and the ordinate at the origin (Sd/R). This procedure is repeated for several water content values to determine the dependence of Sd and qst with the water content.

Estimated life of the delipid flours of Rhynchophorus phoenicis larvae

The life expectancy of the defatted flours of *Rhynchophorus phoenicis* larvae in polyethylene bags was estimated using the Heiss and Eichner equation (1971).

$$ts = \frac{\ln\left(\frac{Me-Mi}{Me-Mc}\right)}{Ks\left(\frac{A}{W}\right)\left(\frac{Po}{S\varepsilon}\right)} \qquad (10)$$

Where

ts is the potential life of the product (time in days required for the packaged product to degraded due to microbial and biochemical deterioration with loss of sensory quality);

A is the surface of the package (m^2);

W is the weight of the product (dry basis, Kg);

P_0 is the vapour pressure at the storage temperature (Pa);

S is the slope of the sorption isotherms (assuming that the interval between Xe and Xc is linear);

Ks is the permeability of the package (kg H20 µm/ m^2/day/Pa)

ε is the thickness of the package (microns)

Me is the equilibrium water content (g/100g MS);

Mc is the safe water content for storage (g/100 g MS);

Mi is the initial water content of the matrix during packaging (g/100g MS);

The packaging used was high density polyethylene available at a lower cost in Cameroon. The safety water content was equal to the water content of the product at a water activity of 0.7 and the highest relative humidity was stopped at (90%). The packaging used was 0.1474 m^2, with a permeability of 0.000155 Kg of water micrometers/m^2.Pa.jr and a product mass of 200 g (Chuzel and Zakhia, 1991).

Statistical analyses

The analysis was carried out in triplicate and the results were presented as an average ± standard deviation. The results of the physical, nutritional and functional analyzes were subjected to the Analysis of Variance (ANOVA) and Duncan test was used to compare the averages at the 0.05 probability threshold using Microsoft Excel software. The adsorption isotherm curves were plotted using Microsoft Excel 2013 and the model parameters were obtained using the solver function of Microsoft Excel 2013. The models were considered statistically valid when the R^2 coefficient of determination ≥ 0.9, the Relative Average Error ≤ 15% and the standards Errors ≤ 1%.

Table 1: Water activity based on temperature and concentrations of H_2SO_4 solutions (Perry and Green, 1997).

Diluted sulphuric acid (Kg. kg^{-1})	Water activity		
	20°C	30°C	40°C
0,20	0,878	0,873	0,878
0,25	0,816	0,817	0,824
0,30	0,749	0,747	0,753
0,35	0,665	0,666	0,674
0,40	0,568	0,565	0,574
0,45	0,458	0,461	0,470
0,50	0,355	0,355	0,366
0,55	0,258	0,260	0,267
0,60	0,167	0,170	0,178
0,65	0,093	0,097	0,102
0,70	0,043	0,045	0,049

RESULTS

Morphometric parameters of larvae used and production yield of larvae flour

The morphometric parameters of the la rvae of *Rhynchophorus phoenicis* (Fabricius, 1801) studied were mass, length of the cephalic capsule, length and width of the abdomen. This study showed that the average length of a cephalic capsule is 7.80 ± 0.88 mm, the average mass of a larva is 2.08 ± 0.80 g with an average abdominal length of 28.6 ± 4.4 mm and an average width of 10.2 ± 1.6 mm.

Chemical characterization of larval flours of *Rhynchophorus phoenicis*

The proximate composition of larvae flour showed low amounts of moisture (7.07±0.02 g/100g of flour), lipids (8 ±0.11 g/100 g of flour), ash (2.58 ± 0.20 g/100 g of flour) and carbohydrate (12±1g/100 g of flour) content. On the contrary, the protein content was high (69.4 ± 0.1 g/100 g of flour).

Functional properties of larval flours of *Rhynchophorus phoenicis*

The functional properties evaluated in this work were the bulk density, water and oil absorption capacity, swelling power, foam capacity and stability. The bulk density of *Rhynchophorus phoenicis* (Fabricius, 1801) larvae flours was 1.52 ± 0.10 g/mL. The water absorption capacity (WAC) and the oil absorption capacity (OAC) were respectively 1.90 ± 0.02 mL/g and 2.58 ± 0.01 mL/g. As for the swelling capacity, it value was 3.83 ± 0.01 g/g of flour. When hydrated, they occupy almost 4 times their initial volume. This corroborates the results obtained from water absorption capacity of the flours (1.74 ± 0.01 mL/g). The foaming power of larvae flours of *R. phoenicis* was 5.84± 0.03% and the foam stability was 67.78 ± 0.03%.

Adsorption isotherms of larvae flours of *Rhynchophorus phoenicis* and modeling

The adsorption isotherms of *Rhynchop horus phoenicis* (Fabricius, 1801) larvae flours are shown in Figure1. These flours were stored in enclosures of relative humidity ranging from 1% to 90% at temperatures of 20, 30 and 40°C. Hygroscopic equilibrium was obtained after an average of 10 days. Each isotherm has 3 parts: a first part with water activity from 0 to 0.2; in

this interval, the equilibrium water content of the flours increases progressively with the increase in water activity and decreases with the increase in storage temperature. A second part whose water activity varies between 0.3 and 0.7. In this area, the equilibrium water content of flours varies little with increasing water activity. The water is in a freer form than in the first part. And a third part with water activity between 0.8 and 1. In this interval, the water contents of the flour increase rapidly with the increase in water activity. On the first parts of the curves, Van der Waals forces are very intense, partially preventing the molecular movement from being expressed by a saturated vapour pressure. The water is in a bound state, "rigid" and the hydrogen bonds are very strong. The second part is the site of intermediate water activities, where adsorption takes place at less active sites. In the third part, linkage forces are weaker and water is in freer form active sites.

Choice and validation of the best model

The adequacy of the experimental results (water content = f (aw)) with the models tested was evaluated by calculating the following statistical coefficients: the coefficient of determination (R^2), the relative average error (RAE) and the standard error (EST). Table 3 shows the values of statistical parameters used to judge the validity of the models used. Figure 2 show the adjustment of the test points by the BET and GAB models.

The storage behavior of the flours can be modeled at 20°C by the BET and GAB equations. At 30°C, it can be described by the GAB model. Finally, at 40°C, OSWIN, PELEG, SMITH, BET and GAB models can be used to predict their storage behavior. These models have the largest coefficients of determination (R^2) (≥90) combined low with RME ≤ 15% and TSE ≤ 1%. Indeed, large coefficient of determination values, low TSE and RME values mean that the model can explain variations in experimental data (Basu et al., 2006). In general, the GAB and BET models are those which best describe the behaviour of flours at the three storage temperatures studied. These two models also make it possible to determine the water content of the monomolecular layer M_0. The water content of the monomolecular layer of *Rhynchophorus phoenicis* larvae flours were determined from the GAB equation. The water contents of the monomolecular layer vary between 1.5 and 2.5 g/100 g of dry matter. Most dried foods have their greater stability at water content close to the water content of the monomolecular layer.

Isosteric heat of adsorption

The GAB equation was used to determine the net isosteric heat of sorption, adsorption entropy and estimated shelf-life of flour. Figure 3 shows the net isosteric heat of adsorption of *Rhynchophorus phoenicis* larvae flour at different initial water levels. The net isosteric heat of adsorption decreases sharply with increasing water content to 5 g/100 g dry matter. It indicates the energy released when water is attached to the adsorption sites. This is the binding energy or intermolecular force between water vapour and the surface of the adsorbent (Touil et al., 2015).

Approximate shelf life of *Rhynchophorus phoenicis* larvae flours

The Heiss-Eichner model (Heiss and Eichner, 1971) estimated the shelf-life of *Rhynchophorus phoenicis* larvae flours based on storage conditions. 200 g of flour were packed in polyethylene with permeability Ks=0.000155 Kg water, m/m².Pa.jr, surface S=0.1474 m², thickness ε=80 micrometres and stored at a relative humidity of 90%. The critical water activity was set at 0.7. The initial moisture content ranged from 0.6 to 6 g/100 g dry matter. The approximate shelf-lives of the flours are shown in Figure 6. In general, the life spans of the flours decrease with increasing storage temperature and initial water content. The lifetime of *R. phoenicis* varied from 140 to 309 days for temperatures of 40°C and 20°C.

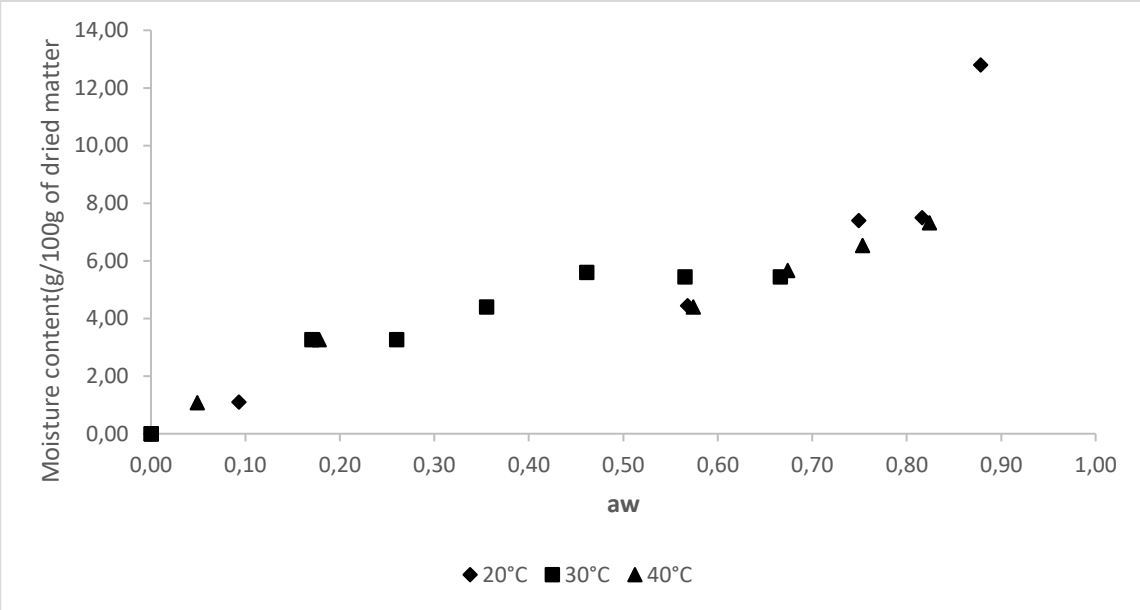


Figure 1: Effect of storage temperature on adsorption isotherms of *Rhynchophorus phoenicis* larvae flour.

Table 2: Adsorption isotherm models and validity areas (Labuza and Altunakar, 2008).

Models	Equations of model	Range of validity	Sources
BET	$M = \frac{MoCaw}{(1-aw)(1-aw+Caw)}$	0.05 - 0.5	(Brunauer, Emmett et Teller, 1938)
Smith	$M = A + Blog(1 - aw)$	0.3–0.9	(Smith, 1947)
Oswin	$M = A(\frac{aw}{1-aw})^B$	0.05– 0.9	(Labuza et al., 1971)
GAB	$M = \frac{MoCKaw}{(1-Kaw)(1-Kaw+CKaw)}$	0.05– 0.95	(Van den Berg, 1984)
Peleg	$M = K1aw^{n1} + K2aw^{n2}$	0,75	(Peleg, 1993)

In the BET and GAB models, Mo represents the water content of the monolayer and C represented the energy constant related to the net heat of adsorption.
In the GAB model, K is the energy constant associated with the multilayer
In the Peleg model, K1, K2, n_1, and n_2 are constants
In the Oswin model, A and B are constants.
In Smith's model, A and B are constants

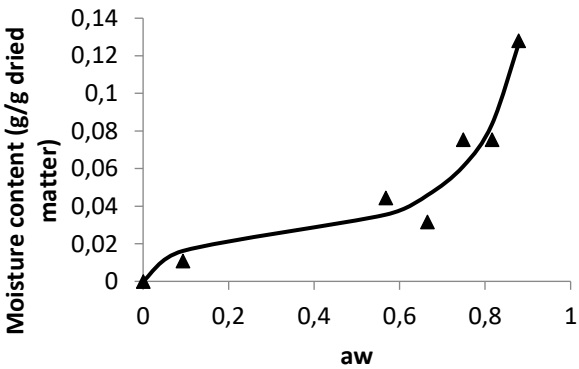
Table 3: Validation statistics and model constants studied.

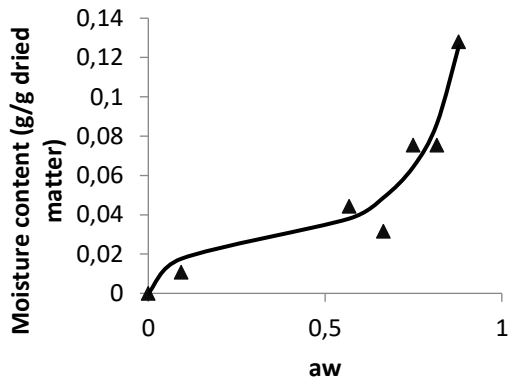
MODELS	R^2	EMR (%)	EST	M0	C	A	B	C1	C2	K
20°C										
BET	0,9	14,6	0,012	1,5	200					
GAB	0,9	11,8	0,012	1,7	200					0,99
PELEG	0,9	22,2	0,015			0,18	3,09	3,43	107,19	
OSWIN	0,9	15,7	0,012			0,75	0,03			

SMITH	0,9	21,6	0,017			-0,01	-0,13			
30°C										
BET	0,8	21,5	0,025	1,9	200					
GAB	0,8	13,2	0,025	2,5	200					0,94
PELEG	1	28,4	0,030			0,17	-5,81	1,56	98,79	
OSWIN	0,8	16,5	0,026			0,51	0,05			
SMITH	0,8	16,9	0,026			0,01	-0,14			
40°C										
BET	0,9	14,0	0,009	1,5	200					
GAB	0,9	15,4	0,007	2,1	200					0,89
PELEG	0,9	6,9	0,008			0,08	-16,55	0,61	54,45	
OSWIN	1	8,2	0,005			0,34	0,04			
SMITH	0,9	11,5	0,006			0,02	-0,08			

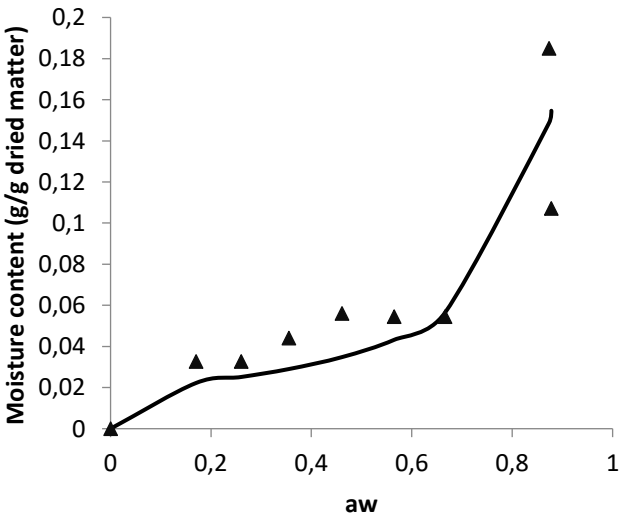
BET **GAB**

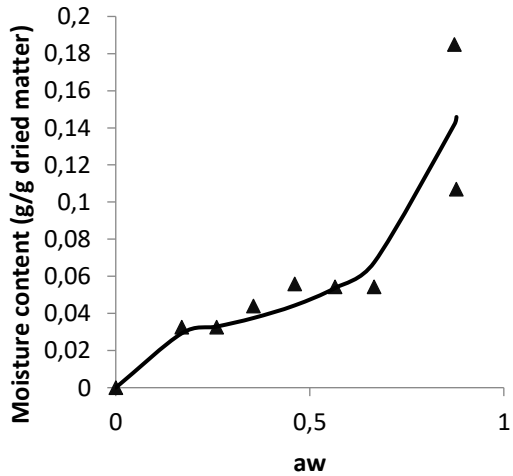
20°C





30°C





40°C

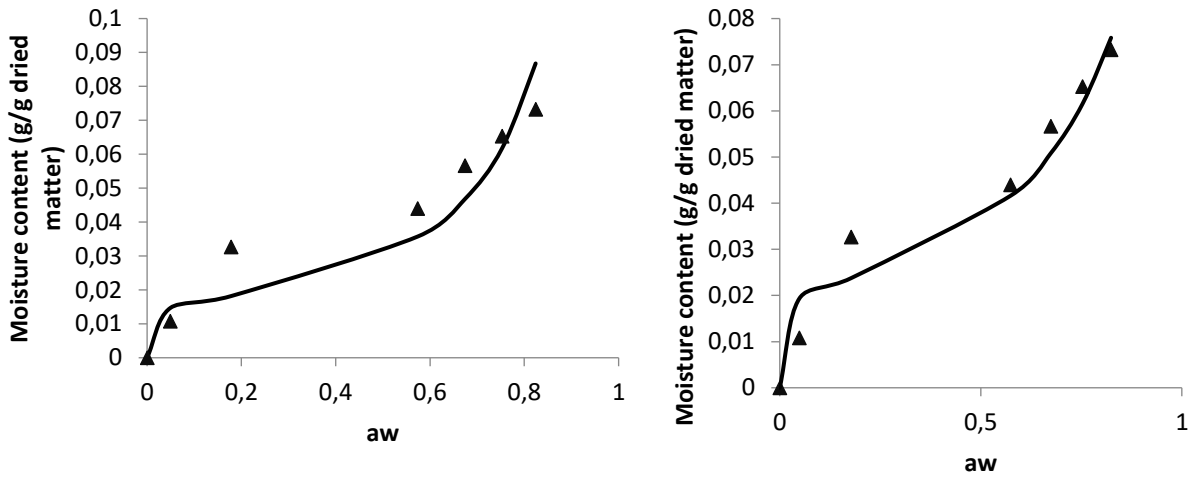


Figure 2: Adjustment by BET and GAB model.

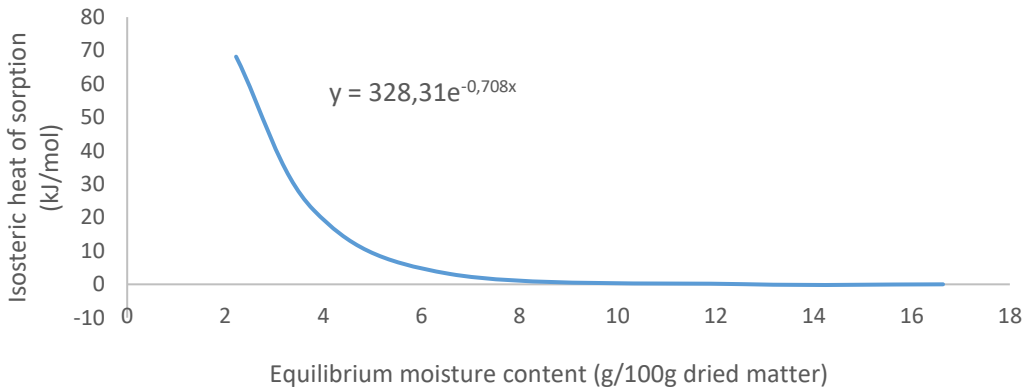


Figure 3: Change in the net isosteric heat of adsorption of *Rhynchophorus phoenicis* larvae flours as a function of water content.

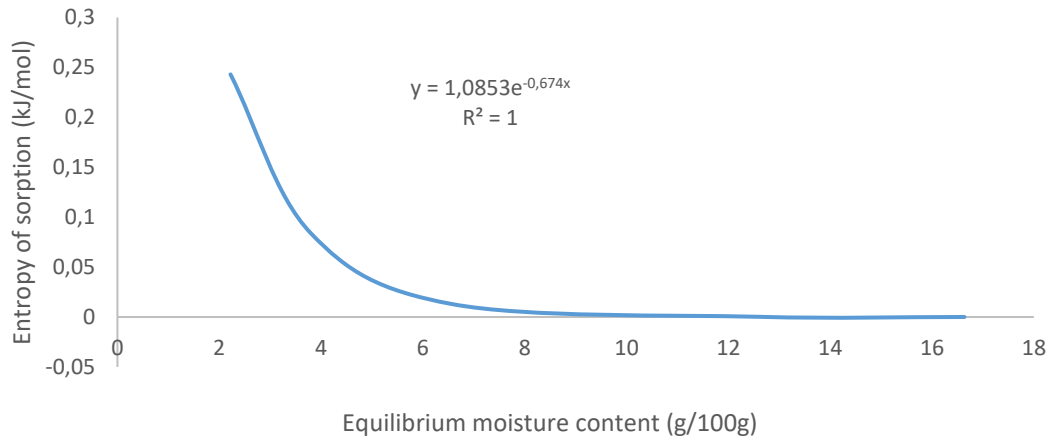


Figure 4: Variation of entropy of adsorption of larvae flours of *Rhynchophorus phoenicis* as a function of water content.

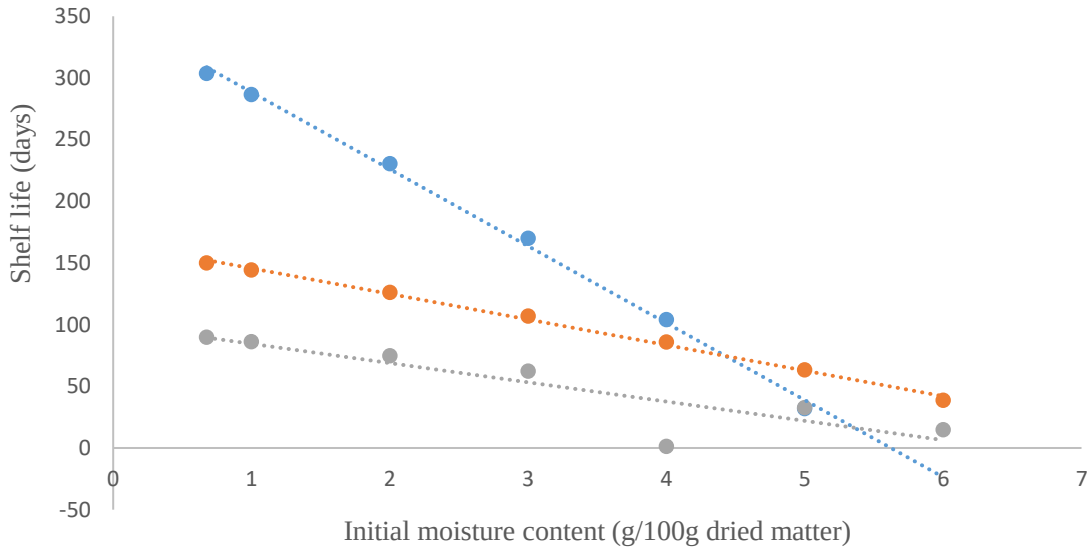


Figure 5: Estimated shelf-life of *Rhynchophorus phoenicis* larvae flours based on initial water content and storage temperature.

DISCUSSION

The morphometric parameters of *Rhynchophorus phoenicis* larvae give indication on their development stage. Those of this study were smaller than those obtained by Lenga et al. (2012) (mean weight 7.33 ± 0.57g, mean length 49.5 ± 2.12 mm, mean width 24 ± 1,73 mm and cephalic capsule length 11 ± 0,97 mm) but close to those found by Omotoso and Adedire (2007) for young *R. phoenicis* larvae respectively 6.5 ± 0.04 mm, 2.6 ± 0.1 g, 25.4 ± 0.04 and 7.7 ± 0.04 mm (for cephalic capsule length, mass, the length and width of the abdomen respectively). This suggests that the larvae used in this study were in their early stage of development. According to Fogang et al. (2018), they could be rich in lipids since his work revealed a positive relationship between morphometric parameters and lipid content.

Proximate analysis of the studied flour showed low water and lipid but high protein contents. The water content of *R. phoenicis* flour (7.07±0.02 g/100g of flour) obtained in this study was lower than that found by Omotosso and Adedire (2007) for young dried larvae of *R. phoenicis* (11.94%) and higher than the one of *Imbrasia oyemensis* flours (5.56%) (Diomande et al., 2017). It is below the maximum limit (12%) for the development of microorganisms (Linder et al., 2004) and is consistent with that recommended for fish flour (10%) (Ifremer, 2008). The water content of food products is one of the parameters that should be taken in consideration for foods preservation. As a reaction medium, water is the site of chemical, biochemical and enzymatic reactions (Belitz et al., 2009) leading to food degradation. Low moisture content is desirable in food as it limit food degradation (Omotosso and Adedire, 2007). These results suggest that the cooking-pressing process used in this study could be used as a method of conservation and/or transformation of *R. phoenicis* larvae.

As for the lipid content (8 ±0.11 g/100 g of flour), it is lower than those found by Womeni et al. (2012), Fogang et al. (2018) and Ukwo et al. (2021) respectively 66 g/100 g of flour, 71 g/100 g of flour and 50 g/100 g of dry matter. These differences could be explained by the fact that those authors did not extract fat from the ground larvae, as it was done in the

present work. Reducing the lipid content of flour has two main advantages: lower caloric energy and reduced lipid oxidations (Okaraonye and Ikewuchi, 2008). This leads to a limitation of the deterioration of the flours by oxidations. The cooking-pressing process thus reduced the lipid content of larvae flours of *R. phoenicis* below the lipid content of fish flour (10%) (Ifremer, 2008).

Concerning crude proteins content, the value recorded in this study (69.4 ± 0.1 g/100 g of flour) was higher than that obtained by Okaraonye and Ikewuchi (2008) (44.3 g/100g of lipid free dry matter) and Ukwo et al. (2021). However, it is close to that of Womeni et al. (2012) (67.1 ±0.3 g/100 g of lipid-free dry matter) for the lipid-free flours of *R. phoenicis* larvae. The protein content of the flour from this study is also higher than that of Diomandé et al. (2017) for *Imbrasia oyemensis* flour (33.57 g /100 g of flour). The protein content of the flours studied is comparable to that of vegetable concentrated (65-90%) (Guéguen et al., 2016), and makes them classified as high in protein. These larvae flours of *R. phoenicis* could therefore be used as an ingredient in the formulation of foods that help combat protein deficiency.

The ash content obtained in this study (2.58 ± 0.20 g/100 g of flour) was lower than those presented by Ekpo and Onigbinde (2005), Okaraonye and Ikewuchi (2008), Womeni et al., (2012) respectively 17.4 g/100g, 16.7 g/100g and 5.9 g/100g of the defatted flours and Ukwo et al. (2021) (5.8, 3.8 and 5.6 g/100 g dry matter respectively for young larvae, late larvae and adult flours). However, these values are closed to those obtained by Fogang et al. (2017) and Omotosso and Adedire (2007) with whole flours of *R. phoenicis*, namely 2.8 g/100g of dried matter and 2.4% of the mass of young dried larvae respectively. The ash content reflects the mineral richness of the food and suggests that larvae meal from *R. phoenicis* could be a source of minerals. It would therefore be interesting to make a qualitative and quantitative analysis of the minerals present in this flour to better appreciate its potential.

The proportion of carbohydrates are lower than those obtained by Womeni et al. (2012) and Okaraonye and Ikewuchi (2008) respectively 17.6 and 49.9 g per 100 g of defatted flour. However, they are higher than those found by Ukwo et al. (2021) for the flours of young *R. phoenicis* larvae (9.9 g/100 g dry matter).

Functional properties are physicochemical properties of an ingredient that affects its process ability in food systems. The bulk density of the *R. phoenicis* larvae flour of this study (1.52 ± 0.10 g/mL) was greater than that obtained by Womeni et al. (2012) for flours of *R. phoenicis* (0.25 to 0.52 g/ml), independently of the cooking and preservation processes applied by the latter. It is also higher than that of *Imbrasia oyemensis* (0.54±0.01 g/cm^3) (Diomande et al. 2017). This density is nevertheless close to the result of El Hassan et al. (2008) with cooked flours of *Tettigonia viridissima* (1.5 g/mL). The cooking-pressing process could therefore make it possible to obtain high density flours. Density is a very important parameter for packaging and transport requirements in food industry but also in the formulation of food. The bulk density of this flour could imply that less quantity of the food samples could be packed in constant volume ensuring an economical packaging. It nutritionally promotes well digestibility especially among children who have immature digestive system diseases (Shittu et al., 2005). The high densities of *R. phoenicis* larvae flours would therefore make them good ingredients for infant feeding.

The WAC of the studied flour (1.90 ± 0.02 mL/g) was lower than those obtained by Womeni et al. (2012) which ranged from 2.25 mL/g to 3.25 mL/g, for grilled and boiled larvae of *R. phoenicis* respectively. This WAC capacity was also lower than that of boiled and fried flours of *Tettigonia viridissima* respectively 2.93 and 2.47 mL/g (El Hassan et al., 2008). The WAC obtained in this work was however closed to that of Ekpo (2010) which was 1.27 mL/g for *R. phoenicis* larvae and that of *Imbrasia oyemensis* flour (1.59 mL/g) (Diomande et al. ,2017) for. The WAC of a flour indicates the degree of hydration of the

proteins. It depends on the nature of the amino acids and the conformation of the proteins (Belitz et al., 2009). The water absorption capacities of the studied flours show that they could therefore be easily incorporated into aqueous food formulations (Ekpo, 2010).

Larvae flours of *Rhynchophorus phoenicis* from this study had a lower oil absorption capacity (OAC) (2.58 ± 0.01 mL/g) than those obtained by Womeni et al., (2012) for flours of *R. phoenicis*. This OAC is however higher than that found by El Hassan et al., (2008) for boiled grasshoppers (*Tettigonia viridissima*) (1.0 mL/g). The OAC of these flours assumes that they could have a strong palatability, present pleasant texture and flavours to the palate that promote food pleasure since lipids acts as flavour sensors.

The swelling of the flours is linked to the capacity of the flour constituents to fix water and therefore to the hydrophilic nature of the constituents. Larvae flour of *Rhynchophorus phoenicis* had a swelling capacity of 3.83 ± 0.01 g/g of flour. When hydrated, they occupy almost 4 times their initial volume. This corroborates the results obtained from water absorption capacity of the flours (1.74 ± 0.01 mL/g).

The foaming power of the studied flour was greater than that of *Imbrasia oyemensis* and *Thunnus albacares* fish flours respectively (2.21 ± 0.08%) and (4.65 ± 0.01) (Diomande et al., 2017). This value is less than that found by Akpossan et al. (2015) for *Imbrasia oyemensis* fat free flour (18 ± 2.37%). The flours in this study have more stable foams than the whole and defatted flours of *I. oyemensis* which are 27.19 ± 0.98% (Akpossan et al., 2015) and 2.31 ± 0.01% (Diomande et al., 2017) respectively. In many foods, proteins act as foaming agents and foam stabilizers (Belitz, 2009). The foaming capacity of a flour is related to the nature and structure of the proteins in the flour, while the stability of the foam is related to the ability of the flour to cope with gravitational and mechanical stresses (Diomande et al., 2017). The high foaming capacity of *R. phoenicis* larvae flour may be due to the low presence of globular proteins that are less resistant to surface tension (Belitz et al., 2009). Since foam is used to improve the texture, consistency and appearance of foods, the foaming power and high foam stability of *R. phoenicis* larvae flour could make it a good ingredient in food formulations.

The adsorption isotherm of *R. phoenicis* larvae flour was evaluated at 20, 30 and 40°C. All of the have a characteristic appearance of type II isotherms which is a sigmoid shape obtained in the presence of multilayers at the internal surface of the flours but, also in the presence of soluble substances in the matrix studied (Labuza and Altunakar, 2008). The overall appearance of isotherms is similar to that obtained by Fogang (2018) for whole flours of *Rhynchophorus phoenicis* larvae stored at 40°C and for *Imbrasia truncata* and *Imbrasia epimethea* flours stored at 20, 30 and 40°C. This similarity with the isothermal appearance of the studied flour may be due to the fact that the flours of these caterpillars have high protein contents (62.9 and 68.6 g/100g dried matter of *Imbrasia truncata* and *Imbrasia epimethea* respectively) as the flours of this study (69.35 g/100g of flour) since the nutritional content influence the shape of isotherms.

Figure 1 shows the adsorption isotherms of *Rhynchophorus phoenicis* larvae flours at 20, 30 and 40°C. It revealed that water activity increased with increasing water content. Many authors (Fogang, 2018; Kamau et al., 2018) found an increase in the equilibrium water content with increased water activity and decreased storage temperature. For Touil et al. (2015), if water activity is kept constant, an increase in temperature results in a decrease in the amount of water adsorbed. This observation indicates that the adsorption force of water molecules increases with increasing temperature.

The water contents of the monomolecular layer is the moisture content when all the ionic and polar groups of the adsorbent have been occupied by water molecules. The one of this study (1.5 and 2.5 g/100 g of dry matter) are lower than those found by Fogang (2018) for whole flours of *R. phoenicis* larvae (5.6, 4.5 and 3.3 g/100g of dried matter at 20, 30 and 40°C respectively).

They are however close to those of black soldier flies larvae (*Hermetia illucens*) 2.6 g/100 g at 35°C, and compare well with those of other foods that are rich in protein (Kamau et al., 2018). The Moisture content of the monomolecular layer is the moisture content for utmost stability of a food/feed material. At this very low moisture content, chemical reactions that depend on solvation are expected to be rather slow but deteriorations arising from lipid phase reactions such as oxidative rancidification may be enhanced.

The net isosteric heat of adsorption decreases with increasing water content. They have the same appearance as locust flours (*Acheta domesticus* (L)) and black soldier fly larvae (*Hermetia illucens*) (Kamau et al., 2018); decreasing sharply with increasing water content to 5 g/100 g dry matter. The isosteric heat of adsorption indicates the energy released when water is attached to the adsorption sites. This is the binding energy or intermolecular force between water vapour and the surface of the adsorbent. The increase in isosteric heat at low water contents indicates the high water binding energy characteristic of adsorption on the monomolecular layer. Maximum values of the adsorption enthalpy indicate that the most rigid binding sites and the greatest water-solid interactions are covered (Kamau et al., 2018). This results in coverage of less favourable sites and the formation of multilayers (Oliveira et al., 2009). Decrease in isosteric heat with increasing water content indicates progressive weakening of water-solid interactions. At equilibrium moisture content of about 8 g/100 g, isosteric heat of adsorption approached zero meaning that total isosteric heat of sorption approximated latent heat of vaporisation of water. Thus above 8 g/100 g equilibrium moisture, water existed in free liquefied form. Such water can support profuse microbial, chemical and biochemical deterioration

The entropy of adsorption of *Rhynchophorus phoenicis* larvae flour decreased with increasing moisture content (Figure 4). It can be associated with binding or repulsive forces in the system as well as spatial arrangements at the water-adsorbent interface. It characterizes or defines the degree of order or disorder existing in the water-adsorbent system and assists in the interpretation of processes such as dissolution, crystallization and swelling (Oliveira et al., 2009). This decrease in entropy reflects the molecular disorder that occurs when water is attached to flour. Thermodynamic parameters such as isosteric heat and differential adsorption entropy determine the end point or final level at which food must be dehydrated to obtain a stable product with an optimal water content and to achieve the minimum amount of energy required to remove a specific amount of water from the food.

The lifetimes of *R. phoenicis* larvae flour (309 to 140 days maximum for temperatures of 20 and 40°C respectively) are shorter than those of locust and black soldier flies larvae flours found by Kamau et al., (2018) that is about 400 days maximum for each of these two flours. Flour from *R. phoenicis* larvae can therefore be stored for long periods when well dried and stored below 40°C. However, the life expectancy estimate was made by neglecting the permeability of the package to oxygen and carbon dioxide which may be responsible for oxidation.

Conclusion

The aim of this study was to determine the nutritional, functional, thermal characteristics and the shelf-life of *Rhynchophorus phoenicis* larvae flours obtained by cooking-pressing, and it is appeared that the production yield of these flours (25%) is close to that of fish flour. With regard to nutritional properties, cooking-pressing makes it possible to obtain flours rich in proteins (69 g/100 g of flours) with a reduced lipid content (8 g/100 g of flours). Their moisture content (8 g/100 g of flour) are lower than the maximum limits for fish flour. These flours can be considered as sources of protein and can be used to combat protein deficiencies in the diet of children and other vulnerable people. As regards functional properties, the flours obtained have high densities (1.17 g/mL) and are therefore easily transportable. Their ability to absorb water, oil and swell, and the

foaming power and stability of these foams suggest that these flours can be used as an ingredient in many food formulations. Finally, the storage behaviour of *R. phoenicis* larvae flour showed that flour adsorption isotherms resembled type II isotherms. Equilibrium moisture content decreased with increasing storage temperature. These equilibrium moisture contents increased with cooking temperature. The most appropriate models for predicting these water levels were BET and GAB. These models have made it possible to determine the moisture contents of the monomolecular layer (between 1.3 and 2.5 g/100 g of dry matter) under different storage conditions. The shelf-life of these flours was estimated at 300 days when they were packed in polyethylene and stored at 20°C in a 90% relative humidity enclosure.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

NNSEN did the conception of the project, the conduction of analysis, the writing of the manuscript. RAMF revised the project and the manuscript. He also helped in data analysis. PM and GK supervised the project.

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REFERENCES

A.O.A.C. 1996. *Official Methods of Analysis of AOAC International* (16th edn). Association of Official Analytical Chemists, Gaithersburg MD: USA.

Akpossan RA, Digbeu YD, Koffi MD, Kouadio JPEN, Due AE, Kouamé PL. 2015. Protein fractions and functional properties of dried *Imbrasia Oyemensis* larvae full fat and defatted flour. *International Journal of Biochemistry Reasearch and Review*, **5**(2): 116-126. DOI: http://dx.doi.org/10.9734/IJBCRR/2015/12178

Badanaro F, Hoekou Y, Gbogbo KA, Amevoin K, Amouzou K. 2021. Microbiological analysis of the *Cirina forda* (Lepidoptera: Saturniidae) commercialized in North Togo. *Int. J. Biol. Chem. Sci*., **15**(1): 34-40. DOI: http://dx.doi.org/10.4314/ijbcs.v10i3.6

Balinga P. 2003. Contribution des insectes à la sécurité alimentaire. Rapport FAO, département des forêts.

Basu S, Shivhare US, Mujumdar AS. 2006. Models for Sorption Isotherms for Foods. A Review. *Drying Technology*, (24): 917–930. DOI: https://doi.org/10.1080/07373930600775979

Belitz H D, Grosch W, Schieberle P. 2009. *Food Chemistry* (4th edn). Springer: Berlin Heidelberg. DOI : 10.1021/ed2007372

Diomande M, Koko CA, Kouame BK. 2017. Propriétés physicochimiques et fonctionnelles des farines de chenilles (*Imbrasia oyemensis*) et de poisson (*Thunnus albacares*). *Current Research Journal of Biological Sciences*, (3): 189-194.

Ekpo KE, Onigbinde AO. 2005. Nutritional Potentials of the Larva of *Rhynchophorus phoenicis*. *Pakistan Journal of Nutrition*, **4**(5): 287-290. DOI: http://dx.doi.org/10.3923/pjn.2005.287.290

Ekpo KE. 2010. Nutrient composition, functional properties and anti-nutrient content of *Rhynchophorus Pheonicis* (F) Larva. *Annals of Biological Research*, (1): 178-190. DOI: http://dx.doi.org/10.4316/fens.2021.003

El Hassan NM, Hamed SY, Hassan AB, Eltayeb MM, Babiker EE. 2008. Nutritional Evaluation and Physicochemical Properties of Boiled and Fried Tree Locust. *Pakistan Journal of Nutrition*, **7**(2): 325-329. DOI:

http://dx.doi.org/10.3923/pjn.2008.325.329

Fogang MAR, Kansci G, Viau M, Ribourg L, Fogoh M J, Hafnaoui N, Le Gall P, Genot C. 2017. Growing conditions and morphotypes of African palm weevil (*Rhynchophorus phoenicis*) larvae influence their lipophilic nutrient but not their amino acid compositions. *Journal of Food Composition and Analysis*, (69): 87–97. DOI: https://doi.org/10.1016/j.jfca.2018.02.012

Fogang MAR. 2018. Étude de quelques propriétés nutritionnelles de trois insectes consommés au Cameroun en vue de leur valorisation alimentaire. Thèse de doctorat, Université de Yaoundé 1, Yaoundé, p.291.

Fogoh JM, Gnetegha AA, Gall P, Levang P. 2015. *Exploitation, trade and farming of palm weevil grubs in Cameroon*. Bogor, Indonesia: Centre for International Forestry Research Content. DOI: http://dx.doi.org/10.17528/cifor/005626

Gbangboche AB, tognibo BAC, Kayode P, Zannou, Codjia JTC. 2016. Croissance et valeur alimentaire des larves de *Oryctes monoceros. Int. J. Biol. Chem. Sci*., **10**(3): 983-992. DOI: https://dx.doi.org/10.4314/ijbcs.v15i1.4

Guéguen J, Walrandb S, Bourgeois O. 2016. Les protéines végétales : contexte et potentiels en alimentation humaine. *Cahiers de Nutrition et de Diététique*, (51) : 177-185. DOI : https://doi.org/10.1016/j.cnd.2016.02.001

Heiss R, Eichner E. 1971. Moisture content and shelf life 11. *Food Manufacture*, (46): 37-42.

IFREMER. 2008. La farine de poisson et autres produits d'origine aquatique. Fiche aquaculture du site web aquaculture. 8p.

Kamau E, Mutungi C, Kinyuru J, Imathiu S, Tanga CAH, Fiaboe KKM. 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: http://dx.doi.org/10.1016/j.foodres.2018.01.012

Kinsella JE. 1976. Functional properties of protein foods. *Critical Review of Sciences and Nutrition*, (1): 219-229. DOI: https://doi.org/10.1002/jctb.280340307

Labuza TP, Altunakar B. 2008. *Water Activity Prediction and Moisture Sorption Isotherms: Water Activity in Foods Fundamentals and Applications*. Blackwell Publishing and the Institute of Food Technologists: Iowa; 109-154. DOI: https://doi.org/10.1002/9781118765982.ch7

Leach HW, Mc Cowen LD, Schoch TJ. 1959. Structure of the starch granules. In: swelling and solubility patterns of various starches. *Cereal Chemistry*, (36): 534 – 544. DOI: https://doi.org/10.4236/ojas.2015.52022

Lenga A, Mafute KC, Kinkela T. 2012. Conservation et étude de la valeur nutritive des larves de *Rhynchophorus phoenicis* (Curculionidae) et *Oryctes rhinoceros* (Scarabeidae), deux coléoptères d'intérêt alimentaire au Congo-Brazzaville. *Int. J. Biol. Chem. Sci*., **6**(4): 1718-1728. DOI: http://dx.doi.org/10.4314/ijbcs.v6i4.28

Lin M J, Humbert ES, Sosulski FW. 1976. Certain functional properties of sunflower meal products. *Journal of Food Sciences*, (39): 368-370.

Linder M, Fanni J, Parmentier M. 2004. Extraction, fractionnement et concentration des huiles marines. *Oléagineux, Corps Gras, Lipides*, **2** (11): 123-30. DOI : http://dx.doi.org/10.1051/ocl.2004.0123

Okaraonye CC, Ikewuchi JC. 2008. *Rhynchophorus phoenicis* (F) larva meal: nutritional value and health implications. *Journal of Biological Sciences*, **8**(7): 1221-1225. DOI: http://dx.doi.org/10.3923/jbs.2008.1221.1225

Oliveira EG, Rosa GS, Moraes MA, Pinto LAA. 2009. Moisture sorption characteristics of microalgae *Spirulina platensis*. *Brazilian Journal of Chemical Engineering*, **1**(26): 189–197. DOI: http://dx.doi.org/10.1590/S0104-66322009000100018

Omotoso OT, Adedire CO. 2007. Nutrient composition, mineral content and the solubility of the proteins of palm weevil, *Rhynchophorus phoenicis* (F). (Coleoptera: Curculionidae). *Journal of Zhejiang University Science B*, **8**(5): 318-322. DOI: http://dx.doi.org/10.1631/jzus.2007.B0318

Shittu TA, Sanni LO, Awonorin SO. 2005. Use of multivariate techniques in studying flour-making characteristics of some Cassava Mosaic Disease resistant cassava clones. *African Crop Science Con Proceeding*, (7): 621–630.

Touil A, Litaiem J, Zagrouba F. 2015. Isothermes de sorption et propriétés thermodynamique de l'*Allium sativum*. *Journal of the Tunisian Chemical Society*, (17): 105-114.

Ukwo S, Udo M, Ekong E. 2021. Nutrient content of *Rhynchophorus phoenicis* and its Application in complementary food Formulation. *Food and Environment Safety*. **1**(20): 21–34. DOI: http://dx.doi.org/10.4316/fens.2021.003

Van Huis A, Itterbeeck J, Van Klunder H, Mertens E, Halloran A, Muir G, Vantomme P. 2014. *Edible Insects. Future Prospects for Food and Feed Security*. Food and Agriculture Organization of the United Nations: Rome.

Womeni HM, Tiencheu B, Linder M, Nabayo MC, Tenyang N, Tchouanguep F, Parmentier M. 2012. Nutritional Value and Effect of Cooking, Drying and Storage Process on Some Functional Properties of *Rhynchophorus Phoenicis. International Journal of Life Science and Pharma Research*, **2** (3): 203–219.

Zielińska E, Baraniak B, Karaś M, Rybczyńska K, Jakubczyk A. 2015. Selected species of edible insects as a source of nutrient composition. *Food Research International*, **77**: 460–466. DOI: http://dx.doi.org/10.1016/j.foodres.2015.09.008